Manual of **Intrauterine Insemination** and **Ovulation Induction**

Edited by
Richard P. Dickey
Peter R. Brinsden
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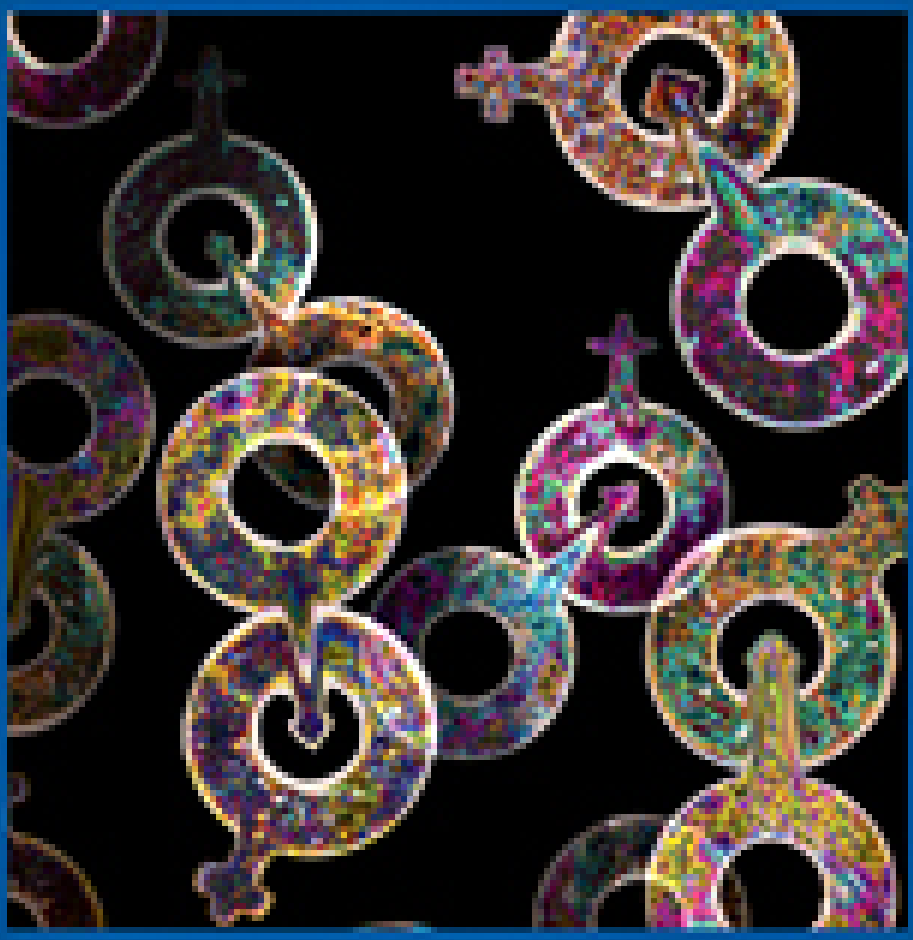

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Contents

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Preface

This manual is intended for the general or family practitioner, as well as for gynecologists and specialists in infertility treatment. While treatment by in-vitro fertilization (IVF) and other advanced techniques attract the most attention and shape the public perception of infertility treatment, far more infertile couples achieve pregnancies with ovulation induction (OI) or combined ovarian stimulation and intrauterine insemination (IUI), the two techniques described in detail in this book, and which form the first line of infertility treatment. Moreover, they can be performed in almost any office or clinic, thus allowing patients to be treated by physicians and nurses already familiar with their general health, without the need to travel to distant specialty clinics. Many practitioners believe, from their time in training, that evaluation of infertile couples is too time-consuming and unrewarding, and that treatment is too complex to be part of their general or obstetric and gynecological practices. Nothing could be further from the truth. Infertility should be viewed, not as a diagnosis, but as a symptom of an underlying medical problem, affecting either one or both partners, which, if left untreated, will eventually affect their general health and emotional well-being.

The role of IUI and OI in the present era of IVF and intracytoplasmic sperm injection (ICSI) is the subject of Chapter 1. Although IVF and ICSI have made donor insemination unnecessary for many couples with male-factor infertility, it is still the method of choice for many couples when they are confronted with the cost and complexity of IVF and ICSI treatment, or if there is a total absence of sperm. Donor insemination is easy to perform, requires no special equipment, and may be the only procedure performed by many physicians who use this manual. Websites are listed in the chapter on donor insemination that provide access to United States and European sperm banks that ship internationally.

The initial step in effective infertility treatment is to make a diagnosis. This can be accomplished with a few simple and relatively inexpensive tests, as explained in the early chapters of this manual. Chapter 2 is written by an excellent team of medical university urologists who explain the causes and treatment of male-factor infertility and erectile dysfunction. Chapter 3, by two clinical reproductive endocrinologists with many years of experience, describes easy and effective methods of diagnosing female-factor infertility. Chapter 4 describes equipping an office laboratory to perform semen analysis and to prepare sperm for insemination.

Chapter 5 reviews those sperm qualities that are necessary for pregnancy when IUI is performed, and describes in detail the methods for performing basic and more complex semen analysis. Chapter 6 describes and compares four methods of sperm preparation for IUI and an additional fifth method for selecting predominantly Y-chromosome-bearing sperm, concluding with a section on handling specimens from men positive for human immunodeficiency virus (HIV) and hepatitis C virus (HCV).

Chapters 7 and 8 review the pharmacodynamics of oral drugs and gonadotropins and describe in detail their use for OI. Chapter 9 complements the chapters on OI with an atlas of ultrasonographic pictures of follicle and endometrial changes throughout the cycle.

Chapter 10 describes the indications for insemination, how to time insemination to achieve the best results, insemination techniques including IUI and tubal perfusion, and concludes with a section on management of complications of IUI. Chapter 11 describes indications for cryopreservation of patient sperm and methods of cryopreservation and storage. Chapter 12 is devoted to donor insemination, and includes a detailed description of how to use cryopreserved specimens.

Chapter 13 is concerned with the many roles that nurses play in the treatment of infertile couples, in a chapter written by nurse practitioners themselves.

Chapters 14 and 15 describe methods of reducing the incidence of multiple births and of preventing and treating ovarian hyperstimulation syndrome

(OHSS) and other complications of OI, and have been contributed by physicians who have written extensively on these subjects.

Chapter 16, by a clinical psychologist, explains how counseling helps infertile couples to deal with issues they face, such as feelings of inadequacy, loss and depression, and includes a list of printed and internet resources for both medical personnel and patients.

Chapter 17 deals with some of the legal and religious issues that confront physicians and couples – and single women – who wish to use donor sperm. The chapter includes a table that describes the laws relating to husband/partner and donor insemination in 57 European, Asian, Middle Eastern, North and South American and African nations.

Throughout this manual we have endeavored to address practical aspects of treatments that will result in optimal results in terms of pregnancy outcome and safety, but we have tried not to neglect the pharmacological and physiological reasons for their use. We believe and hope that both generalists and specialists will find this manual helpful to their practices, and that it will therefore also benefit their patients.

Richard P. Dickey
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Chapter 1

An overview of intrauterine insemination and ovulation induction

Peter R. Brinsden and Richard P. Dickey

Introduction

Sperm preparation methods developed for in-vitro fertilization and embryo transfer (IVF-ET), such as the wash, swim-up and swim-down techniques, and the use of density gradients, have led to a resurgence of interest in intrauterine insemination (IUI). The use of washed prepared sperm for IUI has also resulted in a significant reduction in the side effects associated with the use of neat semen for IUI (which never should be used), such as painful uterine cramps, collapse and infection [1,2]. In view of the fact that IUI is a relatively simple procedure compared with in-vitro fertilization (IVF), its popularity as a treatment option for certain diagnostic groups of infertile couples is increasing, since it is intermediate between the simpler ovulation induction (OI) and the more "high tech" IVF. This is particularly so in developing countries, where facilities for IVF may be limited and the cost of treatment by IVF is a major issue.

The term "artificial insemination" (AI) covers a range of techniques for insemination: it may be intravaginal, intracervical, intrafallopian, intraperitoneal or intrauterine. AI has been used for many years for a number of different indications, and either the husband/partner's sperm (AIH) or donor sperm (AID) may be used. It is almost 200 years since John Hunter advised a man with hypospadias to inject his seminal fluid into his wife's vagina with a syringe, resulting in a normal pregnancy [3]. In the nineteenth century, Sims artificially inseminated six women who had negative postcoital tests. He used their husbands' semen obtained from the vagina after intercourse; one pregnancy was achieved [4]. The first reported case of human donor insemination was by William Pankhurst from Philadelphia in the United States in 1884 [5].

The rationale for the use of IUI instead of intravaginal insemination (IVI) or intracervical insemination (ICI) is to reduce the effect of factors such as vaginal acidity and cervical mucus hostility and to benefit from the deposition of a bolus of prepared motile, morphologically normal sperm as close as possible to the oocytes at the time of ovulation. There continues to be discussion in the literature about whether or not IUI should be complemented by OI – either with the oral medications clomiphene citrate (CC) or tamoxifen (TMX), or with the injectable gonadotropins. Most practitioners are of the opinion that IUI with OI does increase success rates, and many will initially try with CC or TMX, and move on to gonadotropins if there is no success within a few cycles of CC/IUI. The most appropriate time to move on from IUI to IVF is also a matter for debate, but most practitioners agree that the change should be made after no more than 4–6 cycles of IUI (see Chapters 7 and 8).

Before proceeding to artificial insemination, couples should undergo a complete assessment, of which a full description is given in Chapters 2 and 3. This includes a thorough medical history, clinical examination and appropriate investigations for any possible causes of a couple's infertility, such as tubal damage, ovulatory disorder or a male factor. It is essential that couples should receive adequate counseling prior to starting treatment, especially when donor sperm is to be used. Couples should also be assured of complete confidentiality, and informed that all sperm donors are now comprehensively screened for genetic and infective conditions. Couples will wish to know how the donor is to be matched to their own characteristics, the cost of treatment, the probability of success, the potential for complications to occur and the likelihood of their occurrence. Medical professionals and couples can now make use of the internet to find a sperm donor that matches their desired physical, educational, religious and even national and ethnic

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characteristics (see Chapter 12). In countries with strict regulatory systems, such as the United Kingdom, couples using donor sperm must be made aware of any rules that may affect them when using donor sperm, particularly about parentage, registration of the birth of children and, in some countries, the removal of the right to anonymity of donors and therefore the right of a child to discover the identity of his or her true genetic father on reaching the age of 18 (see Chapter 17). The attitude of societies in general, certainly in the developed countries, towards the issue of single women and women in same-sex relationships having children has changed dramatically in the past decade or two. The demand for fertility treatment from these women is increasing, and practitioners are now more willing to provide donor IUI services to them. However, in many countries, mainly those in which the Roman Catholic or Islamic faiths predominate, donor insemination is forbidden.

The development of oocyte–sperm micromanipulation procedures, such as intracytoplasmic sperm injection (ICSI) and its introduction into IVF-ET programs [6], has made it possible to achieve fertilization and pregnancies when only very few spermatozoa are available. Prior to the development of techniques such as microsurgical epididymal sperm aspiration (MESA) [7], percutaneous epididymal sperm aspiration (PESA) [8] and testicular sperm extraction (TESE) [9], men with congenital bilateral absence of vas deferens (CBAVD), surgically unreconstructable vasa or other causes of vasal obstruction had very little chance of fathering their own children. Now, however, if these techniques are combined with ICSI, these men can be offered a very real chance of achieving paternity with their own sperm [10]. These methods have reduced the demand for AID; however, the cost of these procedures puts them beyond the means of many couples, and there is therefore a continuing need for AID, which is most effectively done by IUI using donor sperm.

Indications for intrauterine insemination

There are a number of indications for IUI using the husband or partner's semen; these are summarized in Table 1.1. Ejaculatory failure is the classical indication, since the male partner is unable to ejaculate into the vagina, while cervical mucus hostility is a logical indication for IUI, as it bypasses the mucus in the cervical canal. The most common indications for IUI are the less severe forms of male-factor infertility and idiopathic or unexplained infertility. Other indications, for which conclusive evidence of effectiveness is lacking, are immunological causes of infertility and endometriosis.

Table 1.1. Indications for intrauterine insemination

Effective
Male subfertility
Cervical factor
Ejaculatory failure
Idiopathic/unexplained infertility
Possibly effective
Immunological infertility
Endometriosis

The main indications for donor insemination are (1) gross male infertility or subfertility (azoospermia or severe oligoasthenoteratozoospermia), for couples who cannot afford IVF or reject IVF for other reasons, and (2) familial or genetic disease, such as Huntington's disease, hemophilia and severe Rhesus incompatibility. The use of cryopreserved semen in donor insemination programs is now mandatory in most countries, to minimize the possibility of the transmission of human immunodeficiency virus (HIV) and other infections to the recipients.

Intrauterine insemination: natural and stimulated (OI) cycles

Treatment by IUI may be performed either in a natural or in a stimulated cycle. Many ovarian stimulation protocols have been devised for use with IUI, including: CC alone or in combination with gonadotropins and human chorionic gonadotropin (hCG); TMX alone or combined with gonadotropins; and the use of a gonadotropin-releasing hormone (GnRH) agonist or antagonist combined with gonadotropins. hCG is usually used at the end of the stimulation phase to achieve final maturation of the oocyte(s). Full descriptions of the different regimens for OI are given in Chapters 7 and 8.

The rationale for the use of OI with IUI is both to increase the "efficiency" and likelihood of ovulation and to increase the number of oocytes available for fertilization, and thus to improve the chance of pregnancy. Stimulation also enhances steroid production, which may improve the chance of fertilization and embryo implantation [11]. When considering whether or not to use ovarian stimulation with IUI, the

benefit of a potential increase in success rates that may be achieved, compared with natural cycle IUI, must be balanced against the increased cost of the medication and monitoring, as well as the potential complications of these medications, including ovarian hyperstimulation syndrome (OHSS) and the increased incidence of multiple pregnancies, with their associated maternal and neonatal complications [12] (see Chapters 14 and 15).

There are several methods available to time ovulation in both natural and stimulated cycles. These include simple methods such as the measurement of basal body temperature (BBT), which has been found to be the least accurate, and assessment of the cyclical changes that occur in cervical mucus. Templeton *et al.* showed that in 35% of cycles the optimum mucus score was observed on the day before the luteinizing hormone (LH) surge, in 44% of cycles it was optimum on the day of the LH surge and in 18% of cycles on the day after the LH surge, while in 3% it occurred two days after the LH surge [13]. However, the detection of the serum or urinary LH surge and ultrasound assessment of follicular growth and rupture have proved to be the most accurate methods of monitoring IUI and OI/IUI cycles. Vermesh *et al.* showed that use of a "dipstick" LH test kit predicted ovulation in 84% of cycles in his series [14]. In a stimulated cycle, if hCG is administered when the average diameter of the leading follicle is 18–20 mm, ovulation may be expected to occur 34–40 hours later .

Sperm preparation

The ideal sperm preparation technique is the one which will achieve the largest number of morphologically normal motile spermatozoa in a small volume of physiological culture media, free from seminal plasma, leukocytes and bacteria [15]. There is more information than formerly about sperm quality and quantity necessary in an initial specimen for IUI to be successful, so that an informed decision can be made on whether to perform IUI or, instead, to recommend IVF with intracytoplasmic sperm injection (ICSI) or donor sperm [16]. Although there is no threshold of sperm concentration below which pregnancy is impossible, most conceptions occur when the number of inseminated motile sperm is 4×10^6 or greater (see Chapter 6). The degree of motility and percentage of morphologically normal spermatozoa are other important variables in fertility prognosis.

There are several different sperm preparation techniques for IUI, and each has its own advantages and disadvantages. Sperm preparation using the density gradient technique yields the highest number of motile spermatozoa when compared with simple washing or swim-up or swim-down methods, and significantly reduces bacterial contamination [17], but it should not be used when the initial specimen contains fewer than 15×10^6 motile sperm (see Chapter 6). Equipment and materials necessary for semen analysis and preparation of husband/partner sperm for IUI are described in Chapters 4 and 6 .

Results of treatment by IUI and OI with husband/partner sperm

The results of IUI with husband/partner sperm in terms of pregnancy rates per treatment cycle vary considerably between clinics, and the evaluation of results is difficult because of the heterogeneity of the patient populations and the different ovarian stimulation protocols, if any, used in the studies. Although there are a large number of published studies on IUI, most of these are retrospective and/or on small numbers; only a few are prospective and randomized trials. There is an undoubted need for more large prospective randomized studies to evaluate the real effectiveness of IUI and to elicit which group of patients will benefit most from this treatment. Results of IUI and OI at Bourn Hall and the Fertility Institute of New Orleans are shown in Table 1.2.

Two European Society of Human Reproduction and Embryology (ESHRE) multicenter prospective studies compared ovulation induction alone with ovulation induction in conjunction with IUI, intraperitoneal insemination (IPI), gamete intrafallopian transfer (GIFT) and IVF [18,19]. In the treatment of unexplained infertility, the pregnancy rate achieved from superovulation alone was less than when combined with IUI, IPI, GIFT or IVF [18]. In the treatment of male subfertility, ovulation induction with IUI, GIFT and IVF gave better results than IPI and ovulation induction alone [19]. Martinez *et al.*, in an extensive review of the English-language literature from 1980 to 1991, showed that there was marked variation in the results of IUI between different clinics [20]. Retrospective analyses of IUI data using life-table analysis showed a relatively constant probability of becoming pregnant after each IUI treatment through four gonadotropin IUI cycles [21] or six IUI cycles without OI [22,23], and that thereafter it is hardly increased at all by continuing for longer. Most clinicians are now agreed that further evaluation and discussion of the other treatment options available

Table 1.2. Pregnancy success rates related to cause of infertility

	Bourn Hall Clinic (1989–93) [a]	Fertility Institute of New Orleans (1983–98) [24]	Fertility Institute of New Orleans (1983–98) [25]
Medication	Mixed	Clomiphene	hMG/FSH
IUI cycles	> 1400	3381	4062
Indication	**Pregnancies per cycle (%)**	**Pregnancies per cycle (%)**	**Pregnancies per cycle (%)**
Ovulatory dysfunction	–	14.6	19.5
Cervical, unexplained	–	10.4	19.2
Idiopathic	12.3	–	–
Cervical	16.4	–	–
Endometriosis	–	8.1	16.1
Immunological	10.0	–	–
Male subfertility	21.0	11.4	16.9
Donor sperm	–	16.5	22.2
Ejaculatory failure	13.3	–	–

[a] Unpublished data
FSH, follicle-stimulating hormone; hMG, human menopausal gonadotropin.

to couples should be carried out after 4–6 cycles of IUI without OI. In IUI with OI, the probability of pregnancy per cycle and the number of cycles in which pregnancy rates remain constant, in patients treated at the Fertility Institute of New Orleans, depended on diagnosis, age, sperm source and the number of preovulatory follicles that developed in response to CC (see Chapter 7, Table 7.1) [24] and human menopausal gonadotropin (hMG) or follicle-stimulating hormone (FSH) (see Chapter 8, Table 8.4) [25].

The United Kingdom results for donor insemination are reported yearly by the Human Fertilisation and Embryology Authority (HFEA) on their website (www.hfea.gov.uk). The data from all clinics show live birth rates for 2006 of 14.5% per donor insemination treatment cycle for women below the age of 35, reducing to 9.9% for women aged 35–39, 5.4% for ages 40–42 and just 1.2% for women aged 43–44, with no live births for women over the age of 44. Data from Bourn Hall Clinic for 2007, for all age groups combined, show a 22% clinical pregnancy rate per cycle started for IUI with husband/partner sperm and a 26% clinical pregnancy rate per cycle started for IUI with donor sperm (www.bourn-hall-clinic.co.uk). The majority of cycles are stimulated with CC or gonadotropins .

Cost-effectiveness of IUI and OI

In a large retrospective analysis of 45 published reports on the treatment of couples with unexplained infertility, Guzick *et al.* looked at the cost-effectiveness of no treatment, CC alone, CC+IUI, hMG alone, hMG+IUI, IVF, and GIFT [26]. Analysis of the data showed that CC+IUI was the most cost-effective treatment for idiopathic infertility (US$10,000 per pregnancy), compared to hMG+IUI (US$17,000 per pregnancy) and IVF (US$50,000 per pregnancy). In their conclusions, they state that "on the highest level of evidence found in the data," their recommendations for the cost-effective management of idiopathic infertility are: IUI does not appear to be effective without some form of superovulation; CC+IUI appears to be more cost-effective than hMG+IUI or IVF; IVF and GIFT are effective for couples who have not conceived after superovulation + IUI.

In a study from the Netherlands by Goverde *et al.*, 258 couples with a diagnosis of idiopathic or mild male-factor infertility were divided into three equal groups: (1) IUI alone, (2) IUI with mild ovarian stimulation using low-dose FSH, and (3) IVF [27]. Their conclusion was that stimulated IUI was as effective as IVF in achieving a pregnancy (31% vs. 33%), and was more cost-effective than IVF – cost per pregnancy resulting in a single live birth was US$4,511–5,710 for stimulated IUI versus US$14,679 for IVF. They concluded that patients should be counseled that IUI for these two diagnostic groups offers as good a chance of achieving a pregnancy as IVF and is more cost-effective. More controversially, they suggested that non-stimulated IUI should be the first-choice treatment, as it carries

fewer health risks, even though it is not as effective as stimulated IUI. Cohlen reviewed the literature looking at the evidence of the efficacy of IUI with "mild ovarian hyperstimulation" (MOH) as a treatment for cervical mucus hostility, moderate male-factor infertility and unexplained infertility [28]. His conclusion on the treatment of these three groups of patients was that "When multiple pregnancies are kept to a minimum, MOH/IUI is more cost-effective compared with in-vitro fertilization and embryo transfer."

Complications of treatment

There are few complications to treatment by IUI. Failure of the treatment could be said to be the most frequent, since pregnancy rates per cycle are reported at anywhere between 5% and 25%. The complication which causes couples the most concern, but which is almost certainly very rare, is the possibility that the patient might be inseminated with the wrong semen sample. Other complications include the possibility of transmission of venereal disease, HIV or hepatitis B or C. Proper adherence to protocols and the establishment of clinical and laboratory quality systems should almost eliminate these possibilities. Painful uterine contractions which may occur during insemination can usually be minimized by inseminating slowly. Intrauterine infection and anaphylaxis rarely may also occur, especially if neat semen is used – which it should never be.

The two most serious complications of OI are multiple pregnancy and ovarian hyperstimulation. There is increased awareness of the dangers of multiple pregnancy, including twins. Singleton birth is now the "gold standard" of IVF, and many countries in Europe now mandate single embryo transfer for most IVF patients. Twins occur in 10% of CC and 20% of gonadotropin OI cycles with or without IUI, and triplet and higher-order multiple pregnancies occur in as many as 20% of hMG/FSH IUI pregnancies in women younger than 32 who develop seven or more follicles [25]. Occurrence of multiple pregnancy and OHSS can be minimized by careful management and monitoring of treatment cycles and, if necessary, abandoning the cycle or converting it to an IVF cycle (see Chapters 14 and 15).

Conclusion

IUI is an effective, non-invasive, relatively simple and cost-effective method of treatment for certain diagnostic groups of infertile couples. It can be provided more easily to more infertile couples in office practices and general hospitals than can the more specialized techniques such as IVF, if there are adequate facilities for semen preparation and cycle monitoring and the clinic is staffed by adequately trained physicians and laboratory scientists. However, careful selection of patients suitable for IUI is important. Those who will benefit most are young women with patent fallopian tubes, with no ovulatory disorder, no endometriosis of more than moderate or severe degree and no severe degree of male-factor infertility in their partners. All couples require in-depth advice and counseling about the method, the effectiveness and the potential complications of treatment.

The main advantages of IUI over IVF are its simplicity and relative inexpensiveness. However, there are many advantages of IVF over IUI – principally those of a higher pregnancy rate, the ability to avoid multiple pregnancies by transferring a single embryo and cryopreserving any spare embryos generated in the IVF cycle, and the knowledge gained about the ability of the sperm to fertilize the oocytes. IVF or ICSI are the only realistic treatments for couples with severe male-factor infertility, as well as for severe endometriosis and infertility due to severe tubal damage. Although IUI can be performed outside of specialist units, a clinic with IVF facilities offers the best setting in which to perform IUI in case complications, such as an excessive follicular response or ovarian hyperstimulation, occur. If they do, then patients can be offered the chance to convert to IVF, with the chance to freeze any surplus embryos.

Finally, in an interesting paper on "Patients' preferences for intrauterine insemination or in-vitro fertilization," van Weert *et al.* [29] concluded that, when couples knew their cumulative chances of pregnancy at each stage, both at the start of treatment and after three cycles of IUI, the majority of couples wished to continue with IUI, but their preference changed to IVF after six cycles of IUI, in the knowledge that their chances of success were very much less at this stage [29].

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Chapter 2

Male causes of infertility: evaluation and treatment

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Introduction

The male is solely responsible for the failure to conceive in about 20% of infertile couples, and contributory in another 30–40% [1]. Reduced male fertility can derive from congenital or acquired urogenital abnormalities, infection of male accessory glands, increased scrotal temperature, endocrine disturbances, genetic abnormalities or immunological factors [2]. However, no demonstrable etiology can be diagnosed in 48.5% of cases of male infertility. An abnormal semen analysis (SA) suggests the presence of a male factor; however, a normal SA does not preclude a male factor being present.

The goals of the evaluation of the infertile male are to identify [3]:

- potentially correctable conditions
- irreversible conditions amenable to assisted reproductive technologies (ART) using male gametes or donor insemination if the male partner's sperm is not procurable
- life- or health-threatening conditions that may underlie infertility and require medical attention
- genetic abnormalities that may affect the health of offspring if passed on via advanced reproductive techniques.

Traditionally, evaluation of infertility is postponed until one year of unprotected intercourse. However, it is justified to initiate an evaluation earlier if one of the following conditions is present [3]:

- defined male infertility risk factors
- female infertility risk factors, including advanced age (> 35 years)
- the male or female partner requests an earlier evaluation.

Initial evaluation of the male usually consists of two semen analyses separated by at least a month, a medical and reproductive history, and a focused physical examination by a urologist/andrologist. Additional tests may be required to further investigate underlying pathologies.

Semen analysis provides information on ejaculate volume, spermatozoa concentration, motility and morphology and also round-cell number (additional tests include determining fructose levels and staining for white blood cells in selected cases). Detailed laboratory protocols for each of these assays have been published by the World Health Organization (WHO) [4].

Except for patients with bilateral vasal agenesis or clinical signs of hypogonadism, patients with low ejaculate volume (< 1 mL) need their postorgasmic urine screened for retrograde ejaculation [3].

Hormonal evaluation, consisting of total testosterone and follicle-stimulating hormone (FSH) levels, is warranted if abnormally low sperm concentration, impaired sexual function or other clinical findings suggestive of specific endocrinologic dysfunction are identified. Any detected hormonal disturbance needs further confirmation and corroboration [3].

Imaging studies are not often required for a male infertility evaluation. In the presence of symptoms suggestive of ejaculatory duct obstruction, i.e. low ejaculate volume with severe oligoasthenospermia or azoospermia, transrectal ultrasonography is often performed. Similarly, in patients in whom physical examination of the scrotum is somewhat difficult or a testicular mass is suspected, scrotal ultrasonography has a high specificity for identifying scrotal pathology [3].

Specialized clinical tests (e.g. assays for antisperm antibodies, sperm viability or sperm–cervical mucus interaction) can be useful in a small cohort of patients.

Manual of Intrauterine Insemination and Ovulation Induction, ed. Richard P. Dickey, Peter R. Brinsden and Roman Pyrzak. Published by Cambridge University Press.

They are reserved for special cases of unexplained infertility [3].

Men with non-obstructive azoospermia or severe oligospermia must be counseled for potential genetic abnormalities that may be transmitted to their offspring, and offered formal genetic screening prior to ART [3].

Normal spermatogenesis and ejaculation

The sperm cycle is a complex series of events that requires approximately three months from the beginning of spermatogenesis to the antegrade ejaculation of semen. The physiology of sperm production and delivery is paramount to the diagnosis and treatment of male-factor infertility.

Spermatogenesis and the testicle

Spermatogenesis occurs in the seminiferous tubules, which are the microscopic ducts made up primarily by Sertoli cells, germ cells and peritubular myoid cells [4]. The seminiferous tubules make up the bulk of the testicular cortex. Sertoli cells form the walls of the tubules and are connected by tight junctions; these form the blood–testis barrier that prevents macromolecules from entering the tubules from the lymph system, thus allowing spermatogenesis to occur in an immunologically privileged site [5]. Sertoli cells also play an important role in supporting the germ cells with nourishment and supplying high levels of androgens (20–50 × serum levels) in the seminiferous tubule lumen [6].

Germ cells lie within the tubule in an ordered fashion, beginning with the spermatogonia at the basement membrane, progressing to the mature spermatid at the lumen. Spermatogonia undergo several mitotic divisions to produce a large supply of stem cells, allowing for indefinite production of spermatozoa. The diploid spermatogonia undergo further mitotic division to produce the primary spermatocytes. After the first meiotic division the spermatocytes cross the blood–testis barrier into the adluminal compartment, becoming diploid secondary spermatocytes. Next, within the adluminal compartment, the secondary spermatocytes undergo the second meiotic division, each producing two round spermatids that mature by spermiogenesis into elongated flagellar cells before entering the seminiferous tubule lumen. Each primary spermatocyte produces four mature spermatids, which have compressed chromatin and acrosome in the head, with a flagellar tail containing tightly packed mitochondria in the proximal portion. Spermatogenesis is heavily dependent on FSH and testosterone for initiation and maintenance [7,8].

The epididymis

After release into the lumen, spermatids continue to mature. They are moved to the epididymis by active peristalsis by the peritubular myoid cells. When entering the epididymis, sperm are non-motile and unable to fertilize the oocyte via IUI. The maturation of spermatids within the epididymis largely occurs as a result of micro-environmental influence, and hence there is negligible protein synthesis within the spermatid itself. Most of the changes occur on the cell membrane by altering protein content, immunoexpression, net surface charge, integrity and fatty acid content [9]. These changes prepare the sperm for fertilization and are androgen-dependent. The epididymis also serves as a favorable storage environment for mature sperm.

The vas deferens

After leaving the tail (or cauda) of the epididymis, sperm enter the thick, muscular, vas deferens. The vas is the major storage location of mature sperm prior to ejaculation, although it is not capable of preserving viability to the same degree as the epididymis. The physiology of the vas results in the recommended two-day optimal interval for intercourse, because more frequent intercourse produces less than optimal sperm counts and less frequent intercourse produces decreased sperm viability [10].

Seminal vesicles and prostate

The seminal vesicles and prostate are the sexual accessory glands responsible for contributing to the fluid environment for sperm, with their secretions making up 95% of the normal ejaculate volume [5]. The seminal vesicles produce phosphorylcholine, ascorbic acid, flavin, prostaglandins, fructose and clotting factors. Prostaglandins relax the myometrium of the uterus and cervix; fructose is an energy source for sperm . The prostate is under regulation by androgens, specifically the potent dihydrotestosterone.

Penis physiology

The penis is responsible for effective transportation of sperm and semen from the vas in an antegrade fashion to the cervical os, a process requiring erection and ejaculation. Erections occur from genital or central

stimulation. Genital stimulatory erections may be preserved in lesions above T10, although erections in spinal cord injury patients are usually short and uncontrolled [11]. Central-originating erections involve contributions from many different areas of the brain, involving memories, fantasy, visual and auditory stimuli. The flaccid penis is under constant sympathetic stimulation, causing constricted sinusoidal spaces within the corpora cavernosa. When parasympathetic stimulation increases from the pelvic plexus (nervi ergentes) these sinusoids relax under the influence of nitric oxide, causing an increase in the flow of blood into the penis, while simultaneously compressing the emissary veins (activation of the veno-occlusive mechanism) and decreasing blood outflow [12]. These processes produce a physiologic erection.

Ejaculation

Ejaculation is a very quick and complex series of events that is crucial in the ability to deposit sperm in the vaginal canal. Ejaculation is the result of a combination of central and genital nervous stimulation. Penile sensory information is passed through the dorsal nerve to the spinal column, where it is integrated and sympathetic efferent signals are generated to initiate the ejaculatory reflex [5]. The threshold for this process can be lowered or raised by central modulation. Activation of the sympathetic ejaculatory pathway results in contraction of the vas deferens, bladder neck, seminal vesicles and prostate. Concurrent with seminal emission is a sense of general and localized pleasure, or orgasm. After emission of sperm into the posterior urethra, rhythmic contractions of the perurethral muscles result in an involuntary projectile ejaculation of the seminal fluid.

Hypothalamo–pituitary–testicular axis in the adult male

The hypothalamo–pituitary–testicular axis is essential for human reproduction, especially spermatogenesis and erectile function. Gonadotropin-releasing hormone (GnRH), synthesized in the hypothalamus, is released into the portal system in a pulsatile fashion, stimulating the pituitary to synthesize and release FSH and luteinizing hormone (LH). LH stimulates the Leydig cells of the testicle to produce testosterone. The anterior pituitary production of LH is negatively inhibited by serum testosterone. Testosterone increases secretory production in the seminal vesicles and is converted by 5α-reductase in the prostate cells to stimulate growth and secretion. Within the testicle, testosterone drives spermatogenesis. FSH binds to Sertoli cells, initiating seminiferous tubule development during puberty, and is essential for continued spermatogenesis during adulthood. FSH is negatively inhibited by the Sertoli-cell-produced protein inhibin and, to a lesser extent, by testosterone . Physiologic levels of testosterone are required for adequate libido and spontaneous erections [13].

Pathophysiology of male infertility

Semen quality can deteriorate with any of the recognized pathologies that may interfere with normal spermatogenesis. This section will focus on these pathologies and the treatment options for couples with such problems.

Failure to produce adequate-quality spermatozoa

Production of spermatozoa is complicated by several mechanisms which are discussed below. Endocrine, testicular failure, anatomical, infectious, genetic and immunologic defects can impair the maturation of spermatogonia to produce quality sperm.

Endocrine/hormonal causes

The most common endocrine causes of male infertility are summarized in Table 2.1 [6].

Testicular causes

The most common testicular causes of male infertility are summarized in Table 2.2 [6].

Varicocele

Varicoceles are dilated tortuous testicular veins, classically described as "a bag of worms," within the spermatic cord. They do not transilluminate when a pen light is held against the scrotal skin and usually do not collapse in the supine position. Subclinical varicoceles can be detected with scrotal ultrasound [14]. Varicocele is the most common correctable cause of male infertility, and 90% occur on the left side owing to the anatomy of entering the renal vein rather than the vena cava on the right side. The prevalence of varicocele in infertile men is 20–40% [15]. Authorities theorize that increased temperature, hypoxia and reflux of adrenal and renal metabolites may impair spermatogenesis in patients with varicocele.

Table 2.1. Endocrine causes of male infertility

Disease process	Characteristics
Pituitary disease	Tumors, infarcts, surgery, radiation, infiltrative or granulomatous disease.
Isolated hypogonadotropic hypogonadism (Kallmann syndrome)	Absence of gonadotropin-releasing hormone, associated with anosmia. Fertility can be achieved with LH and FSH replacement.
Fertile eunuch syndrome	LH is normal, but testosterone deficient. Patients have incomplete virilization, gynecomastia and reduced number of sperm.
Isolated FSH deficiency	Normal testes and virilization, does not respond to GnRH stimulation, azoospermic or oligospermic.
Androgen excess	Anabolic steroid abuse: 15% of high-school, 30% of college, and 70% of professional athletes. Temporary subfertility can result. Discontinue steroid use and re-evaluate in 3–6 months. Rarely caused by 21-hydroxylase deficiency characterized by precocious puberty.
Estrogen excess	Usually related to cirrhosis or obesity, which augment aromatase activity resulting in secondary pituitary suppression.
Prolactin excess	Secondary to pituitary adenoma, diagnosed by serum prolactin and CT/MRI of sella turcica. Check prolactin in end-stage renal disease or chronic renal insufficiency.
Thyroid abnormalities	Results in hypothalamic–pituitary dysfunction and alters sex hormone-binding globulin (SHBG) levels; < 0.5% of male infertility.
Glucocorticoid excess	Cushing's syndrome features, decreases spermatogenesis, suppresses LH, fertility improves with correction.

Table 2.2. Testicular causes of male infertility

Disease process	Characteristics
Bilateral anorchia	Secondary to torsion, trauma, infection or vascular injury. No effective ART.
Cryptorchidism	0.8% of boys at 1 year of age; germ-cell abnormalities begin to appear at 2 years of age. Increased risk for infertility and malignancy.
Testicular torsion	If testicle saved, predisposed to immunologic infertility. Contralateral testis is at risk for abnormalities.
Sertoli-cell-only syndrome	Germ-cell aplasia, azoospermia, normal virilization, small testes, elevated FSH. Extensive sampling and biopsy may find sperm suitable for ART.
Myotonic dystrophy	Adult-onset muscular dystrophy, cataracts, muscle atrophy, various endocrinopathies, elevated FSH and LH. Fertility has been reported.
Chemotherapy/ radiation	Dose-dependent, inverse relationship between radiation and sperm counts, primarily affecting spermatogonia; sperm counts rebound after therapy, no increased incidence in congenital defects. Alkylating agents are the most gonadotoxic chemotherapy agents, cytotoxic to spermatogonia, but no increased incidence of congenital defects. Patients should be advised to avoid conception until 6 months after the end of treatment. Consider sperm cryopreservation prior to treatment.
Medications	Discontinue all unnecessary medications: ketoconazole, spironolactone and alcohol inhibit testosterone synthesis, cimetidine is an androgen antagonist and some pesticides have estrogen-like activity.

Semen analysis in patients with varicocele demonstrate decreased motility, decreased sperm concentration and increased amorphic cells [16]. The majority of men who have varicoceles are fertile, but any subfertile man with varicocele should be considered for repair, as 70% of men receiving surgical repair have significant improvement in semen parameters. Motility improvements are most common (70%), followed by improved sperm densities (51%) and improved morphology (44%), all of which increase the overall success rate of intrauterine insemination (IUI) [15]. Conception rates following varicocele repair average 40–50% when female factors are not present or have been appropriately treated.

Infection

Infection of the male reproductive tract may be present in up to 23% of men seeking infertility evaluation [6]. Pyospermia is defined as $>1 \times 10^6$ leukocytes/mL of

Table 2.3. Genetic causes of male infertility

Disease process	Characteristics
Klinefelter's syndrome (XXY)	Most common genetic defect with azoospermia, 1/500 males, small firm testes, gynecomastia. Virilization and fertility may respond to testosterone.
XX male	Absent spermatogenesis and azoospermic.
XYY syndrome	Oligospermia or azoospermia with increased FSH and LH; biopsy demonstrates arrest of maturation of Sertoli cells.
Noonan's syndrome	Also known as male Turner's syndrome, fertility possible if descended testes, but cryptorchidism present in 75%.
Y-chromosome microdeletions	7% of men with low sperm counts have Y microdeletions, usually the long arm. Often these men are fertile with ART, but will likely pass on DNA to their male offspring.
Cystic fibrosis	Azoospermia secondary to congenital bilateral absence of the vas deferens (CBAVD); 80% of men with CBAVD have mutations in the cystic fibrosis transmembrane regulator (*CFTR*) gene.

semen. Although no specific bacterial organisms are clearly related to infertility, leukocytes produce reactive oxygen species, which are harmful to sperm cells. Orchitis, specifically mumps orchitis, can be a specific etiology, and occurs in 30% of postpubertal males who contract mumps parotitis [6].

Genetic

The most common genetic causes of male infertility are summarized in Table 2.3 [6].

Immunologic infertility

Immunologic infertility is present in 10% of infertile couples [17]. Sperm are highly antigenic cells, but coexist in the male body because of the blood–testis barrier. Tight junctions in the seminal tracts provide the immunoprotective environment for haploid sperm cells. Antisperm antibodies (ASA) form when the barrier is compromised, commonly through trauma, testicular biopsy, vasectomy or infection. ASA can be detected in semen, serum and cervical mucus, usually using the immunobead test, which is available at most specialized andrology laboratories. ASA interfere with sperm transport in the female reproductive tract, and with the egg–sperm interaction. They are found in 3–12% of men presenting for infertility evaluation. Specifically relating to IUI, in theory sperm can be washed to prevent clumping and agglutination, but fertility rates are similar to matched controls [18]. Treatment with steroids for both partners has been attempted, with mild improvement in pregnancy rates and high rates of side effects (60%) [19]. Intracytoplasmic sperm injection (ICSI) provides fertilization rates in ASA-positive couples comparable to those in ASA-negative couples [20].

Failure to deposit ejaculate into the prostatic urethra

In 7–14% of cases of azoospermia or severe oligospermia, despite normal spermatogenesis, the cause is obstruction of the seminal ducts [21]. While epididymal obstruction is the most common etiology, occurring in 30–67% of cases of azoospermia in men with normal FSH levels, intratesticular obstruction has been reported in up to 15% of cases.

Ejaculatory duct obstruction is rare, found in 1–3% of cases of obstructive azoospermia. Patients suffering from obstructive infertility may present with less conventional histories, such as a hematospermia, post-ejaculatory pain, recurrent urethritis or prostatitis, obstructive or irritative urinary symptoms, scrotal swelling, infection or pain, prior genitourinary procedures, inguinal herniorrhapy, trauma and chronic pulmonary infections.

Diagnostic steps in these patients include measurement of fructose levels in semen, ultrasonographic imaging and a standard infertility evaluation, to determine the presence and level of obstruction. Scrotal ultrasound evaluates the testis and epididymis, whereas transrectal ultrasound (TRUS) images the distal components of the ejaculatory ducts.

Ejaculatory duct obstruction

Ejaculatory duct obstruction can be caused by inflammation or prostatic cysts. Calculi obstructing both ejaculatory ducts have been reported [22]. Although controversial, prostatic cysts are usually classified into the following two groups: (1) urogenital sinus cysts, in which one or both ejaculatory ducts drain into the cyst; (2) Müllerian cysts, in which ejaculatory ducts

are displaced and compressed by an external cyst. Post-inflammatory obstructions are usually a result of prostatitis or urethritis. Functional obstruction or mega-seminal vesicles are rarer causes of ejaculatory duct obstruction that can occur in men with diabetic neuropathy or polycystic kidney disease, where the seminal vesicles are dilated without any demonstrable obstruction [21].

Partial obstruction of the ejaculatory duct is also a controversial topic. Although there are signs suggestive of ejaculatory duct obstruction, such as dilated seminal vesicles, low-volume ejaculate, seminal vesicle stasis, medial prostatic cyst or intraprostatic hyperechoic foci, no definitive obstruction can be identified.

If TRUS evaluation reveals obstruction of the ejaculatory ducts secondary to fibrosis, calcification or compression by a superficial midline cyst, the preferred therapy is transurethral resection (TURED) or unroofing of the ejaculatory duct region [22,23]. Relief of the obstruction is confirmed by efflux of copious cloudy material or dye from the ejaculatory ducts. Complications can arise on occasion due to reflux of urine back into the ejaculatory ducts and genital tract, leading to a possible infection and deterioration of sperm function from exposure to urine. Transurethral resection also carries a 4% risk of scarring severe enough to cause azoospermia. Overall, TURED results reveal an improvement of 55% for semen parameters and 27% for pregnancy rates. Patients with partial or congenital obstructions, or those secondary to midline cysts, tend to have better outcomes after TURED. Even in cases where natural pregnancy is not achieved, improvement in semen parameters may allow the reproductive endocrinologist improved IUI results.

Alternative treatment options proposed for distal ejaculatory duct obstruction include decompression of the ejaculatory ducts via aspiration of a midline cyst under TRUS guidance, transurethral balloon dilation of the strictured area, antegrade seminal vesicle lavage or laser drilling of the ejaculatory duct region. However, because of low success rates, none of these procedures has become the standard of care.

TRUS may also be employed in azoospermic men with obstruction distal to the seminal vesicles for sperm retrieval by seminal vesicle aspiration for use in ICSI. Although well described as successful in the literature, this is not a commonly performed procedure, as testicular extraction (TESE) and/or epididymal aspiration (MESA) are the preferred methods for sperm retrieval. TRUS-guided aspiration of the seminal vesicles is often suitable for patients undergoing concurrent TRUS-guided aspiration of a midline cyst [24].

Epididymal and vasal obstruction

Vasectomy is the most common cause of acquired obstructive azoospermia, with 2–6% of vasectomized men requesting vasectomy reversal later in life. Unfortunately, 5–10% of such patients will have a concomitant epididymal obstruction secondary to epididymal blow-out, necessitating epididymovasostomy [25]. Vasovasostomy can be performed with loupes or microscopically, though high-power operating microscopic procedures have better pregnancy outcomes. The success of vasovasostomy is inversely correlated with the obstruction interval. Important prognostic factors for pregnancy following the vasovasostomy include sperm quality, partner's age and presence of antisperm antibodies (ASA). Approximately 20% of the patients who undergo successful vasovasostomy experience a deterioration of semen parameters within a year. Patients with low sperm quality or ASA are candidates for IUI or ICSI, depending on sperm quality. Epididymovasostomy is a challenging operation that demands advanced training in microsurgery. The ultimate pregnancy rate is lower (20–30%), and it is reasonable to perform MESA/TESE concurrently for cryopreservation of gametes as a back-up for ICSI in case of failure of the reversal. When performing epididymovasostomy for congenital obstructions, spermatogenesis may be demonstrated by testicular biopsy [26].

Although reconstructive reversal procedures are the more cost-effective treatment options, ART can be used with sperm retrieval techniques in selected cases, e.g. the presence of ASAs or advanced age of the partner [27]. Testicular sperm extraction (TESE), testicular sperm aspiration (TESA), microsurgical epididymal sperm aspiration (MESA), percutaneous epididymal sperm aspiration (PESA) and vasal sperm aspiration (VSA) are routinely used sperm retrieval techniques. Pregnancies using vasal sperm aspiration in combination with IUI have been reported, but the success rate (14.3%) is lower than with ICSI [28].

Ejaculatory dysfunction

Although erectile dysfunction (ED) occupies a prominent position in public awareness and interest, ejaculatory dysfunction (EjD) is the most common sexual dysfunction. Forty-six percent of men aged between 50 and 80 years have some type of ejaculatory

disturbance [29]. Encompassing a broad spectrum of conditions, EjD includes premature ejaculation (PE), anejaculation (AE) and retrograde ejaculation (RE). Despite the large proportion of affected men, EjD remains poorly studied or understood and its treatment does not share the same level of success as that of ED. Although PE is the most prevalent form (30–40%) of EjD, this section will focus on AE and RE, as they are more likely to interfere with fertility.

Inhibited ejaculation / anejaculation

According to guidelines published by the European Association of Urology (EAU) in 2001, anejaculation (AE) is defined as *the failure of expulsion of semen that is usually associated with normal orgasmic function*, whereas inhibited ejaculation (IE) is defined as *the persistent or recurrent difficulty, delay, or absence of attaining orgasm following sufficient sexual stimulation* [2]. Retarded ejaculation, delayed ejaculation, as well as psychogenic AE, are often used synonymously with inhibited ejaculation. This can be a lifelong condition, occurring in all sexual encounters, or acquired, occurring only in specific situations. Some men with IE are able to achieve orgasm via masturbation or, alternatively, nocturnal emission into a condom may provide a potential source of semen for IUI [30].

Anejaculation occurs in 0.14% of the general population, while in patients seeking infertility treatments it is at 0.39%. Anejaculation is more common in older men, likely due to altered sensation and diminished erectile capacity.

Although the etiology of IE has not been elucidated, it has been attributed to psychosocial issues, including cultural and religious beliefs, concurrent psychopathologies such as unconscious aggression and unexpressed anger, insufficient sexual arousal, preconditioning for inhibited ejaculation due to preference for masturbation over partnered sex, and fear of pregnancy or sexually transmitted disease [30].

Some surgical procedures are recognized to cause ejaculatory dysfunction, which must be addressed as part of preoperative informed consent. Any retroperitoneal procedure risks damage to either the sympathetic nervous system or the hypogastric plexus. Retroperitoneal lymph node dissection (RPLND) for testicular cancer, abdominal aortic aneurysm repair, aortic bypass grafting with exogenous materials and abdominal perineal resection have all been implicated in postsurgical AE. Since RPLND is often performed in younger patients who have yet to start families, it carries the greatest potential consequences. Surgeries that damage the outlet of the ejaculatory ducts can also cause anejaculation (as well as retrograde ejaculation – see below). Transurethral resection of the prostate (TURP), bladder neck reconstructions and ablation of posterior urethral valves are all potential causes of AE.

Disease processes or lesions affecting either the peripheral nerves or the central nervous system can cause ejaculatory delay or absence. Diabetes mellitus, a common metabolic condition that causes autonomic neuropathy, inhibits neural outflow via the sympathetic tracts to the bladder neck. Lack of a closed bladder neck during ejaculation may lead to anejaculation or retrograde ejaculation. Spinal cord lesions, whether congenital, acquired or traumatic, commonly induce ejaculatory dysfunction. Lesions above T10–T11 cause anejaculation, whereas lower lesions may merely inhibit the expulsion phase of ejaculation, leaving emission intact. Partial spinal cord injuries may only cause IE. In spinal cord injury (SCI) patients, the reported incidence of the ability to ejaculate during sexual intercourse or masturbation is 0–55% (median 15%) [31]. Multiple sclerosis commonly causes a number of sexual dysfunctions, including an inability to achieve orgasm or ejaculation in up to 61% of patients.

A wide array of medications can induce ejaculatory dysfunction, through a variety of mechanisms. Antihypertensive medications, including α-methyldopa, thiazide diuretics and clonidine, have been implicated. Numerous psychiatric medications from psychotropics to antidepressants, including haloperidol, tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs) and monoamine oxidase inhibitors, have recognized ejaculatory side effects. When possible, discontinuation of the offending pharmaceutical agent is the optimal option. Prolonged alcohol abuse may also lead to AE or IE. Alpha-adrenergic blockers such as tamsulosin were previously believed to cause retrograde ejaculation; however, it has recently been determined that they cause anejaculation via a central mechanism.

Treatment of psychogenic AE or IE is controversial. The decision may depend on patient preference as well as partner age, as a more aggressive approach is warranted in a patient with an older partner. In general, restoring normal ejaculation to facilitate spontaneous pregnancy in patients with anejaculation and inhibited ejaculation can be difficult, with high treatment failure rates. If the goal is only fertility, a number of options exist. Since many patients with IE continue to

have nocturnal emissions, wearing a condom at night to collect a specimen for IUI is a simple, non-invasive method for sperm collection. Some authorities suggest behavioral therapy to restore spontaneous ejaculation as the first treatment option. In case of failed behavioral therapy, penile vibratory stimulation (PVS) is a relatively non-invasive and inexpensive method. Electroejaculation (EEJ) and surgical sperm retrieval procedures are reserved as last resorts for obtaining sperm for later use with IVF-ICSI, because they are invasive and require anesthesia [32]. It should be emphasized that semen quality is somewhat diminished when retrieved with EEJ compared to spontaneous ejaculation, although the difference is not statistically significant in neurologically intact men [33].

Medications used to facilitate ejaculation, including bupropion, buspirone, yohimbine and cyproheptadine, act through central dopaminergic or antiserotoninergic pathways. Unfortunately, most of the literature consists of small uncontrolled series or case studies, and no placebo-controlled studies have been performed from which to draw satisfactory conclusions [30].

In cases where AE or IE is secondary to organic etiologies, such as in SCI patients, PVS is recommended before EEJ with a rectal probe [31]. PVS is successful in the majority of cases with lesions above T10 (54%), when the ejaculatory reflex arc is intact, whereas the success rate drops significantly with lesions below T10 or with lower motor neuron lesions [34]. Pharmacological interventions have been proposed to enhance the success rate of PVS, including the use of physostigmine and midodrine [35].

The mechanism of PVS involves initiation of reflex spinal cord activity and leads to ejaculation. Before the procedure, the bladder is filled with a sperm-friendly culture media (e.g. Ham's F-10 [Sigma, St. Louis, MO]). The vibrator is applied to the frenular area of the penis, while the patient is seated or in a supine position. The stimulation has a frequency of 60–120 Hz and the peak-to-peak amplitude of excursion ranges from 1.5 mm to 4.5 mm. A maximum of six stimulation cycles of three minutes, with resting periods of two minutes between, are applied. In general, the duration of stimulation until ejaculation occurs is between 10 seconds and 18 minutes. Criteria to discontinue stimulation are blood pressure exceeding 200 mmHg systolic or 130 mmHg diastolic, severe headaches, or signs of autonomic dysreflexia. For prevention of autonomic dysreflexia in SCI patients (especially in patients with spinal cord lesions at T6 or higher), nifedipine is administered prior to the procedure. In addition to collecting the antegrade ejaculate, the bladder is emptied and the fluid processed to recover any retrograde specimen. If the PVS produces a successful antegrade ejaculation without complication, home intravaginal insemination might be offered to couples. Roughly one out of four couples will have a pregnancy using home insemination within two years [36].

Although the success rate of PVS in psychogenic AE or IE is unpredictable, semen is retrieved in 55% of the patients with SCI lesions [36]. The overall success rate of PVS does not differ markedly between different types of anejaculation; however, psychogenic anejaculation patients have higher rates of antegrade ejaculation. SCI patients with lesions above T11 have a significantly higher response to PVS than SCI patients with lower-level lesions [37].

EEJ also stimulates the nerves for ejaculation. The patient is premedicated as for PVS to avoid autonomic dysreflexia and positioned in the right lateral decubitus position. The rectal probe is placed against the rectal wall at the level of the seminal vesicles and a stimulus is applied in an increasing pattern until ejaculation occurs. Wave voltage ranges from 16 to 22 V at 200–500 mA. Possible complications of EEJ include rectal injury, autonomic dysreflexia and vomiting. Men with pinprick sensation in the sacral or L4–5 dermatome require general anesthesia for EEJ [37]. With SCI patients, EEJ recovers semen samples in 95% of PVS failures. Pregnancy rates of 9% per cycle and 32% per couple have been reported when EEJ is used in combination with IUI [36]. However, success is correlated to the total motile sperm count retrieved. The success rate per cycle drops to around 1% when the total motile sperm number is below 4×10^6, and exceeds 17% when the total motile sperm count is greater than 40×10^6.

Despite these encouraging outcomes for sperm retrieval with PVS and EEJ, 28% of SCI infertility centers lack the equipment or experience with PVS and EEJ, and 34% do not have IUI capabilities.

Retrograde ejaculation

Retrograde ejaculation (RE) is the misdirected propulsion of semen from the posterior urethra into the bladder. RE can either be complete, with a total absence of ejaculate, or partial, with preservation of a minimal amount of antegrade ejaculate. In the absence of antegrade ejaculation, RE is the most common ejaculatory dysfunction, accounting for 0.3–2% of cases of male infertility [37].

Presentation of RE is similar to AE: orgasm without ejaculation. A postorgasmic urine analysis that reveals a significant number of sperm after centrifugation is diagnostic of retrograde ejaculation [38].

For antegrade ejaculation to occur, the prostate, bladder neck, external sphincter, seminal vesicles, vas deferens and perineal musculature must all be anatomically and functionally intact and follow a coordinated series of events to propel semen in an antegrade direction through the urethral meatus. Medications, neurological conditions (e.g. diabetic autonomic neuropathy, SCI) and surgical procedures (e.g. transurethral resection or incision of the prostate, pelvic or anterior spinal surgery) that interfere with this mechanism result in retrograde ejaculation. In a significant portion of RE patients, no causative factor can be determined. Fortunately, idiopathic cases respond well to therapy [38].

Treatment of RE aims either to restore antegrade ejaculation or to retrieve sperm for IUI or IVF-ICSI. Restoring antegrade ejaculation focuses on increasing the sympathetic tone of the bladder neck or decreasing parasympathetic activity. If successful, the return of antegrade ejaculation may be sufficient to allow for natural conception, or may provide enough quality sperm to use with IUI.

Imipramine, given as a daily dose of 25–50 mg for seven days prior to planned intercourse, has been successfully used to treat RE, with the return of antegrade ejaculation in 65–100% of patients and a rate of spontaneous pregnancy of 40% [39]. Anticholinergics, α-adrenergic agonists or combinations may also be used to modulate bladder neck activity, but they are not as effective as imipramine, which should be considered the first-line therapeutic agent for RE.

Surgical interventions to restore bladder neck integrity, including endoscopic injections of bulking agents at the bladder neck to promote closure or open surgical restoration (Abrahams procedure), have been demonstrated to have limited success and are not recommended by most authorities in the era of IVF-ICSI.

Attempts can be made to harvest sperm from urine for later use with IUI if medical management fails to return antegrade ejaculation. Since the acidity and high osmolarity of urine is detrimental to the motility and viability of spermatozoa, two different techniques are commonly employed to adjust the urine pH and osmolarity within the bladder. The less invasive method involves alkalinization of the urine by drinking sodium bicarbonate solutions until the urine has a ph of 7.6–8.1 and an osmolarity of 300–500 mOsm/L. The patient then masturbates and urine is collected for sperm harvesting. Alternatively, the bladder can be drained and filled with a sperm-friendly culture media (e.g. Ham's F-10 [Sigma, St. Louis, MO]) via a small-diameter catheter prior to ejaculation. The patient then masturbates and sperm is obtained through voiding or catheterization after ejaculation.

Failure to deposit ejaculate in the posterior fornix

Erectile dysfunction

Erectile dysfunction (ED), defined as *the inability to achieve or maintain penile rigidity sufficient for intercourse*, is more prevalent among infertile men than their fertile counterparts (28% vs. 11%). Psychological stress, one of the risk factors of ED, is especially recognized as a cause of ED in cultures that put great emphasis on fertility. Also, the long period of diagnostic studies and treatment regimens may have a negative impact on sexuality [40].

First-line therapy for erectile dysfunction consists of oral pharmacotherapy and vacuum erection devices. Second-line therapy options include intracavernosal injection of vasoactive agents and intraurethral application of alprostadil. Surgical implantation of penile prosthesis is considered a third-line therapy in patients who are unresponsive to medical treatment.

In the USA, oral pharmacotherapy approved for the treatment of erectile dysfunction consists of sildenafil, vardenafil and tadalafil, all of which are phosphodiesterase type 5 (PDE5) inhibitors. PDE5 is an enzyme that hydrolyzes cyclic guanosine monophosphate (cGMP) in the cavernosal tissue of the penis. Increased levels of cGMP lead to smooth muscle relaxation and ultimately to penile erection.

Sildenafil was the first approved PDE5 inhibitor in this class. It is administered in 25, 50 and 100 mg doses and is effective 30–60 minutes after administration. Efficacy may be maintained up to 12 hours and adverse effects are mild in nature, including headache and flushing.

Vardenafil, administered in 5, 10 and 20 mg doses, is effective 30 minutes after administration. In vitro it is 10-fold more potent than sildenafil, although this does not necessarily apply to clinical efficacy. Adverse effects observed include headache, flushing and nasal congestion.

Tadalafil, effective at 1–2 hours after administration, is long acting, with efficacy maintained for up to 36 hours. Its side-effect profile includes headache, dyspepsia and back pain.

No PDE5 inhibitor has proven superior, due to lack of head-to-head studies. Patients will often make the ultimate decision on which PDE5 inhibitor to take, based on their own experience.

Drug interactions are uncommon for PDE5 inhibitors. One known major interaction is with nitrate preparations used to treat angina, which may result in unpredictable drops in blood pressure. Additionally, the use of PDE5 inhibitors with α-blockers may cause orthostatic hypotension and syncope.

PDE5 inhibitors may have an effect on spermatozoa. PDE5 is present in high concentrations in sea urchin spermatozoa, and significantly localized in the sperm flagella, where inhibition by sildenafil increased sperm motility. PDE11 is highly expressed in the prostate gland, in Leydig cells and in developing germ cells in the testis. Genetic deficiency of this PDE isoform ($PDE11^{-/-}$) results in diminished motility, concentration and viability of spermatozoa in rats. PDE11 may play a role in the functional quality of human sperm. In-vitro studies have demonstrated a significant benefit of PDE5 inhibitors on sperm motility, without effects on cellular integrity. Clinical studies with acute or chronic use of sildenafil and tadalafil in healthy subjects demonstrated no adverse effect on semen characteristics and showed improvement of motility in patients under sildenafil treatment. In infertile young men, sildenafil caused an improvement in sperm motility, although median sperm count was diminished after tadalafil [41]. Some researchers hypothesize that premature acrosome reaction may be induced by PDE5 inhibitors, but there is not enough published literature concerning the effect of PDE5 inhibitors on fertility to draw solid conclusions. Therefore, a mild caution is advised with PDE5 use in the subfertile population [42].

Hypospadias

Hypospadias is an abnormal opening of the urethral meatus, located anywhere from the glans penis to the perineum. It is a relatively common congenital defect, affecting 1 in 250 newborn males. A more proximal urethral meatus may hinder proper deposition of the ejaculate into the posterior fornix, thereby affecting fertility. Surgical correction of hypospadias in such circumstances can restore fertility, or IUI can be used. While the risk of underlying testicular abnormality is low in men with isolated hypospadias, testicular dysfunction is observed in 57.1% of patients with accompanying micropenis, cryptorchidism or ambiguous genitalia [43].

Use of ultrasound in male infertility

While abdominal ultrasound (US) is used to evaluate concurrent urological anatomical abnormalities (e.g. renal agenesis in Wolffian duct developmental abnormalities), scrotal and transrectal ultrasound (TRUS using a 7.5 MHz frequency bipolar probe) are the preferred methods of evaluation in more common causes of male-factor infertility, such as varicocele or obstruction [44]. A dilated seminal vesicle with an anteroposterior diameter greater than 15 mm, or a dilated ejaculatory duct with a diameter greater than 2.3 mm, is suggestive of ejaculatory duct obstruction. However, TRUS has a low positive predictive value in diagnosing distal ejaculatory duct obstruction. Obstruction observed using TRUS was confirmed in only 48% of cases undergoing seminal vesicle aspiration [45]. Relying only on the TRUS findings, more than half of patients would have undergone unnecessary surgical intervention. TRUS is also used for non-surgical treatment of obstruction, or to aspirate seminal vesicles to obtain spermatozoa to be used with assisted reproductive techniques [24].

The most common abnormalities detected with scrotal ultrasound in infertile men are varicocele (35.5%), hydrocele (16.7%), testicular microlithiasis (9.8%) and epididymal enlargement (9%) [46]. The examination is performed in a warm and relaxing environment with the patient in a supine position using a high-frequency linear probe (7.5–10 MHz). Testicular size, presence or absence of any masses, epididymal structure and the diameter of the largest vein in resting state and during Valsalva maneuver are noted during this examination. In adults and adolescents, testis volume should be equal bilaterally, with the differential normally not greater than 2 mL or 20% of the volume.

A loss in testicular volume with accompanying ipsilateral varicocele is usually considered to be an indication for surgical correction. Different cutoff values for vein diameter have been proposed to diagnose varicocele; however, none has gained universal acceptance. It has been demonstrated that repair of larger varicoceles results in significantly greater improvement in semen parameters than repair of smaller varicoceles, and also that repair of clinically non-palpable but

ultrasonographically demonstrated varicoceles does not improve fertility rates [47].

Color Doppler ultrasound allows reversal of venous flow in spermatic veins while the Valsalva maneuver is being performed to be demonstrated, improving diagnostic accuracy when examining for the presence of varicocele. There are two competing systems used to classify the severity of reflux observed in testicular veins during Doppler ultrasound examination [48,49]. Both systems agree on the clinical importance of a reflux that lasts more than two seconds and has a plateau aspect throughout the Valsalva maneuver. The patient's chance of improvement after repair is maximum when varicoceles are greater than 3 mm or have a reversal of flow [50]. Additionally, a reflux at the inferior pole of the testis seems to predict a better outcome after surgical correction than reflux only in the supratesticular venous channels [51]. Even though US has proven its effectiveness, current guidelines suggest physical examination as the only tool necessary to evaluate varicocele in infertile patients, reserving US for patients with previous scrotal surgery or those in whom physical examination is difficult due to unfavorable body habitus [52].

Conclusion

A wide range of disorders involving the male reproductive tract may cause deterioration of seminal parameters. Any correctable pathology needs to be treated prior to the use of assisted reproductive techniques, to improve semen quality. This approach might enable the patient to conceive spontaneously, or allow the use of less expensive assisted reproductive techniques, such as IUI, instead of more advanced methods, such as ICSI.

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Chapter 3

Female causes of infertility: evaluation and treatment

Richard P. Dickey and Peter R. Brinsden

> Conception requires ovulation of a mature oocyte, normal fallopian tubes, the presence of progressively motile sperm in the female reproductive tract, and an endometrium favorable for implantation.
>
> RPD, PRB, 2009

Introduction

The incidence of female infertility is age- and parity-related. In a national government survey conducted in 2002, 7.4% of all married women in the United States, aged 15–44, reported difficulty becoming pregnant during the previous year [1]. The incidence of infertility ranged from a low of 4% in previously pregnant women aged under 30, to 27% in never-pregnant women aged 40–44. In a previous survey of the same population, 35% of women who sought medical help were treated with ovulation induction (OI), 13% with husband or donor intrauterine insemination (IUI) and 1.7% with in-vitro fertilization (IVF) or other advanced assisted reproductive technology (ART) [2]. Similar infertility rates are reported elsewhere in the Western world, with the availability of infertility treatment facilities and the use of IVF varying both between and within countries. In 2003, IVF was responsible for 6.5% of live births in Denmark and 3% of all live births in Europe [3], compared to 1.2% of live births in the United States [4]. Patients in the United States with private health insurance were four times more likely to receive OI and three times more likely to have IUI than patients without insurance, but no more likely to have IVF than patients without insurance [2].

The causes of infertility in 14,141 couples, from reports compiled in 1995, were ovulatory disorder 27%, abnormal semen 25%, tubal occlusion 22%, endometriosis 5% and unexplained 17% [5]. Other lists of infertility causes include cervical mucus factor, immunological factor and endometrial factor in 5–15% of cases. Results of IVF and preimplantation genetic diagnosis (PGD), performed because of "unexplained" infertility, suggest that many cases of infertility are due to aged or defective gametes. The primary cause or causes of infertility in over 12,000 pregnancies established following infertility treatment since 1976 at the Fertility Institute of New Orleans (FINO) are shown in Table 3.1. Causes of infertility in couples referred to FINO after three or more cycles of clomiphene citrate (CC) treatment elsewhere are shown in Table 3.2. Over 60% of CC treatment failures were found to have cervical factor or undiagnosed male factor, both treatable by IUI. A thin endometrial lining while taking CC, unsuspected tubal disease or endometriosis, insulin resistance and hypothyroidism were other contributing factors.

The goal of evaluation of the infertile female is to identify the cause of infertility or subfertility, which may be due to a medical or surgically correctable disorder in either partner. Modern infertility treatment with OI, IUI and IVF makes it possible to circumvent most of the "causes" of infertility. However, failure to diagnose and treat the primary cause may impair the patient's future fertility and general health, and can harm the developing fetus.

Ovulation disorders

The World Health Organization (WHO) classifies ovulation disorders into three groups:

- **Group I**: hypothalamic–pituitary failure (hypothalamic amenorrhea, hypogonadotropic hypogonadism). Characteristics: amenorrhea, low follicle-stimulating hormone (FSH) and estrogen, normal prolactin (PRL). Common causes are stress, weight loss, exercise, anorexia nervosa, Kallmann syndrome and isolated pituitary gonadotropin deficiency.

Manual of Intrauterine Insemination and Ovulation Induction, ed. Richard P. Dickey, Peter R. Brinsden and Roman Pyrzak. Published by Cambridge University Press.

Table 3.1. Causes of infertility in 12 000 pregnancies established following infertility treatment at the Fertility Institute of New Orleans

Cause	Incidence[a]	Tests
Male	40%	Postcoital test, semen analysis
Ovulatory	35%	BBT, serum progesterone, ultrasound, endometrial biopsy
Uterine/tubal	25%	HSG, SHG, laparoscopy
Peritoneal	15%	Laparoscopy
Cervical	10%	Postcoital test
Endometrial	10%	Ultrasound, endometrial biopsy

[a] 40% of infertile couples have more than one cause.
BBT, basal body temperature; HSG, hysterosalpingogram; SHG, sonohysterogram.

Table 3.2. Infertility factors found in 100 couples referred to the Fertility Institute of New Orleans because of failure to conceive after three or more cycles of clomiphene citrate

Factor	Incidence
Cervical	39%
Peritoneal (endometriosis)	31%
Male	25%
Tubal	24%
Insulin resistance	12%
Endometrial (thin endometrium)	10%
Hypothyroidism	5%

- **Group II**: hypothalamic–pituitary dysfunction. Characteristics: irregular or anovulatory menses, normal FSH, estrogen and PRL. Common causes are polycystic ovary syndrome (PCOS), and androgen disorders.
- **Group III**: ovarian failure. Characteristics: amenorrhea, low FSH and estrogen, normal PRL.

The WHO classification does not provide for ovulatory dysfunction unrelated to the hypothalamic–pituitary dysfunction, such as endocrine disorders, or ovarian disorders other than premature ovarian failure, as causes of infertility. Types of ovulation dysfunction include:

- anovulation, with heavy irregular menstruation (menometrorrhagia)
- ovulation with infrequent menses (oligomenorrhea), due to polycystic ovaries (PCO), PCOS, decreased ovarian reserve
- ovulation with low luteal-phase progesterone levels, also called luteal insufficiency (LI)
- pseudo-ovulation, apparent ovulatory cycles without release of an oocyte, also called luteinized unruptured follicle (LUF) .

The characteristic laboratory findings and treatment of endocrine, metabolic and ovarian disorders that can result in amenorrhea or anovulation are shown in Table 3.3.

Hypothalamic–pituitary (HP) causes of amenorrhea and ovulatory dysfunction

Primary HP amenorrhea is rare. If Kallmann syndrome (congenital HP amenorrhea associated with anosmia or hypo-osmia) is suspected, genetic counseling should be carried out before attempting pregnancy. Secondary HP amenorrhea due to stress, exercise and eating or weight disorders is mediated through the hypothalamic centers for gonadotropin-releasing hormone (GnRH). Treatment should be aimed at correcting the underlying condition. Secondary amenorrhea due to pituitary infarction or blood-loss shock (Sheehan's syndrome) is associated with adrenocorticotropic hormone (ACTH) and thyroid-stimulating hormone (TSH) deficiency, which should be corrected before attempting pregnancy. Obesity is a common cause of anovulation in developed countries. It has been estimated that 50% of women who are more than 20% over their ideal weight will be anovulatory or have luteal insufficiency. Often a 20% reduction in body weight is all that is required to restore fertility. Because obesity is associated with PCO, PCOS and insulin resistance, these disorders must be ruled out or treated first before attempting OI.

Table 3.3. Ovulation dysfunction

Cause	Test	Finding	Treatment
Hypothalamic[a]	FSH/LH	< 5 mIU/mL	Diet/gonadotropin
Hyperprolactinemia	Prolactin	> 20 ng/mL	Bromocryptine
Polycystic ovaries	LH : FSH	2 : 1 ratio	Clomiphene
Adrenal hyperplasia	DHEAS	> 180 µg/dL	Dexamethasone
Insulin resistance	Insulin	> 20 µU/mL	Diet/metformin
Hypothyroid	TSH	> 2.5 µU/mL	Levothyroxine
Menopause	FSH	> 20 mIU/mL	Estrogen
Premenopausal	FSH	> 10 mIU/mL	Clomiphene
Luteal insufficiency	Progesterone	< 18 ng/mL	Clomiphene

[a] Stress, starvation, anorexia, incarceration, excessive exercise, hypothalamic lesion (rare).
FSH, follicle-stimulating hormone; LH, luteinizing hormone; DHEAS, dehydroepiandrosterone sulfate; TSH, thyroid-stimulating hormone.

Treatment with CC is effective only in patients with sufficient serum estradiol levels (see Chapter 7). After correction of underlying problems, including those related to stress, exercise and eating disorders, treatment with gonadotropins is effective in patients with low FSH levels, but it must be started at a low dose because of the possibility that ovaries unaccustomed to FSH will be hyperstimulated (see Chapter 8).

Hyperprolactinemia

Prolactin-secreting pituitary adenomas may cause primary amenorrhea or secondary amenorrhea, but are fortunately rare. Approximately 30% of women with secondary amenorrhea will have elevated prolactin levels, which may or may not be accompanied by galactorrhea and may be due to drug-induced lactotroph cell hyperplasia, rather than an adenoma. Prolactin levels of 20–100 ng/mL usually cause anovulation rather than amenorrhea, and may be secondary to hypothyroidism. Rarely, growth-hormone-secreting pituitary tumors will present as amenorrhea. Between 4% and 16% of women with elevated prolactin will be found on MRI to have an empty sella turcica, a benign condition. Pituitary adenomas are classified as macro (> 10 mm), and micro (< 10 mm). Dopamine agonist drugs are highly effective in treatment of both macro- and microadenomas. Once pregnancy occurs, dopamine agonists may be continued or stopped. In either case patients should be monitored with repeated prolactin levels and visual fields examinations (not MRI or x-ray), lest an enlarging pituitary macro-adenoma damages the optic nerves. Patients may require CC in addition to dopamine agonist drugs to induce ovulation.

Polycystic ovaries (PCO), polycystic ovary syndrome (PCOS)

Polycystic ovaries (8–10 follicles < 8 mm per ovary) are the most common recognizable cause of ovulation dysfunction. PCO may be found in 10–25% of women with normal ovulation, and this causes a problem for infertility treatment, primarily because of the increased risk of multiple pregnancy during OI. Ovulation occurs, but is often late. The exact cause of delayed ovulation is unknown, but it may be because multiple follicles are competing for the same FSH pool. When polycystic ovaries are accompanied by excess androgens the condition is labeled PCOS. PCOS was originally described by Stein and Leventhal in 1935 as a variant of PCO, which previously had been believed to be associated only with excess estrogen [6]. Wedge resection, removing 50–75% of each ovary, was introduced by Stein and Leventhal and remained the standard treatment for PCOS amenorrhea until the introduction of CC in the 1960s and, later, laparoscopic ovarian drilling. PCOS is caused by a defect in the rate of conversion of androgen precursors to estrogens (aromatization), as demonstrated by in-vitro culture of ovarian tissue from PCOS patients with radiolabeled progesterone and androgen precursors [7]. The concentration of serum LH is increased, and the ratio of LH to FSH, which is normally 1 : 1, is 2 : 1 or greater. Treatment with CC is highly effective for PCO and PCOS.

Insulin resistance, metabolic syndrome

Insulin resistance (IR) is the cause of up to 25% of PCOS. It is differentiated from "classic" PCOS by elevated insulin levels, a normal LH-to-FSH ratio and failure to respond to clomiphene until insulin levels return to normal. Hyperinsulinemia, if left untreated, leads to hypertension, an increased risk of cardiovascular disease and gestational diabetes. Insulin resistance is considered to be one component of a condition formerly called syndrome X and now labeled metabolic syndrome. In addition to insulin resistance and obesity, the metabolic syndrome requires there to be three or more of the following [8]:

- hypertension 130/85 mmHg or higher
- triglyceride levels 150 mg/dL or higher
- HDL cholesterol levels less than 50 mg/dL
- abdominal obesity: waist circumference greater than 35 inches (89 cm)
- fasting glucose 110 mg/dL or higher.

Laboratory findings in IR are: fasting insulin levels greater than 20 μU/mL, and a fasting glucose-to-insulin ratio less than 4.5 [9]. Due to wide variability among different ethnic groups, the 2-hour glucose/insulin response to a 75 g glucose load is considered more reliable. A 2-hour insulin level of 100–150 μU/mL indicates probable IR; 150–300 μU/mL is diagnostic of IR; and above 300 μU/mL indicates severe IR. A 2-hour glucose of 140–199 mg/dL indicates impaired glucose tolerance. A 2-hour glucose above 200 mg/dL indicates non-insulin-dependent diabetes (type II diabetes). Patients with IR should not be labeled as type II diabetes unless, in addition to elevated insulin, the 2-hour glucose is also elevated. The first line of treatment for women with borderline and mild IR should be weight loss. Metformin 500–1,000 mg twice a day with meals is indicated for anovulatory women who continue to have elevated insulin levels after weight loss. Addition of CC is often necessary for ovulation [10].

Adrenal hyperplasia

Congenital adrenal hyperplasia is an inherited autosomal recessive enzyme defect that results in metabolic disorders and masculinization of newborn females. It is fortunately rare. A milder form, with onset at or following menarche, is variously labeled late-onset, adult-onset, acquired, partial, attenuated and non-classical adrenal hyperplasia. The most common form is due to 21-hydroxylase deficiency; other forms are due to 11β-hydroxylase deficiency and 3β-hydroxysteroid dehydrogenase deficiency. Clinical signs include mild hirsutism, increased skin sebum causing mild acne, increased scalp sebum making daily hair washing necessary, and mild hypertension. The diagnosis is confirmed by 17-hydroxyprogesterone (17OHP) levels ≥ 200 ng/dL or dehydroepiandrosterone sulfate (DHEAS) levels ≥ 180 μg/dL, which may also originate in the ovary. Elevated DHEAS is more common in mild cases and can be measured first. 17OHP should be measured first if there is virilization (hirsutism, male-pattern baldness or clitoral enlargement). Treatment for either defect is low-dose corticosteroid (0.5 mg dexamethasone or 5 mg prednisone) daily at bedtime. The addition of CC is often necessary for ovulation. Corticosteroids should be discontinued after ovulation, because of the risk of birth defects. Amenorrhea and excess androgen may be due to Cushing's syndrome or acromegaly. Rapid development of virilization may be due to an androgen-producing tumor.

Thyroid causes of ovulation dysfunction

Hypothyroidism and hyperthyroidism are associated with menstrual dysfunction, with hypothyroidism being more common. TSH levels < 0.4 μU/mL are abnormal and indicate the need for additional studies to diagnose hyperthyroidism. Although TSH levels ≥ 4.5 μU/mL are generally accepted as diagnostic of subclinical hypothyroidism, some medical and reproductive endocrinologists consider TSH levels ≥ 2.5 μU/mL as abnormal and contributing to ovulatory dysfunction. Hypothyroidism during pregnancy is linked to miscarriage and mental retardation in children. Patients with TSH levels ≥ 4.5 should be treated with levothyroxine (LTX) 50–75 μg per day before attempting pregnancy. Treatment with LTX 25–50 μg per day may be considered for anovulatory patients when TSH levels are between 2.5 and 4.5 μU/mL. While pregnant, patients with TSH levels ≥ 2.0 μU/mL should be retested monthly during the first trimester and again postpartum. Newly pregnant patients already using LTX should have the dose increased by 20–50% (at least 25 μg) as soon as pregnancy is confirmed. TSH levels are approximately 30% lower when measured while patients are fasting, and should not be obtained at the same time as tests (insulin, glucose, growth hormone) that require fasting.

Premenopause, menopause

The diagnosis of menopause is customarily made when a woman is amenorrheic for six months or more and

serum FSH is ≥ 20 mIU/mL. Care should be taken not to label a patient as menopausal and therefore infertile on the basis of a single FSH value ≥ 20. FSH levels vary widely during the perimenopausal years: the first author has seen FSH levels as high as 26 mIU/mL followed by ovulation and conception during the same cycle. As a general rule, patients with FSH levels ≥ 12 will respond poorly to gonadotropin stimulation and will not become pregnant. CC has been found to be as effective or more effective than gonadotropins for OI in IUI patients aged 38 and older [11]. When cycle day 3–5 FSH levels are in the range of 8–12 mIU/mL, the CC challenge test (CCT) described in this chapter [12] will help to differentiate patients who can become pregnant on their own or can benefit from fertility treatment from those unlikely to become pregnant.

Luteal insufficiency and luteinized unruptured follicle syndrome

One of the most common ovulatory disorders in patients over the age of 28 is luteal insufficiency (LI). Patients with LI ovulate but pregnancy fails to occur or ends in early miscarriage. At one time the diagnosis was made by endometrial biopsy, a procedure that was uncomfortable and risked injury to an implanted embryo. Today the diagnosis is more often based on serum progesterone levels and the appearance of the endometrium on ultrasound (US). Although some textbooks and laboratory manuals report serum progesterone concentrations of 5–10 ng/mL as evidence of normal ovulation, many infertility experts believe that levels lower than 16–18 ng/mL are associated with failure to implant or early miscarriage. On ultrasound, the mid-luteal phase endometrium should have a thickness ≥ 9 mm and the endometrial pattern should be homogeneous and hyperechogenic (Fig. 9.10). A low progesterone concentration and inadequate endometrial development, despite ovulation, is most often due to inadequate follicular development in the proliferative phase of the cycle. In the luteinized unruptured follicle syndrome (LUF), ovulation does not occur despite an LH surge, an increase in progesterone concentration, physiological changes normally associated with ovulation (shift in basal body temperature, cervical mucus thickening) and a change in the endometrial pattern. However, there is no change in size of the dominant follicle on ultrasound, and no operculum (stigma of ovulation) is found at laparoscopy. The underlying defect may be either inadequate follicular development or a weak LH surge. Treatment of LI with CC is preferred to progesterone supplementation with oral, vaginal or intramuscular progesterone because it corrects the underlying defect of inadequate follicular development. CC is also an effective treatment for LUF, but some patients may require human chorionic gonadotropin (hCG). Treatment with dexamethasone has been tried with less success.

Tubal disorders

Tubal obstruction and tubal adhesions are responsible for 25% of infertility. Causes of tubal disease include infection (primarily *Chlamydia trachomatis*, but also tuberculosis in some countries), endometriosis, pelvic surgery and rarely uterine fibroids. Methods used to evaluate tubal patency are the hysterosalpingogram (HSG), the sonohysterogram (SHG; described in Chapter 9) and laparoscopy. HSG and SHG provide detailed information about the uterine cavity that laparoscopy, without the addition of hysteroscopy, cannot. HSG and SHG are performed in an outpatient setting and may be therapeutic. Laparoscopy provides detailed information about external tubal, ovarian and pelvic anatomy that HSG and SHG cannot, and allows ablation of endometriosis and lysis of adhesions, but is more expensive and requires general anesthesia.

Peritoneal disorder (endometriosis)

Endometriosis, and tubal occlusion due to adenomyosis, were the primary causes of 15% of infertility (Table 3.1), and were a factor in 31% of patients who failed to conceive after three or more cycles of CC-IUI (Table 3.2). The diagnosis of endometriosis requires laparoscopy, but can be suspected if tender nodular areas are palpable near the insertion of the uterosacral ligaments, if a CA-125 test result is > 35 IU/mL, if there is cornual tubal obstruction on HSG (especially when unilateral), or if there have been increasingly severe symptoms of dyspareunia and dysmenorrhea in the absence of pelvic infection. Endometriosis is found at laparoscopy in 18–50% of women with normal fertility. Moderate and severe endometriosis may cause infertility because of adhesions interfering with oocyte pickup, tubal obstruction, impairment of ovarian function by large endometriomas, and presence of blood products from superficial endometriotic implants in the fallopian tubes, which can also occur in minimal and mild endometriosis.

The infertility evaluation is incomplete until HSG, SHG or hysteroscopy and a diagnostic laparoscopy have been performed, but the question is when in the

course of the infertility workup it should be done. In the absence of clinical findings or symptoms of pelvic pathology, tubal evaluation may be reasonably postponed until after three or four cycles of IUI alone or CC-IUI. Tubal evaluation should be performed before beginning gonadotropins, a more costly and intrusive procedure. The decision about whether to perform HSG or laparoscopy before starting any form of infertility treatment is aided by testing for serum chlamydial IgG and CA-125 early in the diagnostic workup. Laparoscopy should be performed by a surgeon trained and equipped to treat peritoneal endometriosis by laser or electrocautery and to resect (not drain) endometriomas laparoscopically.

Medical treatment with GnRH agonists or antagonists and attenuated androgens (danazol) should be reserved for patients with obstructed fallopian tubes due to endometriosis or adenomyosis. When medical treatment is used because of tubal occlusion it should be for three months only, followed by HSG. During medical treatment, whether with modified androgens (danazol) or GnRH agonists, estradiol must be suppressed to menopausal levels (< 15 pg/mL) in order to be fully effective [13].

Cervical factor

Cervical factor was the primary cause of 10% of infertility (Table 3.1), and was a factor in 39% of patients who failed to conceive after three or more cycles of CC-IUI (Table 3.2). The appropriately timed Sims–Huhner postcoital test (PCT) evaluates coital technique, mucus quality and quantity, and sperm morphology and competence. The presence of any sperm with rapid progressive motility (PM) argues against significant cervical factor as a cause of infertility [14], and the presence of large numbers of PM sperm is presumptive evidence that IUI is unnecessary and will not increase the possibility of pregnancy [15]. The PCT using ≥ 5 PM sperm per high-power field (HPF) as a cutoff point has been a standard part of infertility evaluation in the United States for many years. A 1998 report of a survey of Board-certified reproductive endocrinology and infertility (REI) specialists in the United States found that 92% in private practice and 73% in academic practice employed the postcoital test in their initial evaluation of infertile couples [16]. In a report from the Netherlands of 956 cases of infertility, which is the largest report to date from a single center, results of the PCT were scored as negative (no sperm visible), non-progressive (only immotile or locally motile sperm) or progressive (one or more PM sperm in the entire sample 9–12 hours after intercourse) [17]. Cumulative pregnancy rates after one year with no additional treatment were 15% for couples with no sperm, 24% for couples with only immotile sperm, and 41% for couples with ≥ 1 PM sperm. Women with ≥ 1 PM sperm on a 9–12 hour PCT had a monthly fecundity rate 5.5 times greater than women with no sperm.

Table 3.4. Sensitivity and specificity of postcoital test according to sperm criteria

Motile sperm per field (× 400)	Sensitivity	Specificity
≥ 1	0.62	0.80
≥ 5	0.84	0.44
≥ 20	0.90	0.30

Studies with 200+ couples, weighted averages. Adapted from Oei *et al*. 1995 [14]. Sensitivity measures a test's ability to identify infertile couples; specificity measures its ability to identify fertile couples.

Recently, there has been a trend in the United States and Europe to bypass the postcoital test and treatment of mucus disorders and proceed directly to IUI in couples with unexplained infertility and patients treated with OI drugs. The value of the postcoital test has been questioned because of inability to predict fertility (specificity) or non-fertility (sensitivity) in 100% of couples, because of the lack of standardization as to the number of sperm that constitute a positive test and because it is possible to recover peritoneal sperm despite a poor postcoital test [18]. The first two of these arguments are answered in part by a review that included 3,093 cycles, in which sensitivity ranged from 0.62 to 0.90 and specificity from 0.30 to 0.90, depending on whether the criteria for a positive test was set at ≥ 1, ≥ 5, or ≥ 20 PM sperm per HPF (Table 3.4) [14]. Therefore the predictive power of a PCT exceeds that of semen analysis except when sperm numbers are severely depleted [19]. With few exceptions, peritoneal sperm recovery studies have involved intracervical insemination of sperm and small numbers of patients.

The PCT should be performed 9–12 hours after intercourse, the length of time presumed to be required for capacitation and fertilization, in order to evaluate sperm survival and behavior, and 1–3 days before ovulation occurs, in order to evaluate mucus quality when it is at its peak [20]. Directions for performing the PCT are provided in Chapter 5. In a normal PCT,

Table 3.5. Postcoital test: abnormal findings, possible causes, effects and treatments

Finding	Possible cause	Effect on fertility	Treatment
Thick mucus	Low estrogen	Sperm unable to penetrate mucus	Estrogen, guanefesin (cough-syrup ingredient)
Scant mucus	Low estrogen	Sperm fail to enter cervix	Estrogen, alkaline douche (60 g/4 tablespoons sodium bicarbonate per 1000 mL/1 quart warm water)
Bacteria	Coagulase-positive *Staphylococcus aureus*	Sperm rendered immobile	Cephalosporin antibiotics (Duricef® 500 mg daily, Vantin® 400 mg) twice daily, for 7 days).
Acidic pH (< 5.5)	Diet, *Lactobacillus*	Sperm rendered immobile	Diet, calcium carbonate antacid pills (Tums® 2 × 4 times a day) for 2–5 days before ovulation
Polymorphonuclear (white blood cells)	Non-specific infection	Sperm may be phagocytized	Doxycycline
Immotile/shaking sperm	Infection/antibodies	Sperm unable to progress through mucus	Antibiotics, IUI, IVF-ICSI
Bent neck/coiled sperm	Ureaplasma/ T-mycoplasma	Sperm unable to progress or fertilize oocyte	Doxycycline 100 mg 2 × day or erythromycin 500 mg 4 × day, men × 10–14 days & women × 7 days
< 1–19 progressively motile sperm in best field (× 400)	Low sperm count, scant mucus, coital technique	Too few sperm reach fallopian tubes	Alkaline douche (60 g/4 tablespoons sodium bicarbonate per 1000 mL/1 quart warm water), IUI

the mucus should be clear, with egg-white consistency. Abnormal findings in the PCT, their probable causes, their potential effects on sperm survival and passage and suggested treatments are shown in Table 3.5. When the PCT is abnormal and the semen analysis is normal, treatment of the cervical mucus should be tried before resorting to IUI or IVF in couples with unexplained infertility.

Endometrial factor

Endometrial factor was the primary cause of 10% of all infertility (Table 3.1), and was a factor in 10% of patients who failed to conceive after three or more cycles of CC-IUI (Table 3.2). Normal wall-to-wall endometrial thickness is ≥ 9 mm at the end of the proliferative phase. Pregnancies rarely occur in IVF or OI-IUI cycles when endometrial thickness is less than 6 mm, and are reduced or result in early pregnancy loss when thickness is 6–9 mm [21,22]. In a retrospective study, endometrial thickness before ovulation was less than 6 mm in 9% of spontaneous IUI cycles, 9% of CC-IUI cycles and 2% of gonadotropin IUI cycles [21]. Endometrial thickness is adversely affected by CC. Endometrial and uterine abnormalities that affect endometrial thickness or may have an adverse effect on implantation include intrauterine synechiae (Asherman's syndrome), endometrial polyps, endometrial hyperplasia, endometrial fluid and submucosal fibroids. Preovulatory endometrial thickness and follicle development should be evaluated during the infertility workup, during initial OI cycles and before the initial IUI procedure (see Chapter 9).

Infection

Chlamydia trachomatis is the leading cause of tubal occlusion in developed countries. Non-pulmonary tuberculosis is a leading cause of tubal occlusion in some developing countries. Other infectious agents proposed as causes of infertility and miscarriage include *Toxoplasma gondii*, bacterial vaginosis, *Mycoplasma hominis* (*Ureaplasma*), *Listeria monocytogenes*, *Campylobacter*, cytomegalovirus, and herpesvirus. Use of an intrauterine contraceptive device (IUD) has been linked to unilateral and bilateral tubal obstruction due to actinomycosis infection in developed and developing countries. Tubal factor should be suspected if an IUD was removed because of pain, bleeding or infection. Hepatitis B, hepatitis C, human immunodeficiency virus (HIV) and genital herpes do not cause infertility but may worsen during pregnancy and may be transmitted to the baby during delivery. Other viral diseases such as rubella and cytomegalovirus may cause birth defects.

Patients with a history of infectious disease or miscarriage, and those who have been exposed to diseases that may cause infertility and birth defects, or may be transmitted during delivery, should be screened, and treated if necessary, before beginning infertility treatment.

Infection causing tubal adhesions or obstruction may be introduced at the time of surgical (dilation and curettage, hysteroscopy, biopsy) or diagnostic radiological procedures. Prophylactic antibiotics should be used for all intrauterine procedures. Antibiotics are customarily added to IUI media, but nevertheless practitioners should ensure that this has been done and, if not, provide patients with antibiotic coverage. They may also wish to provide additional antibiotics if bacteria are present in the initial sperm specimen or cervical mucus.

Immunological disorders

Autoimmune and alloimmune disorders have been linked to recurrent pregnancy loss in the second and third trimester, and to intrauterine growth restriction (IUGR). There is no verifiable evidence that they are a cause of infertility. Systemic lupus erythematosus (SLE), the most frequently diagnosed immunological disorder, is associated with a 20% pregnancy loss rate in the second and third trimester, almost always in conjunction with antiphospholipid (APL) syndrome. The prevalence of APL syndrome in women with recurrent late abortion is low (3–5%). Patients known to have lupus or who have had recurrent late abortions should be screened for APL before being treated with IUI or OI. Tests for lupus anticoagulant and anticardiolipin antibodies are relatively inexpensive. Treatment with aspirin (75–85 mg daily) beginning with the start of attempting pregnancy, and unfractionated heparin (5,000–10,000 units subcutaneous twice daily) or low molecular weight heparin (enoxaparin [Lovenox®] 40 IU daily) beginning at eight weeks, has been found to be effective in reducing pregnancy loss due to SLE.

Antisperm antibodies (ASA) in the male are discussed in Chapter 2. Antibodies form in the male when the blood–testis barrier is compromised, commonly through trauma, testicular biopsy, vasectomy or infection. ASA can be detected in semen, serum and cervical mucus. They are found in 3–12% of men presenting for infertility evaluation. The presence of agglutination in semen specimens is presumptive but not definitive evidence for antisperm antibodies, since spontaneous agglutination of sperm can occur. The presence of immotile and shaking sperm in cervical mucus is presumptive evidence of antisperm antibodies in the female. The most widely used assay for detection of ASA is the immunobead test (see Chapter 5). The immunobead test is a method of quantifying the percentage, pattern and class (IgG, IgA) of surface antibodies. It should be considered when greater than 1+ agglutination of motile sperm is observed on initial microscopic examination or shaking sperm are observed on a postcoital test. Immotile sperm may be due to other causes, which should be eliminated first. Immunobead binding restricted to the tail is normal. The test is considered positive if 50% of motile sperm heads are coated with immunobeads. Sperm penetration into cervical mucus and in-vivo fertilization are not significantly impaired unless 50% or more of motile sperm have antibodies [23]. ASA resulting from infection in the seminal vessels or epididymis may disappear after the infection is treated. The role of IUI in the treatment of immunological infertility due to antisperm antibodies has yet to be confirmed [24] (see Chapter 10). IVF with intracytoplasmic sperm injection (ICSI) provides fertilization rates in ASA-positive couples comparable to those in ASA-negative couples [25].

Unexplained infertility

The diagnosis of unexplained infertility should not be made without diagnostic laparoscopy and luteal insufficiency, endometrial factor and cervical factor have been eliminated as causes. Studies that report more than 10% of infertility as unexplained omit one and sometimes two or more of these tests. When these potentially correctable factors have been ruled out there will remain a small percentage of couples whose infertility can only be explained when IVF is performed. The findings in these cases are often either oocytes of poor quality, failure of fertilization or embryos that cease to develop or fail to implant.

Organizing the infertility workup

When to start evaluation

In the absence of obvious causes of infertility, evaluation should be started after one year of unprotected intercourse when the woman's age is under 35. Evaluation should be started earlier if the woman is older than 35, or if there is a history of irregular menses, pelvic pain, pelvic surgery, sexually transmitted disease or testicular injury. Evaluation can be started at any age in patients who wish to know their chance of pregnancy

before marriage (vetting). When the woman's age is under 35, 80% of fertile couples will conceive within 12 months, 60% will conceive within six months and 25% will conceive in the first month of trying [26].

Initial tests

At the beginning of infertility evaluation, prenatal screening tests customarily performed on newly pregnant patients and a TSH test should be ordered; the exact makeup will depend on regional differences in endemic diseases. Rubella has now been eradicated in some countries; toxoplasmosis is prevalent in some countries and rare in others. Nearly all countries require serology for syphilis, hepatitis B and C, HIV, blood type and Rhesus blood type. To these should be added TSH, because subclinical hypothyroidism effects reproduction and the fetus. Women should start taking prenatal vitamins. Both sexes should stop smoking, reduce their alcohol consumption and avoid cannabis.

Basic workup

The basic infertility workup will discover a cause in 80% of couples without tubal or pelvic disease, and requires only two tests, both accomplished in one cycle: a postcoital test performed 1–4 days before expected ovulation and mid-luteal-phase serum progesterone, which is measured 4–8 days after ovulation.

Additional tests

Additional tests are ordered based on the findings in these two tests, and on the patient's history and symptoms.

- If the PCT is negative or < 5 progressively motile sperm per HPF, or if the male partner has a history of testicular injury or genitourinary tract infection, a semen analysis (SA) is ordered. If an IUI is necessary the SA can be performed on the IUI specimen.
- If the progesterone is < 18 ng/mL, or if the female partner has infrequent or irregular menses, FSH, LH, estradiol, and pelvic US are performed on day 3–5 of her menstrual cycle. If indicated, ovulation induction can be started the same day. These tests, plus prolactin, can be done on any day of the cycle in amenorrheic patients.
- Additional endocrine tests are drawn at the initial visit as follows. If androgen excess is suspected, serum DHEAS is ordered (17OHP, free and total testosterone may also be ordered). If galactorrhea is present, prolactin is ordered. If there is a family history of type I or II diabetes (adult-onset controlled by oral medications), a fasting glucose/insulin ratio or 4-hour glucose tolerance test with fasting insulin is ordered (note that TSH should not be measured while fasting).

Advanced (invasive) tests

- If the patient has dysmenorrhea, dyspareunia, a history of pelvic inflammatory disease, more than usual pain during bimanual pelvic examination or hydrosalpinx on ultrasound, serum for *Chlamydia trachomatis* and CA-125 (for endometriosis) are ordered during the initial interview and, if positive, either HSG or diagnostic laparoscopy are performed before initiating infertility treatment
- If serum progesterone is normal, there are ≥ 20 progressively motile sperm on a postcoital test, and menstrual cycles are regular, the probability of hidden tubal or peritoneal disease is high enough that HSG or diagnostic laparoscopy should be performed as part of an initial investigation of infertility; otherwise HSG and laparoscopy may be postponed until after insemination and ovulation induction, if indicated, have been performed for 2–4 cycles without pregnancy.

Length of treatment

The majority of couples who do not have obvious causes for infertility (anovulation, low sperm count, symptoms or history of pelvic pathology) are subfertile rather than infertile. Most will become pregnant within four cycles of treatment with IUI or CC, alone or together. Pregnancy rates per cycle decline markedly after the fourth cycle of CC-IUI in patients with endometriosis, and non-obstructive tubal disease, and after the third cycle in those with poor sperm quality [27]. Patients not pregnant after the third or fourth cycle of IUI or CC-IUI should have a diagnostic laparoscopy (if not done previously) followed by gonadotropin IUI or referral for IVF if no cause is found. If mild-moderate tubal or peritoneal pathology is found and treated, IUI or CC-IUI should be restarted and continued for three cycles before gonadotropin IUI in patients aged under 38. Because of lower fecundity, patients aged 38 or over should be moved rapidly to gonadotropin IUI or IVF if they do not develop at least four preovulatory follicles when taking CC. In the authors' practices the chances of pregnancy with OI-IUI versus IVF are discussed with patients aged 38 and older, with emphasis on the much

Table 3.6. Possibility of spontaneous pregnancy during the next 24 months

	Normal response		Decreased ovarian reserve	
Age	Patients	Pregnancy rate	Patients	Pregnancy rate
≤ 30	95%	66%	5%	13%
31–33	93%	57%	7%	13%
34–36	87%	43%	13%	5%
37–39	80%	39%	20%	5%
≥ 40	56%	10%	44%	0%

Clomiphene challenge: FSH ≥ 11 mIU/mL cycle day 3–5 and after clomiphene citrate 100 mg × 5 days. Adapted from Scott *et al*. 1995 [12].

better chance with IVF; however, 2–3 cycles of OI- IUI are offered if the semen quality is satisfactory, so long as couples are fully aware of their chances of success.

Prediction of future fertility (vetting)

Patients starting infertility treatment deserve to know, and indeed frequently ask, about their chance of becoming pregnant in the first cycle or during a course of treatment. The probability of becoming pregnant in a single cycle (cycle fecundity) should be determined early in an infertility evaluation so that patients are not subjected to repeated cycles of treatment that have little chance of success. Physicians may also be asked to evaluate the chance of pregnancy at some time in the future (vetting) by women who are contemplating an educational or career change that may delay their starting a family.

The methods most often used to predict fecundity are antral follicle count (AFC), cycle day 3–5 FSH, and the change in FSH in response to CC, named the CC challenge test (CCT). An AFC < 4 is predictive of poor ovarian response. In the CCT, baseline serum FSH is measured on the third to fifth cycle day, CC 100 mg is taken for five days, and FSH measurement is repeated [12]. An FSH level ≥ 10 mIU/mL on the third to fifth cycle day or following five days of CC is a sign of decreased ovarian reserve and is associated with decreased probability of spontaneous pregnancy (Table 3.6). Cycle day 3–5 FSH levels are unreliable if estradiol levels are above 50–80 pg/mL.

The probability of pregnancy within 1–4 cycles of CC-IUI can be predicted if antral follicles are measured at the start of the CCT and the number of preovulatory follicles are measured 10 days later (see Table 7.1 and Fig. 7.5D). Other methods to predict fecundity include the anti-Müllerian hormone (AMH), inhibin B concentration and ovarian volume on the third to fifth cycle day. The sensitivity, specificity and predictive value of these tests have been reviewed [28]. In a single cycle, the predictive ability was: inhibin B 71%, FSH 77%, AFC 80%, CCT 81%, AMH 92%. Results of all tests must be interpreted to the patient with caution, because patients with abnormal levels in one cycle may become pregnant in a later cycle.

The CCT with AFC method of predicting current and future fecundity per cycle, based on the number of antral follicles and response to CC, can be used to predict pregnancy in controlled ovarian hyperstimulation (COH)-IUI and IVF cycles, as well as in CC-IUI cycles. The number of antral follicles, and the capability of antral follicles to respond to OI and develop into preovulatory follicles, diminishes with age. In IVF cycles, the number of good oocytes per preovulatory follicle, and the likelihood that a good embryo will develop, implant and result in a live birth also diminishes with age. The relationship of patient age, AFC and FSH the cycle before IVF to numbers of preovulatory follicles ≥ 10 mm, mature eggs, good-quality embryos, implantation rate per transferred embryo and continuing or delivered pregnancy rate in a patient's first IVF cycle at the Fertility Institute of New Orleans during 2005–8 is shown in Table 3.7. The prognostic factors most strongly related to live births, in order of importance, were (1) age ($r = -0.20$), (2) AFC ($r = 0.15$), (3) FSH ($r = -0.04$). Other factors were a previous pregnancy ($r = 0.06$) and previous birth ($r = 0.08$).

Between age 25 and 40 the average number of antral follicles and eggs decreased by approximately 3.5% per year, while the number of preovulatory follicles ≥ 12 mm, good-quality embryos and implantations per embryo transferred decreased by approximately 4% per year. All possible good embryos were not transferred in

Table 3.7. Relationship of age, FSH and antral follicles to eggs, embryos and implants: first IVF cycle

Age	25–29	30–34	35–39	40–44	All
Number of cycles	115	218	203	72	608
FSH resting cycle	6.2	6.1	7.0	8.4	6.8
Antral follicles	13.6	10.3	9.0	6.0	10.9
Preovulation follicles ≥ 10 mm	22.6	19.7	15.4	8.8	18.5
% antral follicles	166%	191%	171%	147%	170%
Mature eggs	12.0	10.6	9.6	5.7	9.9
% antral follicles	88%	103%	107%	95%	91%
% preovulation follicles	53%	54%	62%	63%	54%
Good-quality embryos	6.3	5.5	4.8	2.6	5.1
% antral follicles	46%	53%	53%	43%	47%
% preovulation follicles	28%	28%	31%	30%	28%
% mature eggs	52%	52%	50%	46%	52%
Sacs per embryos transferred (%)	44%	36%	26%	18%	32%
Pregnancy per cycle (%)	58%	54%	43%	28%	48%
Continuing pregnancy per cycle (%)	48%	42%	30%	17%	40%

Relationship of live births to prognostic factors: patient age $r = -0.20$; number of antral follicles $r = 0.15$; FSH $r = -0.04$.

younger patients, and the miscarriage rate increased with age so that the live birth rate decreased by more than 4% per year. A high AFC (≥ 20) along with a low FSH in a woman 20–30 years old predicts not only that she has a high chance of pregnancy in a single IVF cycle but that she may have ≥ 10 antral follicles 10 years later and still a good possibility of pregnancy. An AFC < 8 at age 20–30 predicts a good chance of pregnancy at her current age but a low possibility of pregnancy 10 years later. Pregnancy rates in COH-IUI cycles are approximately half those of IVF cycles for patients of the same age. Pregnancy and implantation rates in CC-IUI cycles and COH-IUI cycles are the same per preovulatory follicle ≥ 12 mm [11].

Conclusion

Accurate diagnosis of the cause of subfertility or infertility in a female patient is not possible without a "proper" workup. What constitutes a "proper" workup is open to wide interpretation among reproductive endocrinologists worldwide. Some will insist on a "full" workup, performing every available test, while others perform only a "minimal" workup, before pressing on with treatment. There is an increasing tendency to move directly to the "high-tech" assisted reproductive technologies, which is not always in the patient's best interests. There is no "right answer," but we have presented in this chapter what we believe to be a reasonable overview of what tests and treatments should be done, on whom and why.

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Chapter 4

Clinic and laboratory design, personnel and equipment

Roman Pyrzak

Introduction

The equipment, the personnel and the space requirements needed to perform intrauterine insemination (IUI) and ovulation induction (OI) will depend on the number and type of procedures to be performed, and on the availability of other infertility services in the area. A gynecology or general practice office or clinic that routinely performs speculum examinations and only intends to perform IUI with cryopreserved donor sperm will already have all the necessary equipment if it has a microscope and either performs ultrasound or has it available nearby. Larger offices and clinics that plan to offer IUI with husband's or partner's sperm will need appropriate counter space for equipment to perform semen analysis, sperm processing and other tests if required. If they also plan to cryopreserve sperm, they will need additional space for liquid nitrogen tanks and storage space for Dewar cryogenic storage tanks. Clinics that monitor their own reproductive hormone levels during the cycles require additional equipment and a substantial increase in counter and laboratory space.

This chapter describes the requirements for clinic layout and personnel in general terms, and provides lists of equipment, disposable supplies and safety items that may be needed, depending on the specific procedures that an infertility office or clinic plans to perform.

Facilities

Clinics that perform therapeutic fertility treatments should be designed in such a way that they are capable of providing the best patient care (Fig. 4.1). Small private clinics as well as large clinics need sufficient lobby space and a friendly, welcoming atmosphere. The lobby should be spacious, furnished with journals, magazines, periodicals, professional literature and brochures, with access to the internet, a television and a beverage station. Patient privacy is of the utmost importance, and required by law in the United States. Consultations in the hall or at the nurses' station are no longer tolerated. Physicians and nurses require an adequate number of rooms to consult with their patients when planning and monitoring IUI and OI cycles. The treatment room in which IUI and gynecological examinations are performed should have a counter with a sink and sufficient room to accommodate the examination table, the patient, the patient's companion, the nurse and the physician. In clinics that specialize in infertility diagnosis and treatment a 1 : 1 ratio of consultation rooms to treatment rooms works well. If a sufficient number of IUI or OI cycles is performed, a special room or rooms should be designated for ultrasound scanning to monitor follicular growth, with a private waiting area away from the lobby (Fig. 4.2). A small room or screened space should be dedicated to drawing blood, testing blood pressure and checking patient weight.

Semen collection room

If semen processing is to be performed, it is very important that a private room dedicated for specimen collection be available in the clinic setting. It should have sufficient space, and should be well ventilated. It should be sufficiently isolated to prevent outside noise from being heard in the room, as any distraction may hinder specimen collection. The collection room needs to be comfortable and have a reclining chair or a small sofa. Patients may be accompanied by their partners to the collection room. Each room should also have a sink and a toilet. Visual aids such as erotic magazines and videos should be available in the room for the patients. An "occupied" sign should be placed on the outside door to indicate that the room is in use. The semen collection room can be located adjacent to the laboratory, so that the collected specimen can be placed through a small opening in the wall directly into the laboratory.

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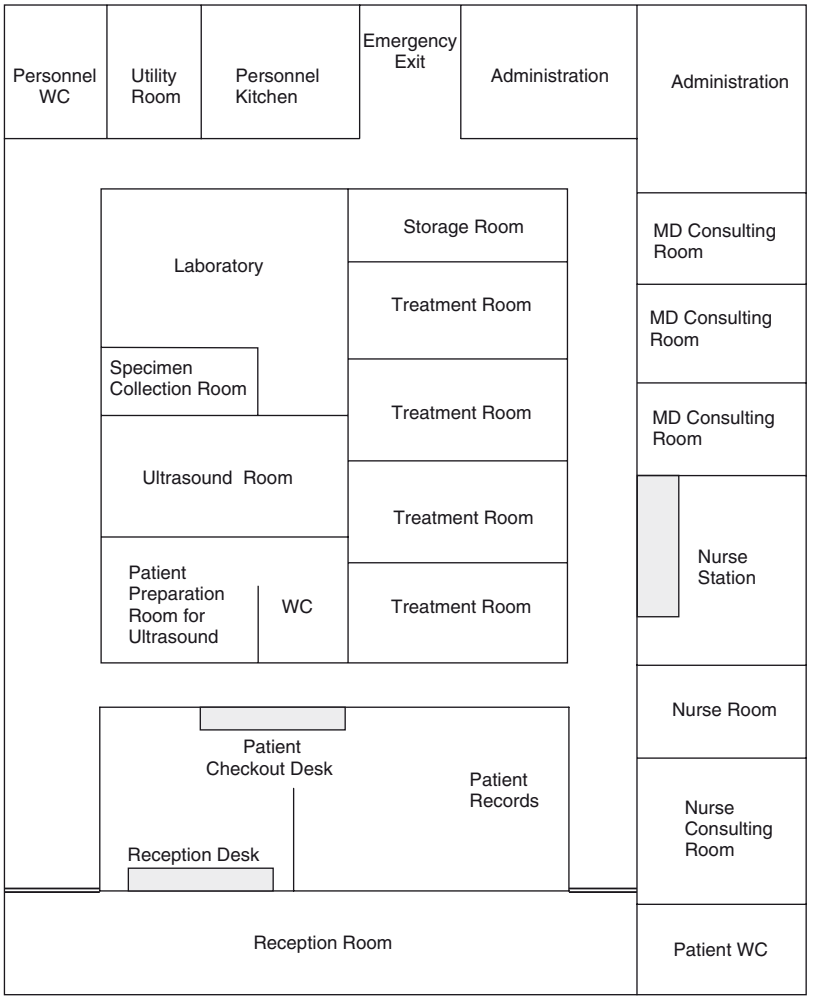

Figure 4.1 Example of clinic design.

Clinic and laboratory personnel

The staff, medical staff, nurses, laboratory technicians, ultrasonographers, support office staff and administration team are the most important components of a successful infertility treatment program. Their skills, and collaboration between them, are essential to the success of infertility services. The number of employees required in each clinic depends on the number of different services it offers, and the numbers of patients it serves. A single nurse designated the "infertility nurse" or "IUI nurse" will be overwhelmed in a large IUI program and can not always be available. It is preferable that all nurses be familiar with OI and IUI procedures.

In the United States, each technologist performing andrology tests and sperm preparation procedures must be Board-certified. Technologists are required to have continuing education, to keep abreast of the newest developments and constantly to improve their expertise. If more than one technologist is working in the laboratory, test results must be comparable. All US laboratories are required to undergo yearly inspections and certification by the Clinical Laboratory Improvement Amendments (CLIA). Therefore, laboratory procedures and documentation must exactly follow what is outlined in the laboratory manual, without exceptions.

IUI procedures may take place during weekends and holidays; therefore, staff need to be available or "on call" at such times to perform all their required duties. For a small office clinic, a nurse and a physician are required to be on hand, while in a larger clinic, additional staff may be required – for example, a receptionist, ultrasonographer and laboratory technician. Ordinarily, the staff are required to be in the clinic for only a few hours in the morning during weekends and holidays.

Laboratory design

The laboratory should have adequate space to follow good laboratory practices. Important issues include bench height, adjustable chairs, microscope eye height, efficient use of space and surfaces, sufficient air circulation and air-conditioning system, enough light and proper storage. All these contribute to a good working environment that minimizes distraction and fatigue. The location of equipment such as incubator and centrifuge should be logically planned for efficiency and safety within each working area (Fig. 4.3).

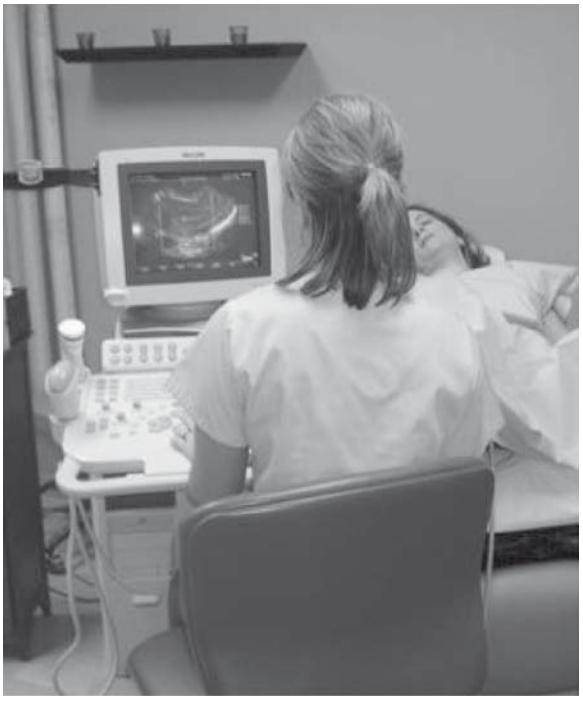

Figure 4.2 Ultrasound room.

Equipment

Most instruments and supplies have more than one manufacturer and model. The specific brand to be used depends on cost, on the preference of laboratory and medical personnel, on the location of the manufacturer, and on the availability of after-sales training and service. A detailed comparison of different equipment manufacturers, suppliers and price quotes needs to be conducted before any equipment is purchased for the laboratory. Other factors that need to be considered when purchasing new equipment include payment method, price of consumables, technician support, service warranty and service contract cost after the first year. Large and sensitive machines or equipment should be delivered to the clinic by the manufacturers or their agents, and should be installed without additional cost.

The essential items of equipment required for a laboratory are shown in Table 4.1, and a selection is illustrated in Figure 4.4. Among the most important of these are: a binocular phase contrast microscope with × 20, × 40 and × 100 objectives and × 10 eyepiece; a bench centrifuge for sperm processing, calibrated in *g* rather than revolutions per minute (see Chapter 6); a standard-size kitchen refrigerator (340–450 L, 12–16 cubic feet) or small under-the-counter refrigerator for the purpose of storing media, reagents and other materials that require refrigeration (the freezer compartment [–15 to –20 °C, 5 to –5 °F] of the refrigerator is used for storage of blood samples that require to be frozen before shipping to referral laboratories, and for storage of

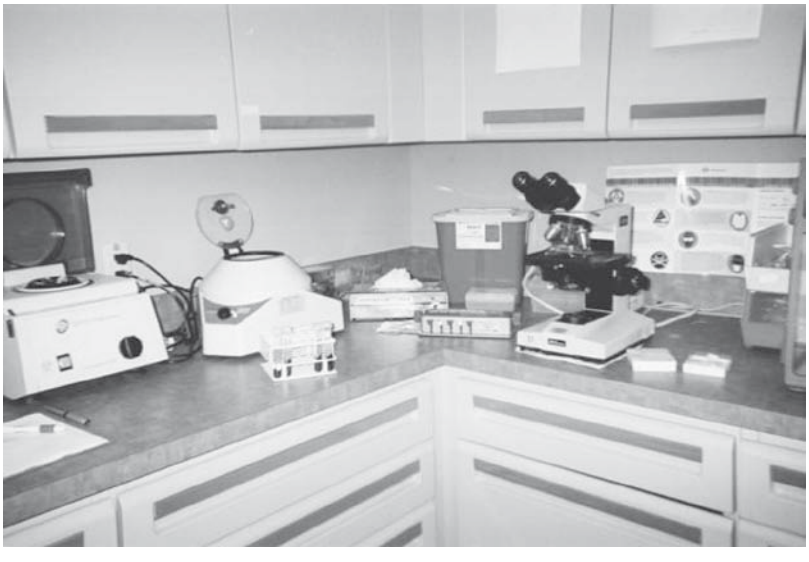

Figure 4.3 A medium-sized infertility clinic laboratory.

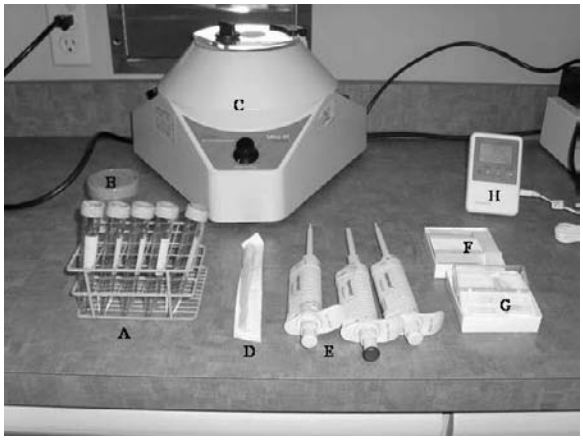

Figure 4.4 Some essential items of equipment: (A) test tube rack and 15 mL conical centrifuge test tubes; (B) semen specimen cup; (C) centrifuge; (D) 1 mL transfer pipettes; (E) adjustable-volume pipettes, 10–100 µL and 100–1,000 µL; (F) microscope slides; (G) cover slips for slides; (H) thermometer.

Table 4.1. Essential equipment

Phase contrast light microscope	Heat block
Bench-top centrifuge (50–1000 *g*)	Calculator
Computer (preferably networked)	Laboratory counter
pH meter	Refrigerator
Incubator	pH test paper
Micropipettors 10–100 µL, 100–1000 µL	Slide warmer
Hood	Sperm counting chamber
Test tube racks	Spirit burners
Timer	Thermometers

cryoprotectant media); a small incubator (55–85 L, 2–3 cubic feet) adjusted to 35–37 °C (94–98 °F), to be used for liquefaction of semen samples, for warming media and for the swim-up method of sperm processing (CO_2 gas is not required); a bench-top autoclave (Fig. 4.5). In addition, either a horizontal (counter-top) or vertical (class II) laminar flow hood is recommended. The hood reduces contamination of semen samples as well as offering protection to laboratory personnel. A horizontal hood is sufficient in a small laboratory (Fig. 4.6). Equipment required for performing sperm freezing is shown in Table 4.2, and in Figures 11.1–11.3.

Disposable items and supplies

Disposable and consumable materials used in the laboratory and clinic include those listed in Tables 4.3 and 4.4. It is more convenient and safer to use sterilized disposable items throughout the practice. In the laboratory, all supplies and materials must be disposable, and must be tested as being non-toxic to sperm (preferably by the supplier). Some disposable materials used in the clinic may appear not to be cost-effective, but are used nevertheless, because they are safer and prevent cross-contamination. Where reusable items are used, they must be cleaned and sterilized using an autoclave before being reintroduced into the clinic for use.

Figure 4.5 Bench-top autoclave.

Standard buffer solution used to prepare wash media for the sperm wash procedure and sperm processing should be pharmaceutical grade, sterile and cell-culture-tested. Although sterilization and cell-culture testing can be performed in one's own laboratory – and some large laboratories do so to save cost – it is easier for most IUI laboratories to purchase buffer solutions and media that are sterile and already cell-culture-tested.

Cryopreservation requires a ready supply of liquid nitrogen (LN_2) and its own special supplies (see Chapter 11).

Clinic and laboratory safety

Safety items required in the laboratory are listed in Table 4.5. Patients who come to the clinic for treatment should be considered as potential sources of infection or contamination; therefore, gloves must always be worn by staff during treatment. The staff in each clinic and laboratory must be acquainted with and adhere to the safety regulations, in order to prevent health hazards, and they must observe the Universal Precautions for Prevention of Transmission of HIV and Other Bloodborne Infections [1]. The medical and nursing staff must have uniforms to be used only in the clinic or laboratory. In the laboratory, the areas in which

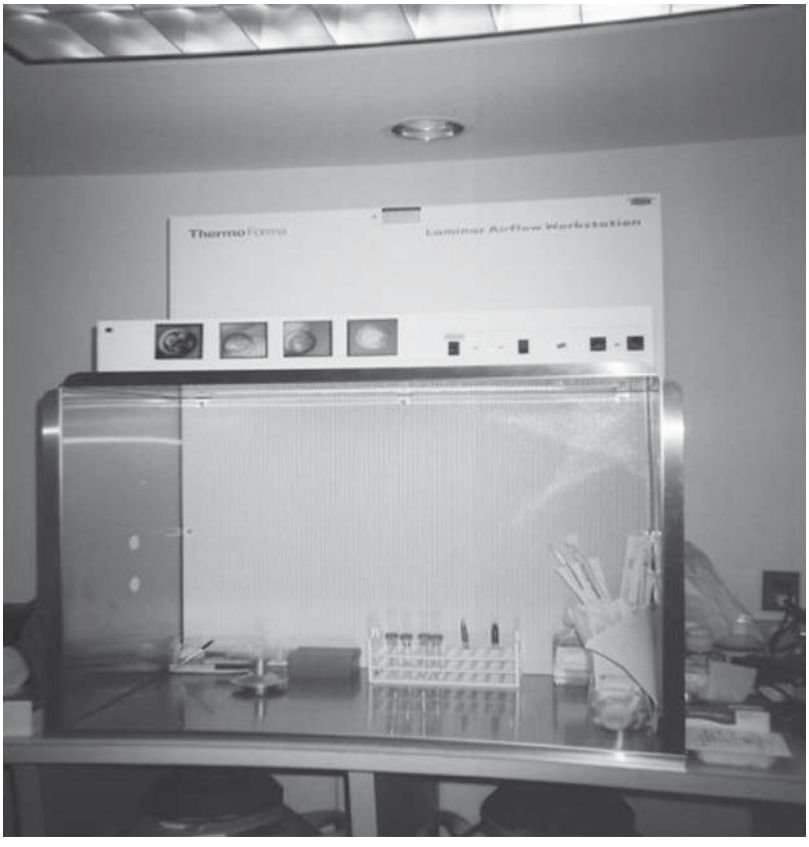

Figure 4.6 Horizontal laminar flow hood.

Table 4.2. Equipment and supplies for sperm cryopreservation

Liquid nitrogen cylinder	Dewar liquid nitrogen cryogenic storage tanks
Dry shipper tank	Cryovials with screw top and white label
Freezing media	Cryosleeves, brown paper or clear plastic
Goblets (storing straws)	Cryostraws (if samples are frozen in straws)
Aluminum cryocanes	Protective gloves and face mask
Freezing machine (if required)	Thermos

biohazard materials are processed or stored must have a tag which identifies the kind of biohazard material. Furthermore, each laboratory must have a material safety data sheet (MSDS) binder containing data sheets about the materials used in that specific laboratory. Each individual MSDS sheet provides the pertinent information about the specific product, including toxicity, health effects, first-aid specifications, reactivity, storage, material disposal and spill handling

Table 4.3. Disposable materials required in the laboratory for semen analysis and sperm processing

Culture media	Buffer media
Plastic syringes (1, 5, 10 mL)	19–21G hypodermic needle
50 mL polystyrene tissue culture flasks	5 and 15 mL centrifuge tubes
70% ethanol-impregnated wipes	Water wash bottles
Human serum albumin	Gloves
Microscope cover slips	Microscope slides
Plastic sterile pipettes	(2 mL) pH paper
Pipette tips (for 100 and 1000 µL)	Soap for washing hands
Polystyrene pipettes (1, 5, l0 mL)	Pipette controller
Garbage bag (regular and biohazard)	Office disposables
Tissue paper	Towel paper
Sharp-safe bin	Biohazard bags

Table 4.4. Reusable and disposable items required in the clinic for intrauterine insemination

Reusable items[a]	Disposable items
Speculum	Speculum
Ring forceps	Cotton wool swabs
Tenaculum	Paper hand towel
Kidney dish	Kidney dish
Alice clamp	Drapes
	Latex- and powder-free gloves
	Insemination catheter
	Syringe 1.0 mL
	Wash media (saline)

[a] Reusable equipment is used only if the clinic has an autoclave for sterilization

Table 4.5. Safety items in the laboratory

Goggles	Face mask or face shield
Eye-wash unit	Laboratory coat
Gloves	First aid box
Fire extinguisher	Fire alarm system
Flashlight	Exit sign
Sharp-safe bin	Biohazard bags
Biohazard labels	Biohazard cabinet
MSDS (material safety data sheet) file	Safety procedure manual
OSHA (Occupational Safety and Health Administration) manual (USA)	Blood-borne pathogen manual

Figure 4.7 Eye-wash station above sink .

procedures. The MSDS binder needs to be placed in an area where it is readily available to the staff in case of an accident. The laboratory should have an eye-wash station (required in the United States; Fig. 4.7). If a serious incident does occur, the employee should be treated immediately, an incident report completed and the rest of the staff informed about the occurrence and how to prevent it from happening again.

Conclusion

In this short review it has been possible to cover only a few of the most important points relating to the equipment, the personnel and the space requirements for an office or clinic that performs IUI and OI. In practice, the details will depend on the number and type of procedures to be performed. As in the delivery of the service, collaboration between staff is essential, and it is important for the physician to be aware of the benefits of input from nursing, laboratory and ultrasound personnel when designing a clinic and selecting equipment.

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Chapter 5

Semen analysis: semen requirements for intrauterine insemination

Roman Pyrzak and Richard P. Dickey

Introduction

The semen analysis (SA) in the form in which it appears today is a relatively recent invention [1]. The definition of a "normal" semen analysis was not standardized until publication of the World Health Organization (WHO) *Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interactions* in 1980 [2]. Between 1869, when Sims reported that sperm needed to be present in cervical mucus for conception to occur [3], and 1951, the Sims–Huhner postcoital test [4] and the presence of motile sperm on a microscopic slide were considered the only tests necessary to evaluate male fertility. The postcoital test was used as part of their initial evaluation of infertile couples by 92% of infertility specialists in private practice in the United States according to a survey taken in 1998 [5]. Examination of a droplet of ejaculate under the microscope remains a highly accurate, easy-to-perform "qualitative" test of male fertility.

In 1951, MacLeod and Gold analyzed the sperm counts of 1,000 fertile and 1,000 infertile men stratified at intervals of 20×10^6/mL. They observed that 19% of infertile men had counts of less than 20×10^6/mL, compared to 8% of fertile men [6]. On this basis they stated that men with sperm counts above 20×10^6/mL were fertile, and men with counts below that concentration were subfertile, even though they had not further stratified counts of less than 20×10^6. They proposed that a minimum of 50×10^6 total sperm, 30% "active" sperm and 55% normal morphology were necessary for normal male fertility [7]. Although reports with lower thresholds were subsequently published by others, and had been published previously, MacLeod and Gold's values were used to define male infertility for the next 30 years.

In 1980 the WHO published new standards for normal semen parameters. These were 20×10^6/mL, 40×10^6 total count, 50% forward progression and 30% normal forms [2]. In the fourth edition of the WHO *Laboratory Manual* there were two important changes (Table 5.1) [8]. The first was that 25% rapid progressive motility was to be considered normal. The second "suggested" that 15% normal forms by the strict criteria developed by Kruger *et al.* [9,10] should be considered normal. Significantly, the Kruger definition of normal was derived from the shape and measurements of progressively motile sperm found in the cervical mucus 9–12 hours after coitus [9]. When evaluated by strict criteria, sperm with 15% or more normal forms were observed to have normal fertilization capability in vitro. Sperm with 5–14% normal forms had intermediate, and sperm with < 5% normal forms had poor fertilization capability in vitro [10]. Semen reference values for normal, intermediate and subfertility were independently defined in 2001 [11] and comprehensively reviewed in 2009 [12].

Semen quality necessary for normal pregnancy rates in IUI cycles differs from the WHO standard for semen analysis with respect to concentration (count per mL) and total sperm count, because the entire volume is used to prepare the insemination specimen. The sperm are placed directly into the uterine cavity and fallopian tubes, thus bypassing the cervix, and the preparation procedure removes the constituents of seminal plasma that stabilize the sperm membrane and prevent capacitation [13]. At the Fertility Institute of New Orleans, sperm criteria for normal pregnancy rates in IUI are: minimum concentration 5×10^6 sperm per mL, 10 million total count, 5×10^6 total motile sperm (TMS) with 30% progressive motility and 5% normal forms by strict criteria. This is based on analysis of fresh semen performed before preparation for timed IUI in 4,056 spontaneous, clomiphene, or gonadotropin cycles (Table 5.2) [14]. Pregnancy rates per cycle averaged

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Table 5.1. World Health Organization reference values for normal semen

Parameter	Reference value
Volume	≥ 2.0 mL
Sperm concentration	≥ 20×10^6/mL
Total sperm count	≥ 40×10^6
Motility	≥ 50% motile, or 25% with progressive motility
Morphology	≥ 30% normal forms, or ≥ 15% using strict criteria
Total motile sperm (TMS)	≥ 25×10^6
Grade of progression	3–4
Agglutination	0–1
Liquefaction	Complete in 20–30 minutes
Viscosity	0–1
pH	7.2–7.8
Viability	≥ 75%, or > 12–15% difference from % motility
White blood cells	< 1×10^6/mL

Total motile normal sperm (TMNS), derived from above, ≥ 3×10^6.
Adapted with permission from *WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction*, 4th edn, 1999 [8].

8% for these values, compared to less than 2.5% when values were lower. Pregnancy rates were 50% higher when the total count was more than 80×10^6, forward motility was greater than 50%, or TMS was more than 40×10^6. Pregnancy rates did not increase when there were more than 5% normal forms. We consider 5×10^6 TMS, 30% progressive motility and 5% normal forms before preparation to be "threshold" levels for sperm to be used in IUI procedures.

The purpose of a comprehensive semen analysis, including differential morphology, is not only to determine if male factor is a contributing cause of infertility, but also to determine whether IUI offers a reasonable possibility of success. If abnormalities are discovered in the initial semen analysis, further evaluation and treatment of the male is indicated. An abnormal SA by WHO criteria does not necessarily mean that IUI would not result in pregnancy if the sperm meet threshold levels. Conversely, a normal semen analysis does not mean that IUI is unnecessary, because the infertility may result from cervical factor (see Chapter 3). A normal SA may also mask DNA fragmentation or chromosome abnormalities. A recent study found that when sperm counts were above 20×10^6/mL, a DNA fragmentation index (DFI) greater than 30% was found in 17% of sperm specimens in couples with unexplained infertility, in 16% of sperm specimens in couples with tubal obstruction and in 32% of poor-quality sperm specimens used for intracytoplasmic sperm injection (ICSI) [15].

Numerous chemicals used in industry and agriculture, and many therapeutic medications, can adversely affect spermatogenesis or fertilization ability [16,17]. A list is shown in Table 5.3. Chemicals and medications that affect fertilization may do so despite a normal SA. It is necessary to read the detailed information found under the heading "impairment of fertility" on the drug information sheet or in the US *Physicians Desk Reference* (PDR) concerning all drugs the patient is using. Excessive exercise and endurance training has a suppressive effect on LH levels, leading to a decrease in circulating testosterone, an increase in serum cortisol and a decrease in spermatogenesis [16].

Morphology is especially important, and can indicate the cause of abnormal sperm numbers or unexplained infertility. Abnormal chromosome complement must be considered when the count is less than 20×10^6/mL, although ordinarily chromosome analysis is not ordered unless there are fewer than 1×10^6 sperm per mL if no other infertility factors are found

Table 5.2. Relation of initial sperm quality to per-cycle pregnancy rate

Sperm variable	Number of cycles	Number of pregnancies	Pregnancy rate per cycle (%)	*P*-value[a]
Sperm concentration (× 10^6/mL)				
< 5	121	3	2.5	
5–10 (T)	221	19	8.6	< 0.04
10–20	434	38	8.8	
20–40	794	83	10.4	
≥ 40	2486	306	12.3	
Total sperm count (× 10^6)				
< 10	102	1	1.0	
10–20 (T)	183	15	8.2	< 0.2
20–40	352	29	8.2	
40–80	647	55	8.5	
≥ 80	2772	349	12.6	
Sperm progressive motility (%)				
< 20	80	1	1.2	
20–30 (T)	194	7	3.6	< 0.001
30–40	555	54	9.7	
40–50	955	123	12.9	
≥ 50	2272	264	11.6	
Percent normal forms (modified strict criteria)				
< 5	11	0	0.0	
5–10 (T)	34	3	10.7	< 0.45
10–20	127	16	12.7	
20–30	248	29	11.7	
30–60	1804	209	11.6	
≥ 60	1719	175	10.2	
Total motile sperm count (× 10^6)				
< 5	175	4	2.3	
5–10 (T)	193	16	8.3	< 0.02
10–20	402	33	8.2	
20–40	658	59	9.0	
≥ 40	2626	337	12.8	

Adapted from Dickey *et al*. 1999 [14].
[a] Less than threshold value vs. threshold value; Fisher's exact test.
T, threshold level.

and IUI is unsuccessful after three attempts. In a population of Tunisian males with non-obstructive sperm disorders, chromosome aberrations were detected in 14% overall [18]. The incidence of chromosome abnormalities was 24% in azoospermia males (52% of whom were XXY), 9% when there were less than 5 × 10^6 sperm per mL, and 7% when there were 5–20 × 10^6 sperm per mL.

Table 5.3. Occupational gonadotoxins and drugs that affect spermatogenesis or sperm function

Industrial gonadotoxins
Pesticides
Carbamates (e.g. carbaryl)
Organochlorines (e.g. dichlorodiphenyltrichloroethane [DDT])
Organophosphates (e.g. dichlorvos)
Fumigants (e.g. ethylene dibromide, also used in antiknock gasoline)
Dibromochloropropane, a nematocide
Herbicides/fungicides (e.g. benomyl)
Organic solvents (e.g. ethylene glycol, benzene, perchloroethylene, ethylene dibromide 'EDB', toluene)
Heavy metals (e.g. lead, manganese, cobalt, cadmium)
Others
Hexanedione
Nifurtimox
Nitrous oxide
Phthalate esters
Drugs and medicines that suppress the hypothalamic–pituitary–gonadal axis
Alcohol
Anabolic steroids
Androgens
Antiandrogens
Antipsychotics
Cocaine
Cimetidine
Cyclosporine
Estrogens
Gonadotropin-releasing hormone agonists e.g. leuprolide acetate
Marijuana
Opiates
Phenothiazines
Progesterones and progestins
Spironolactone
Testosterone
Tricyclic antidepressants
Drugs and medicines that are gonadotoxic
Alcohol
Ascorbic acid
Chemotherapeutic agents (adriamycin, alkaloids, busulfan, carboplatin, cisplatin, cyclophosphamide, nitrogen mustard, procarbazine, triethylenemelamine)
Cigarettes
Cocaine
Erythromycin

Table 5.3. *Continued*

Drugs and medicines that are gonadotoxic
Gentamicin
Ketoconazole
Nitrofuran
Radiation therapy
Spironolactone
Sulfasalazine
Valproic acid
Drugs that affect sperm function and fertilizing ability
Allopurinol
Calcium channel blockers
Colchicine
Minocycline (toxic to sperm at any concentration)
Nitrofuran
Tetracyclines

Adapted with permission from Hakim and Oates 1997 [14] and Nudell *et al.* 2002 [17].

All abnormal semen analyses should be repeated to confirm or exclude the abnormal finding. As a rule the repeat count should be performed after 10 weeks, the length of time required for spermatogenesis. However, that time may be shortened if the abnormality is low motility and there is a possible cause such as recent heat stress, or a low count could be due to high-fever illness more than 10 weeks earlier. A low count, but good motility, may be due to a short interval since the previous ejaculation; conversely, low motility but a good count may be due to an extended time since the last ejaculation. As a rule, total sperm count and volume increase over a period of 7–10 days, but motility may begin to decrease after 3–8 days. The optimum time to repeat a semen analysis can depend on which parameter, total count or motility, is lower.

Semen and sperm assessment for IUI

Semen analysis can be separated into three categories of increasing complexity that determine where it is typically performed – a physician's office, a physician's independent laboratory, reference laboratories or any combination. In the United States, any detailed analysis of semen parameters is categorized as a high-complexity test that can be performed only by a qualified person or laboratory. In Europe, the European Society of Human Reproduction and Embryology (ESHRE) is also in the initial stages of requiring certification of all technicians who perform assisted reproductive technologies (ART).

- **Standard semen analysis (SSA).** Parameters evaluated include appearance, pH, liquefaction, volume, motility, concentration and total count. SSA can be performed in a small space at the physician's office. The equipment and materials required are microscope, pipettes and tips, hemocytometer or Makler chamber for sperm counting, counter, slides and cover slips.
- **Comprehensive semen analysis (CSA).** Parameters additional to SSA include morphology, viability, agglutination, white blood cells and non-spermatozoal cell count. It can also include bacterial culture and antibiotic sensitivity, although this is usually done in referral laboratories. Additional equipment and materials required are a centrifuge, a warming plate or water bath, and stain kits for sperm morphology and viability, and to distinguish between white cells and round cells.
- **Advanced semen analysis (ASA)** tests that may be done as part of a CSA or sent to a reference laboratory include DNA fragmentation, fructose test, immunobead test, sperm–cervical mucus in-vitro tests, zona-free hamster oocyte test, viral

Figure 5.1 Small andrology laboratory: (A) warming block; (B) thermometer; (C) pass-through window to sperm collection room; (D) laboratory counter; (E) test tube rack and pipettes; (F) phase contrast microscope; (G) microscope slides and cover slip; (H) centrifuge; (I) hemocytometer.

tests, sperm chromosome analysis, Y-chromosome deletion.

Facility

A small andrology laboratory equipped to perform a standard semen analysis is shown in Figure 5.1. There should be a private room dedicated for specimen collection in the hospital, laboratory or office setting. It should have proper space and ventilation, it should be adequately sound-proofed, and it should be equipped with comfortable furniture such as a reclining chair or small sofa, a sink and a toilet or wall urinal. The patient may be accompanied in the collection room by his partner. The room should have visual aids such as erotic magazines and videos available for those who require them. When the room is in use, the outside door should have a sign indicating that the room is occupied. If the room is next to the andrology laboratory, it is preferable to have a small window in the wall (12 × 12 inches or 30 × 30 cm), in order that, after collection, the specimen with required documentation can be placed in the window.

Specimen collection

Specimen collection is an important factor in the evaluation of the semen and sperm parameters. Erroneous SA results have resulted from improper attention to specimen collection and handling. Proper methods of specimen collection should be discussed with the patient prior to collection; in addition, the instructions for specimen collection should be posted in the collection room. In order to detect all causes of infertility, patients should abstain from sexual activity for the number of days that would normally occur between episodes of coitus (whether one or several days) before providing a semen specimen. The specimen can be obtained by masturbation and ejaculation into a non-toxic plastic specimen container. Patients are told not to use lubricants. In order to obtain a clean sample, patients are instructed to urinate prior to collection and to wash hands with water only before collection. Care should be taken to prevent contamination of the semen sample during collection. Resident bacterial flora of the urethra, glans penis and hands can contaminate the ejaculate produced by masturbation.

If a patient finds he is uncomfortable collecting the specimen at the facility he can collect at home and deliver it to the office or laboratory within an hour after ejaculation. If longer travel periods are required, the specimen should be ejaculated into transport media (identical to wash media; for instructions in preparing wash media see Chapter 6) provided beforehand. Note that wash media must be used within 24 hours for sperm preparation and cryopreservation, but a longer period can be allowed when it is used for semen analysis. The volume of wash media provided to the patient is recorded, so that the semen volume and count per mL can be adjusted accordingly. Exposure of the specimen to cold in the winter can

be prevented by carrying it in an inner coat pocket. For patients who prefer to collect a specimen during intercourse (as required by some religions) and bring it from home, or from a hotel/motel room, special non-spermicidal silicone rubber condoms designed for semen collection during coitus are available. The specimen should not be collected by coitus interruptus without a condom, because of contamination from vaginal secretions.

Documentation is of paramount importance to maintaining an error-free IUI program. Each patient should check that his first and last names and those of his partner are on the container. It will reduce errors if ages or dates of birth are also indicated. The patient is asked to fill out a form describing the length of abstinence, whether the specimen collected is complete or partial (part of the ejaculate may have been spilled or missed the specimen container), time of collection, whether collected into media, whether there has been any illness within the past 10 weeks and any medications he is taking, including non-prescription remedies (Form 5.1).

Split ejaculate

A split ejaculate (collection of the ejaculate into two separate containers) may be indicated in cases of retrograde ejaculation, or when a large number of bacteria or white cells have been found in a previous SA specimen, and there is a question of where the infection originates. Usually the first part of the split specimen will contain more bacteria. Except in these cases, we have not found it necessary or desirable to have a patient collect a split ejaculate. Collecting a split ejaculate when it is unnecessary increases the chance of spillage and loss of part of the specimen .

Semen analysis procedure

(Procedures adapted and modified from the *WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction*, 4th edition [8].)

Immediately on receiving the semen sample, confirm that the patient's name corresponds with that written on the specimen container and the request form. Gloves should be worn at all times while handling specimen containers and performing the analysis. Results will be recorded on the form shown in Form 5.2, or a similar form.

Standard semen analysis (SSA)

Equipment for performing standard and comprehensive SA is listed in Tables 4.1 and 4.3.

Appearance

The color of the specimen is assessed visually. Normal semen which contains spermatozoa is grey to white. Yellow discoloration indicates urine contamination, and suggests bladder neck dysfunction. Red or brownish discoloration indicates blood contamination, and may indicate inflammation.

pH

The pH of liquefied semen is measured using litmus paper with a range of 6.0–8.0. The pH should be between 7.2 and 7.8, one hour after ejaculation. A pH higher than 8.0 indicates acute prostatitis, vesiculitis or epididymitis. A pH below 7.0 may also indicate infection.

Liquefaction and viscosity

Following ejaculation, seminal fluid forms a coagulum due to the presence of coagulating factors produced by the seminal vesicles. Enzymes produced in the prostate gland liquefy this coagulum. Normal samples should liquefy within 20–30 minutes at room temperature. Increased viscosity adversely influences the determination of other semen parameters. An extremely viscous sample can make the sperm counts and motility determination difficult. Under these conditions, culture or buffer media should be added until the semen viscosity is reduced, and only then should the sperm count be determined. A very viscous specimen may be indicative of an infection in the genital tract, prostate or seminal vesicles. Note the time of arrival of the specimen in the laboratory and whether liquefaction has occurred. If so, proceed with the analysis; if not, note the fact and place the specimen on the counter, checking from time to time. Note the time when liquefaction occurs on the analysis form. Record the length of time since the collection on the semen analysis form.

Volume

The volume is measured in a calibrated 15 mL plastic tube before further evaluation. Normal volume is 2–4 mL. A smaller volume may be a cause of infertility, even though the total sperm count is normal. A small volume may also be due to loss of part of the specimen, retrograde ejaculation, abnormality or infection of accessory sex glands, or ejaculatory duct obstruction. An extremely high volume (> 5 mL) may indicate inflammation or urine contamination and is associated with lower conception rates. IUI is highly effective in cases of either high or low volume

if the total sperm count and motility are otherwise normal.

Motility and progression

Sperm motility is normally assessed at room temperature, between 20 and 23 °C, after complete liquefaction. Sperm motility and progression are evaluated by placing 10 µL of semen on a microscope slide under a cover slip. A minimum of 200 sperm should be counted. Progressive motility is subjectively estimated, and the percentage of each grade recorded according to the following criteria:

4 – rapid forward progression
3 – good forward progression
2 – moderate, definite forward progression
1 – poor, weak or sluggish forward progression, movement in place
0 – immotile.

Sperm count

The sperm count is performed after complete liquefaction using either a Makler chamber or a standard hemocytometer (Fig. 5.2), and the results are recorded on the semen analysis form.

Sperm count using the Makler chamber

(Procedure supplied by manufacturer)

The Makler chamber allows sperm numbers to be counted on an undiluted semen sample, and this is the easier procedure to perform [19]. Briefly, 100–150 µL of specimen is transferred to a clean test tube, and the sperm are immobilized by inserting the tube into a water bath at 60 ± 3 °C for 2–2.5 minutes. A drop or 25 µL of the immobilized specimen is placed in the counting chamber and the number of sperm in 10 squares is counted (there are a total of 100 squares). The number of sperm counted represents the concentration in millions (10^6) per mL. If initial microscopic inspection indicates a low count, all 100 squares must be counted. The number of sperm counted then represents the concentration in one-hundred-thousands (10^5) per mL.

Sperm count using the hemocytometer

A hemocytometer has two chambers, and each chamber has a microscopic grid etched on the glass surface. The chambers are overlaid with a special heavy glass cover slip that rests on pillars exactly 0.1 mm above the chamber floor. The main divisions separate the grid into 9 squares. Each square has a surface area of 1 mm^2, a depth of 0.1 mm and a total volume of 0.1 mm^3 or 10^{-4} cm^3. The central square contains 25 large squares, and each of these contains 16 smallest squares.

As a first step, 10 µL of specimen is placed on a microscope slide under a cover slip and evaluated at 400 × magnification. The number of sperm counted per high-power field (HPF) is used to determine the dilution ratio (semen + diluent) and number of large squares that need to be counted to find the sperm concentration per mL (Table 5.4). The sperm are immobilized with a diluent consisting of 5% $NaHCO_3$ and 1% formalin. For example, if the number of sperm per HPF is 40–200, a 1 : 20 dilution is made by mixing 50 µL of liquefied specimen with 950 µL of diluent. Two separate preparations of diluted sperm are made, so that the count can be performed in duplicate.

A special cover glass is laid on top of the hemocytometer. Approximately 10 µL of completely mixed diluted specimen is loaded into the V-shaped grooves, at the top and on the bottom of the hemocytometer, by gently touching with a pipette, allowing the chambers under the cover slip to fill by capillary action. After waiting five minutes for sperm to settle, the hemocytometer is placed on a microscope stage, and sperm are counted in the number of chambers determined by the dilution ratio shown in Table 5.4. When counting sperm that touch the gridlines, count only those that touch the top and left side of the square, and do

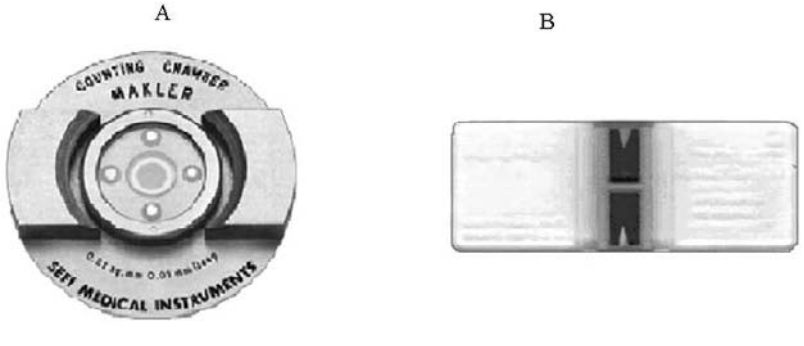

Figure 5.2 (A) Makler counting chamber. (B) Hemocytometer.

Table 5.4. Dilution and conversion factors for performing a sperm count using a hemocytometer.

Sperm per 400 × field	Dilution ratio (semen + diluent)	Conversion factors		
		Number of large squares counted		
		25	10	5
< 15	1 : 5 (1 + 4)	20	8	4
15–40	1 : 10 (1 + 9)	10	4	2
40–200	1 : 20 (1 + 19)	5	2	1
> 200	1 : 50 (1 + 49)	2	0.8	0.4

Adapted with permission from *WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction*, 4th edn, 1999 [8] .

not count sperm that touch the bottom and right side. The sperm count in the original semen sample is determined by calculating the average of the two sides and using the conversion factor for the dilution ratio and squares counted shown in Table 5.4. Results are expressed as count × 10^6.

If the number of sperm is low (< 3/HPF), add diluting media to the specimen (dilution ratio 3 : 1), and centrifuge the media/semen mixture at 300 *g* for 10 minutes. Remove the majority of the top fraction, leaving 0.5 mL of fluid. Mix the pellet with the remaining 0.5 mL and count in the hemocytometer. After counting, correct the final concentration to the initial volume of the specimen. If the specimen was collected into media for transportation, concentrate the contents by centrifugation as above, remove the excess, leaving 2–3 mL, and perform the sperm count from this volume.

Computer-aided sperm analysis

Computer-aided sperm analysis (CASA) offers the advantages of requiring less technician time and providing quantitative data on sperm kinetics. The equipment is expensive and is used primarily in research facilities. Additional information may be found in the WHO *Laboratory Manual* [8] .

Comprehensive semen analysis (CSA)

Morphology

Sperm morphology is highly variable, with marked differences in the size and shape of the head and mid-piece, and in the length and configuration of the tail (Fig. 5.3).

Preparation of smears

Two smears of the initial semen specimen are made using frosted-end slides and allowed to air-dry (one slide will be used as a backup). The frosted end is labeled with patient name and date with a pencil. If the sperm count is > 40 × 10^6/mL, the semen is diluted with buffer media to approximate a concentration of 40 × 10^6/mL. If the sperm count is < 5 × 10^6, the specimen is diluted with buffer and centrifuged, and the resulting pellet is suspended to make the morphology slide. Papanicolaou stain, Shorr stain and the rapid staining method Diff-Quik (Roche Diagnostics) are all equally suitable. With these stains, the head will appear pale blue in the acrosomal region and dark blue in the post-acrosomal region. The mid-piece may or may not show some red staining. The tail will stain blue or red. Cytoplasmic droplets located behind the head and around the mid-piece stain green with the Papanicolaou stain.

Assessment of morphology and reporting results

The stained slide is placed under a microscope and a drop of oil applied. Using the × 100 objective, 100–200 sperm are counted on each slide and the percentage of each classification, broadly subdivided as shown on Form 5.2, is calculated. According to strict criteria, the length of the head should be 4.0–5.0 μm and the width should be 2.5–3.5 μm with a length/width ratio of 1.50 to 1.75 [9,10]. There should be a well-defined acrosomal region comprising 40–70% of the head area. The mid-piece should be slender, less than 1 μm wide, about 1.5 times the length of the head and attached axially (Fig. 5.3). Cytoplasmic droplets should be less than half the size of a normal head. The tail should be straight (uncoiled), thinner than the mid-piece and approximately 45 μm long. For a spermatozoon to be considered normal, the head, neck, mid-piece and tail should all be normal.

Storage

It is advisable to store morphology slides for between two and five years, in case any questions arise in the future about their interpretation. This is required by some regulatory agencies. Storage can also be accomplished by digital imaging technology, but any applicable laws should be checked first. Digital storage allows a picture of the slide to be printed and appended to the semen analysis report, incorporated into an electronic medical record, or compared with previous morphology images. Additional equipment required for this technology is a digital camera that is attached to the microscope and a computer operating system.

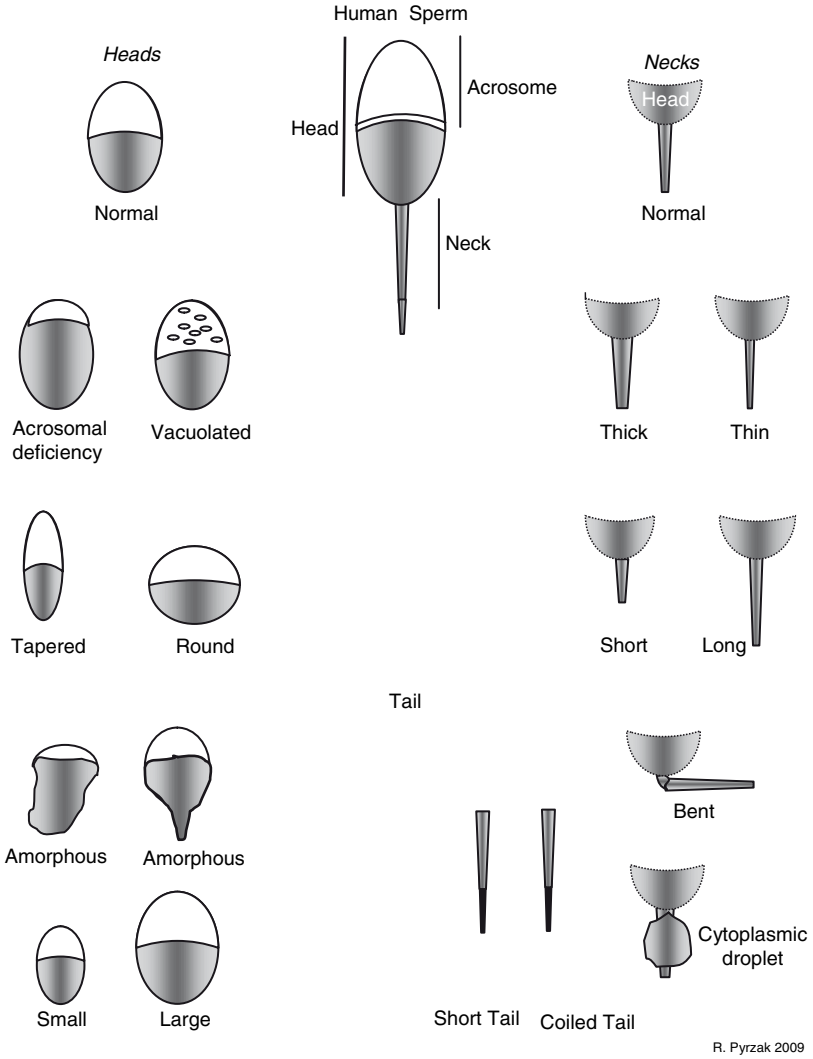

Figure 5.3 Sperm morphology. Adapted with permission from *WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction*, 4th edn, 1999, p. 20 [8].

Vitality

Sperm vitality tests are performed when the percentage of immotile sperm exceeds 50%, in order to determine whether lack of motility is due to defects in the flagellum or to cell death. Vitality can be determined by either dye exclusion or hypo-osmotic swelling. The eosin–nigrosin dye exclusion test is the simpler of the two to perform and is described below. Results can be quantified; however, training is required to distinguish between live and dead sperm. The hypo-osmotic swelling test requires an extensive training, and the results can not be stored.

Eosin–nigrosin dye test

Eosin Y (red color) and nigrosin (black color) differentiate between live and dead sperm. If sperm are dead, eosin will penetrate the sperm membrane and stain the sperm red. If sperm are alive, eosin is unable to penetrate the membrane and the sperm remain white. Nigrosin is used as a background on the slide to make it easier to differentiate between live and dead sperm. This procedure is easy to perform and takes only a few minutes. A kit is available (VitalScreen™; Conception Technologies, San Diego, CA, USA).

Procedure

Mix 10 μL of 1% eosin with 10 μL semen. After 30 seconds add 30 μL of 10% nigrosin and mix. After 30 more seconds put 10 μL of the eosin–nigrosin stain mix on a microscope slide and make a smear. Allow to dry at room temperature for 15–20 minutes. Examine the

slide under oil immersion (× 1,000). At least 200 total sperm should be counted.

Interpretation

Results are reported as percent live sperm. The percent of dead sperm should not exceed the percent of immotile sperm, allowing for counting error. Sperm vitality that is 12–15% greater than percent motility is abnormal and may be due to motility dysfunction. Abnormal vitality could also be due to technical factors: improper specimen collection, or improper handling of the specimen by the patient before and after ejaculation.

Agglutination

Agglutination of motile sperm may be head to head, tail to tail or mixed head to tail. Any other adherence of immotile sperm to each other or to mucus threads, cells or debris is considered non-specific aggregation. Agglutination assessment is graded on a scale from 0 to 4, where 0 is no agglutination and 4 is gross agglutination. The presence of agglutination in semen specimens is presumptive, but not definitive, evidence for antisperm antibodies, since spontaneous agglutination of sperm can occur. Surface antisperm antibodies can result from infection in the seminal vesicles or epididymis and disappear after the infection is treated.

Leukocytes and immature germ cells

Microscopic examination of the semen often shows large numbers of round nucleated cells, which may be either leukocytes or immature sperm cells. The presence of a large number of leukocytes (leukocytospermia or pyospermia) often indicates inflammation of the accessory sex glands or reproductive tract due to infection, which requires treatment. Pyospermia is associated with decreased sperm count and quality that improves after antibiotic treatment [20]. In a normal semen analysis, the number of leukocytes should not exceed 1 per 10^6 per mL. More than 3% immature forms (round cells) in an ejaculate are a sign of stress. Larger numbers of immature forms may be the result of primary or secondary damage to the seminiferous tubules.

Leukocytes (neutrophils, eosinophils, basophils) or "polymorphonuclear leukocytes" (PMNs) and macrophages (monocytes) are distinguished from immature sperm cells by the presence of the enzyme myeloperoxidase, which reacts to hydrogen peroxide (H_2O_2), releasing oxygen. Peroxide-positive cells stain brown in the presence of hydrogen peroxide. A commercial kit is available to stain for peroxides (LeucoScreen, Conception Technologies). The assay should be performed if more than two round cells are observed per HPF during semen analysis.

Semen evaluation when retrograde ejaculation is suspected

Few or no sperm in the initial semen analysis may be due to retrograde ejaculation. In such cases the volume of the ejaculate may be very low or non-existent. One method of obtaining sperm for semen analysis in cases of retrograde ejaculation, which can also be used to obtain sperm for IUI, is the following. On the day prior to sperm collection, the patient is instructed to take sodium bicarbonate (one tablespoon, about 1.5 g, dissolved in 250 mL [8 oz] of tap water). On the morning of semen collection, 2–3 hours before the collection, the patient is instructed to take another tablespoon of sodium bicarbonate dissolved in tap water. A half hour before producing a specimen by masturbation the patient should urinate. Two specimen cups are used for semen collection. The patient should try to ejaculate semen into the first specimen cup. After ejaculation, the patient empties the bladder by urinating into a second specimen cup. Both cups are submitted as soon as possible to the laboratory for immediate analysis or preparation for IUI.

Advanced semen analysis (ASA)

A number of additional tests may be performed as part of a comprehensive SA, or on specimens sent to a reference laboratory. The reasons for using these tests are described below. Specific details of how to perform each procedure may be found in the references cited for the test, in the WHO *Laboratory Manual* or in instructions included with commercially available kits.

DNA fragmentation

DNA fragmentation tests are able to distinguish which patients with unexplained infertility should be directed to IVF or ICSI and should not have IUI.

There are more than a dozen tests for assessment of sperm DNA fragmentation [21]. The two used most often in clinical laboratories are the sperm chromatin structure assay (SCSA) and the sperm chromatin dispersion (SCD) tests. The SCSA is based on staining of sperm nuclei with acridine orange to evaluate the ratio of single to total (single- and double-stranded) DNA in sperm with an impairment of their chromatin structure [22]. Normal double-stranded DNA displays green fluorescence, while denatured single-stranded DNA

displays red fluorescence when exposed to 488 nm laser light. Flow cytometry (FCM) is used to determine the ratio of red to total fluorescence intensity, which is reported as the DNA fragmentation index (DFI). The SCSA requires expensive equipment and specially designed computer software. In a study of nearly 1,000 consecutive cycles in couples with unexplained infertility and sperm counts > 20 10^6/mL, the pregnancy rate per started IUI cycle was 24% when the DFI was < 30%, compared to 3% when the DFI was > 30% [15].

The SCD test consists of mixing sperm with melted agarose, which is placed on a glass slide. After drying, the sperm cells are treated with acid and lysed, and 300–500 cells are examined under bright field or fluorescence microscopy. Sperm with fragmented DNA fail to produce the characteristic halo of dispersed DNA loops that is observed in sperm with non-fragmented DNA, following acid denaturation and removal of nuclear proteins [23]. The SCD test is inexpensive and can be performed without additional equipment other than a microscope. Reagents are available in kits (Halosperm, Halotech). In a series of 622 IVF and ICSI couples, a sperm fragmentation rate of 18% was predictive of fertilization but not of pregnancy or live birth [24].

Fructose test

Fructose in semen reflects the secretory function of the seminal vesicles, and it is used in cases of azoospermia to determine whether absence of sperm is due to obstruction or failure of spermatogenesis. A kit is available for determining the presence of fructose (FertiPro, www.fertipro.com). Because it is performed infrequently, many clinics prefer to send specimens to a reference laboratory.

Immunobead test

The immunobead test is a method of quantifying the percent, pattern and class (IgG, IgA) of sperm surface antibodies. It should be considered when greater than 1+ agglutination of motile sperm is observed on initial microscopic examination. Immunobeads (Irvine Scientific, www.irvinesci.com) are polyacrylamide spheres with covalently bound rabbit antihuman immunoglobulins. The test is performed on spermatozoa washed free of seminal fluid by repeated centrifugation and resuspended in buffer. Immunobead binding restricted to the tail is normal. The test is considered positive if > 50% of motile sperm heads are coated with immunobeads. Sperm penetration into cervical mucus and in-vivo fertilization are not significantly impaired, unless 50% or more of motile sperm have antibodies [25].

Zona-free hamster oocyte test

The zona-free hamster test is intended to determine whether spermatozoa are capable of fusing with the vitelline membrane of the oocyte, a necessary step in initiation of the normal acrosome reaction, and penetration of the oocyte. Because this test frequently resulted in false results, it is now seldom used [26].

Sperm–cervical mucus tests

Postcoital (Sims–Huhner) test

An appropriately timed Sims–Huhner postcoital test (PCT) evaluates coital technique, mucus quality and quantity, and sperm morphology and competence. A small television camera can be attached to the microscope and results of the PCT shown to the infertile couple on a monitor (Fig. 5.4). The postcoital test should be performed 12–24 hours after coitus, the normal length of time presumed to be required for capacitation and fertilization, although longer and shorter times will provide useful information. The couple should not use lubricant gels during coitus.

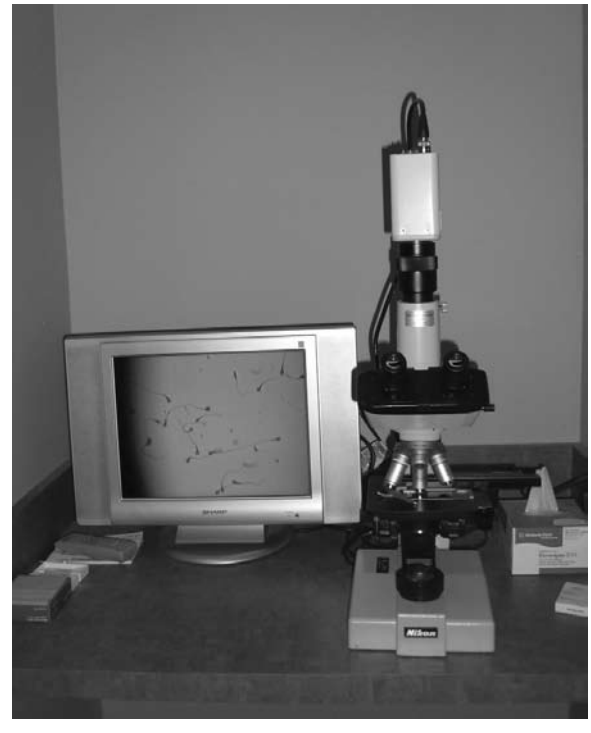

Figure 5.4 Microscope with attached television camera and monitor, with postcoital slide (× 400) shown on monitor.

Method

Obtaining mucus from the cervical canal by aspiration (Aspirette® Endocervical Aspirator; Cooper Surgical, www.coopersurgical.com) or by using forceps is cumbersome and may only collect mucus from the outermost portion of the cervix. A preferred method is to obtain mucus with a cervical cytology brush (CytoSoft™ Cytology Brush; Medical Packaging Corporation, www.medicalpackaging.com). The brush is 3 mm in diameter and has small bristles at the end. After introducing the speculum, the amount and quality of mucus or mixed mucus–seminal fluid in the vaginal pool is noted and recorded. The outer cervix is wiped with a cotton swab to remove debris and external mucus. The brush is gently inserted 1.5–2 cm into the cervix, rotated 260 degrees and withdrawn. A slide for microscopic examination is prepared by touching the end of the brush to the glass slide and gently scraping the mucus off with a 2 × 4 cm glass cover slip while rotating the brush as it is pulled across the slide. As the brush is lifted away from the slide, *spinnbarkeit* (thread making) is estimated in centimeters. pH is measured by touching a pH paper strip to the brush or the edge of the mucus on the slide. The mucus should be clear and of egg-white consistency. Some authors advise making two slides, setting one aside to dry and examine later for crystal formation; however, we do not find this information to be of value.

The slide is examined under the microscope (× 400) for evidence of progressively motile (PM) sperm. There should be at least one PM sperm per microscopic field to consider the test positive; stricter criteria of > 5 and > 20 PM sperm increase the sensitivity (the ability to identify infertile couples with a mucus factor who might benefit from IUI) but decrease the specificity (the ability to identify fertile couples without mucus-factor infertility) [20]. There should be no more than 5% abnormal forms and no more than 20% immotile sperm. Abnormal PCT findings, their probable causes, potential effects on fertility and suggested treatments are described in Table 3.5. If the PCT has been performed several days before ovulation and is abnormal, and there is no evidence of infection, the test should be repeated closer to the time of ovulation.

In-vitro sperm–cervical mucus tests

The purpose of in-vitro sperm–cervical mucus tests is to determine if an abnormal postcoital test (absence of any sperm) is due to defective sperm or to hostile mucus. Tests are customarily performed using donor sperm and/or donor mucus for controls, but can be usefully performed with patient mucus and sperm alone when failure of coital technique or hostile vaginal secretions is suspected. A more complicated quantitative test performed using capillary tubes and a centimeter scale has been described [8].

Quality assurance

A quality assurance program for the andrology laboratory should include record keeping, routine equipment calibration and maintenance, and regularly scheduled proficiency testing of laboratory technologists. In the United States all andrology laboratories must be accredited by a Clinical Laboratory Improvement Amendment (CLIA-88) agency (Centers for Disease Control and Prevention, www.cdc.gov/clia) or by authorized organizations, such as the College of American Pathologists (CAP) (www.cap.org). In the United Kingdom, laboratories are accredited by Clinical Pathology Accreditation (CPA) (www.cpa-uk.co.uk). International standards for medical laboratories are published by the International Organization for Standardization (www.iso.org) as ISO 15189.

Conclusion

The purpose of the semen analysis is not only to determine if a male factor is a contributing cause of a couple's infertility, but also to determine whether IUI offers a reasonable possibility of success. Semen parameters obtained in different laboratories are not always comparable, even when the same methods are used. Each laboratory or clinic should establish its own references ranges, which may be affected by a number of factors, including the ethnicity of the population it serves and their area of residence.

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Male patient questionnaire

General information

Date of visit: _____ Name of your physician: _____

Patient name: _____ Age: _____ ID: _____

Spouse's name: _____ Age: _____ ID: _____

How many days did you abstain from sexual activity before today's test: ____

Is this your initial [ ] or repeat [ ] semen analysis?

Was the specimen collected at home [ ] or at the facility [ ]?

If at home was it collected by:
masturbation [ ], intercourse [ ], other (indicate) _____

Time and date collected: _____. Time and date received in laboratory: _____

Medical information (circle)

1. Have you had varicocele (veins in the scrotum)? YES NO

2. Have you had vasectomy? YES NO

3. Have you had vasectomy reversal? YES NO

4. Do you have any allergies? YES NO

   If YES list: _____

5. Have you had any illness during the last 3 months? YES NO

6. Have you had a higher fever (102°F, 38°C) in the last 3 months? YES NO

7. Are you taking any medication? YES NO

   If YES list: _____

8. Any additional comments: _____

_____

_____

Patient signature: _____ Date: _____

Fertility Institute of New Orleans Form 501: 2008

Form 5.1 Male patient questionnaire.

Semen analysis

Patient information

Patient name: _____ Date reported: ___/___/_____
Patient DOB: ___/___/_____ Age: _____ Patient ID: _____
Spouse name: _____ Physician name: _____
Spouse DOB: ___/___/_____ Age: _____ Technologist name: _____

Specimen information Accession # : _____ Reason for test _____
Collected date/time: ___/___/____, __:__ Collection location: Clinic ___ Home ___ Other _____
Collection method: Masturbation___ Intercourse___ Other_____
Specimen received date/time: ___/___/_____, ___:___ Time test started: ___:___
Days of abstinence:_____
Medications: _____

Semen parameters	*Results*	*Units*	*Normal range*
Sample volume:	_____	mL	2.0–5.5
Count: 1st _____ 2nd	_____	$\times 10^6$/mL	
Mean sperm count:	_____	$\times 10^6$/mL	≥ 40
Motility:	_____	% progressive	≥ 50
Progression grade:	_____	grade 0–4.0	3.0 to 4.0
Total motile sperm (TMS):	_____	$\times 10^6$	≥ 20
Liquefaction:	_____	complete in 20–30 min	
Agglutination:	_____	grade 0–4.0	0 to 1
pH:	_____		7.2–8.0
Vi scosity:	_____	grade 0–4.0	0 to 1
Viability:	_____	%	0 to 15% > than % motility
White blood cells:	_____	$\times 10^6$/mL	< 1.0 (by peroxidase stain)
Non-spermatozoal cells:	_____	%	< 3% of total sperm
Debris:	_____	grade 0–4.0	0 to 1
Total sperm:	_____	$\times 10^6$	≥ 40

Sperm morphology

Normal heads:	_____	WHO criteria 1999:	> 35% normal sperm
Tapered heads:	_____		
Amorphous heads:	_____	WHO strict criteria 1999:	≥ 14% normal sperm
Large heads:	_____		5–13% borderline
Small heads:	_____		<5% abnormal
Double heads:	_____		
Acrosomal abn:	_____		
Mid-piece defects:	_____		
Tail defects:	_____		
Total sperm counted:	_____		

Assessment: _____
Comments and interpretation: _____

Fertility Institute of New Orleans Form 502:2008

Form 5.2 Semen analysis form.

Chapter 6

Semen preparation for intrauterine insemination

Roman Pyrzak

Introduction

The objective of sperm preparation for intrauterine insemination (IUI) is to separate seminal fluid containing prostaglandins from sperm by centrifugation so that the latter can be introduced into the uterus without causing severe cramping. If semen is mixed with a buffered solution supplemented with human serum albumin (HSA) before centrifuging, constituents that stabilize the sperm membrane and prevent capacitation are removed [1]. Advanced preparation procedures described in this chapter select out motile and superior-quality sperm by removing dead and abnormal sperm, immature sperm cells, white cells and debris – thus mimicking the in-vivo process of selecting motile sperm as they progress through the female reproductive tract, while leaving behind dead and immotile sperm and debris. Dead, immotile and abnormal sperm produce 10–15 times more reactive oxygen species (ROS) than motile sperm. High levels of ROS reduce fertilization potential [2].

Many methods have been described in the past for preparation of sperm for IUI [3]. The three principal methods in use today are:

- **Standard sperm wash (SSW)** – which removes seminal plasma from the semen specimen by centrifugation.
- **Swim-up (SWU)** – which uses self-migration of motile sperm from the bottom fraction to the top fraction, followed by centrifugation to remove dead and immotile sperm and debris.
- **Density gradient centrifugation (DGC)** – which separates motile sperm by density, using repeated centrifugation of semen mixed with a media containing a colloidal suspension of silica products. Motile sperm have higher density than non-motile and dead sperm.

A fourth method used by the Fertility Institute of New Orleans, which can be performed with or without centrifugation, is:

- **Swim-down (SWD)** – which employs self-migration of motile sperm from the top to the bottom fraction of media which contains increasing concentrations of HSA.

These four sperm preparation methods, plus a method intended to enrich Y-bearing sperm, are described here in detail. The length of time required for processing, the percentage recovery of total motile sperm (TMS) and the cost of the four methods are compared in Table 6.1. The SSW method is used only for IUI, while the other three methods are also used for in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). Bacteria, dead, immotile and abnormal sperm are separated from normal progressively motile (PM) sperm by SWD and SWU methods. In addition to bacteria, viruses may be removed DGC [4]. The choice of method depends upon the semen and sperm parameters on the day of IUI, which may be different from any previous semen analysis, whether fresh or cryopreserved sperm are to be used, and the training of laboratory personnel (Fig. 6.1). Use of minimal centrifugation forces and a minimum number of steps of centrifugations, for as short a time as possible, reduces damage to normal sperm during the stages of sperm processing, and increases the potential for fertilization. A comparison of SSW, SWU and DGC methods of sperm preparation has been published [5].

Acquisition and initial handling of the sperm specimen

The facility for specimen collection is described in Chapter 4. Because the sperm count is decreased if ejaculation takes place daily, patients should abstain from

Manual of Intrauterine Insemination and Ovulation Induction, ed. Richard P. Dickey, Peter R. Brinsden and Roman Pyrzak. Published by Cambridge University Press.

Table 6.1. Principal sperm preparation procedures compared

Procedure	Standard sperm wash (SSW)	Swim-up (SWU)	Density gradient centrifugation (DGC)	Swim-down (SWD)
Number of centrifugations	2–3	2–3	2	0–1[a]
Speed of centrifugation (*g*)	300–500	300–400	300–650	0–175
Spin time (min)	10	10–15	10–20	0–10
Processing time[b]	60–90	70–150	70–90	50–70
Total motile sperm (TMS) recovery (%)	90–95	10–25	30–45	35–50
Cost[c]	Low	Low	High	Low

[a] Centrifugation is required only in cases when the specimen for IUI needs to be ready in a short time.
[b] Processing time does not include sperm collection and liquefaction time.
[c] Includes only materials and disposables.

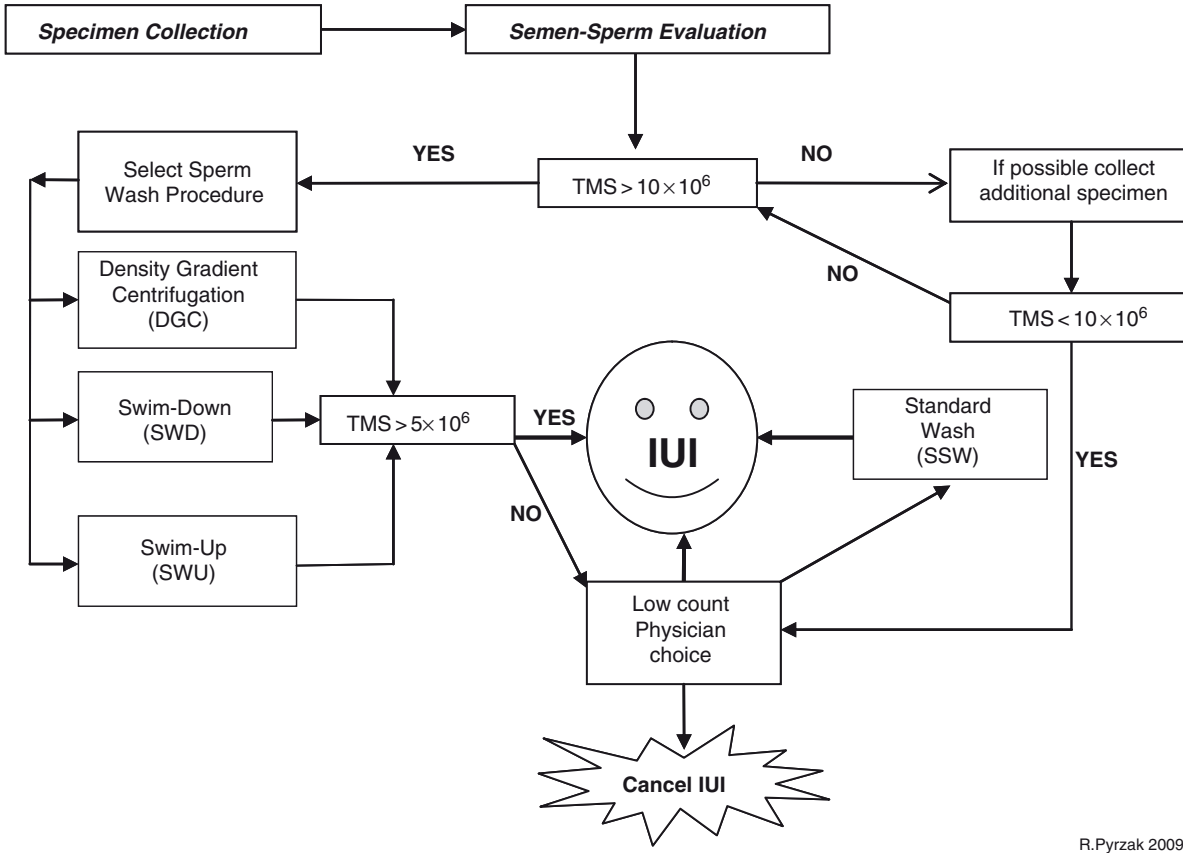

Figure 6.1 Flow chart for selecting the sperm preparation method depending on the total motile sperm (TMS) in the initial semen specimen.

sexual activity for a minimum of two days, but not more than five days, before providing a semen specimen for IUI, and should follow the procedures for clean collection of semen described in Chapter 5. In particular, lubricants (Vaseline, etc.) should not be used. After the semen specimen has been collected, motile sperm should be separated from non-motile sperm as soon as possible. Specimens should be maintained at room

temperature after acquisition by the laboratory before, during and after processing, except for the first 30 minutes after collection, when incubation at 37 °C may be necessary for complete liquefaction. The numbers of sperm with vacuolated nuclei are significantly increased in sperm processed at 37 °C, with a detrimental effect on function. ICSI utilizing sperm with vacuolated nuclei is associated with a low implantation rate, low pregnancy rate and high early abortion rate [6,7]. If it is anticipated that semen processing will be started later than one hour after the time of collection, the specimen should be placed in wash media (described below). While in wash media, sperm quality and motility will remain good for up to 18 hours at room temperature.

Initial evaluation of the semen specimen and selection of a processing method

When the semen specimen arrives at the laboratory and has liquefied, total sperm count, motility, viability and morphology are assessed to determine if an additional sperm sample is required, and if the number of TMS is sufficient to perform the planned preparation procedure. The minimum number of motile sperm inseminated by IUI necessary for a normal pregnancy rate is 4×10^6, with ≥ 30% progressively motile (PM) sperm [8]. Recovery of TMS is 90–95% for SSW, but ranges from 10% to 50% for other methods (Table 6.1). The standard sperm wash method should be used if TMS after preparation for IUI would be less than 4×10^6 after using another method. Average motility of sperm recovered following SWD, SWU and DGC is 84%, compared to 53% before processing, but it is unchanged following SSW. Pregnancy rates following SWD, SWU and DGC are decreased when motility of recovered sperm is < 50%.

Verification

Upon receiving the semen specimen, a nurse or laboratory personnel should verify the names and birth dates of both the person who produced the specimen and the intended recipient, and ascertain that the specimen is appropriately labeled. If semen specimens of more than one couple are being evaluated or processed, the laboratory should have a plan to keep them separate, usually by processing them in different areas.

IUI records

A well-organized IUI laboratory will wish to keep records of each IUI procedure. A form for recording initial and final semen quality, to be filled out by laboratory staff, and patient and cycle characteristics, to be filled out by a doctor or nurse, is shown in Form 6.1.

Laboratory safety

Semen specimens are potentially infectious, dictating that universal precautions for the handling of specimens must be observed. Thorough hand washing, use of sterile non-toxic gloves, sterile consumables and media are basic requirements. If cost is a factor, clean disposable (but non-sterilized) gloves may be used, as long as care is taken that these gloves do not touch the interior of the sterile containers or the semen sample. It is also advised that all the work be performed under a laminar flow hood.

Centrifuge speed

Proper centrifugation speed is critical in sperm processing. It is important to perform quality control at least twice a year to be certain that the centrifuge is working properly. Centrifugation directions are stated in *g* (*g* = relative centrifugal force or RCF). Laboratories may have centrifuges of different models and manufacturers that require different revolutions per minute (RPM) to produce a particular *g*. Excessive *g* can damage sperm, while too little *g* may not provide the desired separation. In the event that the instructions are misplaced or lost, it is possible to calculate the *g*. The RCF of each centrifuge depends on the radius (R), which is the distance in centimeters between the axis of rotation and the end of the tube shield, and the RPM. Nomograms to convert RCF to RPM can be found online but are difficult to use with the necessary degree of accuracy. Instead, the following equations, applicable to any model of centrifuge, should be used:

$$RCF = R \times 11.18 \times (RPM/1000)^2$$

$$RPM = 1000 \times \sqrt{(RCF/R \times 11.18)}$$

Media for sperm processing

Wash media (WM) used for the sperm wash procedure can be any standard buffer solution, such as phosphate buffered saline (PBS), Tyrode's, Earle's Balanced Salt Solution (EBSS) or Ham's F-10. Media should be of pharmaceutical grade, sterile and cell-culture-tested. Culture media that are "ready to use" for IVF procedures, such as human tubal fluid (HTF) (Sigma, www.sigma-aldrich.com), Quinn's Advantage® media

with HEPES (Cooper Surgical, www.coopersurgical.com) or SpermRinse™ (Vitrolife, www.vitrolife.com), may also be used. The above WM must be supplemented with human serum albumin (HSA) to a final concentration of 0.3–0.5%, and with gentamicin solution, 20 µL per 100 mL of media. Although allergic reactions from antibiotics in IUI specimens have been reporteded (see Chapter 8), no patients have been report to be allergic to this low concentration of gentamicin.

Procedures

The equipment and materials required for different procedures are listed in Table 6.2.

After semen is processed, it is best kept in a dark place until IUI. The processed specimen can be kept at room temperature for up to eight hours before IUI and have pregnancy outcomes similar to sperm inseminated within 1–2 hours.

Wash media (WM) preparation

To prepare WM with 0.3% HSA (HSAM), 0.12 mL of 25% HSA is added to 9.88 mL of WM. The required amount of HSAM needed is prepared the same day it is used; at the end of the day the leftover must be discarded. The volume of HSAM needed depends on the number of IUI cases scheduled; in general, each case requires 12–15 mL of HSAM. HSAM needs to be at room temperature before being used.

Standard sperm wash (SSW)

The SSW method recovers the highest number of TMS from a given ejaculate. However, the final specimen maintains a mixture of motile, immotile and immature sperm as well as non-spermatozoal cellular elements commonly found in the semen. These may have a negative impact on the quality of the "normal" motile sperm, and may affect the outcome of IUI.

Procedure

Equipment and supplies for this procedure are listed in Table 6.2, and the procedure is outlined in Figure 6.2.

(1) After liquefaction, place the entire semen specimen in a 15 mL centrifuge tube.
(2) Perform semen analysis, including calculation of TMS and preparation of slides for sperm morphology.
(3) Add WM to the tube to a total volume of 10 mL, and mix.

Table 6.2. Equipment and supplies for sperm preparation

I. Standard sperm wash (SSW)
Equipment
(1) Microscope with ×10, ×20, ×40 and ×100 objectives (any brand)
(2) Pipettes 10–100 µL, 100–1000 µL
(3) Laboratory counter
(4) Hemocytometer or Makler counting chamber
(5) Timer
(6) Tube stands
(7) Centrifuge with speed 50–1000 ***g*** (any brand)
Supplies
(1) Sperm wash media (WM)
(2) Centrifuge tubes 15 mL Falcon 2095 (BD, www.bd.com)
(3) 1 mL sterile transfer pipette (Samco Scientific Corporation, www.samcosci.com)
(4) Pipette tips 10–100 µL and 100–1000 µL
(5) Syringes, 5 mL and 10 mL (BD, www.bd.com)
Safety and biohazard products
(1) Gloves
(2) Protective mask
(3) Biohazard disposable box and bags
(4) Safety glasses; face shield or safe shield
II. Swim-up (SWU)
Equipment required in addition to those used in the SSW procedure
(1) Incubator (any brand)
III. Density gradient centrifugation (DGC)
Supplies required in addition to those used in the SSW procedure
(1) PureSperm® (NidaCon Laboratories, www.nidacon.com) stock solution of 100% (45% and 90% concentrations can be purchased that are ready to use)
(2) Isolate® (Irvine Scientific, www.irvinesci.com) stock solution of 100% (45% and 90% concentrations can be purchased that are ready to use)
IV. Swim-down (SWD)
No additional equipment or supplies required
V. Albumin gradient method of male sex selection
Equipment required in addition to those used in the SSW procedure
(1) Glass culture tubes 10 × 75 mm

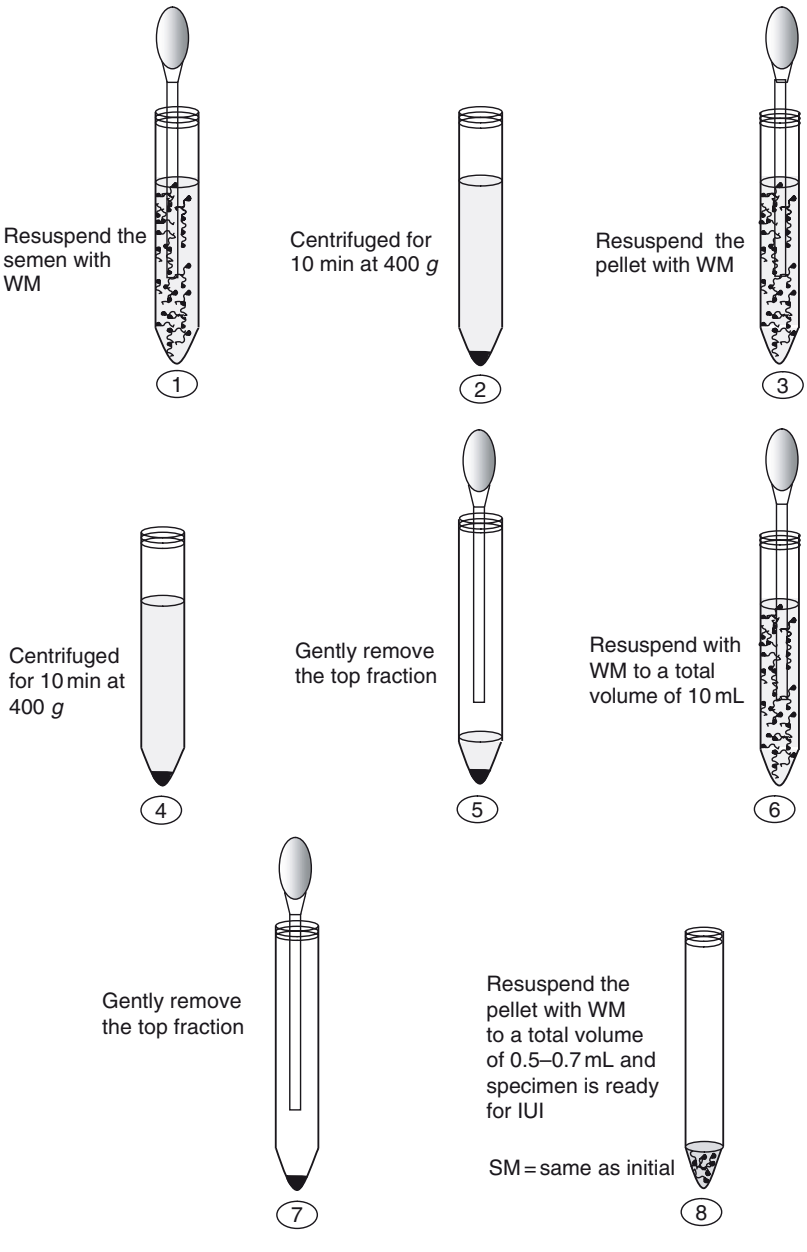

Figure 6.2 Sperm preparation using the standard sperm wash (SSW) procedure. SM, sperm motility (%); WM, wash media.

(4) Centrifuge the semen/WM mixture at 400 *g* for 10 minutes, and discard the supernatant.
(5) Resuspend the pellet with WM to a total volume of 10 mL and centrifuge again at 400 *g* for 10 minutes.
(6) Determine the TMS and progression.
(7) Discard the top fraction; resuspend the pellet with 0.5 mL of WM.
(8) The specimen is ready for IUI.

Note: If the initial specimen is viscous, additional steps of centrifugation may be required.

Swim-up (SWU)

The SWU procedure is based on self-migration of motile sperm. The sperm with the best motility are able to separate from the non-motile sperm and debris by swimming from the bottom to the top fraction of the media. However, the recovery rate of motile sperm is significantly lower than with other methods. Due to low sperm recovery, the SWU procedure should not be considered for IUI if the initial TMS is less than 30×10^6.

Procedure

Additional equipment for this procedure is shown in Table 6.2, and the procedure is outlined in Figure 6.3.

(1) After liquefaction, place the entire semen specimen in a 15 mL centrifuge tube.
(2) Perform semen analysis, including calculation of TMS and preparation of slides for sperm morphology.
(3) Resuspend one volume of semen with two volumes of WM, and mix thoroughly by drawing the sample in and out using a sterile transfer pipette.
(4) Centrifuge the resuspended semen at 400 *g* for 10 minutes and discard the supernatant.
(5) Resuspend the pellet with sufficient WM to achieve a concentration of 20×10^6 sperm per mL, based on initial TMS in the semen specimen.
(6) Place 0.5 mL of the resuspended sperm in the bottom of each 15 mL centrifuge tube.
(7) Very carefully layer 1.0 mL of WM on the top of the resuspended sperm.
(8) Place the tubes in a tube rack at an angle of 45 degrees and incubate for one hour at 37 °C (do not use an incubator with CO_2).
(9) After one hour set the tubes upright and aspirate the top fraction (0.5 mL) with a sterile transfer pipette and collect into a new 15 mL centrifuge tube.
(10) Determine the TMS and progression.
(11) Centrifuge the tube at 300 *g* for 10 minutes, and remove and discard the supernatant. (*Note:* 400 *g* was used in step 4.)
(12) Resuspend the sperm pellet with 0.5 mL of WM.
(13) The specimen is ready for IUI.

Note: Each laboratory may have a slightly different SWU procedure; for example, the time of incubation may vary from 45 minutes to 90 minutes in order to recover a desired number of TMS.

Density gradient centrifugation (DGC)

The DGC method selects sperm on the basis of their density. Motile sperm have higher density than non-motile and dead sperm. Therefore, a subpopulation of highly motile spermatozoa with optimal morphology can be selected; however, the lower recovery rate for DGC remains an issue. DGC media is a colloidal suspension of silica particles in HEPES-buffered human tubal fluid (HTF). Because of low sperm recovery rates, the DGC procedure should not be considered for IUI if the initial TMS is less than15×10^6. The commercial products that are approved for human sperm preparation are PureSperm® and Isolate®. Both products can be purchased as a stock solution of 100%, or with 45% and 90% concentrations that are ready to use. In the past Percoll was extensively used for DGC, but it is not now approved for human use.

Procedure

Additional supplies for this procedure are shown in Table 6.2, and the procedure is outlined in Figure 6.4.

Preparation of density gradient solutions (DGS)

To prepare 10 mL of 90% PureSperm® (PS) or Isolate® (IS) solution from 100% stock solution, add 1 mL of WM to 9 mL of 100% PS or IS solution. To prepare 10 mL of 45% PS or IS solution, make up a 90% solution as described above and mix 5 mL of 90% PS or IS solution with 5 mL of WM. Alternatively, mix 4.5 mL of PS or IS solution with 5 mL of WM.

DGC procedure

(1) After liquefaction, place the entire semen specimen in a 15 mL centrifuge tube.
(2) Perform semen analysis, including calculation of TMS and preparation of slides for sperm morphology.
(3) The number of tubes required to perform DGC depends on the volume and sperm concentration. The total number of sperm in each tube should be no more than 45×10^6, and the total volume of semen should be no more than 1.5 mL per tube. If the sperm concentration is greater than 30×10^6/mL, dilute the specimen with WM to a final concentration of 30×10^6 sperm per mL.
(4) Place 1.0 mL of 90% of DGS in a 15 mL centrifuge tube. With a new pipette, very gently layer 1.0 mL of 45% DGS on the top of the 90% DGS, taking care to avoid mixing the 45% and 90% layers.

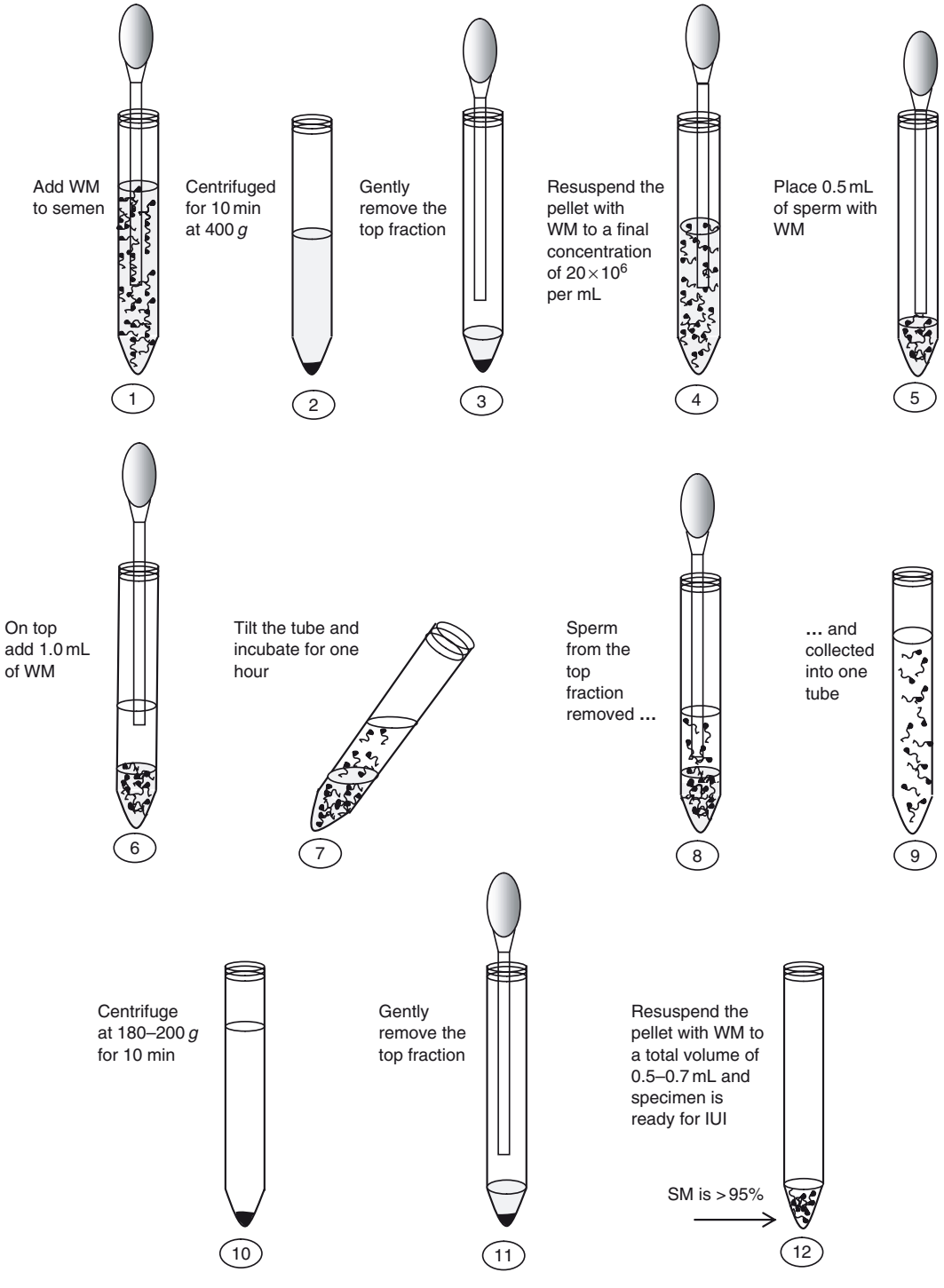

Figure 6.3 Sperm preparation using the swim-up (SWU) procedure. SM, sperm motility (%); WM, wash media.

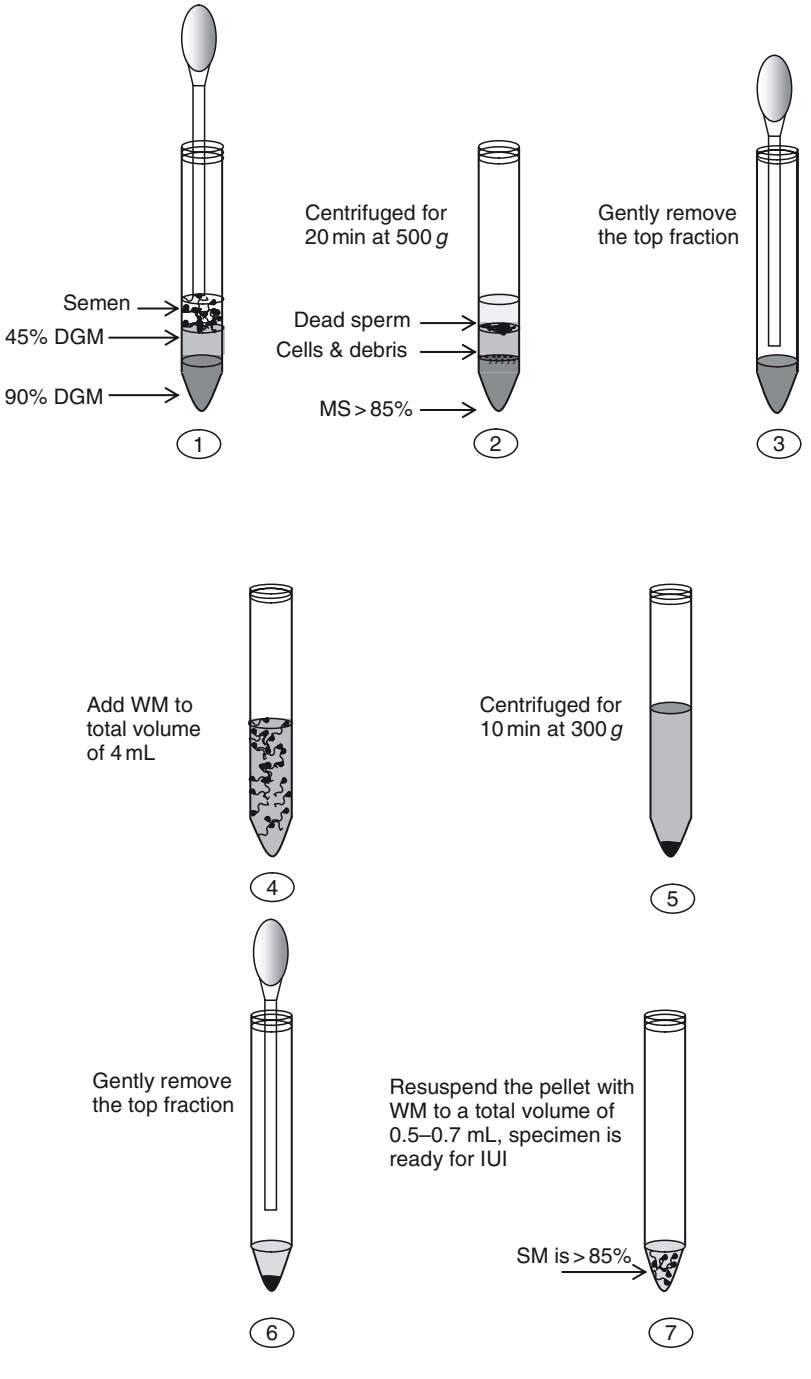

Figure 6.4 Sperm preparation using density gradient centrifugation (DGC) procedure. DGM, density gradient media; SM, sperm motility (%); WM, wash media.

(5) Using a new pipette, very gently layer 1.5 mL of semen on the top of the 45% DGS.
(6) Centrifuge the tubes at 500 *g* for 20 minutes.
(7) With a new pipette, remove the top fraction, which includes the specimen, 45% DGS media and the top of the 90% DGS media (2.5–2.75 mL total). The bottom fraction of the 90% DGS contains the motile sperm.
(8) Add 4 mL of WM into the tube containing the bottom fraction of 90% DGS.

(9) Determine the TMS and progression.
(10) Centrifuge the tubes at 300 *g* for 10 minutes. (*Note:* 500 *g* was used in step 6.)
(11) With a new pipette, remove the supernatant, taking as much of the top fraction as possible, but being careful not to disturb the sperm pellet.
(12) Resuspend the pellet with 0.5 mL of WM, and it is ready for IUI.
(13) If tubal perfusion is planned, resuspend the sperm pellet with 4 mL of WM. Alternatively, for use in tubal perfusion, suspend the pellet in 0.5 mL WM, and pull 3.5 mL of WM into the IUI catheter/syringe first before pulling up the 0.5 mL sperm suspension.

Swim-down (SWD)

The SWD method is based on the ability of motile sperm to orient themselves by gravity and to swim downward through human serum albumin (HSA) media of increasing viscosity at a rate faster than the sedimentation rate of immotile sperm and debris by gravity alone. The fastest-swimming sperm predominate in the lower fractions, while debris and immotile or dead sperm remain on top. Motility following SWD ranges from 90% to 98%, with an improvement in average progression grade from 2.8 to 3.9. The percentage of normal forms is also improved.

The completeness of the separation is dependent on these variables: sperm concentration, progressive motility, total number of sperm applied to each column and the interval of time between starting and finishing the SWD process. The SWD process takes three hours, but by adding a centrifugation step it can be accomplished in 30 minutes or less.

Recovery rates average 45%, but range from 20% to 70%, depending on the initial sperm motility and progression. If motility is less than 35% and progression grade is less than 2.5, recovery will be low. However, when motility is low but within acceptable limits of 35–45%, and only a small amount of debris is present, an additional 10–15 minutes of SWD time is allowed. If the semen specimen contains a large amount of debris, the concentration of HSA should be increased to 12.5% or even 15%.

If the initial specimen contains gel particles (globules), they will settle to the bottom of the tube in 2–3 minutes. The top globule-free fraction is removed and used for processing. If the specimen contains a lot of debris or sperm agglutination, it is diluted 1 : 1 with WM, divided into two tubes and centrifuged at 180–200 *g* for 1–2 minutes. The top fraction is removed and is used for processing.

Procedure

No additional equipment or supplies are required for this procedure, which is outlined in Figure 6.5.

Preparation of human serum albumin media (HSAM) for SWD

WM with 0.3% HSA (HSAM) is prepared as previously described. To prepare 7.5% HSAM, 3 mL of 25% HSA is added to 7 mL of WM. To prepare 12.5% HSAM, 10 mL of 25% HSA is added to 10 mL of WM.

Rapid SWD procedure (requires centrifugation)

If IUI must be performed in less then three hours from the time the specimen is collected a centrifugation step is required to concentrate the sperm.

(1) After liquefaction, place the entire semen specimen in a 15 mL centrifuge tube.
(2) Perform semen analysis, including calculation of TMS and preparation of slides for sperm morphology.
(3) Add an equal volume of 0.3% HSAM to make a 1 : 1 solution, and mix with a transfer pipette.
(4) Place 1.5 mL of 7.5% HSAM into eight 15 mL centrifuge tubes.
(5) Using a new pipette, gently layer 0.5 mL of the 1 : 1 diluted semen on top of the 7.5% HSAM. If the volume of 1 : 1 diluted semen is greater than 4 mL, put an equal amount into each of the eight tubes.
(6) Allow the sperm to swim down into the layer of 7.5% HSAM for 10–30 minutes at room temperature in a dark place. The time of swim-down depends on the sperm concentration:
   (a) If TMS in the initial specimen is $< 40 \times 10^6$, the SWD time must be 30 minutes.
   (b) If initial TMS is $40–80 \times 10^6$, SWD time is 25 minutes.
   (c) If initial TMS is $80–120 \times 10^6$, SWD time is 20 minutes.
   (d) If initial TMS is $120–160 \times 10^6$, SWD time is 15 minutes.
   (e) If initial TMS is $\geq 160 \times 10^6$, SWD time is 10 minutes.
(7) After the allotted time for swim-down is completed, gently remove and discard the top fractions using a new transfer pipette. Combine the bottom fractions, which contain the motile sperm, from all eight tubes into a single 15 mL tube. Determine the TMS.

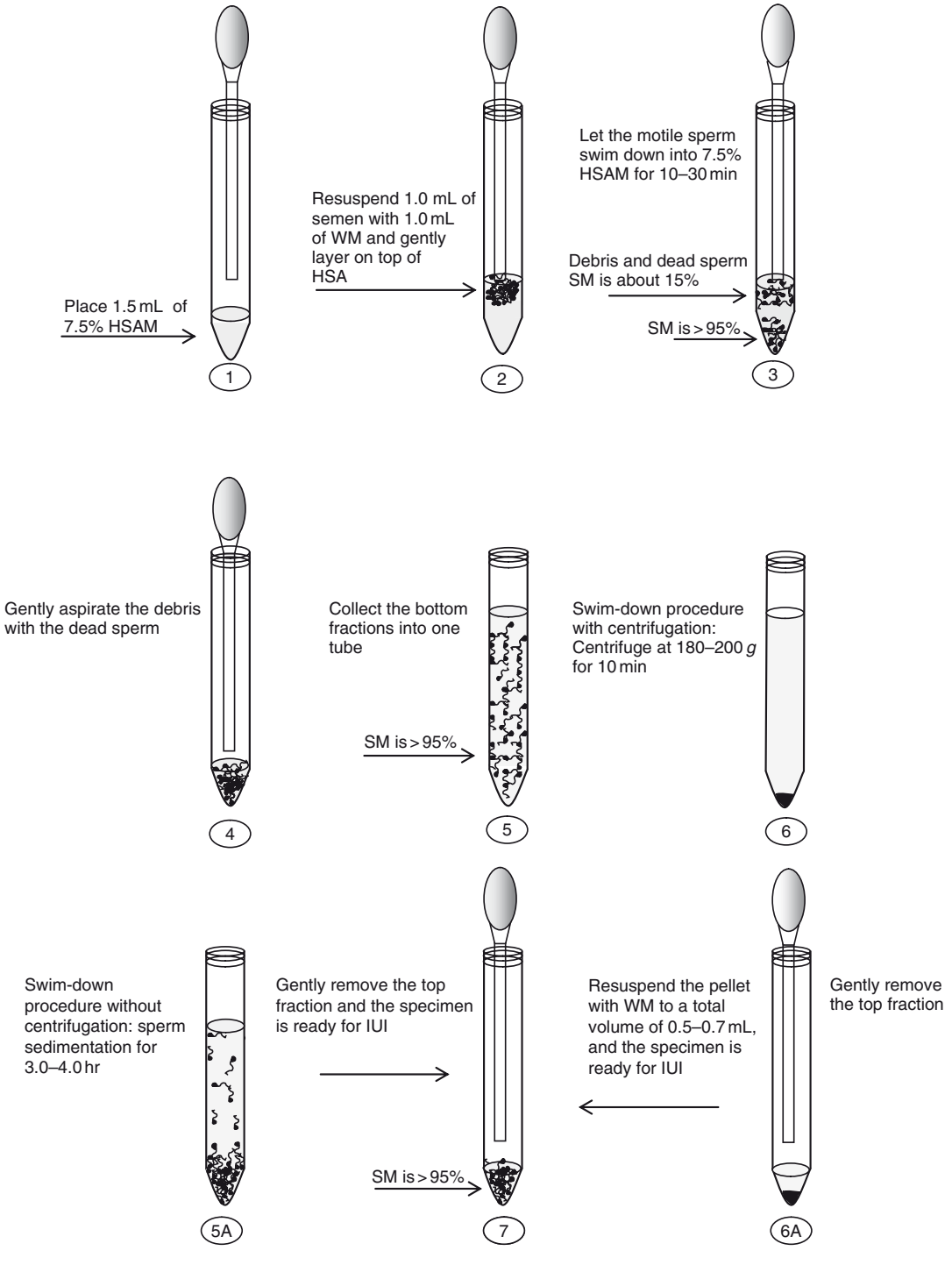

Figure 6.5 Sperm preparation using swim-down procedure, with or without centrifugation. HSA, human serum albumin; SM, sperm motility (%); WM, wash media.

(8) Split the combined specimen into two 15 mL tubes and add 0.3% HSAM to each tube, to a total volume of 10 mL.
(9) Centrifuge the specimen at 180–200 *g* for 10 minutes. This centrifuge step should be performed 25 minutes before performing IUI.
(10) Using a transfer pipette, remove the top fraction without disturbing the bottom of the sperm pellet.
(11) Using a new transfer pipette, combine the two pellets. Add WM if necessary for a total volume of 0.5 mL.
(12) The specimen is ready for IUI.

Slow SWD process (does not require centrifugation)

If IUI is to be performed later than 3–6 hours after collection it does not require the centrifugation step, because sperm will settle on the bottom of the tube due to gravity.

(1) Follow steps 1–8 of the rapid (centrifuge) method.
(2) Instead of centrifuging at step 9, set the two tubes aside in a dark area at room temperature for 3–4 hours or more. Sperm will settle in the bottom of the tube (a white sperm pellet can be seen).
(3) Before insemination, carefully remove and discard the top fractions, using a transfer pipette, without disturbing the sperm pellet.
(4) Determine the TMS and progression.
(5) Using a new transfer pipette, combine the two pellets. Add WM , if necessary, for a total volume of 0.5 mL.
(6) The specimen is ready for IUI.

Preparation of sperm for sex selection

The request for gender selection may be motivated by medical or non-medical issues, including cultural, religious, psychological and economic factors. When sex selection is indicated for medical reasons the most reliable method, IVF with preimplantation genetic diagnosis (PGD), is the method of choice. Often, the motive is a desire to balance or complete the family with a child of a sex different from those already born. When sex selection is used for non-medical reasons, methods that do not require IVF and PGD, even though less reliable, may be chosen because they are less expensive and avoid the requirement for IVF. Ethical considerations of gender selection and the results of different methods used for IUI have been extensively reviewed by Kilani *et al.* [9].

There are two recognized methods of sperm separation for gender differentiation: the human serum albumin gradient method (GM), which is based on the physical and kinetic differences of spermatozoa, and the flow cytometry method (FCM), which is based on the 3% greater amount of DNA in X- compared to Y-bearing sperm. Only FCM has been proven to be effective in selecting X-bearing sperm. Sperm recovery after FCM varies between 0.6% and 1.2% of the sperm processed, with an accuracy of 80–90% for X-bearing sperm and 60–70% for Y-bearing sperm [10]. Sperm recovery following GM is 2–4%, with greater selectivity for male births at the lower recovery rate [11]. Sorted sperm fractions analyzed by fluorescent in-situ hybridization (FISH) after GM show only a slight preponderance of Y-bearing sperm [12]. Nevertheless, a 73% male birth rate for the GM method has been reported [13]. Because of low recovery rates, both methods are unsuitable for oligospermic patients. Only the albumin gradient method, which does not require specialized equipment, is described here. For a detailed description of the FCM see Fugger *et al.* [10]. The use of an albumin gradient for enrichment of Y-bearing sperm was first introduced in 1973 by Ericsson *et al.* [14]. The GM procedure described in this chapter is a modification introduced by Pyrzak that in the author's experience has yielded a 75% male birth rate [11]. It employs repeated swim-down and centrifuge steps. Because the recovery rate is 4%, the initial specimen must have greater than 100×10^6 TMS with a progression score of 2.5 or higher, and no more than a moderate or normal amount of debris.

Albumin gradient method for male sex selection: procedure

The additional equipment required for this procedure is shown in Table 6.2, and the procedure is outlined in Figure 6.6.

Preparation of human serum albumin media (HSAM) for GM

WM with 0.3% HSA (HSAM) is prepared as previously described. HSAM 7.5% and HSAM 12.5% are prepared as described for the SWD method. To prepare 20% HSAM, 8 mL of 25% HSA is added to 2 mL of WM.

GM procedure

(1) After liquefaction, place the entire semen specimen in a 15 mL centrifuge tube.
(2) Perform semen analysis, including calculation of TMS and preparation of slides for sperm morphology.

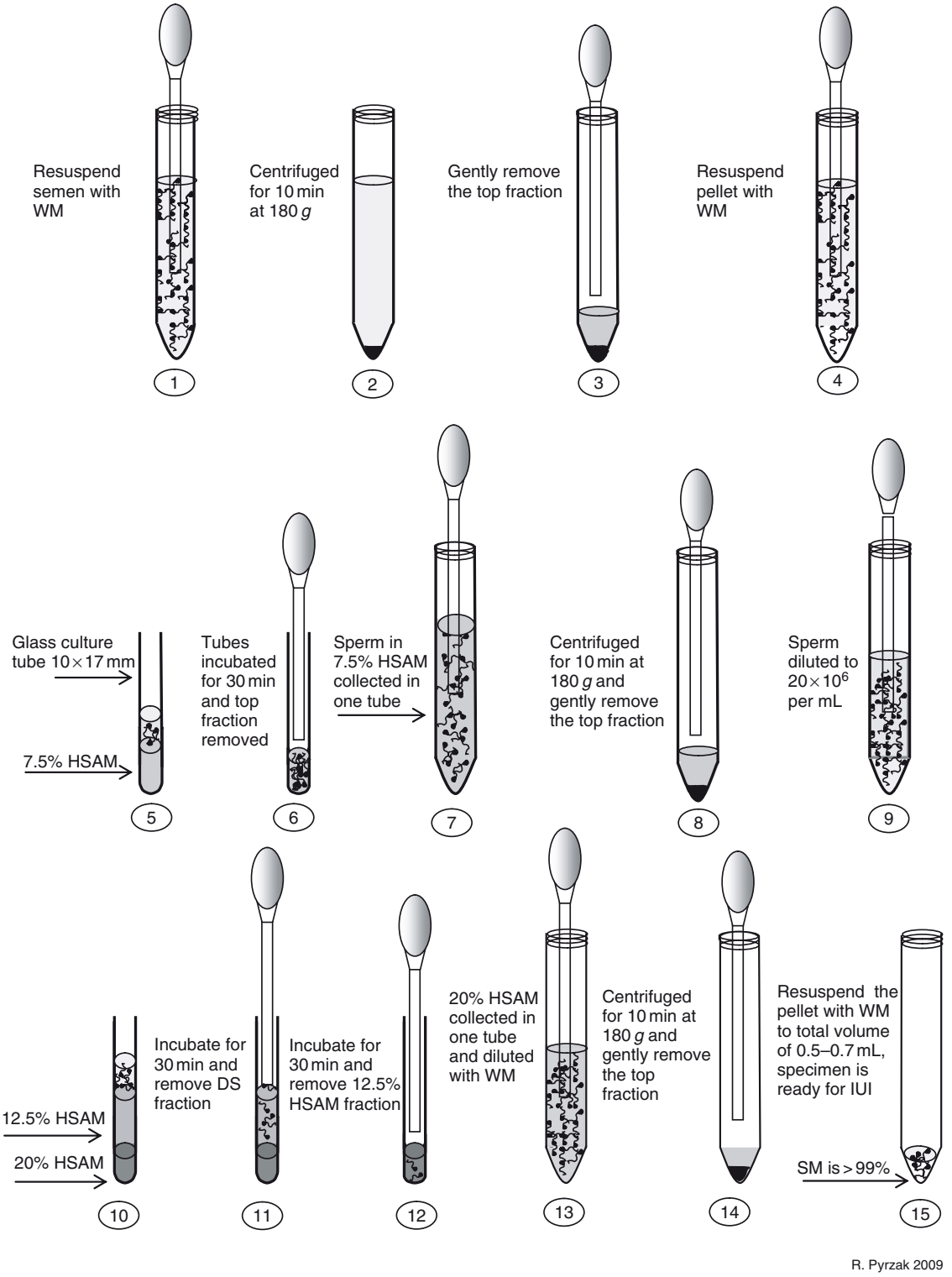

Figure 6.6 Sperm preparation for male sex selection (MSS) procedure using swim-down method. HSAM, human serum albumin media; SM, sperm motility (%); WM, wash media.

(3) Add an equal amount of WM to the specimen, and then centrifuge the specimen at 180–200 *g* for 10 minutes.
(4) Gently remove the supernatant with a transfer pipette, and discard. Resuspend the sperm pellet with WM to a final concentration of 20–25 × 10^6 per mL, based on the initial number of sperm (use a lower concentration if the specimen contains a large amount of debris). Calculate the number of 10 × 75 mm glass culture tubes required for the next step by multiplying the total amount of solution by 2.
(5) Place 1.0 mL of 7.5% HSAM in each glass tube. With a new pipette, gently layer 0.5 mL of the resuspended specimen on the top of 7.5% HSAM.
(6) Place the tubes at room temperature in a dark place for 30 minutes.
(7) After 30 minutes, gently remove the top fraction (0.5 mL) and a very small portion of 7.5% HSAM with a transfer pipette.
(8) Combine the bottom 7.5% HSAM fractions containing motile sperm into one 15 mL centrifuge tube (if the total volume is > 10 mL use additional tubes). Determine the number of TMS.
(9) Centrifuge the pooled specimen at 180–200 *g* for 10 minutes, then gently remove the supernatant with a transfer pipette and discard.
(10) Resuspend the semen pellet with WM to a final concentration of 20–25 × 10^6/mL, based on the TMS before centrifugation. Calculate the number of tubes required for the next step by multiplying the total amount of solution by 2.
(11) Place 0.5 mL of 20% HSAM on the bottom of each glass tube. With a new transfer pipette, very gently layer 1.0 mL of 12.5% HSAM on the top of the 20% HSAM, avoiding mixing the two layers. With a new transfer pipette, very gently layer 0.5 mL of the resuspended sperm on the top of the 12.5% HSAM.
(12) Place the tubes at room temperature in a dark place for 30 minutes.
(13) After 30 minutes, remove the top 0.5 mL with sperm and a small amount of the 12.5% HSAM layer, and allow the tubes to stand for an additional 30 minutes.
(14) After 30 minutes, remove the 12.5% HSAM layer and a minimal portion of the 20% layer.
(15) Combine the 20% HSAM fractions into one 15 mL centrifuge tube and determine the TMS. Dilute the combined fraction to 1 : 1 with WM and centrifuge at 180–200 *g* for 10 minutes, then gently remove the supernatant and discard.
(16) Resuspend the pellet with 0.5–0.7 mL of WM.
(17) The specimen is ready for IUI.

The final fraction should be free of debris, containing 98–100% motile sperm with very good PM. If the initial TMS is > 100 × 10^6, the time of swim-down in each step can be shorter than 30 minutes. This reduces the percentage recovery, but potentially increases enrichment of Y-bearing sperm [13].

Sperm washing for HIV and hepatitis C virus (HCV)

Male-to-female transmission of human immunodeficiency virus (HIV) occurs at a rate of 0.1–0.5% in a monogamous couple. Effective antiviral drugs now allow survival of 20 years or longer after diagnosis for HIV victims, causing patients and physicians to reconsider whether fertility is an option they wish to consider after consultation with their physicians [15]. If so, IUI with washed and migrated sperm offers a method of reducing the risk to the female partner. Through 1995, the United States Centers for Disease Control and Prevention (CDC) recorded only two known cases of HIV transmission following IUI with washed sperm or washed migrated sperm, one of which involved a partner with a high viral load [15]. HIV and HCV are found in the seminal plasma and not in the sperm. Sperm washing may be an effective method of removing both HIV and HCV from seminal plasma if the husband is HIV- or HCV-positive and the wife is negative [3,16,17]. Since 1987, over 3,000 cycles of IUI using washed sperm have been reported in 1,111 sero-discordant couples without an infection [4,18]. However, the estimated rate of contamination would require 30,000 cases to have sufficient statistical power to detect a difference [19]. According to Sauer, semen should be prepared using two methods sequentially, first DGC, followed by SWU [18].

Procedure

No additional equipment or supplies are required for this procedure.

(1) Universal safety precautions should be followed, and IUI should not be attempted while serum viral loads are positive.

(2) First process the semen specimen by the DGC method described above.
(3) After step 10 in the DGC procedure, resuspend the pellet with sufficient WM to achieve a concentration of 20×10^6 sperm per mL, based on initial TMS in the semen specimen.
(4) Process the semen suspension following steps 5 through 11 of the SWU method, and it is ready for insemination using universal safety precautions and disposable instruments.

Note: Due to the required double processing, the final TMS may be too low to achieve pregnancy with IUI. IVF or IVF-ICSI may then be recommended instead.

Conclusion

Sperm preparation for IUI is an absolutely crucial process in any IUI program. It must only be performed by fully qualified scientists in accredited laboratories. Each laboratory team will select their most favored of the sperm preparation methods described in this chapter, according to their experience and the quality of the specimens received. The quality of the sperm achieved in the final insemination specimen is critical to achieving success.

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Sperm preparation for insemination

Patient information

Patient name: _____ Physician name: _____

Patient DOB: ___/___/_____ Age: _____ Technologist name: _____

Spouse name: _____

Spouse DOB: ___/___/_____ Age: _____

Sample information

Accession #: _____

Collected date/time: ___/___/____, ___:___ Sperm source: Partner ____ Donor ____

Received date/time: ___/___/____, ___:__ Allergic to: _____

Days of abstinence:_____

Collection location: Clinic _____Home _____ Other _____

Collection method: Masturbation _____ Intercourse_____ Other _____

Donor (if applicable): Sperm bank name:_____Donor #: _____Date delivered: __/___/____

Sperm preparation method: SSW __SWU __DGC __SWD __None _____Other _____

Notes: _____

Semen parameters	*Pre-wash*	*Normal range**	*Units*	*Post-wash*
Sample volume:	1st _____ 2nd _____	2–5.5	mL	
Count × 10^6/mL:	1st _____ 2nd _____		1st _____ 2nd _____	
Mean sperm count:	_____	≥20	× 10^6/mL	_____
Motility:	_____	≥50	% progressive	_____
Progression grade:	_____	0–1	grade 0–4.0	_____
TMS :	_____	≥20	× 10^6	_____
Normal morphology:	_____	See note	%	_____
PMNs:	_____	< 1.0	× 10^6/mL	_____
Total sperm:	_____	≥ 40	× 10^6	_____
Post-wash recovery of TMS	_____		%	_____

*Normal range of pre-wash specimen

Note: Normal morphology: WHO 1999 >35%. Strict: Normal >14%, Borderline 5–13%, Abnormal <5%

Artificial insemination procedure IUI, cycle #: ___ Vaginal, cycle #: ___ Cervical, cycle #: _____

Physician: _____ Nurse: _____

Diagnosis

Male infertility: _____ Cervical factor : _____ Unexplained : _____

Ovulation disorders / PCO: _____ History of endometriosis: _____ Tubal : _____

Other: _____ _____

LMP date: _____ Cycle day: _____ Stimulating day: _____

Medications: Clomiphene _____ Tamoxifen _____ hMG/FSH _____

Dose: _____

Other: _____ _____

Comments: _____

Outcome: _____

Fertility Institute of New Orleans Form 601:2008

Form 6.1 Sperm preparation report.

Chapter 7

Ovulation induction for intrauterine insemination I: oral drugs clomiphene, tamoxifen, letrozole

Richard P. Dickey

Introduction

Intrauterine insemination (IUI) and ovulation induction (OI) are often combined in order to increase the effectiveness of infertility treatment [1,2]. The reason most frequently given for combining OI with IUI to treat male-factor infertility is to maximize the possibility of pregnancy by increasing the number of preovulatory follicles. However, unless the female partner is anovulatory or has luteal deficiency, there is conflicting evidence on whether oral OI drugs do increase pregnancy rates in IUI cycles. On the other hand, there is undeniable evidence that IUI does improve pregnancy rates in many cases when clomiphene citrate (CC) is used to treat ovulation dysfunction or luteal insufficiency. Whether IUI should be used in all OI cycles, or only if there is either a poor postcoital test (PCT) or semen quality below WHO standards, is arguable. At the Fertility Institute of New Orleans only 20% of 3,400 CC pregnancies have required IUI.

The advantages of oral drugs over gonadotropin for OI include a low incidence of multiple pregnancies and ovarian hyperstimulation syndrome (OHSS), low cost, oral administration and less need for cycle monitoring. However, due to its antiestrogenic nature, CC may adversely affect cervical mucus and endometrial receptivity. Tamoxifen (TMX), a structurally related selective estrogen receptor modulator (SERM) that has a weak estrogenic effect on cervical mucus and the endometrium, and letrozole (LET), an aromatase inhibitor, are used "off label" to replace CC.

Action

CC and TMX are competitive antagonists of ovarian estrogen at hypothalamic receptor sites, and exhibit either antiestrogenic activity (CC) or weak estrogenic activity (TMX) at peripheral receptor sites (Fig. 7.1). Blockade of estrogen receptors in the hypothalamic arcuate nucleus and in the pituitary lead to an increase in gonadotropin-releasing hormone (GnRH) pulse frequency [3] and increased pituitary sensitivity to GnRH [4]. The net effect of these actions is that serum levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) increase three- to fourfold during CC administration [5]. Monofollicular development is facilitated and multiple follicular development is attenuated, when compared to gonadotropin cycles, due to down-regulation of FSH by negative feedback of ovarian estrogen during the follicular phase. Induction of ovulation with competitive estrogen antagonists requires an intact hypothalamic–pituitary–ovarian axis and serum estradiol > 50 pg/mL. CC and TMX are ineffective in women whose ovaries do not produce estrogen because of hypothalamic–pituitary disorders or ovarian failure. CC and TMX are most effective when the total daily dose is taken at one time so as to optimize concentration at hypothalamic and central nervous system receptors beyond the "blood–brain barrier".

CC is an isomeric mixture of ~38% zuclomiphene and 62% enclomiphene. Zuclomiphene is mildly estrogenic, as well as antiestrogenic, while enclomiphene is entirely antiestrogenic. Serum levels of zuclomiphene remain at least 10% of peak levels 28 days after ingestion of a single 50 mg tablet, and small amounts of zuclomiphene continue to be excreted for at least six weeks (Fig. 7.2a) [6]. The effect of repeated administration of a single 50 mg tablet at 28-day intervals is cumulative, with basal levels of zuclomiphene increasing by 50% per month (Fig. 7.2b) [6]. TMX has

Manual of Intrauterine Insemination and Ovulation Induction, ed. Richard P. Dickey, Peter R. Brinsden and Roman Pyrzak. Published by Cambridge University Press.

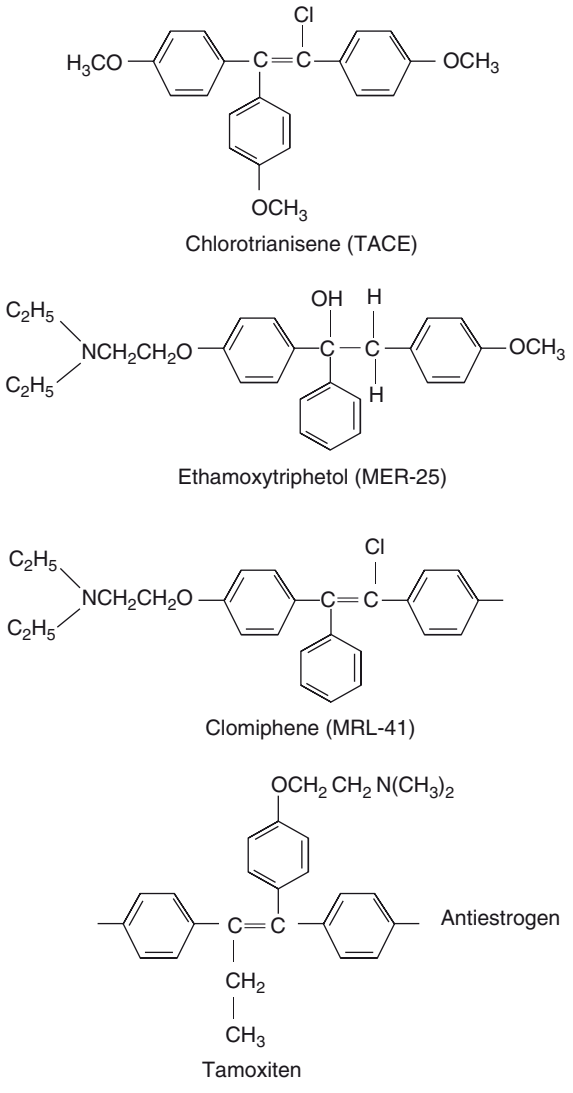


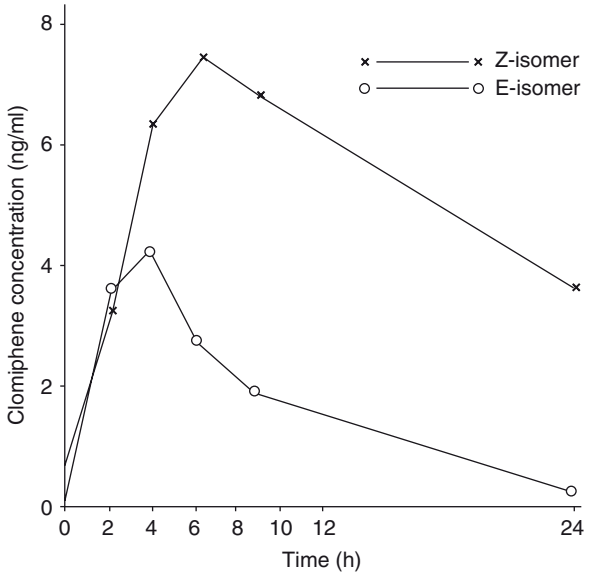
Figure 7.1 Chemical structures of antiestrogens of the triphenylethylene type. From Dickey and Holtkamp 1996 [2]. Reproduced with permission of the author and publisher.

only the z-isomer, with pharmacokinetics assumed to be similar to zuclomiphene. Two results of this prolonged retention in the body are that CC and TMX may be more effective in inducing ovulation during the second and later cycles of treatment, even though the dose remains the same, and that pregnancies as a result of CC and TMX may occur for up to two cycles after treatment has ended.

Following CC-induced, and by inference also TMX-induced ovulation, luteal-phase progesterone and estradiol serum levels are increased in a direct dose–response relationship [7]. During the first trimester of CC pregnancies, serum progesterone level is 200–300% higher and serum estradiol level is 66% higher than in spontaneous pregnancies until the 11th post-ovulation week; after which they decrease gradually, but remain higher than in spontaneous pregnancies through at least the 16th week of gestation [8]. As a result there is less need for supplemental progesterone support in CC than in gonadotropin pregnancies. During the first eight gestational weeks, uterine artery blood flow volume per minute in singleton CC pregnancies is increased approximately 25% compared to spontaneous pregnancies, presumably because of the higher level of progesterone and estradiol [8].

Endometrial thickness is initially decreased in CC cycles, but is later increased compared to natural cycles as rising estrogen concentration overcomes the antiestrogen effect [9]. TMX, which has only estrogenic activity, increases endometrial thickness and enhances mucus quality. Fewer antiestrogen effects on the cervical mucus and endometrium are the most frequent reasons for using TMX instead of CC. Hot flashes are common to both CC and TMX, and are limited to the days they are taken. Scintillating scotoma (moving, flashing lights) occurs more often with CC. Both CC and TMX should be discontinued when scintillating scotoma occurs, but may be restarted at a lower dose in a subsequent cycle.

LET is a third-generation aromatase inhibitor used for treatment of estrogen-positive breast cancer. Aromatase inhibitors act directly on the ovary to decrease production of estrogen by competitive, reversible binding to the aromatase (P450arom) receptor, thereby increasing GnRH secretion, pituitary LH and FSH production, and ovarian follicular development. Intraovarian androgens accumulate because LET competes with androgens for aromatase binding sites. As a result, the number of small follicles is increased in the early follicular phase of the cycle due to enhanced sensitivity to FSH, and mid-sized follicles become atretic late in the follicular phase. Multiple follicular ovulation and multiple pregnancies are reduced compared to CC and TMX; twins occur in fewer than 5% of LET pregnancies [10], and triplet pregnancy is rare. Mid-luteal-phase serum progesterone concentration is significantly lower in LET cycles compared to CC cycles (7.1 vs. 11.1 ng/mL) [11]. Letrozole doses of 2.5, 5.0 and 7.5 mg for five days have been compared to clomiphene 50 mg or 100 mg in small randomized and cross-over trials, with the optimal dose still to be determined. In a meta-analysis of four randomized trials, including



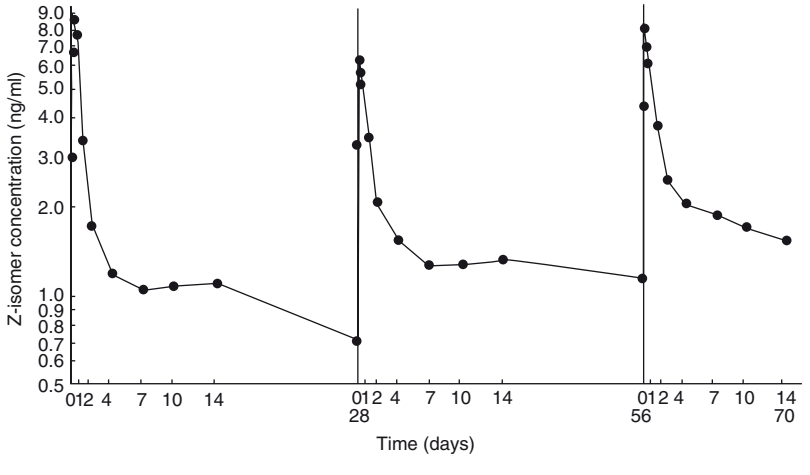


Figure 7.2 (a) Mean plasma concentrations of zuclomiphene and enclomiphene after oral administration of one 50 mg tablet of clomiphene ($n = 23$). (b) Mean plasma concentration of zuclomiphene after administration of one 50 mg tablet of clomiphene at 28-day intervals. From Mikkelson *et al.* (1986) [6]. Reproduced with permission of the authors and the publisher.

662 patients with polycystic ovary syndrome (PCOS), pregnancy rates in patients treated with CC and LET were identical [12].

In 2005, the United States FDA ordered manufacturers to issue a warning that LET is contraindicated for ovulation induction [13]. The order followed a study of 150 babies born following LET which concluded there was an increased risk of cardiac anomalies and skeletal malformations [14]. Subsequently, a retrospective review of 514 babies conceived following LET and 397 babies conceived following CC at five Canadian fertility centers reported a major malformation rate of 1.2% for LET and 3.0% for CC [15]. When administered to animals during pregnancy LET is embryotoxic and fetotoxic and is classified as Pregnancy Category D. The half-life of LET is approximately 30–60 hours, thus it is effectively cleared from the body in OI cycles by the time of embryo implantation [12] .

Preparation for ovulation induction

Before any OI drugs are started it is important to evaluate and treat medical conditions that could decrease the chance of conception or adversely affect mother or baby during pregnancy (Fig. 7.3). As a minimum, thyroid-stimulating hormone (TSH) should be measured. FSH, LH, estradiol, dehydroepiandrosterone sulfate (DHEAS), fasting glucose/insulin and prolactin should be measured if indicated by absent, irregular or infrequent menses, androgen excess or galactorrhea (see Chapter 3). Semen analysis should be performed,

INITIAL EVALUATION

Regular menses	Irregular menses	Amenorrhea	Androgen excess	Galactorrhea
TSH Day 21 progesterone	TSH LH/FSH/estradiol Glucose/insulin	FSH/estradiol Prolactin hCG	DHEAS/17OHP Glucose/insulin LH/FSH/estradiol	Prolactin MRI

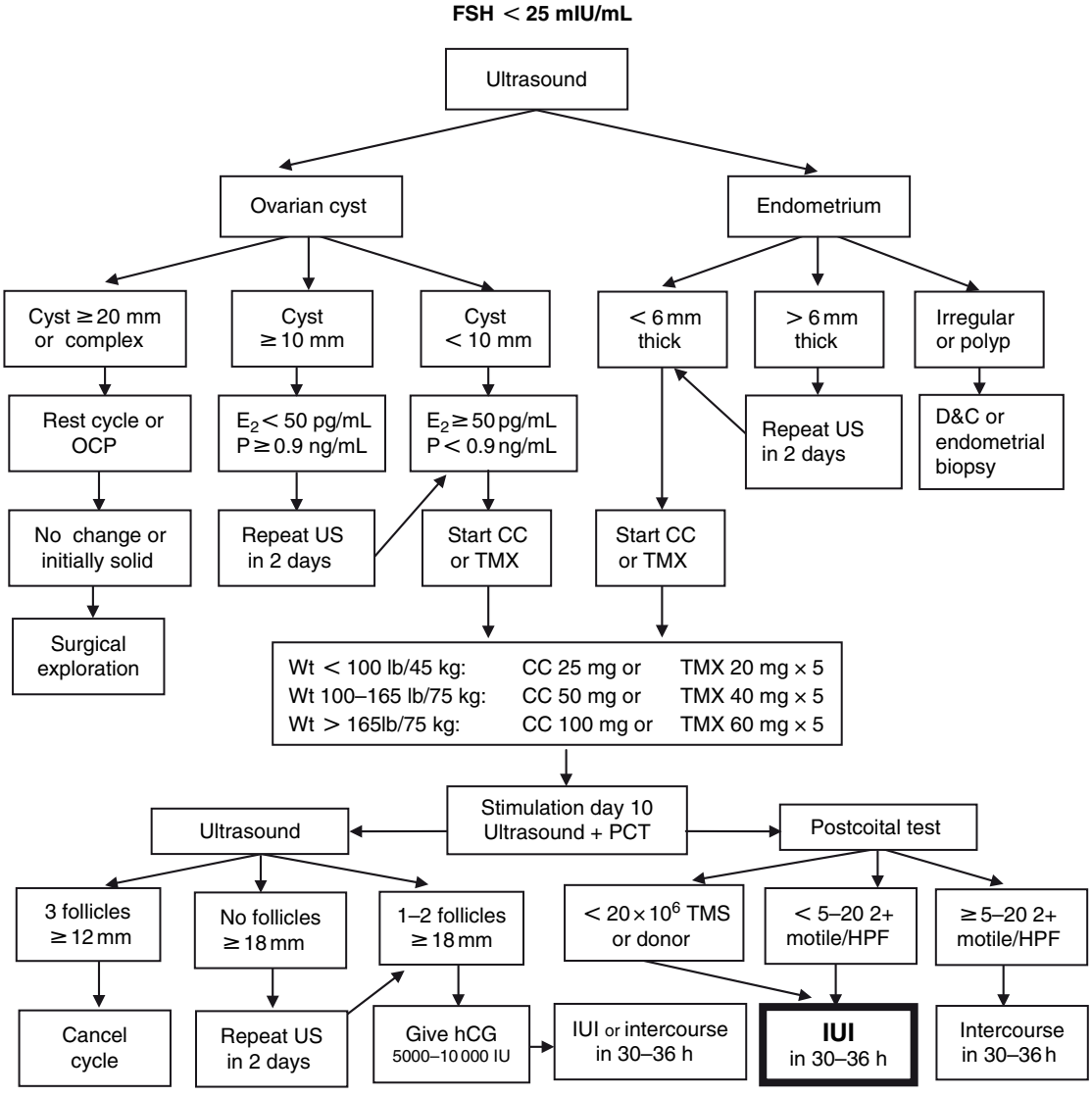


Figure 7.3 Ovulation induction with oral drugs: flowchart showing evaluation and treatment protocols.

and if it does not meet threshold levels for IUI, partners should be referred for treatment (Table 5.2). Ultrasound (US) should be performed to rule out ovarian cysts and endometrial or uterine pathology. Pre-existing ovarian neoplasm, endometriomas, hemorrhagic cysts and corpus luteum (CL) cysts should be ruled out before starting OI lest they be blamed on the use of OI drugs. Ovarian cysts larger than 4 cm should be explored surgically and removed, not drained. Smaller cysts without cancer characteristics (wall thickness > 3 mm or inclusions) may either be followed until they resolve or suppressed with oral contraceptives (OCs). Clear cysts

less than 1 cm can be ignored unless they continue to be present in later cycles.

US should be repeated before starting the second OI cycle when pregnancy does not occur. If no significant cysts are found, US is not necessary before starting OI in succeeding cycles unless the dose of CC or TMX is increased. Development of clear follicular cysts is common in OI cycles and is the result of continued growth of 8–10 mm follicles that have acquired LH receptors but fail to ovulate. Persistent CL cysts are also more common when there has been multiple follicular development.

Vaginal US is preferred, but abdominal US may also be used. If neither is available, bimanual pelvic examination should be performed before starting the first OI cycle and repeated before each subsequent OI cycle. Ovarian cysts less than 2 cm that would be apparent on US may not become detectable by bimanual pelvic examination until the second or third OI cycle.

Clinical management

The basic protocol as used at the Fertility Institute of New Orleans

The basic protocol for OI with CC and TMX is as follows (Fig. 7.3).

Cycle day 3–5 of regular or induced menses:

- Ultrasound is performed to evaluate the endometrium, to determine the number of antral follicles and to rule out ovarian cysts (clear cysts larger than 1 cm, solid or complex cysts of any size).
- Endometrial thickness should be < 6 mm. An overly thick endometrium found on cycle day 3–5 will ordinarily shrink to < 6 mm in 2–4 days. Failure to do so is an indication for further evaluation.
- Antral follicle counts greater than 8–10 per ovary signify increased probability of triplet or higher-order pregnancy and the need to start with a low dose of CC or TMX and repeat US before ovulation.
- If a corpus luteum cyst larger than 1 cm is present, serum progesterone should be measured. Initiating OI when progesterone is > 0.9 ng/mL will result in fewer preovulatory follicles. (For management see advanced protocol.)
- If no significant cysts are present, and endometrium is < 6 mm, a single 50 mg tablet of CC or 20 mg tablet of TMX is taken daily for five days.
- Couples are advised to have sexual relations every other day beginning on the tenth cycle day.
- If menses occur within the usual time frame – cycle day 26–32 – ovulation is assumed to have occurred and the same dose is repeated for two additional cycles.
- If ovulation does not occur and there are no side effects, the dose is increased by one tablet each succeeding cycle to a maximum of three tablets (150 mg CC, 60 mg TMX). All tablets are taken at the same time each day.
- Side effects of CC are hot flashes (11% of patients) and visual symptoms (2% of patients) [16]. Visual symptoms may be blurring or spots and flashes (scintillating scotoma). CC and TMX should be discontinued immediately if visual symptoms occur, but can be restarted at a lower dose in the next cycle.

Use of preovulation US to avoid multiple pregnancy and to time timed intercourse (TI) or IUI

- US is performed 5–7 days after the last tablet of CC or TMX.
- If no more than two follicles are ≥ 12 mm and the lead follicle is at least 18 mm, 5,000–10,000 IU of human chorionic gonadotropin (hCG) or 250 μg recombinant hCG (rhCG) may be given for IUI or timed intercourse (TI) 30–36 hours later.
- If more than two follicles are ≥ 12 mm and age is < 38 years, the cycle is cancelled to avoid triplet or higher-order pregnancy. The couple is warned not to have unprotected sexual relations for five days.

Use of LH testing for TI and IUI

- Patients are instructed to begin LH monitoring on the 10th cycle day using a home LH test kit. When the LH test indicates that ovulation is imminent by a change in color, usually on the 12th or 13th day, the IUI patient notifies the clinic or office and arrangements are made to perform IUI within 24 hours. Alternatively, TI couples should have sexual relations within 24 hours.

Notes regarding the basic protocol

- Whether to start the third or fifth day is based on the length of the patient's untreated cycle, with

the aim of maintaining a minimum of six days between the last pill and ovulation, in order to negate the antiestrogen effect of CC. Thus patients with 28-day cycles are started on the third day and patients with 30-day or longer cycles are started on the fifth day.

- Because TMX does not have an antiestrogen effect on cervical mucus or endometrium, it is not necessary to start as early as CC. TMX can be started as late as the seventh day.
- In patients who are amenorrheic or have a prolonged time between cycles, it may be necessary to induce menses before starting OI. Inducing menses with oral contraceptive (OC) pills or medroxyprogesterone acetate (MPA) is no longer acceptable due to the possibility of fetal masculinization or birth defect if a patient is pregnant. Unmodified progesterone is effective in inducing menses if the endometrial thickness is 6 mm or greater, and will support rather than harm an early pregnancy.
- Progesterone can be administered as a single injection of 50–100 mg in oil, as a vaginal gel or as tablets 90–100 mg 1–3 times daily for 7–14 days, or as 200 mg micronized oral tablets 3–4 times daily for 7–14 days. Menses should occur within 14 days of injection or two days following the last vaginal or oral progesterone.
- The preovulation LH surge normally occurs by the 16th cycle day, or nine days after the last oral pill. If the patient does not detect a change within the expected time, the OI drug may have failed to induce follicular development, follicular development may be delayed but be otherwise satisfactory, or the patient may have failed to detect the LH change. Which of these has happened can be determined by performing a pelvic US and measuring serum LH, estradiol and progesterone. In some cases IUI is still possible, while in others the information will be used to plan treatment in the next cycle.
- When IUI is planned, the basic protocol calls for insemination twice, six and eight days after the last pill. When IUI is timed by LH monitoring or hCG administration, only a single IUI is needed.
- Unless it is a planned IUI cycle, a postcoital test should be performed during the first CC cycle and should be repeated in subsequent cycles if the CC dose is increased. IUI can be advised if the PCT test is abnormal.

The advanced protocol as used at the Fertility Institute of New Orleans

The effectiveness and safety of OI with oral drugs are increased if the starting day is determined by menstrual day 3 estradiol and progesterone levels, if the initial dose is determined by body weight and midluteal progesterone level, and if preovulation follicular and endometrial response are monitored by ultrasound.

Additionally, the day and time of IUI or timed intercourse can be regulated to fit patient and clinic schedules by triggering ovulation with hCG when the preovulatory ultrasound and estradiol level indicate that follicle development is sufficiently advanced.

Use of estradiol and progesterone levels to choose the starting day

CC and TMX require serum estradiol levels ≥ 50 pg/mL to be effective. Ipsilateral follicle development is inhibited when the serum progesterone level is ≥ 0.9 ng/mL. Initiating OI with CC or TMX before these levels are attained will result in no or reduced follicle development. Serum levels usually reach these parameters on the third menstrual cycle day, but may require seven days or longer in patients with PCOS or persistent corpus luteum cysts. Serum estradiol levels normally double every two days, and progesterone levels normally decrease 50% per day during the early follicular phase of the cycle, and do not need to be rechecked unless they would require more than three days to reach the level required to start. Delaying the start of CC or TMX until hormone levels are in the desired range will increase the chance of successful stimulation.

Use of body weight to select the initial dose of CC or TMX

The dose of CC, and by inference TMX, necessary to induce ovulation is proportional to body weight [17,18]. A starting dose of 100 mg CC or 60 mg TMX is recommended for patients who weigh > 165 lb (75 kg). A starting dose of 25 mg CC or 10–20 mg TMX is recommended for women who weigh < 100 lb (45 kg). Other weights should be started on 50 mg CC or 20–40 mg TMX.

Use of mid-luteal progesterone to select the dose of CC or TMX

Progesterone levels in the mid-luteal phase of CC cycles that result in term pregnancies average 37 ng/mL, compared to 22 ng/mL in spontaneous cycles [19]. Progesterone levels in the mid-luteal phase, 5–7 days after ovulation, that are less than 18 ng/mL are evidence of possible luteal insufficiency. Levels less than 15 ng/mL are rarely associated with ongoing pregnancy. When progesterone levels are less than 18 ng/mL following CC, oral or vaginal supplementation (see notes regarding basic protocol) should be considered in the current cycle, and the dose of CC or TMX should be increased in 50 mg and 20 mg increments respectively in subsequent cycles until progesterone levels are ≥ 18 ng/mL.

Effect of increasing the dose of CC or additional days of treatment

Increasing the dose of CC from 50 mg in the first cycle to 100 mg in the next cycle results in minimal increases in average number of small, medium and large follicles (≥ 12 mm from 2.4 to 2.6, ≥ 15 mm from 1.7 to 1.9, ≥ 18 mm from 1.2 to 1.3) [18]. Extending the number of days that 50 mg of CC is taken to 8 or 10 days has been shown to result in ovulation in patients who did not respond to 200 or 250 mg CC for five days in a small series [20]. The benefit of increasing the dose of CC or number of days CC is taken must be balanced against the possibility of increased antiestrogen effect on the endometrium and cervical mucus. The effects of increasing the dose of TMX or extending the length of TMX treatment have not been reported, but they would not be expected to have an adverse effect on endometrium or cervical mucus. When additional numbers of follicles are desired, increasing the dose of CC or TMX will have little effect compared to adding or substituting gonadotropins (see discussion of concurrent and sequential protocols in Chapter 8).

Use of preovulation ultrasound (US) to predict ovulation and multiple pregnancy

Preovulatory US performed 5–7 days after the last CC or TMX allows the ovulation day to be predicted for timed IUI or intercourse, and the number of preovulatory follicles to be assessed in order to cancel cycles if an excessive number of preovulatory follicles is present.

In CC and TMX cycles the lead follicle is usually 18–20 mm in diameter on the day of spontaneous LH surge and 20–24 mm on the day of ovulation. The dominant follicle and others destined to ovulate ordinarily increase at a rate of 2 mm per day from cycle day 10 until ovulation. The size of the leading follicle on cycle day 12–14 can be used to predict when a spontaneous LH surge and ovulation will occur. If predicted to occur at an inconvenient time for performing IUI, ovulation can be induced by hCG (5,000 or 10,000 IU) or rhCG 250 mg if the lead follicle is at least 16 mm and estradiol concentration is consistent with the number of follicles. The serum estradiol level should be 180–250 pg/mL per mature follicle.

The possibility of multiple pregnancy can be estimated from the number of follicles expected to be ≥ 10–12 mm on the day of spontaneous LH surge or hCG injection. This allows sufficient time to proscribe intercourse or cancel IUI if there is risk of triplet and higher-order multiple pregnancy (see Chapter 15) or a desire to avoid a twin pregnancy. The probability of any pregnancy in CC cycles is most closely related to the number of follicles ≥ 15 mm, rather than to smaller or larger sizes [21,22]. Multiple pregnancy rates in CC and TMX cycles are most closely related to the number of follicles ≥ 12 mm on the day of spontaneous LH surge or hCG injection, but follicles as small as 10 mm on the day of hCG can result in pregnancy [21]. Pregnancy rates do not increase appreciably when there are more than four follicles ≥ 15 mm in CC cycles [21].

Use of preovulatory ultrasound (US) to evaluate endometrial receptivity

Preovulatory US enables evaluation of endometrial receptivity by measurement of endometrial thickness and observation of the endometrial pattern. Ideally, endometrial thickness will be ≥ 9 mm, and endometrial pattern will be triple line on the day of LH surge or hCG injection [23]. If the endometrial thickness is < 9 mm on preovulation ultrasound, administration of hCG should be delayed. If the LH surge has already started, vaginal estrogen (2 mg micronized estradiol tablets or the equivalent twice daily), or oral estrogen (2 mg micronized estradiol tablets or the equivalent four times daily), can be used provided that hCG is given to induce ovulation, lest the estrogen suppresses the LH surge. Whether adding estrogen increases endometrial thickness is unproven. Thickness normally increases at a rapid rate in the late proliferative phase of CC cycles as the endometrium escapes the antiestrogen effect of CC, and eventually equals or surpasses thickness in spontaneous cycles [9]. In subsequent cycles endometrial thickness may be improved by using a lower

dose of CC, by switching to TMX, or by taking oral or vaginal estrogen (2 mg daily) concurrently with and following CC.

Use of serum LH monitoring to predict ovulation day

If a baseline LH has been measured at the start of the cycle, a repeat LH measurement on cycle day 12–14 will provide an indication of how soon spontaneous ovulation will occur. Ovulation normally occurs within 24 hours from the time that LH levels are twice the baseline level. A smaller increase above baseline level indicates the beginning of the LH surge and ovulation in 36 hours. A sharp dip in serum LH is often seen one day before the start of LH surge. In PCOS patients, LH levels are often ≥ 20 mIU/mL during the early follicular phase but decrease to < 10 mIU/mL one or two days before ovulation and rise again at the beginning of the LH surge. Repeated LH determinations combined with US and estradiol levels may be needed to determine when the LH surge starts in PCOS patients.

Use of human chorionic gonadotropin (hCG) or recombinant hCG (rhCG)

Use of hCG or rhCG in CC and TMX cycles is seldom necessary to induce ovulation. It is used in CC and TMX cycles to induce ovulation at a time convenient for IUI or TI. Use of hCG or rhCG does not increase the incidence of multiple pregnancies.

Adjunctive treatment

Pretreatment with oral contraceptive

Pretreatment with combination oral contraceptives (OC) the cycle before taking CC significantly increased ovulation rates and pregnancy rates in a systematic review of randomized controlled studies [24]. Multiple pregnancy rates were also increased. Combination OCs containing 30–35 μg estradiol are more effective than combination OCs containing 20 μg estradiol or multiphasic OCs for most patients, but may reduce follicle development in a few patients. Pretreatment with OCs is particularly beneficial in PCOS patients, because they suppress serum and ovarian androgen levels.

Addition of dexamethasone

Adding dexamethasone to CC cycles in patients with or without increased DHEAS concentrations significantly improves ovulation and pregnancy rates compared to CC, according to a systematic review of randomized controlled studies; however, it also increased multiple pregnancy rates [24]. In addition to suppressing adrenal androgen, dexamethasone partially negates the antiestrogen effect of CC on endometrium [25]. Dexamethasone is administered as a single 0.5 mg tablet at bedtime from cycle day 1 until six days after ovulation. At the Fertility Institute of New Orleans dexamethasone is not used routinely in OI cycles, but is added if serum DHEAS levels are ≥ 180 μg/dL.

Addition of metformin

Metformin improved ovulation in women with insulin resistance and hyperandrogenism associated with PCOS who were resistant to CC, according to a systematic review of multiple small prospective randomized studies [26]. However, in a large randomized prospective study of anovulatory infertile women, not previously found to be CC-resistant, metformin alone at a maximum dose of 1,000 mg per day was not effective in inducing ovulation (7% live births) compared to CC (23% live births), or the combination of metformin and CC (27% live births) [27]. Despite conflicting results, the combination of metformin and CC should be tried in patients who are resistant to CC, before switching to gonadotropins, because of its low incidence of multiple pregnancy. The usual dose is 1,000–1,500 mg per day, administered in a single or divided dose with meals.

Treatment results

Pregnancy rates in CC and CC-IUI cycles vary widely, depending on semen source and quality, number of follicles developed during stimulation, reason for treatment, age and how many previous treatment cycles have been performed. In our experience at the Fertility Institute of New Orleans, average pregnancy rates per cycle during four cycles of CC-IUI range from a low 3.2% per cycle for sub-IUI-threshold sperm to 20.4% per cycle for luteal insufficiency, and are 16.5% per cycle when donor sperm is used for IUI (Table 7.1) [22]. Compared to hMG-IUI cycles, pregnancy rates per CC-IUI cycle are 50% lower for ages < 32 and 33% lower for ages 32–38, but equal or higher for ages 38–44 (Fig. 7.4) [21]. The difference in pregnancy rates before age 38 was entirely due to the number of preovulatory follicles in CC-IUI cycles compared to the number of preovulatory follicles in hMG-IUI. On average, 21% of CC cycles were monofollicular, 32% had two follicles and 47% had three or more follicles ≥ 12 mm. By comparison, 8% of gonadotropin cycles were monofollicular, 12% had two follicles and 80% had three or more follicles ≥ 12 mm.

Table 7.1. Relationship of patient characteristics and ovarian response to clinical pregnancy and pregnancy rates in clomiphene citrate (CC)-IUI cycles

	Cycles	Pregnancies (#)	Pregnancies (%)	Odds ratio	*P*-value
Diagnosis[a]					
Ovulatory dysfunction	1075	157	14.6	1.01	ns
Polycystic ovaries	884	118	13.3	–	–
Luteal insufficiency	191	39	20.4	1.67	< 0.02
Endometriosis	1102	89	8.1	0.57	< 0.001
– without tubal involvement	797	63	7.9	0.56	< 0.001
– with tubal involvement	305	26	8.5	0.60	< 0.05
Tubal factor, no endometriosis	279	16	5.7	0.39	< 0.001
Other	354	37	10.4	0.76	ns
Age[b]					
< 30	431	63	14.6	–	–
30–34	604	84	13.9	0.94	ns
35–37	221	26	11.8	0.78	ns
38–42	173	21	12.1	0.81	ns
≥ 43	53	2	3.8	0.29	ns
Semen[c]					
WHO	410	53	12.9	–	–
IUI threshold	527	60	11.4	0.86	ns
Sub-IUI threshold	186	6	3.2	0.22	< 0.001
Donor	492	81	16.5	1.32	ns
Number of follicles > 15 mm[d]					
1	377	37	9.8	–	–
2	286	41	14.3	1.54	ns
≥ 3	215	38	17.7	1.97	< 0.01
Cycle number					
1	1624	168	10.4	–	–
2	887	81	9.1	0.88	ns
3	461	41	8.9	0.84	ns
4	207	18	8.6	0.81	ns
5–6	145	4	2.8	0.24	< 0.005
7–12	57	0	0.0	–	–
Total	3381	313	9.2	–	–

[a] Patients aged ≥ 43 and cycles with total initial motile sperm count < 5×10^6 or motility < 30% excluded.
[b] Patients with endometriosis, tubal impairment and cycles with total initial motile sperm count < 5×10^6 or motility < 30% excluded.
[c] Patients aged ≥ 43, and patients with endometriosis and tubal factor excluded. WHO, initial sperm quality ≥ World Health Organization values for normal sperm of 20×10^6/mL, 40×10^6 total count, 50% progressive motility, 30% normal forms; IUI threshold, initial sperm quality less than WHO criteria but ≥ 5×10^6 total motile sperm and ≥ 30% initial motility [28]; sub-IUI threshold, initial motile sperm < 5×10^6 or motility < 30%.
[d] Patients aged ≥ 43, patients with endometriosis and tubal factor, and cycles with total initial motile sperm count < 5×10^6 or motility < 30% excluded.
Adapted from Dickey *et al*. 2002 [22]. Reproduced with permission of the publisher.

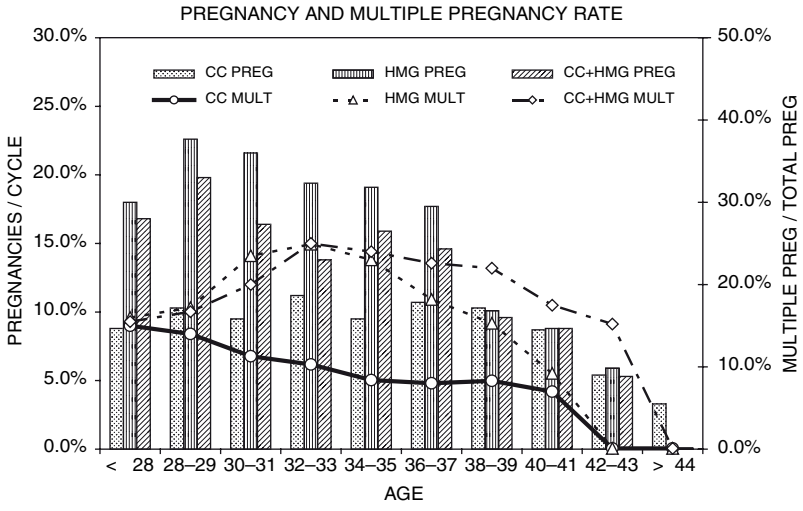


Figure 7.4 Effect of age on pregnancy rate (bars) and multiple implantation rate (lines) per cycle. From Dickey *et al.* 2001 [21]. Reproduced with permission of the authors and the publisher.

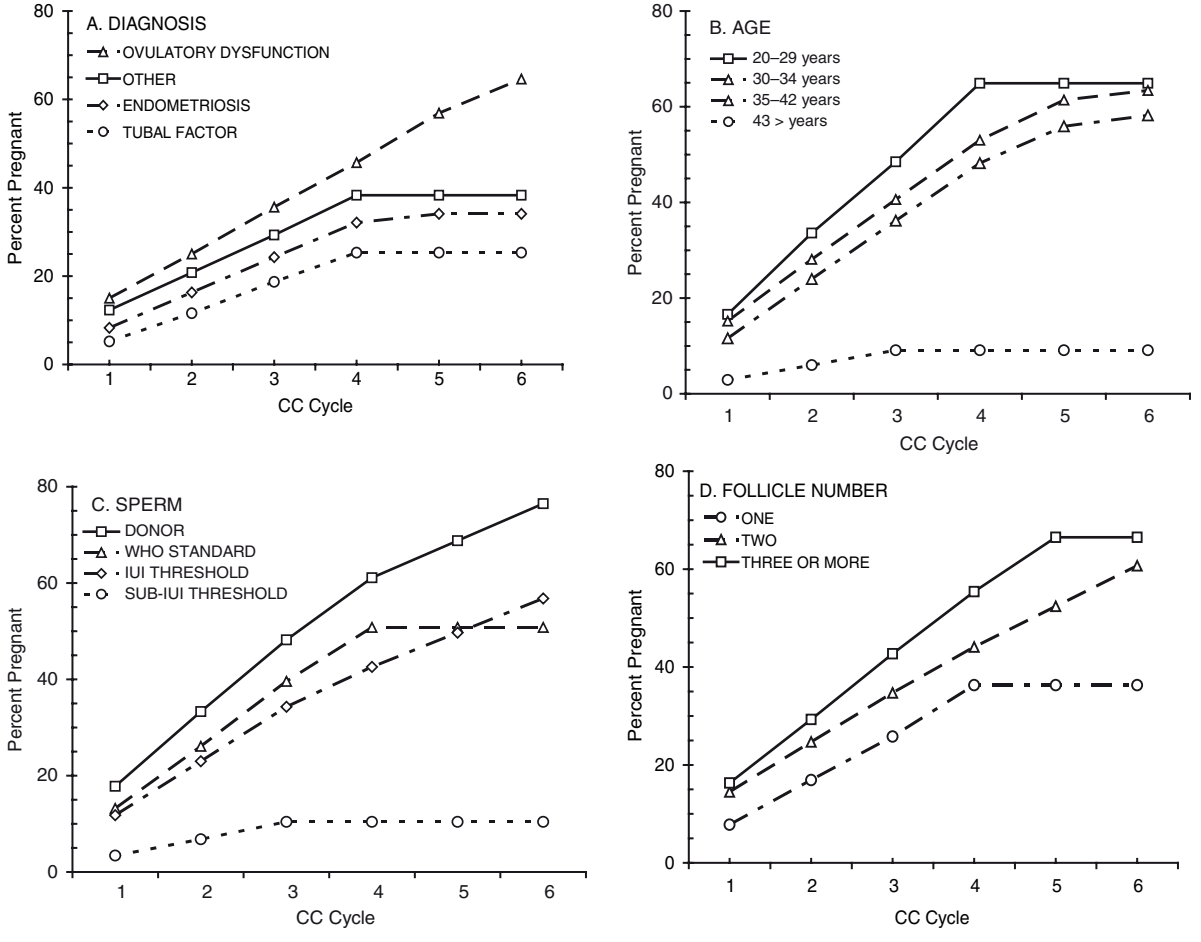


Figure 7.5 Cumulative pregnancy rate, based on: (A) Diagnosis (ovulatory dysfunction = anovulatory, polycystic ovaries or luteal insufficiency; endometriosis = with or without tubal involvement; tubal factor = unilateral tubal obstruction or tubal adhesions without endometriosis; other = cervical factor, male factor or unexplained infertility and normal cycles without endometriosis or tubal factor). Patients aged ≥ 43 and cycles with total initial motile sperm count < 5×10^6 or motility < 30% excluded. (B) Age. Patients with endometriosis, tubal impairment and cycles with total initial motile sperm count < 5×10^6 or motility < 30% excluded. (C) Sperm characteristics (WHO standard = initial sperm quality ≥ World Health Organization values for normal sperm of 20×10^6/mL, 40×10^6 total count, 50% progressive motility, 30% normal forms; IUI threshold = initial sperm quality less than WHO criteria but ≥ 5×10^6 total motile sperm and ≥ 30% initial motility [28]; sub-IUI threshold = initial motile sperm < 5×10^6 or motility < 30%). Patients aged ≥ 43, and patients with endometriosis and tubal factor excluded. (D) Follicle number. Patients aged ≥ 43, patients with endometriosis and tubal factor, and cycles with total initial motile sperm count < 5×10^6 or motility < 30% excluded. From Dickey *et al.* 2002 [22]. Reproduced with permission of the authors and the publisher.

Length of treatment

How long CC or TMX and CC-IUI or TMX-IUI should be continued before evaluating tubal patency, switching to gonadotropin IUI or recommending in-vitro fertilization (IVF) depends on diagnosis, age, sperm quality and the number of preovulatory follicles that develop in response to CC or TMX (Fig. 7.5) [22]. Based on the results from 3,381 CC-IUI cycles, we believe that IUI and CC-IUI should be continued for a minimum of four cycles (three cycles for severe sperm factor or age ≥ 43) before recommending gonadotropins or IVF, and that it can be continued longer than four cycles for some indications if there is a reasonable expectation of success. In agreement with this observation, a 2008 multicenter retrospective cohort analysis of 15,303 cycles of IUI, 51% of which were CC-IUI cycles, with a 5.6% ongoing pregnancy rate per cycle, concluded that there was no rationale for stopping IUI before nine cycles had been completed [29].

Conclusion

OI with CC or TMX, combined with IUI, may achieve improved pregnancy rates over IUI alone. We believe that up to four to six cycles of treatment, depending on the cause of infertility, is well worth trying before resorting to the use of gonadotropins or recommending IVF.

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Chapter 8

Ovulation induction for intrauterine insemination II: gonadotropins and oral drug–gonadotropin combinations

Richard P. Dickey and Peter R. Brinsden

> The guiding principle for treatment of women with anovulatory infertility should be restoration of the feedback system which selects a single follicle for ovulation … Treatment with gonadotropins should be restricted to women who are resistant to clomiphene.
>
> *ESHRE Capri Workshop 2000*

Introduction

Use of gonadotropins for ovulation induction (OI) in intrauterine insemination (IUI) cycles increases pregnancy rates per cycle compared to IUI alone or with clomiphene (CC-IUI) [1,2]; however, whether pregnancy rates per couple are increased is less certain [1]. Gonadotropin use for OI may be complicated by multiple pregnancies, particularly triplets and higher orders, and by ovarian hyperstimulation syndrome (OHSS). Their use as first-line treatment should be limited to those women who have hypopituitary or hypothalamic amenorrhea not correctable by other treatment. Gonadotropins should not be used in the treatment of "unexplained infertility" for patients who ovulate, but fail to conceive, until a minimum of three cycles of CC with IUI have been tried first. Gonadotropins should not be used in place of CC because of its antiestrogen effect on endometrial thickness and cervical mucus before first trying tamoxifen (TMX) 40–60 mg for five days instead of CC. These proscriptions will, if followed, significantly reduce the risk of multiple pregnancy and the risk of OHSS.

The risk of multiple pregnancy is related to the number of preovulatory follicles (more with gonadotropins, fewer with CC and TMX), patient's age and initial cycle of treatment. Young women, aged under 32, who develop 3–6 follicles 10 mm or larger during the first three cycles of gonadotropin IUI have an 18% chance of twins and a 6% chance of triplets. If they develop seven or more follicles they still have a 20% chance of twins but now also have a 20% chance of triplets or more [3,4]. The risk of triplets but not twins is reduced for patients age 38 and older [4], and when a patient has had three or more cycles of CC-IUI and not become pregnant [5]. Women who should not have twins because of health problems, small stature, uterine anomalies, or previous premature birth should not receive gonadotropins. Use of gonadotropins requires frequent ultrasound (US) and hormone determinations with results available the same day. Due to the risk of high-order multiple pregnancy (HOMP) and OHSS, only clinicians who have had special training or extensive experience should employ gonadotropins. Use of gonadotropins for three days following CC, as described later in this chapter, increases pregnancy rates over CC alone and is associated with a low risk of OHSS; however, the risk of twins and HOMP is as high as in longer gonadotropin cycles [6].

Composition of therapeutic gonadotropins

Modern gonadotropin products differ from those available in earlier years, which were urinary-derived mixtures of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), but their use and side effects remain the same. FSH exists in multiple isoforms. In nature, the proportion of specific isoforms in circulation depends on the phase of the menstrual cycle (Table 8.1) [7,8]. Follicular-phase FSH isoforms are more acidic and have long half-lives and high in-vivo bioactivity. Mid-cycle FSH isoforms are more basic, and have shorter half lives and lower in-vivo bioactivity

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Table 8.1. Percentage of FSH with isoelectric point (pI) > 4.25 and percentage of complex, intermediate and simple glycoforms for two recombinant FSH preparations and in the normal cycle

	pI > 4.25 (%)	Glycoforms (%)		
		Simple	Intermediate	Complex
Early follicular phase	36	0	10	90
Late follicular phase	37	0	10	90
Mid cycle	50	1	36	63
Luteal phase	29	0	7	93
Gonal-F (rFSHα)	56	14	77	9
Puregon (rFSHβ)	64	10	79	11

Adapted from Dickey 2004 [8].

Table 8.2. Composition of gonadotropin preparations

Proprietary name	Generic name	FSH activity/mg protein	LH activity/mg protein	Non FSH/LH protein	Manufacturer
Perganol	hMG	~75 IU	75 IU	> 95%	Serono
Humegon	hMG	~75 IU	75 IU	> 95%	Organon
Repronex	hMG	~75 IU	75 IU	> 95%	Ferring
Menopur	hMG HP	na	75 IU	< 0.1%	Ferring
Merional	hMGHP	na	75 IU	< 5%	IBSA
Metrodin	uFSH	~150 IU	< 0.7 IU	< 5%	Serono
Bravelle	uFSH HP	na	≤ 2 IU	< 5%	Ferring
Fostimon	uFSH HP	na	< 0.5 IU	< 5%	IBSA
MetrodinHP/ Fertinex	uFSH HP	~9000 IU	< 0.001 IU	< 1%	Serono
Follistim/ Puregon	rFSHβ	~8500 IU	None	< 0.1%	Organon
Gonal-F	rFSHα	~13600 IU	None	< 0.1%	Serono

na, not available.
Adapted from Dickey 2004 [8].

than follicular-phase isoforms. Postmenopausal FSH isoforms are more acidic than follicular-phase isoforms and have longer half-lives and higher in-vivo bioactivity. Recombinant FSH – rFSHα (Gonal-F) and rFSHβ (Puregon/Follistim) – differ from natural FSH and the urinary gonadotropins human menopausal gonadotropin (hMG) and uFSH in having more intermediate and simple glycoforms, fewer complex glycoforms and fewer sialic acid residues (Table 8.1). Consequently, rFSH products have a more prolonged activity compared to uFSH and hMG in bioactive pharmacokinetic studies [8].

Due to differences in manufacturing procedures, the two recombinant products themselves differ from each other. rFSHβ contains a higher proportion of oxidized FSH (8.9–11.1%) than rFSHα (2.7–7.1%). The specific activity of rFSHβ is lower and more variable (7,200–9,800 IU/mg protein) than that of rFSHα (12,900–14,400 IU/mg protein) (Table 8.2) [9]. However, randomized assessor-blind clinical studies show no significant differences between rFSHα and rFSHβ in efficacy or side effects [10]. Differences in individual responses of patients to exogenous FSH far exceed differences between exogenous FSH products

themselves, rendering such differences as may exist of little consequence.

All hMG products contain human chorionic gonadotropin (hCG) in addition to LH. The relative amount of each is clinically important because the serum half-life of hCG is longer (11 hours fast component, 23 hours slow component) compared to the half-life of LH (0.8–1.2 hours fast component, 11–12 hours slow component). LH is necessary for estradiol production, optimal follicular development, ovulation and luteinization [11]. Serum LH levels less than 0.8–1.2 mIU/mL during OI stimulation are associated with low estradiol levels, poor endometrial quality, and possibly poor oocyte quality. High levels of LH in the late follicular stage may be important for follicular–oocyte–endometrial maturation and for monofollicular ovulation [12]. During preparation of hMG (Repronex, Humegon, Perganol) and preparation of highly purified (HP) hMG (Menopur, Merional) much of the natural LH and hCG activity is lost and must be replaced by adding hCG to bring the LH-to-FSH bioactivity ratio to 1 : 1 [8,13]. The bioactivity of 9 IU hCG is approximately equal to 75 IU LH [13]. In pharmacodynamic studies, rFSH and HP uFSH behave differently from hMG and HP hMG, chiefly because of the absence of LH activity [8].

Pharmacokinetics of natural gonadotropins and therapeutic gonadotropins

Circulating levels of natural gonadotropins represent a balance between pituitary release, tissue binding and metabolic clearance [14]. In humans, natural FSH and LH are synthesized and secreted in response to pulses of gonadotropin-releasing hormone (GnRH) that occur with a frequency of 94, 71 and 216 minutes in the early follicular, late follicular and late luteal phases of the normal cycle respectively. Each GnRH pulse is followed by a 10–15 minute upsurge in serum LH and FSH. The pharmacokinetics of gonadotropins used in OI is different than that of endogenous gonadotropins because they are administered once, or at most twice, a day instead of 15–20 times during a 24-hour period, and because their isoforms do not change according to the phase of the cycle.

Therapeutic gonadotropin serum levels are affected by type of preparation, whether lyophilized powder or in solution, route of administration – whether subcutaneous (SC) or intramuscular (IM), and by body mass index (BMI). Serum levels following injection of 150 IU premixed rFSHβ solution are approximately 18% greater than following injection of 150 IU of dissolved lyophilized rFSHβ [8]. When rFSH is administered SC, the maximal increase over baseline occurs nine hours earlier, and steady state is reached on day 4, one day earlier, compared to IM administration. However, there is no difference in maximum FSH levels [15].

In women with a high BMI, maximum FSH levels and area under the curve (AUC) are significantly decreased, compared to women with a low BMI, following both SC and IM administration [16]. Following SC administration of 225 IU rFSH, the estimated absorption half-life is three times longer (60 hours vs. 20 hours) in women with high BMI [17]. As a result, patients with high BMI take longer to respond to an initial dose of FSH and to a change of dose. During once-daily SC injection of rFSH in GnRH down-regulated patients, the increase in serum FSH per 100 IU of drug, after a steady state of drug level is reached on the third to fifth treatment day, averages 4.8 mIU/mL for patients with a BMI less than 20, compared to 2.1 mIU/mL when the BMI is greater than 35 (Table 8.3). When the BMI is not taken into consideration, patients may be started on doses of FSH or hMG that are either too low or unnecessarily high.

Differences between natural and gonadotropin cycles: threshold hypothesis

In the natural cycle, FSH levels are highest during the early proliferative phase, and decrease in response to the rising level of estrogen produced in developing follicles. Beginning on approximately the seventh cycle day, the largest follicle, usually 8–9 mm, because it has the greatest number of FSH receptors, begins to monopolize the decreasing FSH production and becomes the "dominant" follicle destined to ovulate. The dominant follicle increases in size by approximately 2 mm a day and acquires LH receptors (when it is 8–10 mm), enabling the oocyte to mature and ovulation to occur, while non-dominant follicles, deprived of sufficient FSH to stimulate further development, become atretic. After 5–7 days, when the dominant follicle is 20–24 mm, a surge in LH occurs that results in ovulation approximately 36 hours later. During this time estradiol levels double approximately every two days. Follicle volume exactly parallels estradiol levels and closely parallels

Table 8.3. Relationship of body mass index (BMI) and FSH dose to the increment in serum FSH level on stimulation day 3–5 in GnRH-agonist IVF cycles

FSH dose	100 IU	200 IU	300 IU	400 IU	600 IU
BMI	**Serum FSH on day 3–5 (mIU/mL above starting level)**				
BMI < 20	4.8	9.6	14.4	19.2	28.8
BMI 20–24.9	4.1	8.2	12.3	16.4	24.6
BMI 25–29.9	3.4	6.8	10.2	13.6	20.4
BMI 30–34.9	2.8	5.6	8.4	11.2	16.8
BMI ≥ 35	2.1	4.2	6.3	8.4	12.6

Note: ≥ 7.8 mIU/mL is necessary for monofollicular development, ≥ 11.7 mIU/mL is necessary for multiple follicle development in non-polycystic-ovary patients [18]; R. P. Dickey and R. Pyrzak, unpublished.

inhibin levels. In gonadotropin treatment cycles, a constant dose of FSH is administered, which, if sufficiently high, will enable non-dominant follicles to continue to develop and to acquire LH receptors and ovulate when the LH surge occurs. When gonadotropin administration is discontinued in the late follicular phase, estradiol levels continue to increase for one additional day and the volume of the dominant follicle continues to increase for four days [18].

The threshold hypothesis for FSH proposes that there is a minimum FSH level needed to initiate an ovarian response, and that an increase of 50% above the FSH threshold level induces multiple follicular development [19]. The threshold serum FSH level necessary to induce follicular growth has been found to be 7.8 mIU/mL in WHO Group 1 women and normally cycling women suppressed with GnRH analogues. In WHO Group II women the threshold ranges from 6.8 to 9.8 mIU/mL [20]. The duration and extent to which FSH level is above the threshold level determines the number of follicles that are capable of ovulation [19,20]. According to the threshold theory, constant FSH levels ≥ 11.7 mIU/mL are necessary for multiple follicular development. In a retrospective study of the relationship between serum FSH levels during gonadotropin stimulation and in-vitro fertilization (IVF) outcome, the highest ongoing pregnancy rate per retrieval occurred when steady-state FSH levels were 10.0–15.0 mIU/mL (R. P. Dickey and R. Pyrzak, unpublished). Lower FSH levels were associated with fewer follicles and lower pregnancy rates. Higher FSH levels were associated with lower initial pregnancy rates and higher pregnancy loss rates. Whether the higher pregnancy loss rate was related to use of high doses of FSH per se, or to the reason for using high doses, such as previous poor response, was not determined.

The threshold hypothesis for LH maintains that serum levels of 1.8–2.0 mIU/mL are required for continued steroidogenesis and oocyte maturation, and that if LH exceeds a ceiling level, non-dominant follicles become atretic and development of oocytes may be impaired [21].

Follicle measurement

An important factor in successful ovulation induction is timing the administration of hCG to achieve the final maturation of oocytes. Oocyte maturity is directly related to follicle size. In most gonadotropin IUI programs, hCG is administered when the lead follicle is > 18 mm in diameter. However, hCG can be administered earlier in order to prevent multiple pregnancy, because the majority of follicles 10–12 mm are mature (MII) and able to be fertilized and develop into an eight-cell embryo. In intracytoplasmic sperm injection (ICSI) and IVF cycles, MII oocytes were retrieved from 72% of follicles 13–15 mm in size and from 79% of follicles 16–18 mm, compared to 90% MII oocytes from follicles larger than 18 mm [22]. The fertilization rate and rate of development to the eight-cell stage per mature oocyte was similar (64–72%) for all follicle sizes (Table 10.1).

Preparation for ovulation induction

Before initiating treatment with gonadotropins, it is important to evaluate other medical conditions that could decrease the chance of successful conception, or that might adversely affect mother and fetus – as described in Chapters 3 and 7. When using

gonadotropins for OI, the initial dose should be as low as possible until the ovarian response can be evaluated, in order to avoid multiple pregnancies. Controlled ovarian hyperstimulation (COH), the intentional use of gonadotropins at higher doses and for longer periods to induce multiple follicular development, should be reserved for patients who fail to conceive after three cycles of IUI with low doses of gonadotropins. Gonadotropin stimulation should not be started unless the patient can be monitored with frequent US and serum hormone levels.

Clinical management

Low-dose gonadotropin protocol

The goal of the low-dose protocol is monofollicular ovulation. The basic low-dose gonadotropin protocol for OI is described below.

Cycle day 3–5 of regular or induced menses

- Ultrasound is performed to evaluate the endometrium, to determine the number of antral follicles, and to rule out ovarian cysts (clear cysts larger than 1 cm and solid or complex cysts of any size).
- Corpus luteum cysts larger than 1 cm may be ignored if progesterone levels are < 0.9 ng/mL.
- Endometrial thickness should be < 6 mm. An overly thick endometrium found on cycle days 3–5 will ordinarily shrink to < 6 mm in 2–4 days. Failure to do so may compromise implantation and is an indication for further evaluation.
- Antral follicle counts (AFC) greater than 8–10 per ovary significantly increase the possibility of a triplet or higher-order pregnancy.
- Baseline hormone levels: FSH, LH, estradiol and progesterone are measured.
- Progesterone levels should be < 0.9 ng/mL. Initiating gonadotropins when progesterone is above this level will result in reduced follicle development. (For management see Chapter 7, advanced protocol.)
- If FSH is > 12 mIU/mL, additional gonadotropin stimulation will have little effect. Gonadotropins should not be started. FSH levels should be rechecked every 5–7 days and gonadotropins started when the FSH level falls below 10 mIU/mL.
- If there are no clear cysts > 10 mm, serum progesterone level is < 0.9 ng/mL and FSH is < 12 mIU/mL, gonadotropin stimulation is initiated.
- An initial dose of 37.5–75 IU of lyophilized hMG or uFSH, or of 37.5–50 IU of premixed rFSH in solution, is administered either SC or IM once daily for 5–7 days.
- If the baseline serum LH is < 1.8 mIU/mL, either an hMG product should be used or, if uFSH or rFSH are used, 75 IU rLH, 10 IU uhCG, or 0.3 μg rhCG should be given daily.

Stimulation day (SD) 6–8

- Ovarian response monitoring by measuring estradiol and LH levels starts. Estradiol levels normally double every two days.
- If estradiol is ≥ 250 pg/mL and would be ≥ 2,000 pg/mL when the lead follicle meets criteria for hCG, the dose of gonadotropin is decreased.
- If estradiol has increased two to fivefold above the baseline level and is not expected to reach 2,000 pg/mL before hCG is given, the dose is left unchanged and measurement of estradiol is repeated 2–4 days later.
- If estradiol has not increased after 5–7 days of gonadotropin stimulation, the dose of gonadotropin is increased by 50% and estradiol level repeated again after 5–7 days. This step is repeated until there is evidence of follicular response, or until a maximum dose of 375 IU lyophilized or 300 IU rFSH in solution is reached. Once there is evidence of response, the dose is not increased further. Note that patients with a BMI of 30 or greater may not respond until they are given 300 IU rFSH daily.
- Alternatively, serum FSH can be measured and, if < 7.8 mIU/mL, the dose can be increased by an amount calculated to achieve that level, based on the increase in FSH that has occurred since day 1 as a result of the initial dose.
- When estradiol reaches 250 pg/mL, US monitoring should begin; a dominant follicle ≥ 10 mm may be apparent at this time. Alternatively US monitoring can begin on SD 6–8.
- Cycles should be cancelled at this time if excessive numbers of follicles are present.
- Monitoring with US and estradiol is continued every one or two days until the criteria for administration of hCG are met. The lead follicle normally grows 2 mm per day once it is ≥ 10 mm.

- When one follicle is 18 mm or larger and estradiol is 200 pg/mL per follicle ≥ 14 mm, 5,000–10,000 IU uhCG, or 250 μg rhCG is administered as a single dose 12 to 36 hours after the last gonadotropin dose.
- The time of day the hCG injection is given is not critical, and is chosen so that IUI or timed intercourse (TI) can occur when it is optimal for conception and convenient for the couple and, if IUI is to be performed, the clinician.
- The cycle is cancelled, hCG is not given, IUI is not performed and couples are advised to avoid intercourse or use barrier contraception for five days if:
  (a) two or more follicles are ≥ 10 mm, to prevent twin birth
  (b) three or more follicles are ≥ 10 mm and patient's age is < 38, to prevent triplet and higher-order pregnancy (see Chapter 14).
- Many US practitioners administer luteal support in all gonadotropin cycles. European practitioners tend to administer progesterone for luteal-phase support only when GnRH agonist or GnRH antagonists are used in the treatment cycle.
- Luteal support should be started no earlier than 36 hours after IUI or TI to avoid interference with cervical/uterine/tubal passage of sperm and tubal passage of the oocyte.
- Only pure progesterone should be used. It can be given as a single injection of 50–100 mg in oil, as a vaginal gel or vaginal tablets 90–100 mg 1–2 times daily, or as 200 mg micronized oral tablets 2–4 times daily.
- 14 days after ovulation, a urine pregnancy test is performed by the patient, or a serum quantitative hCG test is performed in the clinic.

Progesterone is continued to 10–12 gestational weeks if the pregnancy test is positive, although many practitioners now stop it at the time of a positive pregnancy test, or when a fetal sac is identified on ultrasound.

Use of the baseline (SD 1) FSH level and BMI to select the FSH dose

Selecting the initial FSH dose according to the baseline FSH level and BMI, and remeasuring FSH levels after 4–5 days to adjust the dose up or down, can reduce the time required to elicit a response and decrease the risk of over- or understimulation.

- The initial FSH dose is chosen based on the FSH level on the day gonadotropins are initiated and the BMI, so that the steady-state (SS) FSH level will be between 7.8 and 11.6 mIU/mL on SD 4 or 5.
- The increment in serum SS FSH levels from SD 1 per 100 IU of rFSH (118 IU of lyophilized FSH) is related to BMI as follows (Table 8.3):
- BMI < 20, FSH increment = 4.8 mIU/mL
- BMI 20–25, FSH increment = 4.1 mIU/mL
- BMI 25–30, FSH increment = 3.4 mIU/mL
- BMI 30–35, FSH increment = 2.8 mIU/mL
- BMI > 35, FSH increment = 2.1 mIU/mL.

Use of the steady-state FSH level to adjust the drug dose on stimulation day 4–5

- The dose of FSH is increased or decreased according to the amount necessary for serum FSH levels to be between 7.8 and 11.6 mIU/mL.
- *Note:* baseline FSH levels decrease as estradiol levels increase, so that more FSH may be needed to keep levels above 7.8 mIU/mL.

Use of US to estimate when the lead follicle will reach the criteria for administration of hCG and risks of multiple follicular development

- US is performed between SD 4 and 6 to determine the number and size of follicles that have responded to FSH since SD 1.
- Follicles should have grown 1 mm/day and estradiol levels should have doubled every two days during this time.
- All follicles that have grown and are ≥ 6 mm on SD 5, or 8 mm on SD 8, will be ≥ 10–12 mm on the day the dominant follicle is 18 mm, and may contain an MII oocyte.
- The dominant follicle is not selected until the lead follicle is 8–10 mm.

Use of frequent serum LH measurements to detect the start of a spontaneous LH surge

A spontaneous LH surge occurs in up to half of gonadotropin cycles. Measuring LH at the start of stimulation and again during the late proliferative phase allows the day of a natural LH surge to be predicated more reliably than by US alone.

- Serum LH is measured on SD 1 and each time E is measured or US is performed
- A doubling of LH over baseline levels signals the start of a spontaneous LH surge.

- Doubling is often preceded one day earlier by a drop in serum LH.
- In polycystic ovary syndrome (PCOS) patients, baseline LH levels may be up to 20 mIU/mL and decrease to less than 10 mIU/mL, before they surge
- hCG should be given, even though a spontaneous LH surge occurs, so as to ensure ovulation occurs and luteal progesterone levels increase [23].

Use of progesterone measurements

Measuring serum progesterone each time estradiol is measured is standard procedure in some clinics. Other clinics measure progesterone only during the final days of follicle growth. Many clinics do not measure progesterone at all. Clinics that measure progesterone believe that elevated levels, greater than 1.2 ng/mL for two or more days before hCG is given, are associated with accelerated endometrial development, poor oocyte quality and low pregnancy rates. The level of LH is unrelated to progesterone levels, but lowering the dose of FSH will result in a decrease in progesterone level. In many clinics, particularly in Europe, where lower doses of gonadotropins are used, stimulated OI and IUI cycles are monitored by US alone, considerably reducing patient inconvenience and cost.

Concurrent low-dose gonadotropin and oral drug protocol

The concurrent protocol is the same as the basic low-dose protocol except that CC 100 mg or TMX 60 mg are started on the same day as FSH or hMG and continued for five days. FSH or hMG are continued until hCG is administered. In some cases, hMG and FSH are started one or two days later than the CC or TMX (overlap protocol). This protocol requires estradiol levels > 50 pg/mL to start, and therefore cannot be used in GnRH-suppressed patients.

Sequential protocol (oral drug followed by gonadotropin)

In the sequential protocol, FSH or hMG is started on cycle day 8–10 after five days of oral CC or TMX. The advantages of the sequential protocol are a lower medicine cost and in most cases the need for only a single US to monitor follicle development.

Cycle day 3–5

- CC 50–100 mg or TMX 20–60 mg are taken for five days.
- The CC/TMX dose depends on weight.
- US should be normal and estradiol level ≥ 50 pg/mL.
- FSH or hMG 75 IU is started on day 8 or 10, after the last CC or TMX, and continued for three days.
- Day 11–13 US is performed and hCG is given if follicle and endometrial criteria are met.
- FSH or hMG may be given for 1–3 additional days until hCG criteria are met.
- Patients are instructed to self-test urine LH daily if hCG is not given, and are instructed to return for repeat US if no LH surge is seen after three days.

The disadvantages of the sequential protocol are that multiple pregnancy rates are as high as for the basic gonadotropin protocol [6]. This protocol effectively rescues non-dominant follicles from atresia. It has no advantage over CC or TMX alone in patients who develop no more than one or two follicles with basic COH.

Adjunctive treatment

The adjunctive use of metformin, dexamethasone and oral contraceptive (OC) pills, described in Chapter 7, also applies to low-dose gonadotropins.

High-dose gonadotropin protocol: controlled ovarian hyperstimulation (COH)

The goal of the high-dose gonadotropin protocol, also called controlled ovarian hyperstimulation (COH), is multiple follicular ovulation. With very few exceptions COH should be used only for IVF and similar procedures, where the number of embryos or oocytes transferred can be limited.

Treatment results

Low-dose gonadotropins

Pregnancy rates in low-dose gonadotropin IUI cycles equal or exceed pregnancy rates in large series of COH-IUI cycles, and have multiple pregnancy rates similar to CC-IUI cycles [23]. In a series of 500 or more patients, totaling 8,370 low-dose gonadotropin IUI cycles, with initial doses of 37.5–75 IU FSH or hMG, clinical pregnancy rates ranged from 10% to 20% per cycle, twin implantations ranged from 6% to

Table 8.4. Relationship of patient characteristics and ovarian response to clinical pregnancy and multiple pregnancy rates in controlled ovarian hyperstimulation IUI cycles 1–3

	Cycles	Pregnancies		2 sacs		≥ 3 sacs		≥ 4 sacs	
	(#)	(#)	(%)	(#)	(%)	(#)	(%)	(#)	(%)
Diagnosis[a]									
Cervical, male, unexplained	553	106	19.2	26	24.5	11	10.4	2	1.9
Ovulatory dysfunction	1068	208	19.5	41	19.5	20	9.5	8	3.8
Endometriosis without tubal involvement	753	121	16.1	26	21.5	6	5.0	2	1.6
Tubal factor, with or without endometriosis	352	36	10.2	5	13.9	1	2.8	0	0
Age[b]									
< 32	1203	129	19.0	46	20.1	24	10.5	10	7.8
32–34	636	120	19.2	28	23.3	7	5.8	1	0.8
35–37	535	86	16.1	19	22.1	6	7.0	1	1.2
38–40	379	49	12.9	5	10.2	0	0.0	0	0.0
41–43	182	11	6.0	1	9.1	0	0.0	0	0.0
Sperm[c]									
WHO	1056	194	18.4	43	22.2	14	7.2	5	2.6
IUI-threshold	971	164	16.9	37	22.6	11	6.7	3	1.8
Sub-IUI threshold	213	19	8.9	4	21.0	0	0.0	0	0.0
Donor	347	77	22.2	13	16.9	12	15.6	4	5.2
Number of follicles >10 mm[d]									
1	204	23	11.3	0	0.0	0	0.0	0	0.0
2	303	40	13.2	9	23.2	0	0.0	0	0.0
3	334	56	16.8	10	17.8	3	5.4	0	0.0
4	304	60	19.7	14	23.3	3	5.0	1	1.7
5–6	377	82	21.8	16	19.5	5	6.1	1	1.2
7–8	264	58	22.0	10	16.9	8	13.6	4	6.9
≥9	366	95	26.0	27	28.4	18	18.9	6	6.3
Estradiol pg/mL[e]									
< 500	538	90	16.7	8	8.9	4	4.4	0	0.0
500–999	798	146	18.3	32	21.9	8	5.5	1	0.7
1000–1499	401	95	23.7	21	23.1	11	11.6	4	4.2
1500–1999	188	43	22.9	9	20.9	6	14.0	4	9.3
≥ 2000	178	45	25.6	14	31.1	8	17.8	3	7.0
Cycle number									
First	2279	373	16.4	70	18.8	27	5.4	7	1.9
Second	1015	143	15.1	25	16.3	6	3.9	5	3.3
Third	431	47	10.9	13	27.6	0	0	0	0
≥ Fourth	302	14	4.6	0	0	0	0	0	0

Table 8.4. *continued*

[a] Patients aged ≥ 38 and cycles with total initial motile sperm count < 5×10^6 or motility < 30% excluded.
[b] Patients with endometriosis with tubal involvement, tubal factor and cycles with total initial motile sperm count < 5×10^6 or motility < 30% excluded.
[c] Patients aged ≥ 38, patients with endometriosis with tubal involvement and patients with tubal factor excluded. WHO, initial sperm quality ≥ World Health Organization values for normal sperm of 20×10^6/mL, 40×10^6 total count, 50% progressive motility, 30% normal forms; IUI threshold, initial sperm quality less than WHO criteria but ≥ 5×10^6 total motile sperm and ≥ 30% initial motility [31]; sub-IUI threshold, initial motile sperm < 5×10^6 or motility < 30%.
[d] Patients aged ≥ 38, patients with endometriosis with tubal involvement, tubal factor and cycles with total initial motile sperm count < 5×10^6 or motility < 30% excluded.
[e] No exclusions.
Adapted from Dickey *et al*. 2005 [4].

15% and triplets from 0% to 1.3% (Table 14.6) [24–29]. Importantly, all of the studies employed protocols in which cycles were cancelled for more than 3–5 preovulatory follicles.

Controlled ovarian hyperstimulation (COH)

In the two largest retrospective COH-IUI studies, consisting of 3,347 cycles in 1,494 infertile couples [30] and 4,067 cycles in 2,272 infertile couples [4], clinical pregnancy rates per cycle were 13.2% and 14.4% respectively, no greater than rates reported in low-dose gonadotropin cycles. Twin rates were doubled and triplet or higher-order pregnancy rates were 15 times greater than in low-dose cycles. In the study shown in Table 8.4, patients with polycystic ovaries were administered 75 IU hMG or FSH, and most other patients received 150 IU. Pregnancy rates and multiple pregnancy rates were related to diagnosis, age, semen quality and source, number of preovulatory follicles, serum estradiol and number of previous cycles. Average pregnancy rates during the first three cycles of treatment ranged from less than 9% per cycle in patients with poor sperm quality, age greater than 40, or endometriosis with tubal involvement, to 18% or more per cycle in patients inseminated with donor sperm or WHO "normal" sperm, aged less than 35, when four or more preovulatory follicles ≥ 10 mm were present and when estradiol was > 500 pg/mL. Twins averaged 18%; triplets and higher orders averaged 4.4%, but were > 17% if there were nine or more preovulatory follicles ≥ 10 mm, or estradiol was ≥ 2,000 pg/mL. No triplet pregnancies occurred after the second cycle. Importantly for users of donor sperm, triplets and quadruplets were doubled compared to WHO and IUI "normal" sperm.

Length of treatment

How many cycles of treatment should be attempted before considering IVF or donor sperm depends on the number of preovulatory follicles, diagnosis, age and sperm quality (Fig. 8.1) [4]. For patients with tubal-factor infertility and very poor sperm quality there is little chance of pregnancy after the second failed cycle; for others, treatment can be continued for six cycles with a reasonable chance of success. Patients who developed nine or more follicles ≥ 10 mm achieved cumulative pregnancy rates of 70% by the third cycle, with no additional clinical pregnancies after the third cycle (Fig. 8.1D). Patients who developed 7–8 follicles and 5–6 follicles also achieved cumulative pregnancy rates of 70% but required four and five cycles respectively to do so, and pregnancy rates per cycle decreased after the third cycle. Patients who developed fewer than five follicles continued to become pregnant through six cycles. Whether to use CC or TMX instead of gonadotropins in poor responders should depend on whether the latter stimulates the development of more follicles. The pregnancy rate is the same per follicle regardless of the stimulation regimen [6](Fig. 14.1). The relationship between follicle number and the number of cycles in which IUI is effective treatment for infertility explained why previous studies, in which follicle number was not considered, have failed to reach a consensus on how many cycles of OI or IUI should be attempted before recommending IVF.

Conclusion

The use of gonadotropins for OI and IUI requires skill and experience in order to achieve the prime aim of follicular stimulation – the development of one or two mature follicles in a controlled and safe way, so as to prevent the two most serious complications of gonadotropin stimulation – high-order multiple births and hyperstimulation. We have presented stimulation strategies in this chapter which we hope will enable colleagues to practice safe and effective OI.

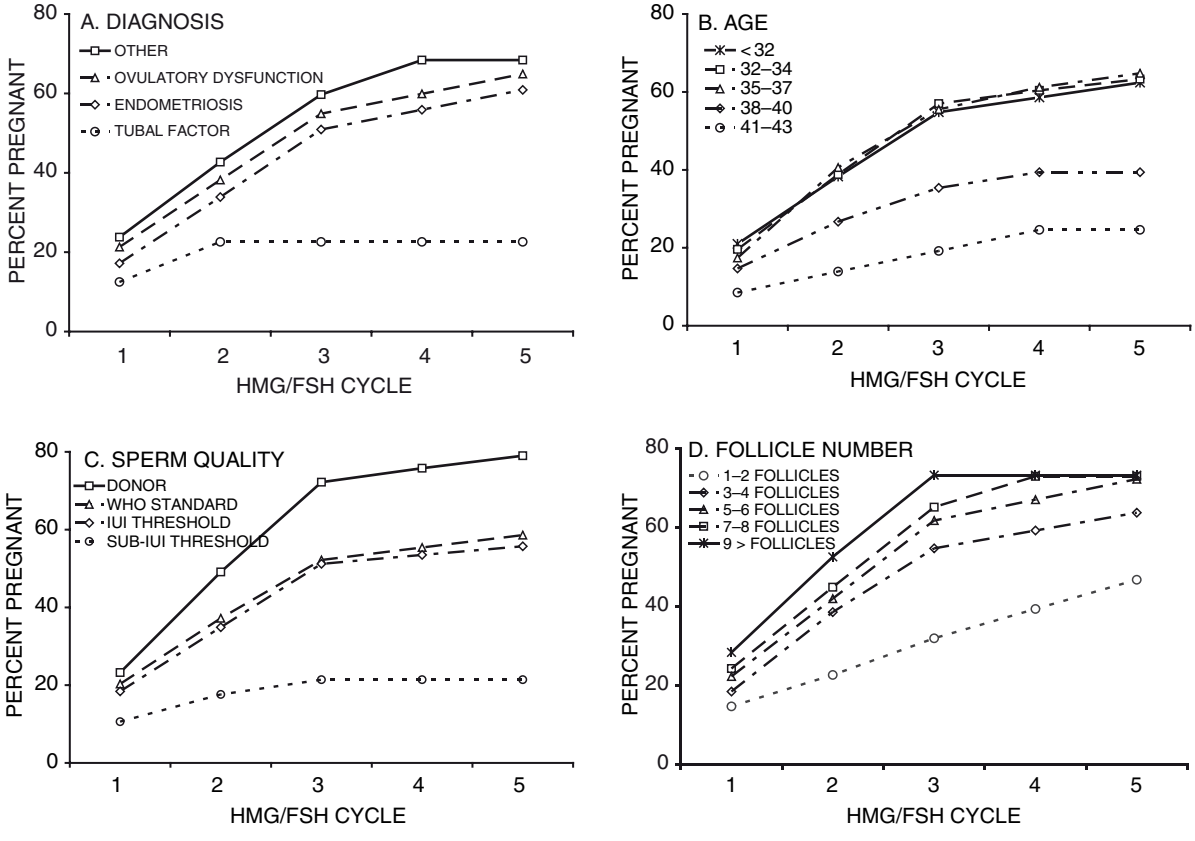

Figure 8.1 Cumulative pregnancy rate, based on: (A) Diagnosis (ovulatory dysfunction = anovulatory, polycystic ovaries, or luteal insufficiency; endometriosis = without tubal involvement; tubal factor = endometriosis with tubal involvement and tubal adhesions or unilateral tubal obstruction; other = cervical factor, male factor or unexplained infertility and normal cycles without endometriosis or tubal factor). Patients aged ≥ 38 and cycles with total initial motile sperm count < 5×10^6 or motility < 30% excluded. (B) Age. Patients with endometriosis, tubal factor and cycles with total initial motile sperm count < 5×10^6 or motility < 30% excluded. (C) Sperm quality (donor = cryopreserved sperm with ≥ 30×10^6 motile count; WHO standard = initial sperm quality ≥ World Health Organization values for normal sperm of 20×10^6/mL, 40×10^6 total count, 50% progressive motility, ≥ 30% normal forms ; IUI threshold = less than WHO standard but ≥ 5×10^6 total motile sperm and ≥ 30% initial motility [31]; sub-IUI threshold = initial motile sperm < 5×10^6 or motility < 30%). Patients aged ≥ 38 and patients with tubal factor excluded. (D) Follicle number. Patients aged ≥ 38, patients with tubal factor and cycles with total initial motile sperm count < 5×10^6 or motility < 30% excluded. Adapted from Dickey *et al.* 2005 [4].

These strategies require the use of low doses of gonadotropins and frequent monitoring of follicle development. As an aid to monitoring follicle development, follicle size and hormone levels (if measured) can be recorded on a "flow sheet" similar to that shown in Form 8.1.

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Ovulation stimulation flow sheet

IUI Partner □ IUI Donor □ Relations □
Physician_____ Patient ID_____ Cycle #____ LMP _____
Patient name _____
D.O.B. ___/___/_____ Age____ HT_____ WT _____ BMI_____
Partner name_____ (or donor #)
Phone (home) _____ (work/cell) _____
G_____ P_____ Ect_____ AB_____ ETOP _____
Patency date_____ R _____ L _____
Diagnosis_____ Allergies _____
Reason for Cx _____
Induction MD_____ Insemination MD_____

Date		LMP				Last OCP date						OCP name					OI drug start day				
Endo. mm																					
Grade																					
Follicle Size RIGHT ovary	1																				
	2																				
	3																				
	4																				
	5																				
	6																				
	7																				
	8																				
Stim. day		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Follicle Size LEFT ovary	1																				
	2																				
	3																				
	4																				
	5																				
	6																				
	7																				
	8																				
Estradiol																					
LH																					
FSH																					
Progesterone																					
OI drug																					
Other meds																					

1st Q hCG ____ D-post procedure____ 2nd QhCG____ D-post procedure____ Gestational sac(s) ____
OUTCOME
Delivered date _____ M__ F__ AB date_____ Ectopic date _____ Elective termination_____

Fertility Institute of New Orleans Form 701:2008

Form 8.1 Ovulation induction form.

Chapter 9

Ultrasonography in the management of ovulation induction and intrauterine insemination

Richard P. Dickey and Sarah C. Romero

Introduction

Ultrasound (US) is an essential part of infertility evaluation of the female, and is indispensable for obtaining optimal results from intrauterine insemination (IUI) and ovulation induction (OI). Use of US for evaluation and management of infertility is of recent origin. The first report of follicle changes throughout a complete menstrual cycle appeared in 1979 [1]. Sonohysterography (SHG), instillation of sonolucent fluid into the uterus in order to improve visualization of the fallopian tubes and soft tissue abnormalities of the uterine cavity, followed in 1984 [2]. Recent developments include the use of three-dimensional US to delineate uterine structural abnormalities, and the adoption of color Doppler blood-flow analysis of uterine and ovarian blood vessels [3].

Transabdominal ultrasound (AUS) is the preferred method for evaluation of pelvic masses larger than 5 cm, and for evaluation of the pregnant uterus after the first trimester. Transabdominal US requires a distended bladder, which is uncomfortable for the patient, particularly when pressure is applied on the lower abdomen during scanning. Accurate delineation of pelvic structures is not possible in all women due to beam scatter by excessive subcutaneous fat and overlying gas-filled bowel loops, or when the ovaries and uterus are located deep in the pelvis. Transabdominal US utilizes frequencies between 2.5 and 3.5 MHz. As frequency is increased, imaging depth decreases and ambiguity occurs because of attenuation. For soft tissue, attenuation in decibels (dB) is approximately 0.5 dB per cm for each MHz of frequency. At a pulse frequency of 5 kHz, ambiguity begins at depths beyond 15 cm. At a pulse frequency of 20 kHz, ambiguity begins at depths beyond 4 cm. AUS is used in IVF to guide catheter introduction during embryo transfer. It can be used for the same purpose in IUI cycles when there is difficulty in introducing the insemination catheter into the endometrial cavity.

Transvaginal ultrasound (TUS) eliminates the inconvenience of having a full bladder and provides better visualization of the ovaries, uterus and fallopian tubes. TUS utilizes frequencies of 5–7.5 MHz; however, structures need to be within 6 cm of the transducer for optimal imaging at these frequencies. Structures larger than 8–9 cm may not be imaged transvaginally, and the use of both scanning routes is often needed for complete evaluation of large pelvic masses. This chapter will describe follicular and endometrial changes in normal and OI cycles. Ovarian and uterine pathologies which are amenable to sonographic diagnosis are also described. Use of Doppler US to evaluate uterine and ovarian blood flow has been described by one of us elsewhere [3]. For a general view of ultrasonography in infertility, and in obstetrics and gynecology, readers are referred to comprehensive textbooks on these subjects by obstetricians, gynecologists, reproductive endocrinologists and radiologists [4,5] .

Measurement technique

When AUS is performed, a water-based ultrasound gel placed on the skin surface acts as an acoustic couplant by obliterating the air interface between the transducer head and abdominal wall. When TUS is performed, the vaginal transducer is covered with a disposable condom to reduce the risk of cross infection, and the ultrasound gel is placed inside the condom tip before pulling it over the transducer. The probe itself is washed with

Manual of Intrauterine Insemination and Ovulation Induction, ed. Richard P. Dickey, Peter R. Brinsden and Roman Pyrzak. Published by Cambridge University Press.

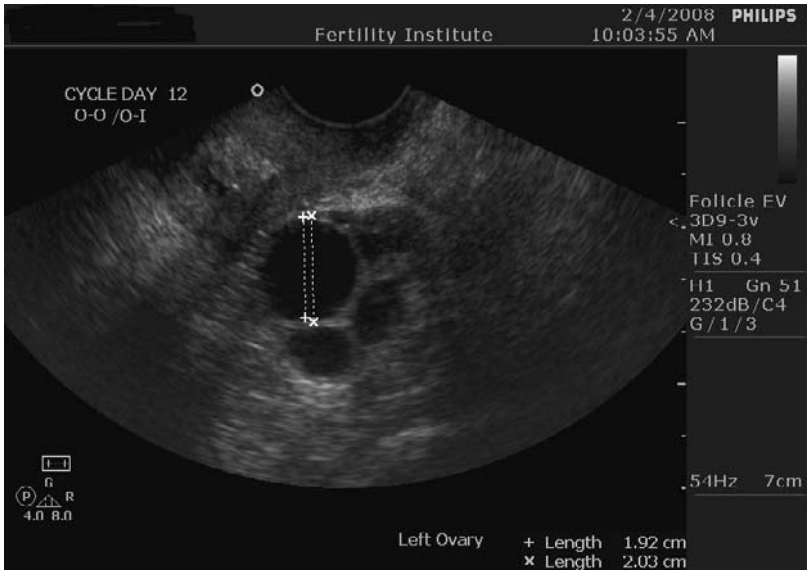

Figure 9.1 Preovulatory follicle. Measurement taken from the outer edge on one side to the inner edge on the opposite side (O-I) and from the outer edge on both sides (O-O).

antiseptic solution and rinsed thoroughly between patients because of the possibility of a condom defect not visible to the naked eye. Latex rather than plastic condoms should be used, because they are less likely to break during use.

The technique for measuring follicle size is not standardized. Measurements may be taken from the outer edge on one side to the inner edge on the opposite side, the most common method, or from the outer edge of both sides (Fig. 9.1). Follicles are rarely spherical, so that the examiner must decide where to take measurements and usually selects two or three positions that seem to most accurately portray the real dimensions. Because the choice of where to measure is subjective, recording three dimensions offers little advantage over recording two dimensions, and is impractical when large numbers of follicles must be measured. Differences in how measurements are taken can account for 1–2 mm differences in recorded follicle size [6].

Endometrial thickness is customarily measured from outside to outside in an anterior–posterior view at the widest point; if measured inside to outside the difference can be as much as 2 mm (Fig. 9.2). The difference in how thickness is measured may explain some of the differences in values deemed critical for successful implantation reported in peer-reviewed publications. In addition to thickness, the endometrial pattern is frequently reported. As initially described in 1984, there were three patterns: type A, a multilayered "triple-line" endometrium consisting of a prominent outer line or layer, a central hyperechogenic line and an inner hypoechogenic or black region; type B, an intermediate isoechogenic pattern, with the same reflectivity as the surrounding myometrium and a non-prominent or absent central echogenic line; type C, an entirely homogeneous endometrium without a central echogenic line [7]. Subsequently, others reversed the ABC pattern [8]. Descriptions of endometrial pattern in the medical literature may follow either classification system. Most present-day authors no longer use ABC classification, but instead use the terms "triple-line" and "homogeneous" to describe the two most common endometrial patterns. A third term, "post-ovulation," is used to describe the bright pattern seen during the mid-luteal phase.

To perform sonohysterography, either a 5.3 French flexible hysterosalpingogram catheter with a 3 mL latex plastic balloon (H/S catheter: Ackrad Co., www.coopersurgical.com), or a pediatric Foley catheter, is filled with 0.9% normal saline and inserted midway into the cervix, which has been cleansed with iodine solution or other antiseptic. The balloon is inflated with water, and the speculum is withdrawn and replaced with the vaginal US transducer. A 30 mL syringe is used to slowly infuse 0.9% normal saline while scanning the uterus, first longitudinally fanning from cornu to cornu and then, turning the transducer 90 degrees in a transverse fashion, from the external cervix to the fundus.

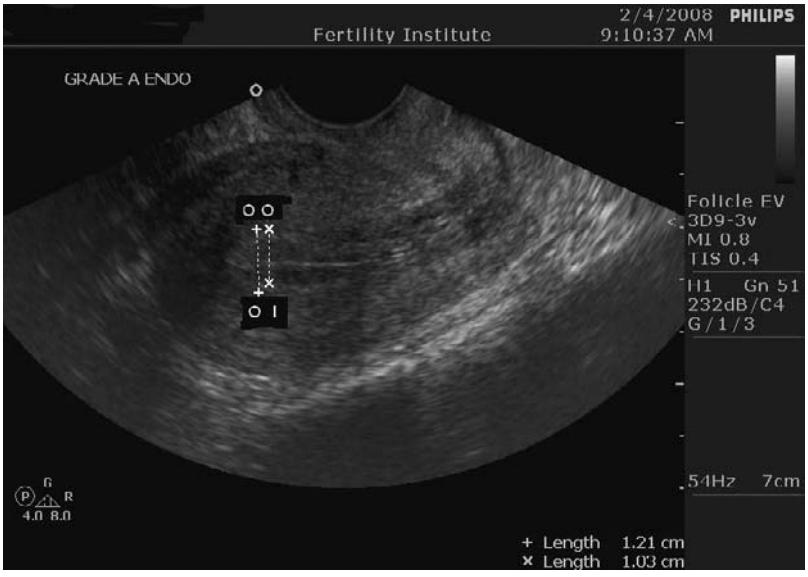

Figure 9.2 Endometrial measurement. Triple-line pattern. Thickness measured in an anterior–posterior view at the widest point from outside to outside (O-O), and from the outer edge on one side to the inner edge on the opposite side (O-I). The difference is 2 mm.

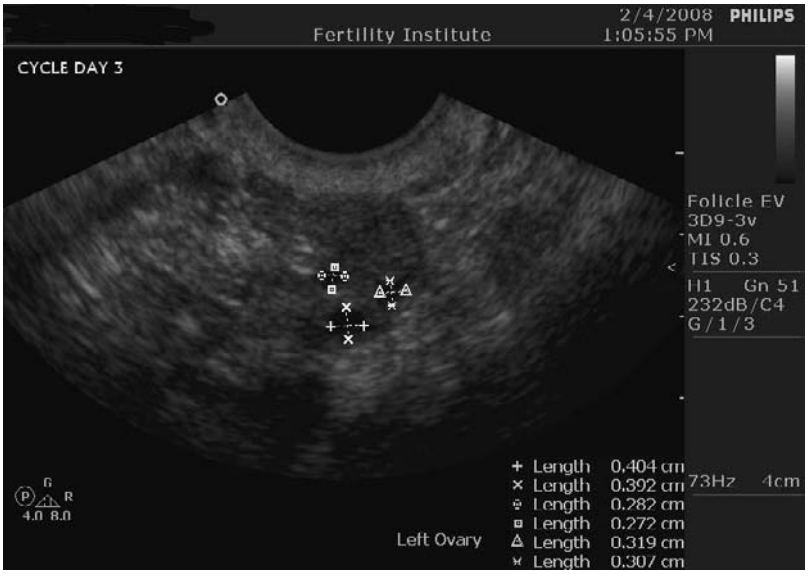

Figure 9.3 Follicular phase day 3. Normal ovary. Antral follicles before day 3 are normally 3–4 mm in diameter, and 4–6 in number.

Follicular and endometrial appearance in the normal and OI cycle

Follicular phase day 3

Ultrasound for OI is ordinarily performed on the third to fifth cycle day, to provide a baseline from which to monitor follicular development, and to rule out the presence of ovarian or endometrial pathology. Antral follicles before day 3 are normally 3–6 mm in diameter and 4–6 per ovary in number (Fig. 9.3). The presence of eight or more follicles in the 3–6 mm range on each ovary, with none larger, signifies the potential development of 10–20 or more preovulatory follicles when gonadotropin stimulation is used. Approximately 25% of women have this type of ovary (occult polycystic ovaries), but without OI will develop only one or two preovulatory follicles due to normal functioning of the ovarian–hypothalamic–pituitary feedback system. The presence of eight or more 6–8 mm follicles in each ovary on day 3 or later in unstimulated cycles is diagnostic

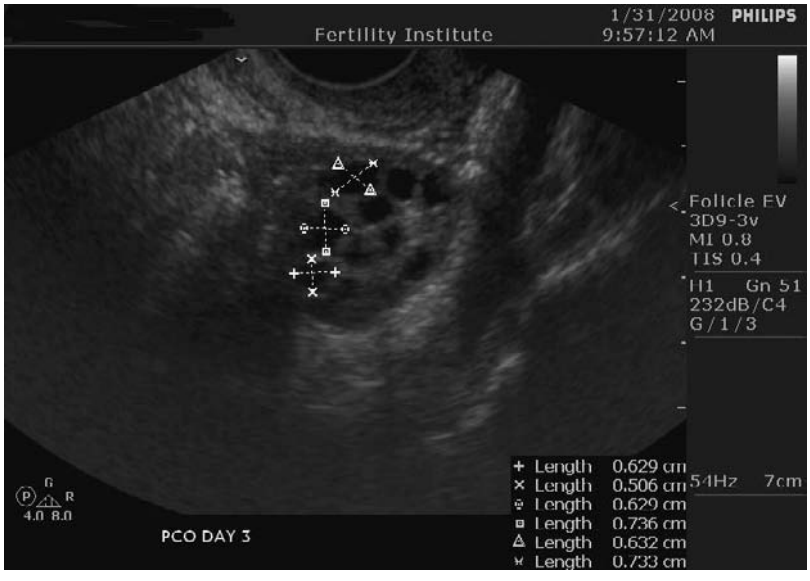

Figure 9.4 Follicular phase day 3. Polycystic ovary. The finding of eight or more 4–7 mm follicles on each ovary on day 3 or later in unstimulated cycles is diagnostic of classical polycystic ovarian disease. If stimulated with gonadotropins or antiestrogens (less often), 7–20 or more preovulatory follicles may develop, placing the patient at significant risk for triplet or higher-order pregnancy.

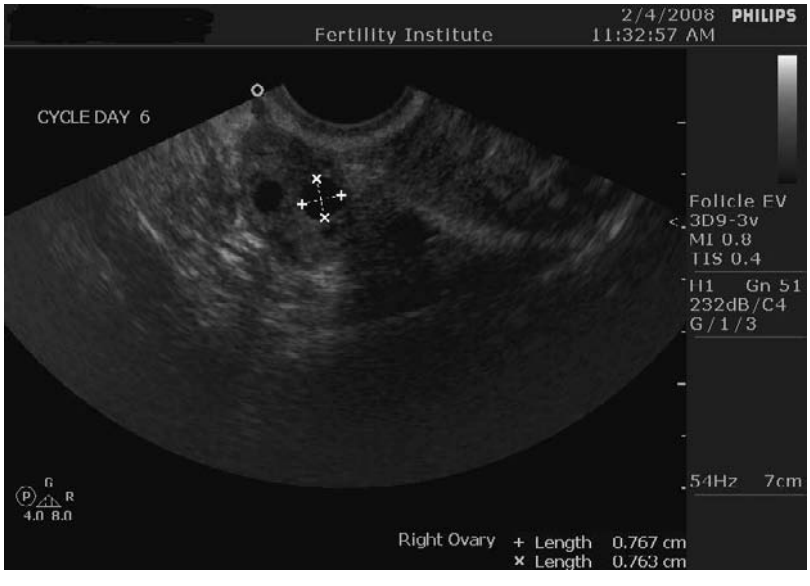

Figure 9.5 Follicular phase day 6. Spontaneous cycle. The lead follicle is 7 mm. The lead follicle normally grows at the rate of 1 mm a day during the first half of the follicular phase of the cycle until it reaches 10 mm, and then grows at a rate of 2 mm per day. Lead follicles may be 7–8 mm by day 6. In gonadotropin cycles, all follicles that are 6 mm or larger on day 6 will be 10 mm or larger and capable of ovulation by day 12–14 when a spontaneous LH surge occurs or hCG is administered for timed ovulation.

of classical polycystic ovarian disease (Fig. 9.4). Other abnormal findings at the beginning of the cycle may include ovarian cysts larger than 10 mm, endometrial hyperplasia and endometrial polyps, described later in this chapter. The endometrial thickness at the completion of menstruation should be less than 6 mm.

Follicular phase day 6: appearance of the dominant follicle

The lead follicle destined to become dominant normally grows at the rate of 1 mm a day during the first half of the follicular phase of the cycle until it reaches 10 mm, and then grows at a rate of 2 mm per day. The lead follicle may be 7–8 mm by day 6 (Fig. 9.5). In gonadotropin OI cycles, US performed on day 6, or after three days of stimulation, will confirm that follicles are developing. As a general rule, all follicles that were previously smaller and are 6 mm or larger on day 6 of gonadotropin OI cycles will be 10 mm or larger by day 12–14, when a spontaneous LH surge occurs or hCG is administered for IUI or timed intercourse (TI). In spontaneous cycles only the lead or dominant follicle will ordinarily continue to develop and ovulate.

Follicular phase day 12: appearance of preovulatory follicle(s) capable of ovulation

By day 12 the dominant follicle should be 16–18 mm or larger and capable of ovulation if an LH surge occurs, or if hCG is administered (Fig. 9.6). In spontaneous cycles further enlargement may occur, with the follicle reaching a size of 22–24 mm immediately before ovulation. An increase of 3 or even 4 mm in 24 hours may occur at this time. In controlled ovarian hyperstimulation (COH) cycles stimulated with hMG or FSH, the follicular size at ovulation is often smaller, ranging from 16 to 20 mm. It is at this time that the decision is made about whether to proceed with IUI, or to withhold IUI and proscribe intercourse for 4–5 days if there is an excessive number of preovulatory follicles. Any follicle that has attained a size of 10 mm or larger may ovulate a mature egg, although most eggs from 10–12 mm follicles will be immature and not ovulate. Follicles which are 8 mm or larger may have acquired FSH receptors and, if they fail to ovulate, may continue to grow and produce estrogen, resulting in ovarian hyperstimulation syndrome (OHSS).

Follicular phase day 12: endometrial pattern

Endometrial thickness and pattern on the day of the spontaneous LH surge or hCG administration are intimately associated with implantation success or failure [8–12]. Both wall-to-wall endometrial thickness and endometrial pattern have been reported to be related to implantation success, but the former may be more important. The endometrial pattern typically changes from an entirely homogeneous hyperechogenic pattern in the first few days of the menstrual period (type C: Fig. 9.7), through an intermediate stage with a thin central line and echogenicity similar to the myometrium (type B: Fig. 9.8), to a triple-line appearance with a clearly demarked center line and echogenicity of the outer lines less than half that of the myometrium (type A: Fig. 9.9) before ovulation. After ovulation, the triple-line pattern becomes obscured by the increasingly hyperechogenic pattern of the post-ovulation luteal-phase endometrium (Fig. 9.10). Implantation does not occur, or occurs at a reduced rate per follicle, if the endometrium lacks a triple-line pattern on the day of hCG administration in COH cycles [12]. Abnormal patterns seen at this time include fluid within the endometrial cavity, which if persistent is incompatible with implantation (Fig. 9.11); fluid collection within the fallopian tube or tubes (Fig. 9.12); and small polyps that were not visible earlier in the cycle. A homogeneous pattern may be an indication of endometrial or uterine pathology. Multiple leiomyomata, synechiae, diethylstilbestrol (DES) anomalies or adenomyosis were found in 94% of IVF patients with a homogeneous endometrial pattern at the end

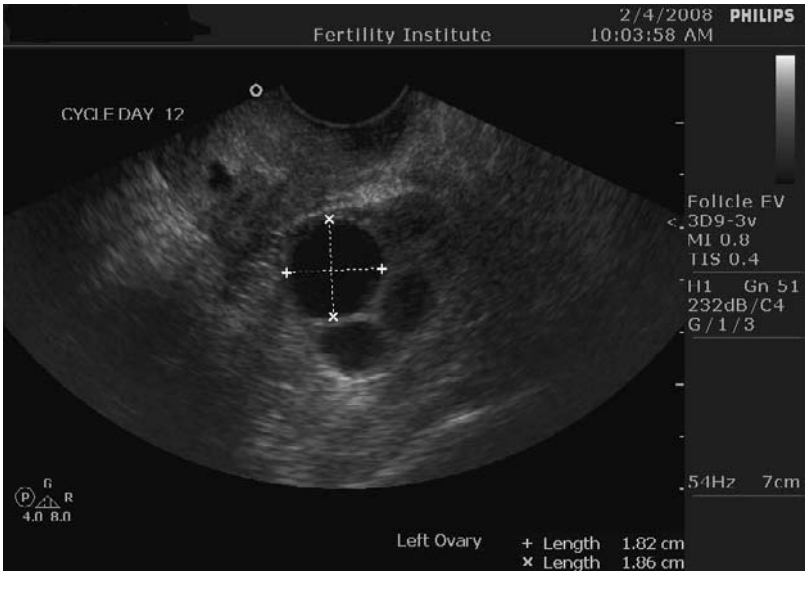

Figure 9.6 Follicular phase day 12. Spontaneous cycle. The dominant follicle is 18 mm and capable of ovulation if hCG is administered. In spontaneous cycles it will continue to grow 2–4 mm a day until a spontaneous LH surge, reaching a size of 22–24 mm immediately before ovulation. In gonadotropin cycles, follicular size may be smaller at the time of LH surge. Any follicle that has attained a size of 10 mm may ovulate a mature egg.

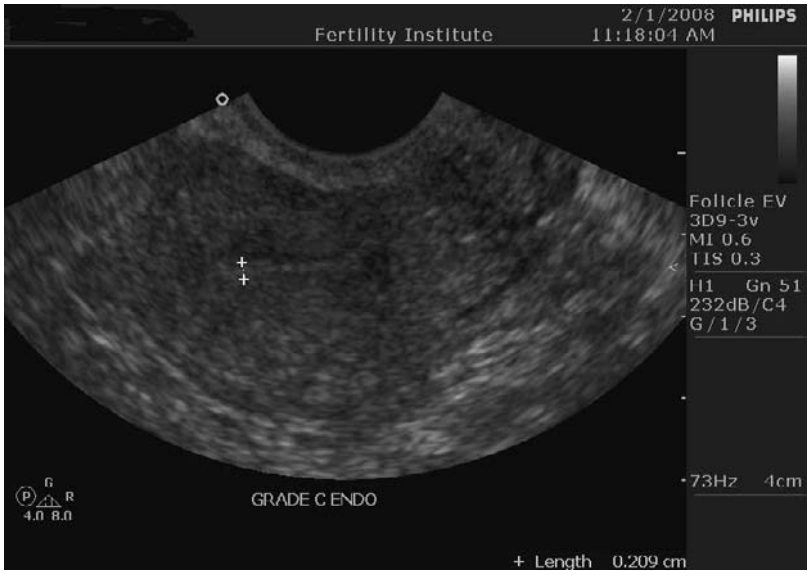

Figure 9.7 Follicular phase day 3. Spontaneous cycle. The endometrial pattern shows an entirely homogeneous hyperechogenic pattern in the first few days of the menstrual period (type C).

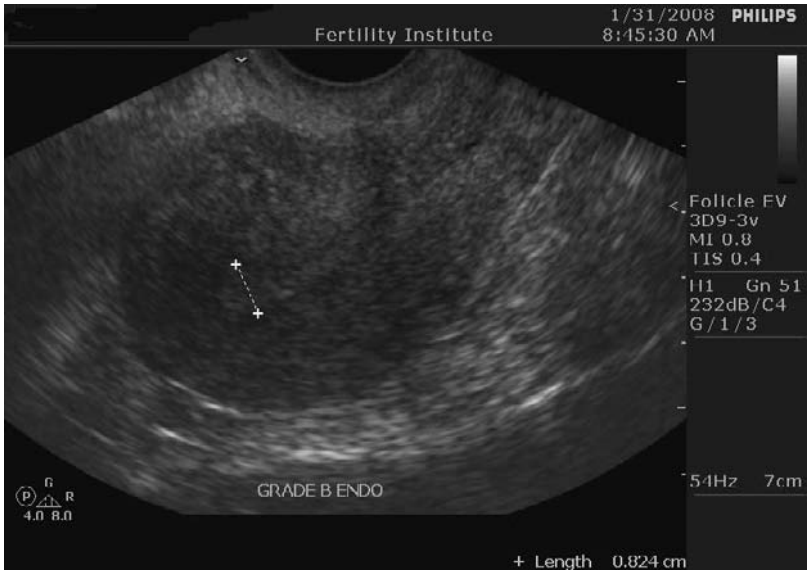

Figure 9.8 Follicular phase day 6–8. Spontaneous cycle. The endometrial pattern is at an intermediate stage with a thin central line and echogenicity similar to the myometrium (type B).

of the proliferative phase, in 30% of patients with triple-line pattern and endometrial thickness < 9 mm, and in 6% of patients with triple-line pattern and thickness ≥ 9 mm [13].

Follicular phase day 12: endometrial thickness

Endometrial thickness measured by TUS correlates well with histological endometrial maturation [14]. In spontaneous cycles endometrial thickness increases from a mean of 4.6 mm during menstruation to 12.4 mm the day of the LH surge [15,16]. The increase in thickness is generally constant, averaging less than 1 mm per day, but it may increase by 2 mm a day in the late proliferative phase. Endometrial thickness from outer wall to outer wall at the widest point ≥ 9 mm on the day of LH surge or hCG injection is associated with a higher pregnancy rate compared to thickness < 9 mm [9,10,13].

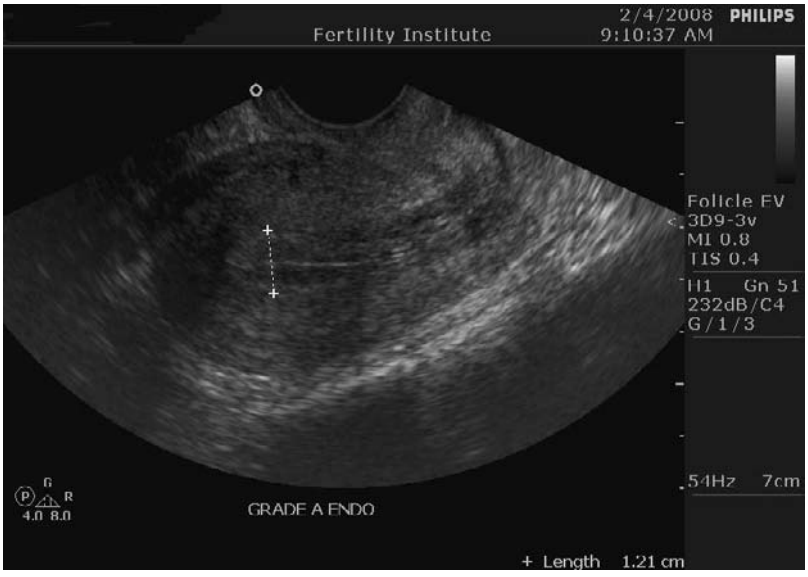

Figure 9.9 Follicular phase day 12. Spontaneous cycle. The endometrial pattern shows a triple-line appearance with a clearly demarked center line and with the echogenicity of the outer lines less than half that of the myometrium (type A) from approximately day 6 before the LH surge until 2–5 days after the LH surge, when the triple-line pattern becomes obscured by the increasingly hyperechogenic pattern of the post-ovulation luteal-phase endometrium. Implantation does not occur, or is reduced, if the endometrium lacks a triple-line pattern on the day of hCG administration in OI cycles for IVF.

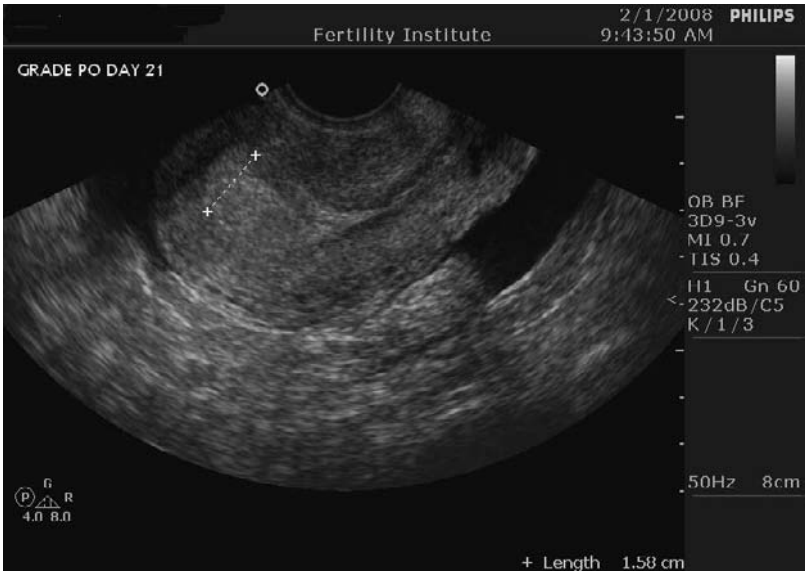

Figure 9.10 Mid-luteal phase day 18–24. Spontaneous cycle. Post-ovulation pattern. The normal endometrial pattern at this time is homogeneous and hyperechogenic.

In spontaneous and OI cycles, implantation rarely occurs when thickness is less than 6 mm (Table 9.1) [9,10,12]. When thickness is 6–8 mm, the incidence of biochemical pregnancies is increased and there is a lower ongoing pregnancy rate than when thickness is ≥ 9 mm [11]. Endometrial thickness < 6 mm is found in 2% of COH cycles and 9% of CC cycles (Table 9.2). Endometrial thickness < 6 mm is also found in 9% of spontaneous ovulatory cycles, where it may be the cause of unexplained infertility. When endometrial thickness is less than 9 mm the deficiency can be corrected in many cases by administration of exogenous estrogen, as described in Chapter 7 .

Luteal phase day 21

Implantation occurs approximately six days after ovulation and seven days after a spontaneous LH surge or hCG injection. The endometrium by this time should

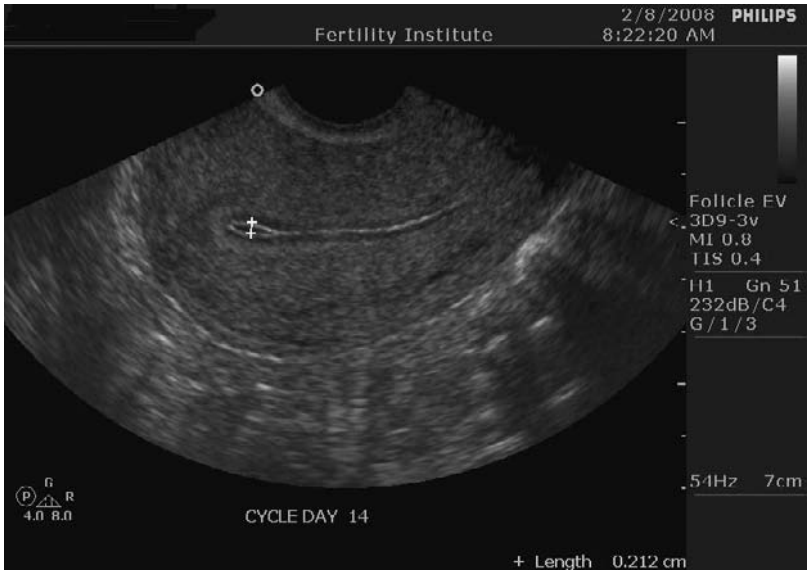

Figure 9.11 Fluid within the endometrial cavity. Gonadotropin cycle. Endometrial cavity with 3 mm of fluid. Fluid in the endometrial cavity on day of embryo transfer in IVF or six days after ovulation is incompatible with implantation.

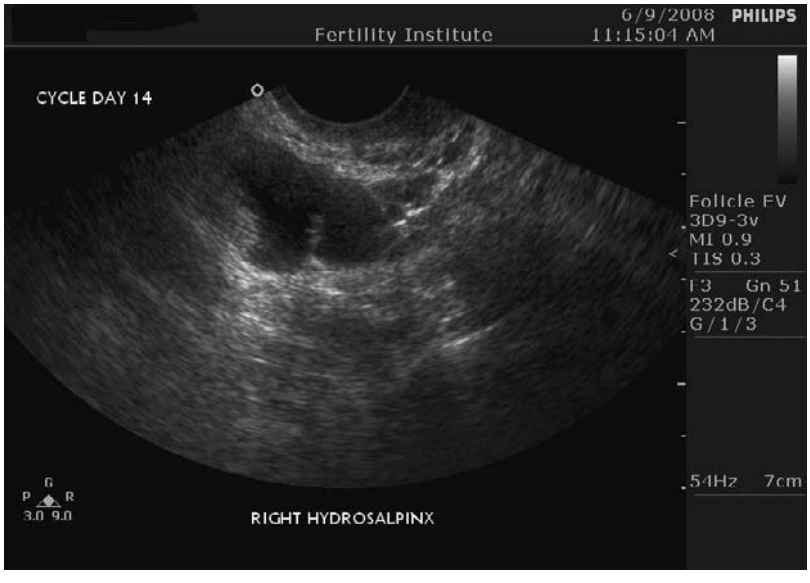

Figure 9.12 Fluid collection within the fallopian tube. Small fluid collection in the fallopian tubes is the probable source of fluid in the endometrial cavity. It is the result of hyperstimulation with gonadotropins, and is to be distinguished from hydrosalpinx due to obstructed fallopian tubes (Fig. 9.20).

show a completely homogeneous hyperechogenic pattern (Fig. 9.10). A mixture of type C post-ovulation pattern and triple-line at the time of implantation 5–6 days after ovulation (Fig. 9.13) is associated with inadequate progesterone (luteal insufficiency) and a lower pregnancy rate. If luteal insufficiency is suspected it can be corrected with administration of exogenous progesterone as described in Chapter 7. Endometrial thickness normally decreases by 0.5 mm the day after the LH surge but then increases an average of 2 mm between ovulation day and 5–6 days later [17]. A decrease in endometrial thickness two days after ovulation, compared to before ovulation, is believed to be detrimental to implantation. Endometrial thickness can be increased by administration of exogenous estrogen even at this late date.

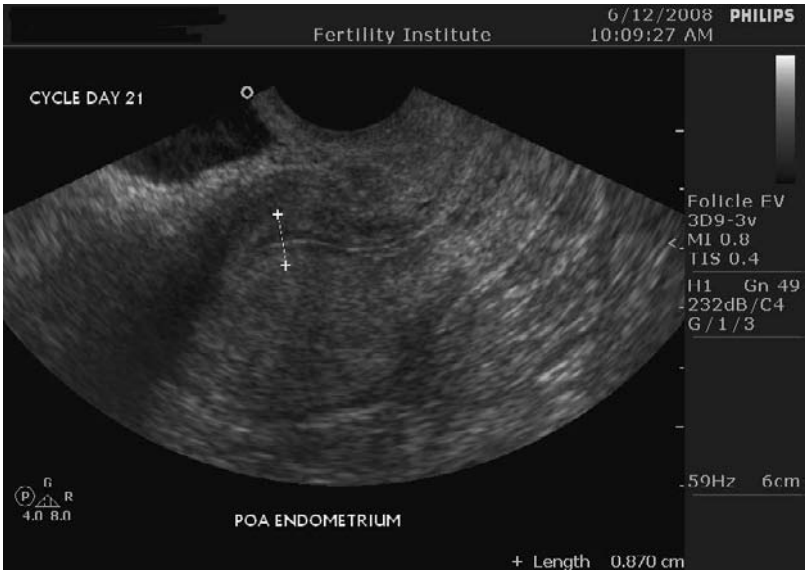

Figure 9.13 A mixture of post-ovulation pattern and type A triple-line pattern (PO/A) 6–7 days after ovulation. The PO/A pattern is believed to be associated with inadequate progesterone (luteal insufficiency) and a low pregnancy rate.

Table 9.1. Preovulation endometrial thickness versus outcome in OI-IUI cycles

Thickness	Percentage of total cycles	Pregnancy rate (%)	Pregnancy outcome (%)		
			Biochemical pregnancy	Clinical miscarriage	Term
< 6 mm	9.1%	0%	0%	0%	0%
6–8 mm	43.6%	8.1%	21.4%	15.4%	62.5%
≥ 9 mm	47.2%	14.0%	0%	12.2%	87.8%

Adapted from Dickey *et al*. 1993 [10,11]. Reproduced with permission of the publisher.

Table 9.2. Preovulation endometrial thickness according to ovulation regimen

Regimen	Cycles	Endometrial thickness		
		< 6 mm	6–8 mm	> 9 mm
None	23	8.7% (2)	56.5% (12)	34.8% (8)
CC	197	9.1% (18)	43.6% (86)	47.2% (93)
hMG	49	2.0% (1)	38.8% (19)	59.2% (29)
CC+hMG	205	11.2% (23)	55.6% (114)	33.2% (68)

CC, clomiphene; hMG, human menopausal gonadotropin; CC+hMG, sequential regimen.
Adapted from Dickey *et al*. 1993 [11]. Reproduced with permission of the publisher.

Pelvic pathology

Ovarian cysts

Clear thin-walled (simple) ovarian cysts (Fig. 9.14) usually represent atretic Graafian follicles that did not ovulate but continued to enlarge, sometimes reaching 3–4 cm in diameter over several months. Hemorrhagic and non-hemorrhagic luteal ovarian cysts (Fig. 9.15) may be confused with endometriomas (Fig. 9.16). They can be distinguished from endometriomas, which have a homogeneous texture, by their heterogeneous nature, and because they are associated with elevated progesterone concentrations as late as the first 3–5 menstrual cycle days. Both types of "functional cyst" occur frequently following OI and do not require treatment, other than a cycle of rest. When they are found on an initial US

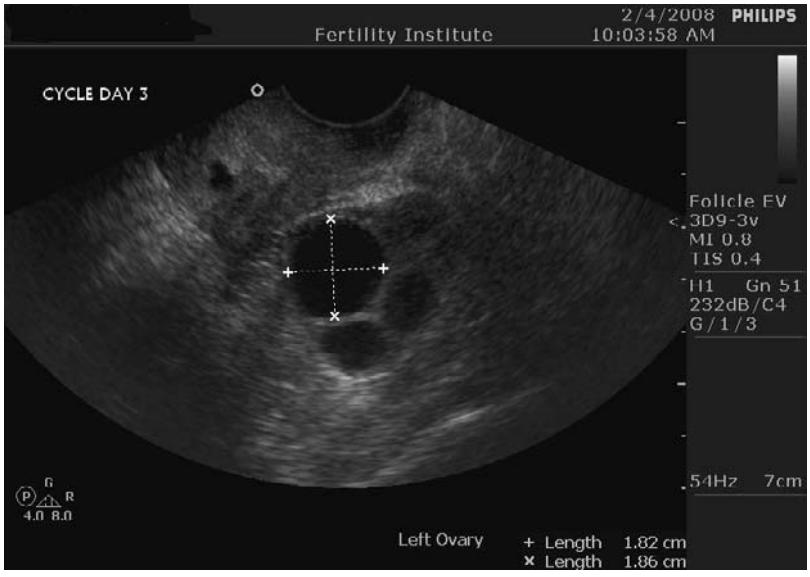

Figure 9.14 A simple ovarian cyst present on a cycle day 3 is distinguished by thin wall and clear fluid center. It may be surrounded by ovarian tissue or be on the surface of the ovary. Several antral follicles are also present.

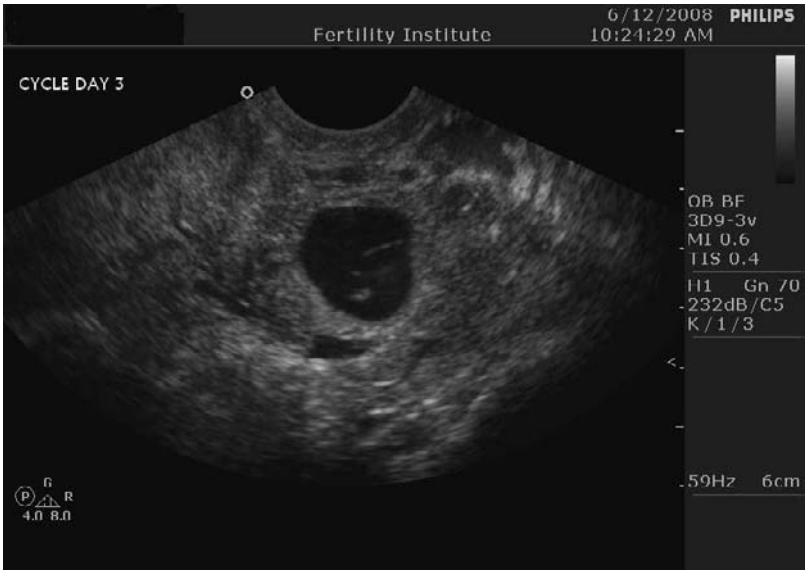

Figure 9.15 Persistent corpus luteum cyst. Hemorrhagic and non-hemorrhagic luteal-phase cysts persisting into the next cycle may have a similar heterogeneous appearance but can be distinguished from each other by measurement of serum progesterone, which will be elevated in the corpus luteum cyst.

they may be treated with oral contraceptive (OC) pills before initiating OI. Ovarian cysts larger than 4 cm should be removed, not drained. Smaller cysts without cancer characteristics (cyst wall ≥ 3 mm or inclusions) may either be followed until they resolve or suppressed with OC pills. Aspiration of single unilateral cysts before superovulation for IVF does not increase the number of preovulatory follicles or the number of oocytes recovered [18].

Functioning hemorrhagic and non-hemorrhagic corpus luteum and simple cysts inhibit follicle development on the ipsilateral side by reason of estrogen and progesterone production. Benign ovarian neoplasms and endometriomas may inhibit follicle development by pressure. In addition, endometriomas may expand and rupture during stimulation. Ovulation induction should not be attempted when endometriomas are larger than 2 cm. When endometriomas are smaller

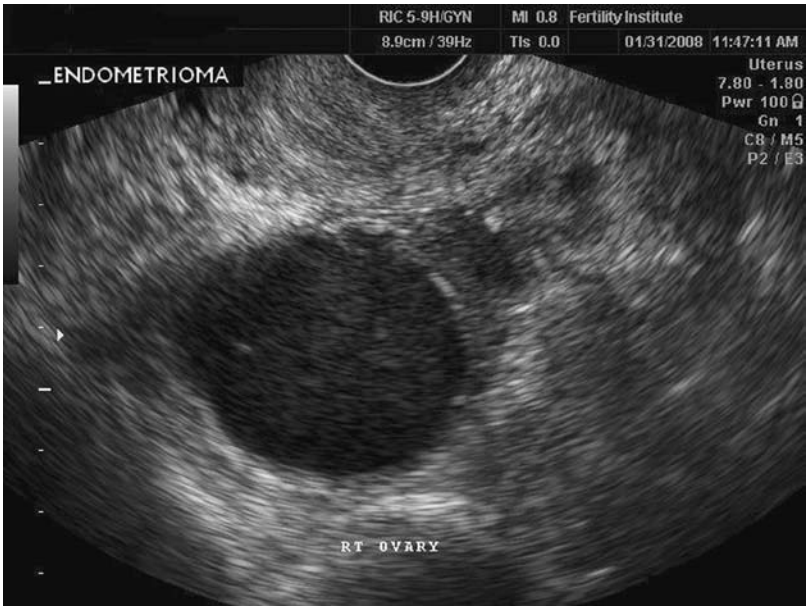

Figure 9.16 Endometrioma. Distinguishable from a persistent hemorrhagic cyst by its homogeneous texture.

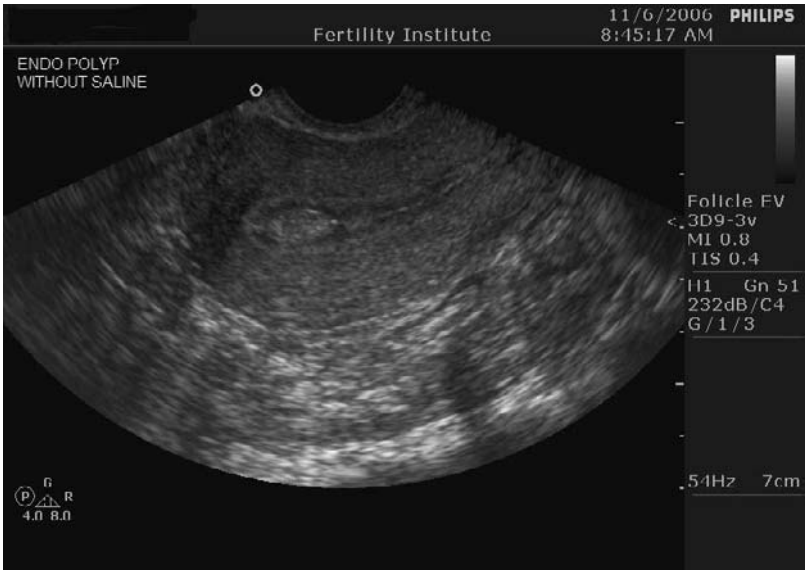

Figure 9.17 Endometrial irregularity which could be either an endometrial polyp or a submucosal fibroid.

than 2 cm, mild OI with oral drugs may be attempted for up to three cycles.

Simple ovarian cysts can be distinguished from periovarian cysts originating from the fallopian tube, nabothian cysts of the cervix and mesothelial cysts by their position. The first two are often similar in size to a developing dominant follicle. Mesothelial cysts are a collection of serous fluid secondary to pelvic adhesions. Mesothelial cysts can become very large but are distinguishable by their position and irregular shape.

Sonohysterography (SHG) for endometrial polyps, endometrial adhesions, submucosal fibroids and hydrosalpinges

An endometrial polyp that appears only as an irregularity on routine US (Fig. 9.17) will be sharply outlined on an SHG scan (Fig. 9.18), and is clearly distinguishable from a submucosal fibroid (Fig. 9.19). Endometrial

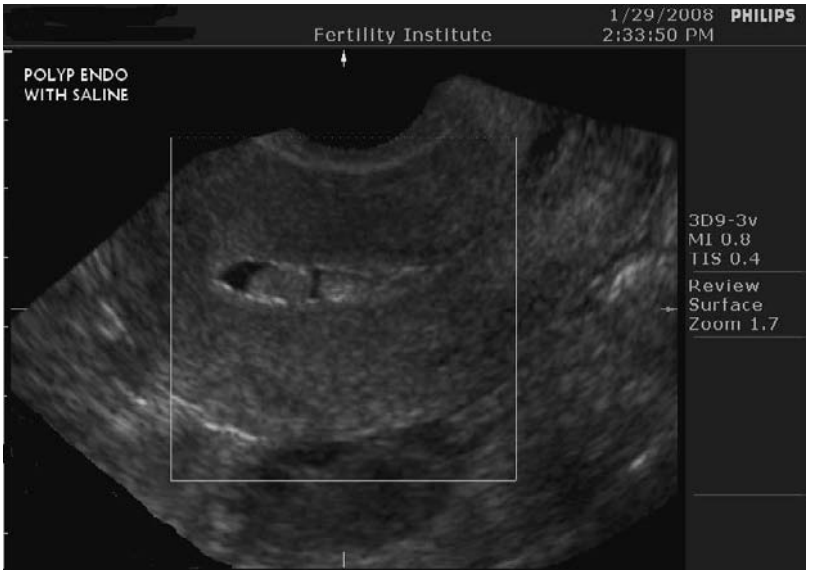

Figure 9.18 The same patient as in Figure 9.17, scanned using sonohysterography (SHG). The endometrial polyp is sharply outlined on the SHG scan, and clearly distinguished from a submucosal fibroid.

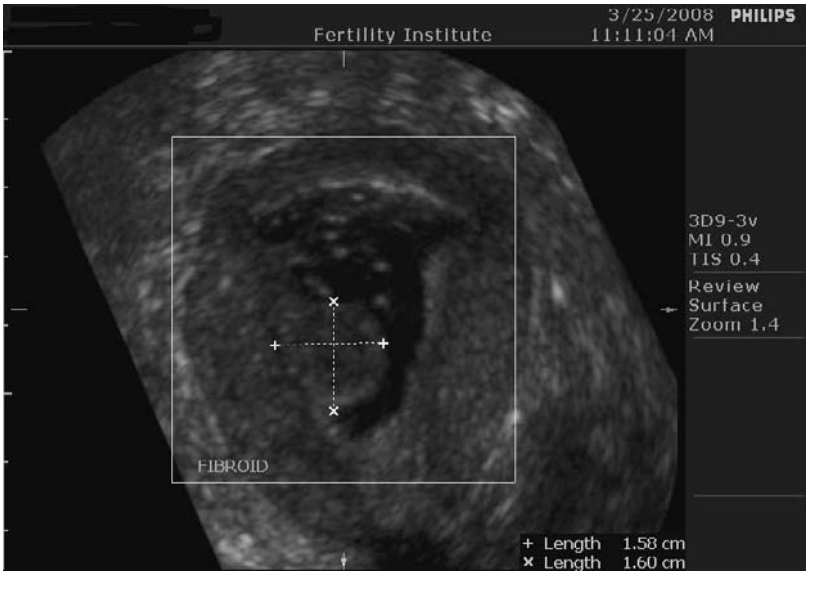

Figure 9.19 Submucosal fibroid seen using sonohysterography .

polyps and submucosal fibroids intruding into the endometrial cavity may impair implantation and development of the embryo and impede passage of the IUI catheter. If a polyp is found it should be removed to improve the chances of pregnancy and to rule out the possibility of malignancy. Endometrial synechiae will be highlighted as dark strips appearing against the bright hyperechogenic saline contrast material. Endometrial synechiae (Asherman's syndrome) may not be apparent on conventional US, but should be suspected if there is marked irregularity of the endometrial lining, accompanied by failure to increase in thickness during OI.

Hydrosalpinges, although best visualized on hysterosalpingogram (HSG), can also be demonstrated on SHG (Fig. 9.20). The possibility of hitherto unsuspected tubal obstruction is immediately raised if more than the customary pressure is required while infusing saline or performing IUI using the tubal perfusion technique. The site of tubal obstruction,

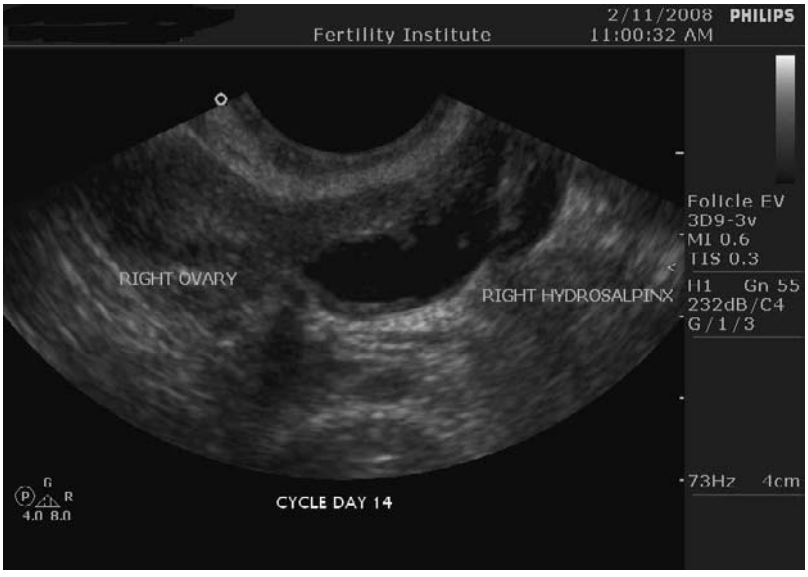

Figure 9.20 Hydrosalpinx. An unsuspected tubal obstruction is confirmed when more than the customary pressure is needed while infusing the saline, and distention of the distal fallopian tubes is observed during sonohysterography performed because of the appearance of the endometrium. The hydrosalpinx appears as a fusiform, often convoluted, anechoic mass tapering towards and enlarging away from the uterus.

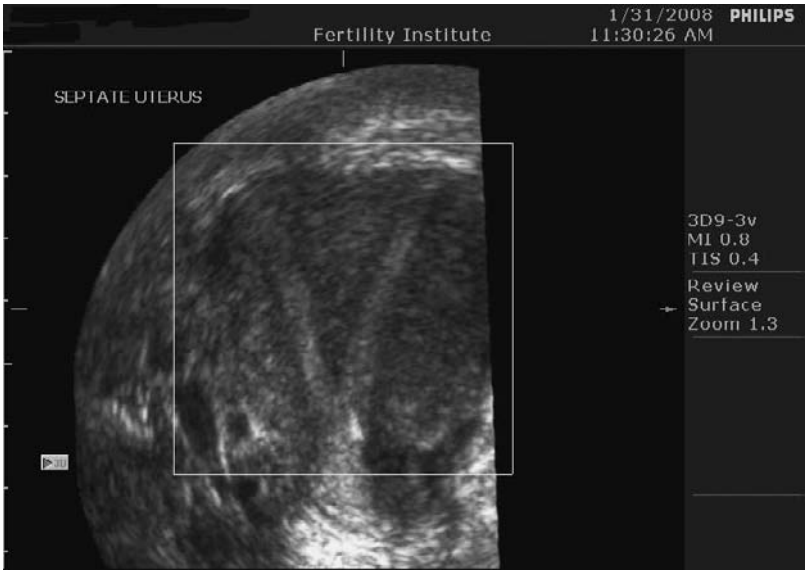

Figure 9.21 Three-dimensional ultrasound image of a uterus didelphys.

whether corneal or distal, will be immediately apparent on SHG. When performing any perfusion of the uterus or tubes, prophylactic antibiotics should be given, and the day of procedure should be selected so that the patient is not menstruating. A customary course of antibiotics for this purpose, if patients are not allergic, is doxycycline 100 mg twice daily beginning before and continuing for four days following the procedure.

Uterine anomalies, uterine leiomyomata: three-dimensional ultrasound

Three-dimensional (3D) ultrasound may provide additional information about the size and position of uterine developmental anomalies and leiomyomata that cannot be obtained with standard AUS and TUS. The size of the septum and uterine wall in a septate or bicornuate uterus is clearly delineated on 3D US,

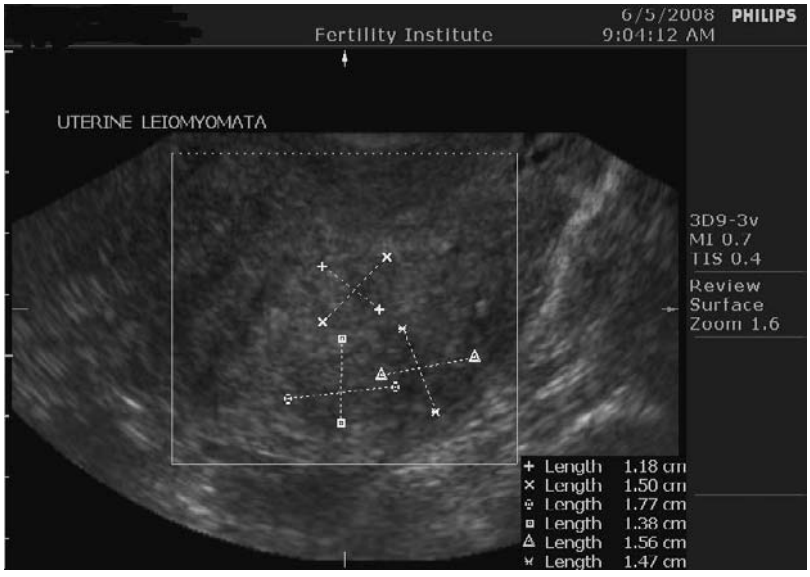

Figure 9.22 Three-dimensional ultrasound image of uterus with multiple leiomyomata, one of which intrudes into the uterine cavity, with another in position to obstruct the cornu.

allowing a decision about whether to attempt hysteroscopic resection or to perform an open metroplasty (Fig. 9.21). Similarly, the size and position of leiomyomata, whether they lie wholly within the myometrium (intramural) where they may obstruct the cornu, or if they intrude into the uterine cavity (submucous), can be clearly determined by a combination of SHG and 3D US (Fig. 9.22).

It is important to detect uterine pathology before initiating IUI and OI. Müllerian duct developmental defects are present in 2–3% of women [19]. Uterine developmental anomalies are associated with poor uterine support and poor placental development, and with increased tendency to miscarriage and premature delivery. Uterine leiomyomata, which occur in more than 20% of women and in all races, do not cause infertility unless by obstruction of the cornu, but are associated with pregnancy loss and prematurity. Uterine anomalies and large uterine leiomyomata are contraindications to multiple pregnancy.

Conclusion

US plays an indispensable part in the pretreatment assessment and in the monitoring of OI and IUI treatment cycles. Experience and skill in US technique, both abdominal and vaginal, are prerequisites to the proper conduct of infertility treatment. Results of follicle, endometrial and uterine measurements are typically reported as shown on Form 9.1, which has space for the large number of follicles that may be present in OI cycles.

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Follicle ultrasound report

Patient information

Date of test:

Patient Name: Patient ID:
Patient DOB: Age: Physician:
LMP Date: Cycle day: Stim day: Ultrasound done at:
Medications: Age: Sonographer:
Type of exam: Report status:

Uterus

Configuration: Size: Longitudinal: mm Internal OS: mm
Texture: AP fundal: mm External OS: mm
Cervical appearance: Cervical length: mm Trans fundus: mm

Endometrium

Thickness: mm Description:
Grade:

Right ovary

Size: mm
Position:

	Follicle size Number	Mean Description
mm	mm	#
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		

Left ovary

Size: mm
Position:

	Follicle size Number	Mean Description
mm	mm	#
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		

Cul-de-sac

Comments _____ _____

_____ _____

_____

Sonographer

Fertility Institute of New Orleans Form 901: 2008

Form 9.1 Follicle ultrasound report.

Chapter 10

Insemination technique and insemination complications

Richard P. Dickey, Peter R. Brinsden and Roman Pyrzak

Introduction

Insemination technique has changed greatly during the past 40 years, from a simple vaginal technique that was little different than that used by John Hunter in 1785 to intracervical (ICI), intrauterine (IUI), fallopian tube sperm perfusion (FSP) and direct intraperitoneal insemination (DIPI) techniques. When Nachtigall *et al*. reviewed the status of artificial insemination in 1979 [1], nearly all inseminations were vaginal and pregnancy rates were generally satisfactory when donor sperm was used or insemination with partner sperm was performed because of physical problems, but very poor when insemination with partner sperm was performed because of low sperm count. The semen specimen was usually fresh for both donor and partner insemination, except in rare cases where multiple samples of poor-quality sperm were cryopreserved in the hope that sufficient "good" sperm would be available when the accumulated specimens were thawed. Those cases were nearly always unsuccessful.

Successful pregnancies following intrauterine insemination of 10 minims (0.6 mL) of fresh unprocessed sperm were reported in 1920 [2], but the procedure was abandoned because of concern about infection. Furthermore, the amount inseminated had to be kept small to reduce uterine cramping, so it was not helpful in cases where the sperm count was low. When insemination techniques were compared in 1957, pregnancy rates for IUI were worse than when the entire sperm specimen was placed in a cervical cup applied to the cervix, or simply deposited in the vagina [3].

In the present day, insemination with both donor and partner semen is nearly always performed by the intrauterine insemination (IUI) technique. However, vaginal insemination is still performed successfully in the office and by couples at home for problems of coital failure, so long as the sperm count and motility are normal and there is no cervical factor. IUI began to be performed more frequently, compared to vaginal insemination, as pregnancy rates began to improve when sperm from specimens with low counts were concentrated and capacitated in vitro using techniques developed for in-vitro fertilization (IVF) [4,5]. Rates further improved when motile sperm were separated from immotile sperm [6]. An important impetus for the change to IUI in donor insemination was introduction of the requirement that sperm sold for anonymous donor insemination (AID) must be cryopreserved and quarantined for six months before it could be used. Cryopreserved sperm is sold in vials or straws containing 10–20 million motile sperm after thawing, considerably less than the number of fresh sperm customarily used for AID in the past. Additionally, cryopreserved sperm is believed to have a shorter life span than fresh sperm.

Indications for IUI

In the past, insemination was limited to donor insemination and treatment of otherwise fertile men with ejaculatory or coital failure. This changed with the advent of IUI. IUI is an effective treatment of cervical-factor infertility and for moderately abnormal male-factor infertility. Fortunately, a high percentage of male-factor infertility falls within the range of moderately abnormal. As a result of being able to achieve pregnancy through IUI and IVF with sperm concentrations that formerly would have necessitated use of donor sperm, donor insemination, which once accounted for 21% of all insemination cycles performed for married couples at the Fertility Institute of New Orleans, now accounts for no more than 7%.

The most successful use of IUI is for cervical-factor infertility, defined as suboptimal or complete absence of motile sperm in cervical mucus extracted

Manual of Intrauterine Insemination and Ovulation Induction, ed. Richard P. Dickey, Peter R. Brinsden and Roman Pyrzak. Published by Cambridge University Press.

from high in the cervical canal 12–24 hours after coitus. Although the diagnostic value of the postcoital test has been questioned recently, much of the controversy is due to defining the parameters used for a normal test. The first author believes that absence of any motile sperm should be considered an absolute indication for IUI, and fewer than 20 motile sperm per high-power field (400 × magnification) should be considered a relative indication for IUI, even though pregnancies may eventually occur without IUI in a small percentage of cases (see Chapter 3, Table 3.6) [7]. In their 1979 review, Nachtigall *et al*. summarized their findings with the statement, "there is neither evidence nor logic to support the use of AIH [husband insemination] in cases where the sperm count, motility and the postcoital test are normal" [1]; this remains true today.

The role of IUI in the treatment of immunological infertility due to antisperm antibodies has yet to be confirmed [8]. Antisperm antibodies have been proposed to inhibit fertility through two mechanisms: inhibition of sperm passage through cervical mucus [9] and obstruction of sperm–zona pellucida binding [10]. Attempts to remove sperm antibodies by repeated sperm washing have met with minimal success [11]. Moreover, IUI has been found to lead to a significant increase in the titer of antisperm antibodies in women with pre-existing antibodies [12].

Sperm requirements for IUI

Many studies reported in the literature only report the number of sperm necessary for pregnancy after processing. We believe that knowing semen quality before processing is important, in order to decide whether to recommend IUI or a more advanced technology. Threshold values before processing for fresh sperm used in IUI necessary to achieve an 8% pregnancy rate per cycle are described in Chapter 5. Briefly, these are 10×10^6 total count, 30% progressive motility, 5×10^6 total motile sperm (TMS) and 5% normal forms (Table 5.2) [13]. Post-processing parameters for an average pregnancy rate of 8% are 4×10^6 total sperm and 50% motility, irrespective of whether sperm was processed by the wash or a more advanced technique. Because the final number of motile sperm is reduced during processing by 5–10% for the wash method, 55–70% for the swim-down and DGC methods and 75–90% for the swim-up method, the effect of processing must be taken into consideration when deciding which method of processing to use and whether referral for IVF with intracytoplasmic sperm injection (ICSI) instead of IUI should be recommended.

Two inseminations on successive days

At one time, two inseminations one day apart may have improved pregnancy rates when there was no accurate method of predicting ovulation. Even then, double insemination was unnecessary when administration of human chorionic gonadotropin (hCG) was used to initiate ovulation. A Cochrane review found no advantage to double insemination over single insemination [14]. However, another study claimed that double insemination may result in increased pregnancy rates in couples with low sperm counts, possibly by increasing the total number of sperm inseminated [15]. Now, with the ready availability of kits that patients can use at home to detect the beginning of the spontaneous luteinizing hormone (LH) surge, and rapid serum LH tests performed in a clinic's laboratory, there is no longer any justification for double insemination. Exceptions will occur where the male partner believes that some of the specimen may have been lost during collection and he is unable to produce a second specimen immediately.

Timing the IUI

Performing insemination at the proper time is the most important factor for success other than the quality of the sperm being used and the number of preovulatory follicles. Sperm may remain viable and capable of fertilizing an oocyte for 4–7 days, but oocytes may remain capable of fertilization for no more than 48 hours, after which zona hardening sets in. When fertilization does not occur within 12 hours the incidence of chromosomal anomalies may be increased. The optimal time for insemination in IVF cycles has been found to be more than four hours after oocyte retrieval, to allow time for completion of the first meiotic division, and less than eight hours after oocyte retrieval. Therefore, insemination with fresh unwashed sperm should be performed 6–8 hours before ovulation to allow time for capacitation, and insemination with washed sperm that have undergone capacitation should be no sooner than four hours after ovulation. Because of their shorter period of viability after thaw, cryopreserved sperm should be inseminated as close to ovulation as possible.

The approximate time that ovulation occurs following a spontaneous LH surge and following an injection of hCG are different and now known. Ovulation usually occurs 36 hours after the start and 24 hours

after the peak of the LH surge. After the administration of hCG, ovulation typically occurs between 30 and 40 hours later. Urine home LH detection kits typically detect a urine LH level equivalent to a serum LH concentration of 20–25 mIU, which is near the peak of the preovulatory LH surge. Some patients with polycystic ovary syndrome (PCOS) and premenopausal patients have chronic serum LH concentrations near these levels, and therefore they may be subject to false-positive test results. Invariably, if ovulation is going to occur in such patients, the LH level drops below 20 miU a day or two before rising again, signaling the start of the true ovulatory surge.

The simplest method of insemination timing is to have the patient monitor her LH with any of the LH kits available in pharmacies or by email, starting on the 10th cycle day. Patients with normal cycles of 30 or 32 days, instead of 28 days, would start testing two and four days later. Patients are told to perform the test on the first morning urine, which is usually the most concentrated one of the day, and to come to the clinic the same day to have IUI, after having informed the clinic and obtained an appointment. Patients who suspect that their ovulation is imminent may test themselves the night before so as to plan the next day's activities. In no case should IUI be delayed for more than 24 hours once an LH surge is detected on a home test kit. There are some differences in the sensitivity and accuracy of the different LH kits, and physicians will want to familiarize themselves with those that are available and select one kit for all patients to use.

hCG timed insemination

Administration of hCG to induce and to time ovulation does not increase pregnancy rates over LH monitoring in natural and oral ovulation induction (OI) drug cycles [16]. However home LH monitoring can be combined with office ultrasound (US) and hCG injection to avoid performing inseminations on weekends or holidays. For example, if ovulation is expected on Saturday, US can be performed on Wednesday and if one or more follicles are 16 mm or larger, hCG 10,000 IU can be given Wednesday night for insemination Friday morning, or given Thursday morning for insemination Friday afternoon to allow an extra day of development. This method can be used both by patients undergoing natural cycles and patients taking oral OI drugs. Use of US, sometimes supplemented by LH to time hCG administration, is mandatory when low-dose gonadotropins or controlled ovarian hyperstimulation (COH) are used. A doubling of the cycle day 3–5 LH concentration indicates that an LH surge has started; in these cases hCG can also be given immediately as a boost to ovulation. If doubling has not occurred, hCG is given for timed IUI, providing at least one and no more than two preovulatory follicles are present on US. Traditionally follicles are believed to contain a mature oocyte when they are 20–24 mm. Because follicle size increases by 2–4 mm after the start of the LH surge, this means that hCG should be given when the largest follicle is 16–20 mm. However, experience with ovum retrieval for IVF has shown that some follicles 18 mm and larger are empty (do not contain oocytes) and some 10 mm follicles contain a mature oocyte. Giving hCG too soon may result in immature oocytes and waiting too long increases the risk of multiple ovulation. Table 10.1, developed for IVF, is a useful tool for estimating the probability that a follicle contains an oocyte capable of fertilization in IUI cycles [17].

Insemination methods

Before insemination, the name on the specimen and the name of the patient being treated must be checked by the physician, the patient and her partner, if present, and by at least one other witness in the physician's or clinic's employ. This is done by showing the test tube or straw to the patient. The names of all those who verified the specimen's identity should be recorded in the patient's chart. If electronic records are used, there should be a paper record of the names.

At the Fertility Institute of New Orleans, insemination is performed in an ordinary gynecologic examination room with clean, not sterile technique. The patient is draped as for a vaginal examination. The physician or nurse performing the procedure is attired in the laboratory coat or other clothing ordinarily worn when performing a gynecologic examination, without cap or mask. Latex gloves are worn and universal infectious disease precautions are followed.

Vaginal insemination (IVI): suitable for high-quality sperm, does not require washed semen

In a randomized comparison of insemination with cryopreserved donor sperm, deposition of sperm into a cervical cap resulted in a pregnancy rate of 15% per cycle, compared to 6% for intracervical insemination (ICI) [18].

Table 10.1. Oocyte maturity for different follicle sizes: percentage of oocytes that are mature (MII) and able to be fertilized and develop into an 8-cell embryo

Follicle size (*n*)	< 10 mm (63)	10–12 mm (100)	13–15 mm (236)	16–18 mm (367)	> 18 mm (890)
MII oocyte	47.6%	53.0%	72.9%	78.7%	89.9%
Immature	52.4%	47.0%	27.1%	21.3%	9.1%

Adapted from Rosen *et al*. 2008 [17].

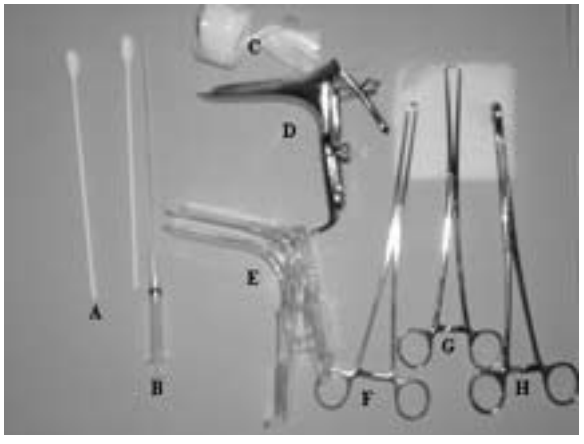

Figure 10.1 Intrauterine insemination tray: (A) cotton swabs; (B) IUI catheter and 3 mL syringe; (C) sponge in plastic wrap (Fertility Pack®); (D) metal bivalve speculum; (E) disposable bivalve speculum; (F) Allis forceps; (G) tenaculum (vulsellum); (H) long-handled ring forceps.

Equipment needed

Bivalve speculum, 3–5 mL syringe, cervical cup (optional), plastic-wrapped sponge (Fertility Pack, Milex Products Inc., Chicago, IL, USA; optional) (Fig. 10.1).

Procedure

A clean bivalve speculum lubricated with warm tap water or saline is inserted in the vagina, exposing the cervix. The specimen is drawn into the syringe and injected high into the posterior fornix of the vagina. The patient remains in a supine position for 15 minutes (if the uterus and cervix are retroflexed the patient lies prone instead). A plastic-wrapped sponge may be inserted to hold the specimen near the cervix before removing the speculum. If inserted it should be removed in 1–2 hours by pulling the attached string. Alternatively, the specimen may be transferred into a cervical cup, which is placed against the cervix. The patient stays supine or prone for 15 minutes. The cup is removed 1–2 hours later by the patient.

Intracervical insemination (ICI): suitable for high-quality sperm, does not require washed semen unless a forceps is used to close the cervix

In a large randomized comparative study with a minimum of 600 cycles for each procedure, the pregnancy rate per cycle in unstimulated infertile couples was 2.0% for ICI versus 4.9% for IUI; in COH couples the pregnancy rate per cycle was 4.1% for ICI versus 8.7% for IUI [19]. Despite the poor results compared to IUI and vaginal-cup insemination, the "high" intracervical insemination with forceps technique is occasionally performed when it is not possible to introduce a catheter into the uterus for IUI.

Equipment needed

Bivalve speculum, 3–5 mL syringe, 18G blunt needle or short plastic catheter or straw, plastic-wrapped sponge (Fertility Pack; optional), long-handled forceps (Allis forceps, Ring sponge forceps or other with a wide end), cotton swabs (Fig. 10.1). Specimen is suspended in 0.4–0.5 mL of wash media in a conical centrifuge tube, with an additional 1–2 mL standard buffer solution (phosphate buffered saline [PBS], Tyrode's, Earle's Balanced Salt Solution [EBSS] or Ham's F-10). Buffer solution should be pharmaceutical grade and sterile, with added gentamicin (20 μL per 100 mL); it does not need to contain human serum albumin (HSA).

Procedure: without forceps

The patient is positioned and draped as for vaginal insemination. A blunt 18G needle or catheter is attached to the syringe. First 0.5 mL of air and then the specimen, suspended in 0.4–0.5 mL wash media, are drawn into the syringe, and air remaining in the needle or catheter is expelled. With the cervix exposed, excessive vaginal secretions are wiped away and the cervical os is cleansed with the standard buffer media using a cotton swab. The end of the needle or catheter is gently introduced into the cervix as far as possible. The specimen

is slowly ejected from the syringe. The air remaining in the syringe is expressed as the needle or catheter is withdrawn. The optional plastic-wrapped sponge is inserted before removing the speculum. The patient remains on the table for 15 minutes and goes home. The sponge, if inserted, is removed two hours latter.

Procedure: with forceps

Before ejecting the specimen an opened forceps is positioned on either side of the cervix and the opposing ends gently squeezed together. Pressure on the forceps is maintained during ejection and for 1–2 additional minutes afterward. This is done to prevent escape of fluid from the external cervical os; as a result a portion of specimen enters the uterus. The forceps technique should only be performed with washed specimens.

Intrauterine insemination (IUI): required for poor-quality sperm and cervical factor, requires washed semen

IUI is the procedure normally used at the Fertility Institute of New Orleans, where pregnancy rates per cycle in patients aged under 35 without tubal factor averaged 19.0% during the first three COH-IUI cycles and 14.1% during the first four clomiphene citrate (CC) IUI cycles when prepared specimens with $\geq 5 \times 10^6$ total motile fresh sperm were used. When frozen donor sperm was used, pregnancy rates averaged 22.2% during the first three COH-IUI cycles for patients aged < 38, and 16.9% during the first four CC-IUI cycles for patients aged < 43 [20,21].

Equipment needed

Bivalve speculum, 3 mL syringe, malleable intrauterine insemination catheter (Insemi-Form™, Cook Medical, www.cookmedical.com; or Frydman IUI Memory Cannula, CCD International, www.ccd-international.com), long-handled forceps (Allis forceps, Ring sponge forceps or other with a wide end), single toothed cervical tenaculum (vulsellum), plastic-wrapped sponge (Fertility Pack; optional), cotton swabs (Fig. 10.1). Specimen is suspended in 0.4–0.5 mL of wash media in a conical centrifuge tube, with an additional 2–4 mL standard buffer solution (PBS, Tyrode's, EBSS or Ham's F-10). Buffer solution should be pharmaceutical grade and sterile with added gentamicin (20 μL per 100 mL); it does not need to contain human serum albumin (HSA).

Procedure

The patient is positioned and draped as for vaginal insemination. The sterile catheter package is opened at one end and the syringe attached without removing the catheter. With the cervix exposed, excessive vaginal secretions are wiped away and the cervical os is cleansed with the standard buffer solution using a cotton swab. The final 3 cm of the catheter is bent into a gentle 15–20° curve without removing the catheter from the package. The catheter with syringe is removed from the package. The catheter package should not be discarded, in case the catheter has to be reshaped.

First, 0.5 mL of air and then the specimen in 0.4–0.5 mL media are drawn into the catheter and syringe. The catheter is gently introduced into the cervix. At a point 3.5 cm from the external cervical os a sharp angle is usually encountered where the cervix and uterus join (the uterocervical angle or internal cervical os) unless the patient has had a previous vaginal delivery. The syringe and catheter are gently rotated as they are advanced in order to pass beyond this point until the catheter enters the uterus. Once past the internal cervical os, the catheter is advanced to a depth of at least 4 cm but no more than 6 cm to avoid trauma to the endometrium. The Cook catheter widens out 5 cm from the tip, and lines 6, 7 and 8 cm from the tip help to determine how far it has been advanced. The Frydman catheter has lines 5.2 cm and 6.2 cm from the tip.

When the catheter is in place, and before ejecting the specimen, the opened forceps is positioned on either side of the cervix and the opposing ends gently squeezed together with just enough pressure to prevent fluid escaping. Pressure on the forceps will be maintained during ejection. Next, the specimen is slowly ejected from the syringe (this should take not less than 30 seconds). The air remaining in the syringe is expressed as the catheter is withdrawn to form an air block in the cervix. During the injection the patient should be told that she may experience mild cramping and that she should tell the person performing the insemination if the discomfort is too severe, so that the rate of injection can be slowed. Pressure on the forceps should be maintained until the cramping subsides, usually within one minute. After the forceps is removed the plastic-wrapped sponge is inserted and the bivalve speculum removed. The patient remains on the table for 15 minutes and then goes home or returns to work. She is instructed to remove the sponge after two hours by pulling on the attached string.

Alternative semi-perfusion IUI procedure

After drawing up 0.5 mL air, 2 mL of buffered solution is drawn up into the syringe before drawing up the specimen suspended in 0.4–0.5 mL wash media. The cervix is cleansed, the catheter inserted as before and the opened forceps is positioned on either side of the cervix and the opposing ends gently squeezed together as before to prevent escape of specimen and buffered solution from the external cervical os. The specimen and buffered solution are injected slowly (this should take not less than one minute). The specimen, propelled by the 2 mL of media, will fill the endometrial cavity and partially fill the fallopian tubes, but not reach the peritoneal cavity. During the injection the patient should be told that she may experience mild cramping. The cramping will be more than when only the specimen is injected but will subside as the specimen and buffered solution in the uterus are pushed into the fallopian tubes. Pressure on the forceps should be maintained until the cramping largely subsides, plus an additional minute. The forceps is removed as above and the plastic-wrapped sponge is inserted. The patient remains on the table for 15 minutes and goes home or returns to work. She is instructed to remove the sponge after two hours.

Difficult catheter insertion

Difficulty with insertion of the catheter occurs if the uterus is retroverted or anteverted. The uterocervical angle becomes more acute when the patient is tense. It is less acute if the patient is relaxed and if she has a full bladder. Techniques to straighten the uterocervical angle, other than reconfiguring the curve of the catheter include: tilting the bivalve speculum up or down to lift or depress the external cervix and having the patient perform a Valsalva maneuver (bear down). Occasionally it is necessary to attach a single tooth tenaculum (vulsellum) to the lip of the cervix and exert traction downward. Rarely the IUI catheter cannot be passed into the uterus and a high cervical insemination must be performed instead, forcing fluid into the uterus.

Post-insemination management

Patients inseminated with fresh sperm are encouraged to have intercourse that evening unless they have had sex selection, firstly to provide additional sperm, which may benefit from media retained in the cervix, and secondly because prostaglandins present in seminal fluid may stimulate tubal transport. Couples using donor sperm and same-sex couples are encouraged to have intercourse so that the partner feels involved. Some physicians allow the patient's partner to inject the specimen for the same reason. Luteal-phase support, if needed, is reviewed with the couple before they leave the clinic. The immediate post-insemination interview is also a good time to remind patients that insemination seldom results in pregnancy the first cycle it is used, and they should expect to continue the indicated treatment for a minimum of three cycles before considering other methods. The couple are instructed to have a urine home pregnancy test in 14 days or, alternatively, a serum quantitative test in 12 days.

Other insemination procedures

Fallopian tube sperm perfusion (FSP); intrauterine tuboperitoneal insemination (IUTPI)

Reported results of FSP vary widely [22,23]. However, a meta-analysis of randomized controlled studies found that, when procedures performed using a Foley balloon catheter were excluded, FSP resulted in significantly higher pregnancy rates than IUI (OR 2.42, 95% CI 1.54–3.80) [24].

FSP involves injecting 4.0 or 6.0 mL of a washed specimen under pressure while sealing the cervix to prevent reflux. Increasing the insemination volume to 10 mL ensures peritoneal delivery of sperm and is better referred to as intrauterine tuboperitoneal insemination (IUTPI) [23]. Elaborate perfusion devices for IUI and FSP have been developed and are sold commercially. These include the Makler® Insemination Device (Sefi-Medical Instruments, www.sefimedical.com) and the Fallopian Sperm Transfer (FAST®) system (CCD International, www.ccd-international.com), designed to seal off the cervix to prevent reflux. A simple pediatric Foley catheter has also been used for the same purpose. The Makler device, which resembles the Sims–Huhner tubal insufflation cannula, uses spring pressure to hold an acorn-shaped cannula against the external cervical os to prevent retrograde leakage of fluid. The FAST system applies a suction cup to the cervix for the same purpose. All these systems accomplish the same purpose, but they are expensive and some of them need to be sterilized for reuse. Concerns have been expressed that FSP might cause the oocyte to be swept into the peritoneal cavity, that fallopian-tube fluid is important for fertilization and might be flushed out, or at least diluted, and that cellular debris might be swept into the peritoneal cavity, with unknown immunological consequences.

Direct intraperitoneal insemination (DIPI)

Concern has been expressed that small numbers of sperm, or the small amount of specimen available for insemination after processing, often less than 0.5 mL, might remain in the uterus and not ascend to the ampullary portion of the fallopian tube, where fertilization of the oocyte occurs. This has resulted in the development of procedures that attempt to place sperm in or near the fimbriated end of the fallopian tube. Early attempts involved insemination of small volumes of sperm directly into the fallopian tube by laparoscopy, similar to gamete intrafallopian transfer (GIFT) [25], and by transvaginal US-guided canalization of the fallopian tube [26]. A more recently described method involves depositing sperm at both tubal fimbria under US guidance using a 17G, 20 cm IVF needle [27]. However, the uterine cavity volume is only approximately 0.4 mL and all IUI techniques result in sperm entering the fallopian tube and possibly the peritoneal cavity. Direct intraperitoneal insemination may have a role in the treatment of patients with severe cervical stenosis.

Insemination complications

Pain

IUI procedures are mildly uncomfortable. However, pain rarely results in the need to abandon the procedure entirely. Prior to the procedure, patients who exhibit more than usual anxiety may be given a mild sedative such as diazepam 10 mg. When a tenaculum is necessary to straighten the cervix, or when cervical stenosis requires dilation, application of lidocaine jelly should be considered. In rare instances, general anesthesia or cervical block may be necessary.

Vasovagal attack

A vasovagal attack, consisting of a transient vascular and neurogenic reaction marked by pallor, nausea, sweating and a rapid fall in arterial blood pressure, may occasionally occur during insemination procedures and result in brief loss of consciousness. No treatment other than psychological support and a cool damp cloth to the forehead is usually necessary. If a patient has a history of epilepsy, that diagnosis must be considered, and particular attention must be given to maintaining her airway.

Infection

Semen is not sterile; therefore, antibiotics are routinely added to the culture media used for sperm preparation and insemination. The incidence of pelvic infection following IUI has been estimated to be less than 0.5% [28], even though bacteria were demonstrated in the peritoneal fluid of patients who have had ICI and IUI [29] A list of bacteria and viruses identified in semen has been compiled by Meniru (Table 10.2) [30]. Sperm preparation methods that include sperm migration or centrifugation through density gradient media remove most, but by no means all, bacteria contained in semen [31].

Genital herpes virus, hepatitis B virus, hepatitis C virus and human immunodeficiency virus (HIV) are all known to have been transmitted through use of donor sperm [28]. The male-to-female transmission of HIV, which has been the most studied of the viruses, occurs at the rate of between 0.1% and 0.5% in monogamous couples, yet there is no unanimous agreement that

Table 10.2. Organisms that can be found in semen

Bacteria, fungi and related organisms
Candida albicans
Chlamydia trachomatis
Escherichia coli
Gardnerella vaginalis
Lactobacillus spp.
Mycobacterium tuberculosis
Mycoplasma hominis
Neisseria gonorrhoeae
Proteus mirabilis
Staphylococcus aureus
Staphylococcus capitis
Staphylococcus epidermidis
Streptococcus milleri
Streptococcus faecalis
Trichomonas vaginalis
Ureaplasma urealyticum
Viruses
Human immunodeficiency virus (HIV)
Human T-cell lymphotropic virus type I (HTLV-1)
Hepatitis B virus (HBV)
Hepatitis C virus (HCV)
Herpesvirus
Cytomegalovirus (CMV)
Human papillomavirus (HPV)

Adapted from Meniru 1997 [30].

sperm itself can act as a vector for HIV [32]. Neither is there a close correlation between the circulatory viral load and the viral load in seminal fluid [33]. Thus far the United States Centers for Disease Control and Prevention (CDC) has recorded only two known cases of HIV transmission following IUI with washed sperm or washed migrated sperm, one of which involved a partner with a high viral load [34]. Sperm wash followed by density gradient centrifugation has been shown to result in sperm specimens that contain no detectable viral load [35].

Allergic reactions

Allergic reactions at the time of IUI are rare: only four cases have been reported, two to bovine serum albumin (BSA) and two to penicillin [30]. However, additional reactions may have occurred, being either unrecognized due to their mild nature or not reported. Gentamicin, used in the sperm processing methods described in Chapter 6, has not elicited an allergic reaction in over 7,000 IUIs performed since 1998 at the Fertility Institute of New Orleans. Most sperm processing procedures, including the ones described in Chapter 6, have replaced BSA with heat-inactivated human serum albumin obtained as a by-product of blood bank processing.

Antisperm antibodies

Although antisperm antibodies (ASA) might be expected, this has not been found to be a significant problem. In a small study of 51 IUI patients who were treated for an average of four cycles, 5.9% (three patients) developed cervical mucus antisperm antibodies and 9.8% (five patients) developed serum antisperm antibodies, but there was no correlation between the number of cycles performed and the development of antibodies [36]. One of the patients who experienced increasing antibody titers during her course of IUI treatment became pregnant in her fourth cycle. Antisperm antibodies coexist with pathologic states of the genital tract (obstruction, infection, trauma, varicocele), and are found in approximately 10% of infertile men in the general population [37]. Another study concluded that IUI did not provoke antisperm antibody formation in women who had not previously been sensitized, but increased the antibody titer in women who already had serum antisperm antibodies [38].

Genetic abnormalities

Moderate oligospermia ($< 10 \times 10^6$ total sperm) and severe oligospermia ($< 1 \times 10^6$ total sperm) are associated with abnormal karyotypes in approximately 1% and 10% of males respectively. These abnormalities include Y-chromosome abnormalities, balanced translocations, Klinefelter's syndrome (XXY) and XY/XXY mosaics [39]. In cases of severe oligospermia, conception as a result of IUI is unlikely. The risk is much greater in IVF-ICSI. Even though the possibility of a child being conceived by IUI with sperm from a male with severe oligospermia is low, it is advisable to obtain a chromosome analysis before proceeding when the total count is less than 10×10^6.

Sex ratio

Whether IUI and/or OI alter the sex ratio at birth has been a recurrent matter of conjecture. Because the normal male-to-female ratio is 52% : 48% at birth a very large number of cases would be needed to detect a difference due to treatment. Several small studies have suggested that IUI without OI performed shortly before or at the time of ovulation results in more male births, and that there are more female births when clomiphene citrate (CC) is used. Our experience at the Fertility Institute of New Orleans is that when singleton conception is the result of coitus the male-to-female ratio has been 48.9% : 51.1% in 1,885 births conceived without CC or gonadotropins, and 49.9% : 50.1% in 904 births conceived by coitus in clomiphene cycles [40]. When timed partner or donor IUI was performed during the cycle of conception, the male-to-female ratio was 55.9% : 44.1% in 59 births without CC or gonadotropin, compared to 46.3% : 53.7% in 149 births when clomiphene was used. Our results suggest that CC has no effect on the sex ratio in cycles of coitus. Although we have too few cases to allow conclusions, when combined with the results of other studies they suggest that IUI alone may increase the proportion of male births, and that this increase may be negated by the use of CC.

Laboratory or clerical error

Laboratory and clerical errors in which patients receive the wrong specimen are rare but catastrophic events for the infertile couples involved. They are avoided by strict adherence to procedures for identifying and handling sperm specimens, as described in earlier chapters .

Conclusion

Intrauterine insemination is an effective treatment of mild to moderate male-factor infertility (TMS $> 5 \times 10^6$ to $< 20 \times 10^6$) and cervical-factor infertility, and when using cryopreserved donor sperm. Pregnancy

rates may be increased with the tubal perfusion techniques.

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Chapter 11

Cryopreservation

Roman Pyrzak

Introduction

The clinical use of frozen-stored human semen or sperm is practical, beneficial, safe and it provides a readily available method for the treatment of infertility and the preservation of fertility. It was known in the eighteenth century that sperm could survive freezing. However, the first published report of successful insemination of cryopreserved sperm did not appear until 1954 [1]. Since then, improvements in cryoprotectant media and freezing protocols have made sperm cryopreservation applicable for all aspects of therapeutic insemination [2]. When human chorionic gonadotropin (hCG) injection or luteinizing hormone (LH) timing and equal numbers of progressively motile donor sperm are used for intrauterine insemination, there is no difference in pregnancy rates between fresh and cryopreserved sperm [3]. Most clinical practices that perform large numbers of inseminations with fresh sperm will wish to be able to cryopreserve patient sperm. If a clinical practice does not itself perform cryopreservation it may still refer patients to clinics or laboratories that do, and arrange for the cryopreserved specimen to be returned to the practice for thaw and insemination, as would be done in a case of donor insemination.

The advantages of frozen sperm over fresh sperm are many (Table 11.1). Cryopreservation provides the means to disassociate sperm collection and processing from ovulation. It is always available for use. For men with low total sperm counts several ejaculates can be frozen and combined later. Conversely, for men with high total sperm counts a single ejaculate can be used in several insemination cycles if they are not always available to furnish a fresh specimen. Cryopreserved sperm can be thawed and partially used, and the remainder can be refrozen [4]. Sperm can be frozen in one location and transported to another location for use. At a biological level there seems to be no practical limit to the length of time that human spermatozoa can remain alive in liquid nitrogen (LN_2), although sperm survival has been reported by some to decrease after three years, and the longest period of storage known to result in pregnancy in domestic animals is 25 years [2]. This information should be kept in mind when counseling couples and single men who have cryopreserved sperm.

Indications

The indications for cryopreservation of patients' sperm for later (autologous) insemination in addition to those suggested above are listed in Table 11.2. The ability to have a cryopreserved specimen as a "back-up" in cases where the male partner is unsure of his ability to perform on demand, or may need to be away at the time of ovulation, is a source of comfort for many couples. This is especially true when couples are separated for long periods due to foreign assignments, especially when the woman is over age 35 and there is concern about lost opportunity for pregnancy. Cryopreservation is indicated in medical conditions requiring treatment that may temporarily or permanently affect sperm quality or cause sterility, such as cancer treatment and certain cardiovascular medications [5]. In the case of cancer, however, sperm quality and count are often low and in-vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI) is recommended. Surprisingly, sperm from men with testicular cancer yield higher pregnancy rates compared to other cancers, and delivery rates of 82% have been achieved [6].

Collection and cryopreservation of sperm for posthumous insemination is possible, with several cases reported. Sperm can be collected for up to 36 hours following accidental death or trauma for later use [7]. Police and others in high-risk occupations may

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Table 11.1. Benefits of sperm cryopreservation

(1) Collection and processing of sperm can be dissociated from ovulation
(2) Frozen oligospermic specimens can be combined, increasing the number of sperm for IUI
(3) Ejaculates with high sperm numbers can be aliquoted and several samples can be frozen
(4) Cryopreserved sperm can be thawed, partially used, and the remainder then frozen
(5) Cryopreserved sperm can be stored almost indefinitely
(6) Cryopreserved sperm can be deposited at one site and shipped to another for insemination

Table 11.2. Indications for sperm cryopreservation

(1) Inability to achieve an erection or ejaculation on demand due to the stress of infertility treatment, or a medical condition (e.g. multiple sclerosis, diabetes)
(2) Work situations where the man may be absent during his partner's ovulation
(3) Situations in which couples who are separated for an extended period may wish to have fertility treatment
(4) Spinal cord injury that requires medical or electrical stimulation for ejaculation, which may not be available when ovulation is occurring
(5) Before beginning treatment which may impair fertility by causing decreased sperm count and function or sterility (e.g. testicular cancer or other cancers that require chemotherapy and/or radiation)
(6) Before beginning treatment with medication that may have a negative affect on sperm quality or sexual function (see Table 5.3)
(7) Posthumous in case of accidental death
(8) Mumps orchitis
(9) Before undergoing vasectomy as insurance against a later change in marital status

proactively request that sperm retrieval be attempted in the event of their death. In these cases, and when sperm is cryopreserved before cancer treatment, it is prudent to have a legal document prepared designating whether sperm is to be used in the event of the donor's death and stating that a child born as a result of posthumous insemination is his legal heir.

A potential use of cryopreservation, although never attempted to the author's knowledge, is preservation of sperm in cases of mumps orchitis, a disease that often occurs in sexually mature young males. In such cases it may be possible to find sperm in the ejaculate for 4–8 weeks after the diagnosis is made, although there is no assurance of viability and the sperm would need to be stored separately from all other sperm, as is done for sperm from men with other viral infections. Lastly, some men wish to have sperm cryopreserved before undergoing vasectomy.

Sperm freezing technique

The objective of any sperm freezing technique is to stop all cellular activity and to maintain the integrity of the sperm so that after thawing the motility and fertilization potential can be restored [8]. Spermatozoa may be cryopreserved by slow freezing or by vitrification (fast freezing). The key step in slow freezing is the removal of intracellular water from the cell so that during the freezing process development of ice crystals, which are potentially lethal, will be prevented. An additional function of cell dehydration is to reduce cell activity. Both functions are accomplished by adding cryoprotectant agent before the freezing procedure. There are two cryoprotectant formulas, one based on egg yolk and 12–15% glycerol in buffered media, the other containing glycerol, glycine and sucrose. Egg yolk is not a cryoprotectant but helps to stabilize the cell membrane. Egg yolk–glycerol media is the more popular for cryopreservation of spermatozoa, and can be prepared on site or obtained from commercial sources. Glycerol cryoprotectant media can be prepared on site or obtained from commercial sources with or without added gentamicin (Irvine Scientific, www.irvinesci.com; Medi-Cult, www.medicult.com; Spermfreeze™: FertiPro, www.fertipro.com). Cryoprotectant allows sperm to be stored in liquid nitrogen (LN_2) at a temperature of –196 °C (–325 °F). At this temperature, spermatozoa lack energy for physiological cellular activity and therefore can be stored indefinitely without causing damage to the sperm or its DNA integrity [9,10].

Slow freezing is still performed manually much as it was originally described by Bunge and colleagues [1]. It has the advantage of being simple to perform and requiring no expensive equipment, but as its name implies it is time-consuming, requiring approximately 150 minutes (Table 11.3).The most time-consuming step in the manual freezing process is the need to hold the sperm/cryoprotectant specimen in a 4 °C refrigerator for 90 minutes before exposing it to LN_2. Larger andrology services and commercial sperm banks now perform slow freezing using automated computerized stage freezers, which require only 30–40 minutes after initial specimen preparation [11]. However, these

Table 11.3. Sperm freezing methods

Methods	Cryoprotectant	Freezing time	Reference
Manual: slow	+	150 minutes	Bunge *et al.* 1954 [1]
Computerized	+	40 minutes	Hammadeh *et al.* 2001 [11]
Manual: fast	+	15 minutes	Donnelly *et al.* 2001 [15]
Vitrification	–	10 seconds	Isachenko *et al.* 2004 [13,14]

are expensive, the least costly being approximately US$20,000. At the other end of the time scale is vitrification, which is almost instantaneous.

Vitrification, originally described in 1942, is performed without first adding cryoprotectant to remove cell water [12]. Due to the fast change in temperature, the development of ice crystals is prevented. However, vitrification without cryoprotectant is only suitable for very small amounts of specimen. For vitrification of spermatozoa, concentrated sperm in 20 ± 2 μL drops of media are placed on copper loops of 5 mm diameter and immediately plunged into LN_2 [13,14]. Cryoloops (Hampton Research, www.hamptonresearch.com) are placed into 2.0 mL cryovials for storage. Vitrification can be performed outside the clinic or laboratory without using special equipment; for example in an operating room at the time of a MESA or TESA procedure. In order to freeze larger volumes of sperm in straws and vials by vitrification the presence of cryoprotectant is necessary. A semi-vitrification method is described in this chapter in which the initial step of bringing the temperature of the semen/cryoprotectant mixture slowly down from room temperature to 4 °C is eliminated; the cryovials or straws are suspended in nitrogen vapor for 15 minutes before plunging into LN_2 [15].

Specimen preparation

The facility for specimen collection and the methods of specimen collection and initial handling are the same as described in Chapter 6. When multiple collections for freezing are planned, they should be spaced a minimum of two days but not more than five days, and patients should abstain from intercourse between collections. Before specimen collection for cryopreservation and storage, patients should have their blood tested for sexually transmitted diseases (STDs) including *Chlamydia* antibody, Cytomegalovirus (CMV), hepatitis B and C, human T-cell lymphotropic virus (HTLV) 1 and 2, human immunodeficiency virus (HIV) and syphilis. The panel of tests may be expanded or reduced according to local custom and laws. If test results are positive, indicating infection, the test should be repeated to confirm or exclude the results. The objective of these tests is to prevent transferring a disease to the child or female partner, and to prevent contamination of specimens from other men that might be stored in the same container. If cryopreservation of the sperm is urgent, the specimen can be frozen and stored in a separate tank (quarantine), until all the tests are completed and the results are known.

The importance of a short time interval between specimen collection and processing cannot be overemphasized. Whenever possible, semen should be collected at the site where it is to be processed. When this is not possible, 5 mL of standard sperm wash media (SSW) should be provided to the couple, to be added to the ejaculated specimen as soon as it is collected.

Sperm intended for use in intrauterine insemination (IUI) can be washed to remove seminal plasma either before or after freezing. If frozen without removing the seminal plasma, the SSW, swim-up (SWU), density gradient centrifugation (DGC) or swim-down (SWD) procedure (described in Chapter 6) is performed after thawing and before use in IUI. If the specimen is processed by SSW before freezing, the specimen is ready for IUI after thawing without any additional processing. If the total motile sperm (TMS) count per vial or straw before cryopreservation is sufficiently high, the SWU, DGC or SWD procedure should be performed after thawing to remove sperm damaged by freezing. Semen specimens with poor sperm quality are washed after rather than before freezing, to prevent sperm loss during the wash procedure. Before freezing, the semen specimen is evaluated according to World Health Organization criteria [16]. If the TMS count is greater than 20×10^6, wash before cryopreservation is recommended.

Sperm storage

After completion of the freezing process, specimens are kept in a Dewar aluminum LN_2 storage tank (Fig. 11.1). Specimens may be cryopreserved in

Figure 11.1 Dewar cryogenic storage tank: side, top and cutaway views. Reproduced with permission.

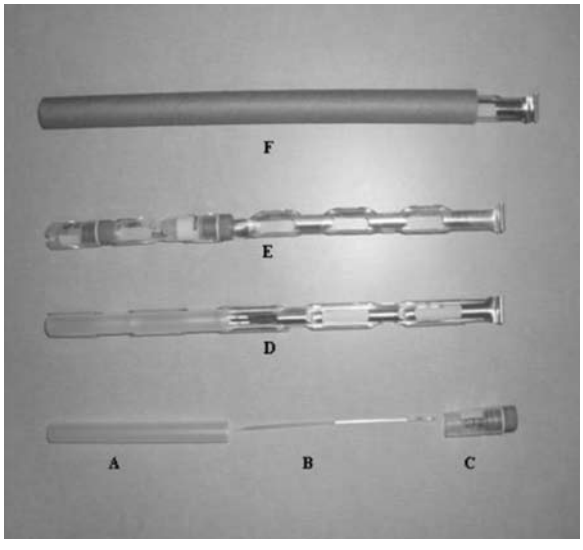

Figure 11.2 Cryopreservation supplies: (A) goblet (cryostraw protective vessel); (B) cryostraw; (C) cryovial; (D) goblets containing cryostraws attached to aluminum cane; (E) cryovials attached to aluminum cane; (F) aluminum cane inside protective sleeve.

polypropylene screw-top 1.0 or 2.0 mL cryogenic vials (Corning Life Sciences, www.corning.com), which can contain 0.5 and 1.2 mL specimens respectively, or in 0.25 or 0.5 mL polypropylene straws (Cryobiosystem, www.cryobiosystem-imv.com), which can contain 0.2 or 0.4 mL specimens. Even though the volume in a vial is different than the volume in a straw, the TMS can be the same. There is no difference in freezing method for specimens in straws or vials; it is just a question of preference.

The LN_2 capacity of a Dewar can range from 20 to 50 liters. Dewar tanks have capacities ranging from 720 to 3,500 straws and 210–1,050 vials. Each tank can hold between six and ten canisters, depending on the model. Each canister can hold 7–21 canes, and each cane can hold a maximum of five vials. Straws are stored in "goblets," with each cane holding one goblet and each goblet holding a maximum of 17 straws (Fig. 11.2). In practice, a separate cane is allotted to each patient, so that the number of canes in each Dewar corresponds to the number of patients whose specimens are stored, and the number of vials or straws is less than maximum capacity. Commercial Dewars are available that store specimens in boxes with a capacity of 100 vials per box, making it possible to store up to 2,000 vials per Dewar.

Viruses such as HIV, hepatitis B and C and other microbial agents can survive in LN_2 for a long period of time. If both contaminated and non-contaminated specimens are kept in the same Dewar there is the possibility that a vial or straw containing an infected sample could be damaged and its contents spill into the LN_2, potentially infecting other specimens. The possibility of cross-contamination is reduced if specimens are suspended in nitrogen (N_2) vapor above the liquid LN_2 [2]. There is no significant difference in recovery of motile sperm suspended in N_2 vapor at –180 to –190 °C compared to LN_2 [17].

The loss rate of LN_2 in the Dewar depends on the static evaporation rate and the frequency of opening. The minimum level of LN_2 should be 10 cm below the end of the neck opening. The preferred method of monitoring the level of LN_2 is to attach the Dewar to an alarm system. Alternatively, the level can be monitored manually by measuring periodically with a graduated rod or yardstick.

Facilities that store sperm must maintain detailed records, including patient identification, results of

blood tests for diseases, sperm parameters before and after freezing, reason for storage, readiness of specimen for insemination (prewashed or not) and storage tank where the specimen is to be found.

Cryopreservation procedures

Slow freezing methods, and vitrification with cryoprotectant, are described. Vitrification without cryoprotectant is used principally for cryopreservation of single or very few cells, e.g. embryos, oocytes and spermatozoa recovered by MESA or TESA. For a further description of vitrification procedures see the references above and in Table 11.3. A consent form for sperm cryopreservation is shown in Form 11.1.

Slow manual freezing method

The procedure for freezing vials is described. The procedure for freezing straws is the same except that volume per straw is less. However, the TMS count is the same per vial or per straw.

(1) Allow the semen specimen to liquefy, and calculate the number of total motile sperm (TMS).
(2) If a standard sperm wash, SWU, SWD or DGC procedure is planned, perform this before proceeding, and resuspend the pellet in a volume of HSAM wash media (see Chapter 6 for method of preparation) sufficient to bring the TMS count to 20–40 × 10^6 (twice the count planned for after addition of cryoprotectant).
(3) Place the entire specimen into one 15 mL centrifuge tube. Add cryoprotectant very slowly to the ejaculate at room temperature in drop-wise fashion and repeatedly mix by aspiration in and out of a 1 mL pipette until a semen-to-cryoprotectant ratio of 1 : 1 is achieved. At least 7–8 minutes is ordinarily required to complete this step.
(4) After mixing, leave the specimen with cryoprotectant at room temperature for 10 minutes.
(5) Calculate and record the TMS/mL after addition of cryoprotectant. According to the TMS count, the number of cryovials needed to freeze the entire specimen is calculated so that each vial contains between 15 and 20 × 10^6 TMS in 1 mL of fluid. Label each vial with patient's name, date of freezing, and distinct identification number (using a cryomarker). Mark the same patient identifications both on the top and on the side of the aluminum cane.
(6) Aliquot between 0.8 and 1.0 mL of sperm/cryoprotectant mixture into each vial. For straws, load 0.3–0.4 mL containing 15–20 × 10^6 TMS into each straw by attaching a small syringe (1.0 mL) into the closed end, which contains a polyvinyl alcohol (PVA) plug that expands when in contact with liquid, and placing the open end in the specimen. Use the 1 mL syringe to create a vacuum to load the specimen into the straw. Leave a small air space at the end of the open straw. Seal the open end of the straw with an electric sealer (Quick-Seal™, National Instruments Corporation, www.ni.com). A system of sealing straws hermetically at both ends that does not employ PVA is available from www.cryobiosystem-imv.com.
(7) Freezing takes place in three steps. In the first, place the vials in a 4 °C refrigerator for 90 minutes.
(8) In the second step, remove the vials from the refrigerator, place them in a shortened aluminum cane and expose them to N_2 vapor for 30 minutes by suspending them 4–5 cm above LN_2 in the neck of a Dewar storage tank (some authors recommend 45 minutes exposure to N_2 vapor instead of 30 minutes).
(9) For the third step, after exposure to N_2 vapor, very quickly remove the vials, clip them to previously labeled aluminum canes, place them in a canister and plunge them into LN_2 in a storage Dewar.
(10) Record the place where the vials are stored, including tank, canister and aluminum cane.
(11) After 48 hours, thaw one vial or straw and determine and record the post-thaw TMS.

Note: Liquid nitrogen is potentially dangerous, and must be handled with care. Eye and hand protection should be worn. Liquid nitrogen spills that saturate a person's clothing require immediate action, i.e. immediate removal of the affected clothing and shoes.

Computerized stage freezing method

Steps 1–6 of the slow freezing procedure are followed, except that straws instead of vials are customarily used in computerized stage freezing. Step 7 will be performed by the program.

After the computerized program is entered into the automated freezer and checked, the straws are loaded into the automated freezer, and the program is started

(*Note:* the program may not be the same if vials instead of straws are used).

(1) Straws are cooled from 22 °C at the rate of –1 °C/min to +5 °C.
(2) Straws are next cooled from +5 °C at the rate of –10 °C/min to –80 °C.
(3) Straws are then cooled from –80 °C at the rate of –25 °C to –140 °C and immediately plunged into LN_2 (in a 1 liter thermos).
(4) Remove the straws from the LN_2 and place them in a previously labeled aluminum goblet. Place the goblet in a labeled canister and then into a storage Dewar, where they are either suspended in N_2 or plunged again into LN_2.
(5) Record the place where the straws are stored, and determine post-thaw TMS after 48 hours, as in the slow procedure.

Fast manual freezing method (vitrification)

(1) Follow steps 1–6 of the slow freezing procedure.
(2) After step 6, attach the vials to a shortened aluminum cane and suspend them in N_2 vapor 6–8 cm above LN_2 in a Dewar or suitable container for 15 minutes.
(3) Remove the cane and vials from the vapor and plunge directly into LN_2.
(4) After 1–2 minutes, remove the canes and vials from LN_2. Quickly attach them to previously labeled regular-length aluminum canes and transfer to a storage Dewar.
(5) Record the place where the vials are stored, and determine post-thaw TMS after 48 hours, as in the slow procedure.

Establishing a donor sperm bank

Practices wishing to establish their own local sperm bank should consider first the laws in their country or state and the laws in the country or state where the donor sperm from the clinic will be used, if not strictly in their own practice. Donor sperm banks are required to freeze and quarantine all sperm specimens and then retest the sperm donor for HIV before releasing sperm for use. Since May 25, 2005, all sperm banks in the United States have been regulated and are required to comply with new Food and Drug Administration (FDA) guidelines. In Europe, with effect from April 7, 2006, sperm banks have been regulated by the European Union (EU) Tissue Directive. Similarly, effective May 25, 2005, donor specimens cannot be imported into the USA if they are not compliant with the FDA regulations. When exporting a donor specimen from the USA to the EU, the specimen needs to comply with EU regulations.

In the United States, the 2006 FDA requirements for testing of sperm donors specify the following tests: HIV-1 and 2, hepatitis C antibody, hepatitis B surface antigen, hepatitis B core antibody (IgG and IgM), serologic test for syphilis, HTLV-1 and 2, CMV (IgG and IgM), serum, urinary, or urethral test for *Neisseria gonorrhoeae* and either a urethral or urinary test for *Chlamydia trachomatis* [18]. In the United Kingdom, 1999 guidelines of the British Andrology Society (BAS) for infectious screening are the same except that they additionally require urinary or urethral tests for *Ureaplasma urealyticum*, *Mycoplasma hominis* and *Trichomonas vaginalis*, and omit serum screening for hepatitis B core antibody and HTLV-1 and 2 [19]. In both countries, depending on ethnicity, other tests are recommended or may be required, such as Creutzfeldt–Jakob disease, Tay–Sachs disease, sickle-cell anemia, thalassemia and cystic fibrosis. Countries and smaller jurisdictions differ in their requirements for infectious-disease and genetic testing.

Screening and selection of a donor involves a number of steps to ensure that the health and quality of the sperm specimen is adequate. The initial step is completion of an extensive questionnaire, which includes the following information: personal, maternal and paternal medical history; genetic and sexual history; alcoholism or use of drugs. Potential donors who have had STDs in the past, who are homosexual, have a history of intravenous drug abuse or have received blood products before the modern use of heat treatment are excluded at the outset. The donor should be under 40 years of age. A donor is not acceptable if there is a history of genetically inherited disorders anywhere in his family, unless these have been excluded by specific genetic testing. The list of genetic disorders is open-ended; Table 11.4 lists the BAS recommendations for genetic screening [19]. In the United States, recommendations are similar, with the exception that the FDA requires rather than recommends screening for cystic fibrosis carrier status for all sperm donors [18]. A semen analysis should show sufficient quality and quantity of spermatozoa. After collection and processing, the specimen is frozen in vials or straws and an aliquot is thawed to ensure that at least 10×10^6 motile sperm per vial or straw have survived the freezing process. Donor semen is collected at the sperm bank facility under strict aseptic conditions. As a general rule, donors do not collect more than two

Table 11.4. British Andrology Society genetic screening guidelines for sperm donors

(i)	The donor should not have any familial disease with a major genetic component:	
	Cleft lip	Congenital heart malformation
	Cleft palate	Hypospadias
	Club foot	Spina bifida
	Congenital hip dislocation	
(ii)	The donor should not have any non-trivial Mendelian disorder:	
	Albinism (general or ocular)	Hereditary hypercholesterolemia
	Hemoglobin disorder	Neurofibromatosis
	Hemophilia	Tuberous sclerosis
(iii)	The donor should not have any familial disease with known or reliably indicated major genetic component:	
	Asthma severe and persisting into adulthood	Rheumatoid arthritis
	Psychosis Epileptic disorder	Severe hypertension
	Juvenile diabetes mellitus	Severe refractive disorder
(iv)	The donor should not be heterozygous for an autosomal recessive gene known to be prevalent in the donor's genetic background:	
	β-thalassemia	Cystic fibrosis (screening is optional)
	Sickle-cell disease	Tay–Sachs disease
	Gaucher's disease	
(v)	The donor should not carry a chromosomal rearrangement that may result in unbalanced gametes	
(vi)	The donor should be < 40 years old	
(vii)	The donor's first-degree relatives (parents, siblings, offspring) should be free of:	
	(1) Familial disease with a major genetic component (as above)	
	(2) Non-trivial disorders showing Mendelian inheritance:	
	(a) Autosomal dominant or X-linked with age of onset extending beyond age of the donor, such as Huntington's disease.	
	(b) Autosomal dominant disorder with reduced penetrance, such as Marfan's syndrome and Alport's disease.	
	(c) Autosomal recessive disorder which is common in the population, such as cystic fibrosis.	
	(3) A chromosomal abnormality, unless the donor has a normal karyotype.	

Adapted from British Andrology 1999 [19].

samples per week. Because of cost, performance of tests for infectious diseases described above is the final step. In the United Kingdom a strict check is kept by the Human Fertilisation and Embryology Authority of the number of children "fathered" by each donor – and that number is restricted to 10. Exceptions may be made for the creation of siblings to existing children from the same donor. In the United States, there are no federal restrictions on the number of children that can be "fathered" by a donor, but American Society for Reproductive Medicine (ASRM) guidelines suggest limiting a single donor to no more than 25 births in a population of 800,000 [18]. The total births allowed in the Netherlands, Spain and France are 25, 6 and 5, respectively [20].

Shipping frozen sperm

Because LN_2 is a hazardous material, shipping of biological specimens in LN_2 is prohibited in most countries. Instead, biological products such as semen are shipped frozen in dry shippers filled with N_2 vapor. Dry shippers are small Dewar flasks that contain just enough LN_2 at the bottom to maintain specimens at –180 °C for 10–12 days. After the specimen arrives at its destination, it must either be used within a few days or transferred into an LN_2 storage Dewar (Fig. 11.3).

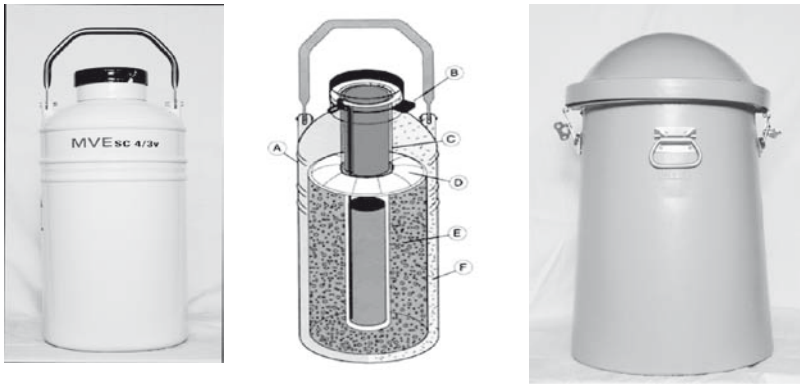

Figure 11.3 Dry shipper, dry shipper cutaway view, dry shipper outer container. (A) Aluminum outer skin; (B) locking tab; (C) high neck tube to reduce nitrogen loss; (D) chemical vacuum retention system; (E) hydrophobic absorbent to repel moisture and humidity while maintaining a –150 °C chamber environment; (F) insulation. Reproduced with permission.

Preparing frozen sperm for shipment

(1) Fill a dry shipper to the brim with LN_2; the LN_2 will be absorbed into the container lining. Monitor the level of LN_2 every hour. If it runs dry, add more LN_2. Continue monitoring until no more LN_2 is absorbed into the shipper. This usually takes two hours.
(2) Place the vials or straws to be shipped on the lowest part of an aluminum cane or in a goblet previously labeled with the patient's or donor's identification, and plunge the cane or goblet immediately into the shipper. Specimens should be moved from the storage tank to the shipper as rapidly as possible without compromising personnel safety.
(3) Update laboratory cryopreservation records, noting the number of vials shipped and the number remaining, the date of shipment and to whom the shipper was sent. Information about sperm quality must accompany the specimen.
(4) Before sealing the shipper, other lab personnel should verify that the contents in the shipper match the information to be sent with the shipper.
(5) Secure the inside shipper cap with a plastic cable tie.
(6) Place the shipper into the outer container and close the container with a security device.
(7) Call the shipping company, for example Federal Express in the USA, for pick-up of the shipping container, with a request for next-day delivery. Shippers must be informed that the shipment contains LN_2 vapor and that the Dewar contains biologic material.

It may sometimes be convenient to freeze patient sperm at one facility and transport the frozen specimen to another facility for insemination. When two facilities are close, the specimen can be thawed at either site and transported at room temperature by the patient, his partner, or facility personnel to the place where it will be used, providing this can be accomplished within two hours after removal from the storage tank. When the two facilities are far apart or the specimen will not be used within a few hours, the specimen can be shipped while still frozen. Prior to shipping his frozen sperm the patient should sign a consent form that gives authorization to release the specimen and transfer it to another (named) facility (Form 11.2). The form should have the name and the address of the facility to which the specimen is to be shipped. The partner's name is not required on the consent-to-ship form.

Thawing

The thawing step is critical for success, because if thawing is done improperly intracellular ice crystals will form, damaging the sperm. The method of thawing varies according to the method used for freezing. As a rule it is best to thaw slowly when freezing slowly and rapidly when freezing rapidly. However, there can be exceptions to the rule. Programs that plan to freeze patient sperm should give a high priority to determining the optimal method of thawing for the sperm they have frozen. Procedures for thawing are described in Chapter 12.

Conclusions

Sperm cryopreservation is a tried and tested procedure, long in use and a very useful part of a full IUI service. Slow freezing is the tried and proven method of maintaining motility and vitality but vitrification, a much quicker process, may be the future.

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Cryopreservation of semen/sperm consent

I, _____, would like to preserve my sperm by freezing (cryopreservation) at _____. I understand that the initial sample taken of my sperm will be frozen according to standard laboratory procedure, and that a small portion of the sample will be removed after 24 to 76 hours to test the sample's post-thaw quality. The results of this test will show the effects of freezing on the sperm sample and the probable usefulness of frozen storage. I further understand that:

1. _____ will make reasonable efforts to keep my frozen sperm sample viable; however, there is no guarantee that the sample will survive long-term frozen storage. Furthermore, the possibility exists that under unusual circumstances or situations beyond the control of _____ (which may include, but are not limited to, power failure), the sperm sample may thaw. At that time, the sperm sample may lose any part or all of its viability. I have been informed of both of these possibilities and am indicating my acceptance of these risks by entering the program and signing this consent.

2. _____ is not obligated at any time to implant my sperm for the purpose of insemination. _____ also retains the right to terminate and cease any sperm cryopreservation procedures already underway for any reason they determine to be appropriate.

3. Should my cryopreserved sperm sample be used for artificial insemination (AI) or assisted reproductive technology (ART), of my wife or any other female designated by me, I agree to indemnify and hold harmless _____ , its agents, employees, and assignees, from complications or adverse events, including but not limited to genetic defects in any or all offspring, arising out of or in connection with any services rendered by _____ that I have contracted for or that are taken on my behalf, including but not limited to, the use of any part or all of my cryopreserved sperm sample in the performance by _____ of the artificial insemination of my wife or any other female designated by me.

4. If I should die or become incompetent, I wish to transfer ownership of my sperm sample(s) to my wife so that she may be inseminated and conceive a child(ren) according to her wishes.

5. I agree to pay in advance for the initial work-up and maintenance of my frozen sperm sample and to pay on a yearly/quarterly basis for the annual maintenance required for such sample. If any fee payment is delinquent after six months, _____ reserves the right to dispose of the sperm samples.

6. I will notify _____ as soon as possible of any address change or relevant change of status, such as, but not limited to, those listed in paragraph 4 above.

7. I will notify _____ by notarized writing if I desire to terminate my storage contract with _____ and/or desire the release of any part or all of my sperm sample to a licensed physician for any use whatsoever.

I have discussed this matter at length with _____ and all my questions have been answered to my satisfaction.

Patient signature _____ Date _____

Physician/Nurse _____ Date _____

Witness signature _____ Date _____

Fertility Institute of New Orleans Form 1101:2008

Form 11.1 Consent for cryopreservation of semen–sperm.

Release to transfer frozen semen specimen to other facility

I, _____ am requesting that_____

release all frozen semen specimens currently being stored in nitrogen to the care of

Dr. _____ at _____.

Further, I the undersigned agree to prepay all applicable shipping charges to be shipped according to _____ guidelines for safe and expedient travel. The _____ staff verifies that the frozen semen specimens will be in the frozen state when they leave the _____.

The _____ makes no warranty, either expressed or implied, as to the post-thaw quality of the frozen semen specimens. It is normal and expected for less than 50% of semen to survive during the freezing process. Additionally, I the undersigned will not hold _____ liable for any equipment failure or transport accidents which could result in loss of any or all frozen semen specimens.

Patient name_____ Witness _____

Signature _____ Signature _____

Date _____ Date _____

Fertility Institute of New Orleans Form 1102:2008

Form 11.2 Consent to transfer frozen semen specimen.

Chapter 12

Donor sperm

Richard P. Dickey and Roman Pyrzak

Introduction

Donor insemination is the only insemination procedure performed by many physicians. It is convenient, easy to perform and requires no special equipment. Unwashed donor sperm can be purchased from commercial sperm banks "ready to use." Commercial sperm banks usually provide specimens in screw-topped polypropylene cryovials containing $10–30 \times 10^6$ motile sperm after thaw in 1 mL media. Specimens may be either prewashed for intrauterine insemination (IUI) or unwashed for vaginal (IVI) or intracervical insemination (ICI). Unwashed specimens can also be used for IUI after they are washed by the recipient clinic. Specimens may also be provided in straws in 0.5 mL of media.

Unwashed specimens need only to be thawed at room temperature or in warm water for 5–10 minutes to be ready for IVI and ICI. IUI specimens require centrifugation to remove the cryoprotectant, even if they were prewashed. Because liquid nitrogen (LN_2) is extremely hazardous if spilled, specimens are usually "dry" shipped in smaller versions of the Dewar LN_2 tanks used for long-term storage (Fig. 12.1). Dry-shipping tanks contain small amounts of LN_2 at the bottom sufficient to maintain the temperature of vials or straws suspended in LN_2 vapor at –180 °C for 10–12 days from the date the shipper was filled. Sperm specimens that can not be used within this time may be transferred to regular larger LN_2 tanks for long-term storage. A few commercial banks still ship donor sperm in dry ice overnight for use the next day.

Donor insemination practice

A 1987 survey commissioned by the US Congress Office of Technology Assessment estimated that, in the previous 12 months, 172,000 women underwent artificial insemination, resulting in 35,000 births from artificial insemination by husband (AIH) or partner and 30,000 from artificial insemination by donor (AID) [1]. Approximately 22% of practitioners performing AIH used fresh sperm exclusively, 25% used frozen sperm exclusively and the remainder used both fresh and frozen sperm. The survey found widespread variability in physicians' screening practices for infection and heritable disorders. The survey also found widespread reluctance to release even non-identifying information about donors to their offspring, and reluctance to offer AID to single women. Similar findings were documented in infertility practices in Canada [2], England [3] and New Zealand [4]. In England, support for maintaining anonymity of the donor was universal among practitioners surveyed, although 43% favored supplying recipients with information concerning physical appearance and 25% with information concerning social background [3]. In New Zealand nearly half of physicians performing AID felt that children conceived by AID should be told of their origins [4].

The principal reasons for performing AID in the USA in 1986–7 were male infertility (81.4%), no male partner (3.7%), impotence of the male partner (3.3%), genetic disorder of male partner (3.1%), exposure of the male partner to mutagens (0.4%), Rh incompatibility of the male partner (0.2%) and male partner with sexually transmitted disease (0.2%) [1]. Most inseminations were IVI or ICI. Since the development of in-vitro fertilization (IVF) in 1978 [5] and intracytoplasmic sperm injection (ICSI) a decade later in 1997 [6], the use of AID for male infertility has decreased sharply. In 1988, 25% of insemination cycles for male-factor infertility at the authors' clinic were performed using donor sperm. By 1997 only 7% of male-factor infertility was treated with AID. One reason that the demand for donor sperm remains high in the USA and in other Western countries is the increase in numbers of single

Manual of Intrauterine Insemination and Ovulation Induction, ed. Richard P. Dickey, Peter R. Brinsden and Roman Pyrzak. Published by Cambridge University Press.

women and same-sex couples seeking and receiving donor insemination services. To a lesser degree, the demand for AID remains high because many couples live where IVF and ICSI are not covered by a national health service or private health insurance. The reasons for the continued high demand can only be conjecture, because there have been no new national surveys since 1987 to provide information about the extent of use of AID in the USA.

Medical reasons for using donor sperm

Indications for AID before the modern era included severe and moderate oligospermia, asthenospermia, teratozoospermia, azoospermia, seminal antisperm antibodies and risk of transmission of genetic disease. In the modern IVF era, because of IUI, ICSI, testicular and epididymal sperm recovery from azoospermic men (see Chapter 2) and preimplantation genetic diagnosis (PGD), the need for donor sperm as a treatment for male-factor infertility has decreased. However, donor sperm is still necessary for men with total failure of spermatogenesis due to mumps orchitis, undescended testes, cancer chemotherapy, radiation or castration, infection transmittable in semen and autosomal dominant genetic disease. It is also the choice of men unwilling or unable to undergo surgical retrieval or vas reversal following previous vasectomy.

Commercial sperm banks

In the 1970s many large infertility practices and a few larger hospitals, particularly those connected with medical schools, maintained their own donor programs. In 1987, 74% of practitioners who performed AID with frozen sperm during the previous year used sperm from commercial sperm banks, 7% used sperm from hospital sperm banks and 26% used sperm from their own sperm banks, with 7% of practitioners using more than one source. The percentage of practices operating their own frozen donor sperm banks ranged from 2% in smaller programs to 62% in programs that performed AID on 100 or more patients. Today the number of practices that maintain their own frozen donor sperm banks is much smaller. In February 1987, the US Centers for Disease Control and Prevention (CDC) introduced a requirement to quarantine sperm for six months. Since then, numerous additional regulations have been added that apply equally to private clinics, hospitals and commercial sperm banks (see Chapter 11, establishing a donor sperm bank). Commercial sperm banks relieve the clinic or practitioner from the tasks of screening for infectious disease and genetic testing, and the need for long-term storage facilities. The wider selection of donors is another advantage appreciated by infertile couples.

Donor choices

Commercial sperm banks may offer videos, audio tapes, pictures of the donor as a baby and as an adult, and even essays written by donors. Large and small commercial banks allow patients to select a donor on the basis of race, hair and eye color, height and weight, education and ethnic origin. Patients can select their donor online if they have a computer, or from a catalogue, with guidance from their physician. At some sperm banks patients who plan to have more than one child by donor insemination can reserve sperm for their own use several years in the future for a storage fee.

A single website (www.spermcenter.com), accessible to physicians and patients for a small semi-annual fee, offers contact and other information for 25 United States commercial sperm banks, many of whom ship sperm internationally, and lists 1,667 donors (as of June 2009). It allows those who access the website to view and select potential donors on the basis of 130 different national or ethnic origins in addition to physical characteristics, educational achievements and religion. Cryos International Denmark (dk.cryosinternational.com) lists over 250 donors at several locations in Denmark.

International shipping of donor sperm

Many commercial sperm banks ship internationally. Most sperm banks ship sperm to patients' homes when authorized to do so by a physician. A small number of sperm banks in the USA ship directly to patients' homes without physician authorization, and provide instructions and "kits" for home insemination. United States commericial sperm banks that ship internationally to medicial practices and physicians, and to patients when authorized to do so by a physician, are listed in Table 12.1, along with their policies on donor identification. Cryos International in Europe also ships to other countries. Physicians considering using sperm from international shippers should check the laws in their own country to determine if there is a prohibition against importation of human sperm.

Table 12.1. United States commercial sperm banks that offer international shipping

Sperm bank	Location	Number of donors	Donor identification releasable	Website
Fairfax Cryobank	Virginia	179	√	www.fairfaxcryobank.com
Repro Lab	New York	25		www.reprolabinc.com
Cryos International	New York	39	√	ny.cryosinternational.com
Fertility Center of California	California	60		www.spermbankcalifornia.com
California Cryobank	California	249	√	www.cryobank.com
New England Cryogenic	Massachusetts	125	√	www.necryogenic.com
Cryobiology, Inc	Ohio	70		www.cryobio.com
Idant Laboratories	New York	16		www.idant.com
Biogenetics Corp	New Jersey	29	√	www.sperm1.com/biogenetics
Xytex Corporation	Georgia	124	√	www.xytex.com
ZyGen Laboratory	California	43		www.zygen.com
Reproductive Resources	Louisiana	33		www.reproductiveresources.com
Cryogenic Laboratories	Minnesota	117	√	www.cryolab.com
Sperm Bank of California	California	79	√	www.thespermbankofca.org
Total		1188		

Source: www.spermcenter.com (as of June 2009), with permission.

In some countries, such as the United States, laws concerning donor insemination and legitimacy of babies born as a result of insemination differ from state to state (see Chapter 17, Table 17.1) .

Donor identification

Identification of the sperm donor is required in an increasing number of countries. The United Kingdom, the Netherlands, Norway, Switzerland, Sweden, Italy and Austria have legislation banning anonymous sperm donation (see Chapter 17, Table 17.1). In other countries, there is a requirement to keep the donor anonymous. In the USA, the majority of sperm banks keep the donor anonymous and withhold identification unless permission is received from the donor. Increasing numbers of commercial sperm banks have donors who agree to have their identity released when the child is an adult (usually age 18 or older). In the USA in 2009, 370 (22%) of all donors, and 265 (22%) of donors at sperm banks that shipped internationally, agreed to be identified. In comparison, in Europe in 2008, fewer than 25 (9%) of donors at Cryos International agreed to be identified. Even when donors do not agree to be identified, many US sperm banks retain their blood or other tissue for a number of years in case it is needed at some time in the future if a genetic or other medical condition affecting a baby conceived through use of donor sperm is discovered.

Number of babies per donor

Most sperm banks keep records on how many children have been born from the same donor, to reduce the chances of inbreeding. In some countries this is required by law. Choosing a donor from a sperm bank in another state or country may lessen the risk of consanguinity, providing the sperm bank follows good practice guidelines for limiting the number of times sperm from a donor can be shipped to the area where the patient resides. International sperm banks have different policies on the number of pregnancies that it is acceptable for a donor to achieve in each area or country. In the United States, American Society for Reproductive Medicine (ASRM) guidelines suggest limiting a single donor to no more than 25 births in a population of 800,000 [7]. In the Netherlands, the United Kingdom, Spain and France, the corresponding numbers are 25, 10, 6 and 5, respectively [8]. Small commercial sperm banks may have fewer than

20 donors and store 50–100 units from the same donor. Larger sperm banks that deliver specimens to worldwide destinations may have 100 or more donors and keep a higher unit inventory of a specific donor, which can reach 400–600 units.

Safety

A major concern of patients and practitioners about the use of donor sperm is the risk of acquiring human immunodeficiency virus (HIV) and other infections. Since late 1987 in the United States and approximately the same time in other countries, donor sperm banks have been required to freeze and quarantine all sperm specimens for at least six months after collection, and to then retest the sperm donor for HIV before releasing his specimens for use. Guidelines for infectious and genetic disease tests of sperm donors in the United States [7] and Great Britain [9] are described in Chapter 11. They are identical for most serum tests. The British guidelines additionally require urinary or urethral tests for *Ureaplasma urealyticum*, *Mycoplasma hominis* and *Trichomonas vaginalis*, and omit serum screening for hepatitis B core antibody [9]. In the United States, sperm from a donor seropositive for cytomegalovirus (CMV) can be used, but only in a patient who is herself seropositive and only if the infection is not recent (IgG-positive, IgM-negative). In the UK, guidelines stress that sperm from CMV-seropositive donors can only be used in exceptional cases, where the donor belongs to an ethnic group with a high prevalence of CMV and a seronegative donor cannot be found.

Using donor sperm

Timing

Since frozen–thawed sperm is presumed to have shorter longevity than fresh sperm, handling of specimens after they are received, specimen preparation and timing of insemination are believed to be critically important to achieving a successful pregnancy. Insemination with frozen–thawed sperm should be timed as closely to ovulation as possible and performed before the oocyte(s) is released. This can be achieved by human chorionic gonadotropin (hCG) timed ovulation or by the patient monitoring her urine luteinizing hormone (LH) level twice instead of once daily, as would be acceptable for insemination with fresh sperm. Ovulation normally occurs 36 hours after the start, or 24 hours after the peak of serum LH, and 36–44 hours after hCG injection. To avoid missing ovulation during the weekend, ultrasound can be performed on Thursday and hCG administered if the lead follicle is at least 16 mm; although follicles of 17–18 mm and > 18 mm will yield slightly higher percentages of mature oocytes [10] (Table 10.1). Insemination on two successive days is not necessary when LH surge testing or hCG timing is used (see Chapter 10).

Handling and thawing the specimen

Donor sperm may be kept either in large storage tanks or in smaller shipping tanks (Fig. 12.1). Dry shipping tanks are small Dewar tanks that contain a minimal amount of LN_2 at the bottom sufficient to maintain specimens at –180° C for 10–12 days. If a specimen cannot be used within a few days after it arrives it must be transferred into a regular LN_2 Dewar tank. After removal of the specimen the shipping tank is returned to the sender. Before thawing the specimen, it is necessary to make sure that the identification of the specimen on the vial or straw is identical to that on the shipping record and also matches the specimen order in the recipient's chart. It is assumed that the recipient and the chart also match, but to be absolutely certain, it is best to check the recipient's birth date, social security number (USA) or other unique identification.

When donor sperm is received from a commercial sperm bank, the thawing procedure recommended by the sperm bank must be followed. The sperm bank

Figure 12.1 Containers for storage and shipping: (A) Dewar liquid nitrogen cryogenic storage tank; (B) liquid nitrogen supply; (C) cryoshipper outer container; (D) dry shipper.

provides information about post-thaw sperm count, motility and total motile sperm (TMS) of the specimen; this information should be stored in the clinic or laboratory. If the documented sperm count is low, additional vials or straws may be thawed if available and the specimens combined until a minimum of 10×10^6 motile sperm are available for insemination. If after thawing the motility is less than 25%, then pregnancy is less likely to occur, regardless of how many motile sperm are present. The sperm bank that prepared and shipped the specimen should be notified, so that a shipping problem, if it occurred, can be corrected. If the documented post-thaw motility of the specimen is less than 20% it should be returned to the sperm bank unused.

Each step in the thaw procedure is important to recover the maximum number of motile sperm, because during the freezing and thawing process the number of motile sperm may be reduced by 50–60% from the initial TMS count. If the initial sperm quality before freezing is poor, recovery of motile sperm can be less than 15%. A deviation from the thawing procedure can cause additional damage and further reduce the fertilization potential. If the specimen was washed before freezing, it is ready to be used for insemination immediately after thawing. If the specimen was not washed before freezing, it is not ready for insemination and needs to be washed using the standard sperm wash (SSW) procedure. If more than one vial or straw is thawed, all should be combined before the wash procedure. If after thawing the TMS is $\geq 15–20 \times 10^6$, then the density gradient centrifugation (DGC), swim-up (SWU) or swim-down (SWD) procedures described in Chapter 6 can be performed instead of SSW, providing the final TMS count will be $\geq 10 \times 10^6$ (refer to Table 6.1 for recovery rates of different wash procedures). If the TMS is $< 15 \times 10^6$, the SSW procedure should be performed.

Thawing vials

Simple method to be used for ICI

(1) Remove the vial or vials from the dry shipper or LN_2 Dewar and place in a 35 ± 2 °C water bath until the sample is thawed. This takes from four to eight minutes, depending on specimen volume in the vial. During the thawing process, the vial should be gently shaken (manually): this will shorten the thawing time and reduce sperm damage. If the specimen was washed before freezing, it is ready for IUI. If it was not washed, it is ready for ICI, but must be washed before using for IUI according to steps 2–9.

Advanced method to be used for IUI: removal of cryoprotectant

(1) After thawing in step 1, transfer the specimen(s) from the vial(s) into a 15 mL centrifuge tube.
(2) Keep the specimen in the centrifuge tube at room temperature for 10 minutes.
(3) After 10 minutes, very slowly add wash media (WM) in drop-wise fashion while mixing by repeated aspiration in and out of a 1 mL pipette until a 1 : 1 semen-to-WM ratio is achieved. The adding and mixing should be continuous and take at least 5–6 minutes.
(4) After mixing and dilution are completed, place a sample of 10 µL on a slide under a cover slip to confirm that sperm motility is the same as stated on the documentation provided by the sperm bank.
(5) Next, centrifuge the specimen for 10 minutes at 150–170 *g*.
(6) Remove the supernatant and dilute the sperm pellet with 0.5 mL of WM, and calculate the TMS.
(7) If the specimen was washed before freezing it is now ready to be used for insemination. If the specimen was not washed before freezing, proceed to step 8.
(8) If the "observed" TMS is $\geq 20 \times 10^6$, a DGC, SWU or SWD procedure can now be performed. If the TMS is $< 20 \times 10^6$ but $\geq 15 \times 10^6$ only a SWD procedure can be performed. If the TMS is $< 15 \times 10^6$ no additional procedure is performed, and the specimen is ready to use for insemination.

Thawing straws

(1) Remove the straw from the dry shipper or LN_2 Dewar and expose it to room temperature for 45–60 seconds.
(2) Wipe the outer surface of the straw to remove condensation water.
(3) Plunge the straw into water at 31–35 ° C for one minute.
(4) Open the hermetically sealed straw end by cutting (with scissors or scalpel blade) 2–3 mm from the sealed area.
(5) Direct the opened end of the straw into the opening of a 15 mL centrifuge tube.
(6) Cut the top end of the straw (the polyvinyl alcohol [PVA] plugged end) 1–2 mm below the plug. By opening both ends, the specimen is released into the tube.

(7) The specimen is ready for ICI. Before using for IUI it should be washed to remove cryoprotectant according to steps 2–9 for thawing vials.

Wash media (WM) used for sperm wash procedures can be any standard buffer solution such as phosphate buffered saline (PBS), Tyrode's, Earle's Balanced Salt Solution (EBSS) or Ham's F-10. For advanced sperm processing (DGC, SWU, SWD), the above WM must be supplemented with human serum albumin (HSA) to a final concentration of 0.3–0.5%, and with gentamicin solution, 20 μL per 100 mL of media.

Conclusion

Donor insemination is safe and easy to accomplish without special equipment. Commercial sperm banks that ship internationally offer couples and practitioners a wide choice of donors. Forms 12.1 and 12.2 show sample consent forms for the use of donor sperm.

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Consent for donor insemination: married recipient

We, _____ and _____ , being husband and wife authorize Dr. _____ or his associates at _____ to perform one or more artificial inseminations on the wife with the sperm obtained from an unknown donor for the purpose of making her pregnant.

We have selected an appropriate donor of our choosing. We also agree that donor sperm that has been frozen for patient safety will be used.

We understand that there is no guarantee that these inseminations will result in a pregnancy. We further understand that within the normal human population, a certain percentage (approximately 4%) of children are born with physical or mental defects, and that the occurrence of such defects is beyond the control of the physicians. We, therefore, understand and agree that _____ and its physicians do not assume responsibility for insemination. We also understand that within the normal population, approximately 20% of pregnancies result in miscarriages and that this may occur after donor insemination as well. Similarly, obstetrical complications may occur in any pregnancy. Although _____ performs extensive tests to avoid the risk of sexually transmitted diseases, including but not limited to gonorrhea, syphilis, herpes, hepatitis, and acquired immune deficiency syndrome (AIDS), we understand and accept that the artificial insemination procedure carries with it the risk of such diseases. This agreement, therefore, is not a contract to cure, a warrant of treatment, or a guarantee of conception. By these presents, we do hereby absolve, release, indemnify, protect, and hold harmless from any and all liability for the mental or physical nature or character of any child or children so conceived or born, and for affirmative acts or acts of omission which may arise during the performance of this agreement, the physicians and staff of _____ .

We understand that if a woman is artificially inseminated with the consent of her husband, the husband is treated in _____ (state) law as if he <u>is / is not</u> (circle one) the natural father of the child/children thereby conceived.

It is further agreed that from conception, I _____ , as husband or partner, accept the act of insemination as my own and agree:

That such child or children conceived or born shall be my legitimate children and heirs of my body, and

That I hereby waive forever any right which I might have to disclaim or omit the child or children as my legitimate heir or heirs, and

That such child or children conceived or born shall be considered to be in all respects, including descent and distribution of property, a child or children of my body.

Husband _____ Wife _____

Date _____ Date _____

_____ o'clock __ .M.

Witness _____ Date _____

Fertility Institute of New Orleans Form 1201:2008

Form 12.1 Consent for use of donor sperm: married recipient.

Consent for donor insemination: single recipient

I, _____ , an unmarried woman 18 years of age or older, authorize Dr._____ or his associates at _____ to perform one or more artificial inseminations on me with the sperm obtained from an unknown donor for the purpose of making me pregnant.

I have selected an appropriate donor(s) whose characteristics I find compatible. I will never seek to identify the donor(s), nor shall the donor(s) be advised of my identity. I understand and agree that it cannot be guaranteed that the same donor will be available for each insemination. I also agree that donor sperm that has been frozen (for patient safety) will be used.

I understand that there is no guarantee that these inseminations will result in a pregnancy. I further understand that within the normal human population, a certain percentage (approximately 4%) of children are born with physical or mental defects, and that the occurrence of such defects is beyond the control of the physicians. I, therefore, understand and agree that _____ and its physicians do not assume responsibility for insemination. I also understand that within the normal population, approximately 20% of pregnancies result in miscarriages and that this may occur after donor insemination as well. Similarly, obstetrical complications may occur in any pregnancy. Although _____ performs extensive tests to avoid the risk of sexually transmitted diseases, including but not limited to gonorrhea, syphilis, herpes, hepatitis, and acquired immune deficiency syndrome (AIDS), I understand and accept that the artificial insemination procedure carries with it the risk of such diseases. This agreement, therefore, is not a contract to cure, a warrant of treatment, nor a guarantee of conception. By these presents, I do hereby absolve, release, indemnify, protect, and hold harmless from any and all liability for the mental or physical nature or character of any child or children so conceived or born, and for affirmative acts or acts of omission which may arise during the performance of this agreement, by the physicians and staff of _____ .

I further agree that I am assuming entire responsibility for any child or children conceived or born. I agree that I will not seek support for the child or children, or any other payment from the donor, physicians, or nurses associated with _____ . I further agree that if the child or children should seek support or any other payment from the donor, physicians, or nurses, I will indemnify and hold harmless the donor, physicians, nurses, and _____ .

It is further agreed that the nature of this agreement is such that it must remain confidential; therefore, I agree that a sole copy of this agreement may be retained in the above-named doctor's files and shall not be disclosed except with my express written permission.

The _____ may use the agreement as necessary in connection with any legal proceeding to which it is relevant.

Signature: _____ Witness: _____

Date: _____ Date: _____

Fertility Institute of New Orleans Form 1202:2008

Form 12.2 Consent for use of donor sperm: single recipient.

Chapter 13

The role of the nurse in intrauterine insemination and ovulation induction

Mary M. Macgregor and Lisa T. Lofton
with the assistance of Gaylyn L. Achary, Wendi S. Dalferes and Wadena M. Saucier

Introduction

The fertility nurse is an essential member of a multiprofessional team whose focus is to ensure the delivery of quality care to patients. Information regarding ovulation induction (OI) and intrauterine insemination (IUI) is introduced to the patient by multiple team members, but most importantly by the nurse. The nurse works with the physicians and other team members, and becomes the primary advocate for the patient.

After the physician designs a plan of treatment based on his or her initial assessment and diagnosis, it is the nurse who coordinates and implements the plan of treatment. During the planning phase, the nurse becomes the primary caregiver and has the majority of interaction with the patient. The nurse's role is to direct couples through their treatment by explaining and defining all procedures and instructions clearly and concisely. It is very important for the nurse to be knowledgeable, compassionate, confident and empathetic.

The nurse implements the physician's plan of treatment by formulating and reviewing the OI patient's cycle calendar, ordering and instructing on the administration of oral or injectable medications, and educating on potential risks and/or side effects. The nurse teaches the patient the purpose of each step in the treatment protocol. The nurse reviews results of ultrasound and labs, and then communicates these results with the physician so as to instruct the patient with her plan of treatment. The nurse has an important role in teaching the patient regarding ovulation predictor kits.

Occasionally the nurse's role includes discussion of costs involved. The nurse ensures that couples understand their treatment plan and that their questions are answered. Appointments are scheduled around the patient's schedule, being as flexible as possible to keep the patient's stress down and to give the patient a feeling of being in control, which is crucial.

Informed consents are vital in each phase of fertility treatment. All appropriate consent forms should be provided and completely explained to the couple. All parties should sign consents, and the nurse should witness them, prior to the initiation of treatment. Documentation of all steps is essential.

Couples are often overwhelmed with information. The nurse reassures them and advises them to call with any questions regarding the information presented to them. There are many avenues to follow regarding knowledge and education for the patient. Referring couples to pertinent websites and providing booklets and pamphlets (available in the United States from the American Society of Reproductive Medicine) will enhance their knowledge. It is important to attempt to give them as much information as possible without being overwhelming. Keeping the couple informed is essential to achieving optimal results. Information is powerful in giving the couple some control in the process.

The relationship between the nurse and the patient can become very close. Listening skills are very important. Reaching out to the patient after procedures and offering emotional support counseling can enhance that relationship. An occasional phone call or hug can make all the difference to the patient. Occasionally, referring a patient to a clinical social worker may be indicated. Support groups may also be helpful.

Patients are usually confused and overwhelmed, and therefore have many questions. Anticipating the usual questions can avoid many phone calls. It is

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important to provide as much information as possible. Usual questions asked are:

(1) What is IUI?
(2) When is it used / Why do we need it?
(3) How does it work?
(4) What does it feel like?
(5) Will the sperm leak out if I stand up?
(6) How long do I have to lie down after IUI?
(7) How much sperm do you need?
(8) Do we have to abstain from sex after IUI, and if so, how long after?
(9) How many IUIs are done before turning to adoption or IVF?
(10) How much does it cost?
(11) What are the risks of IUI?
(12) Can we choose the sex of the baby?
(13) Can it be done at home?

Husband/partner insemination

In many cases the husband's or partner's sperm is used for artificial insemination (AIH) because of low sperm count, low ejaculate volume, cervical factor or difficulty in successfully completing coitus or obtaining a semen specimen. Examples of the latter are inability to maintain an erection, retrograde ejaculation and spinal injury requiring special technique to obtain a semen specimen. The nurse's role in providing emotional support to both partners is especially important in these instances.

Anonymous-donor insemination

In other cases anonymous donor sperm is used for artificial insemination (AID). AID may be used for couples where the husband or male partner has no sperm (azoospermia), extremely low sperm count, motility or normal forms (oligoasthenoteratozoospermia), or for genetic reasons. This step in the fertility process can be emotionally traumatizing. In some venues, AID is increasingly commonly employed for single women and same-sex couples. These patients have other issues as discussed in Chapter 16. The nurse may provide the patient or couple with information about sperm-donor agencies and sperm-bank websites. When indicated, the nurse may also recommend legal resources, after consulting with the physician (see Chapter 17). The chosen agency or sperm bank will provide pertinent information on the selected donor. The nurse should encourage the couple to seek counseling to discuss moral, ethical and legal issues, as well as each other's feelings and emotions.

The IUI nurse will often be the person responsible for seeing that all government regulations and clinic rules regarding laboratory tests required for sperm donors and recipients are completed and informed consents are signed (alternatively, the laboratory director may be responsible for these matters). Once the donor is chosen, the nurse will assist in completing necessary arrangements to have the sperm delivered to the office in ample time for insemination.

Known-donor insemination

Insemination with sperm from a known sperm donor may be planned. Usually the donor is a close friend or family member. The nurse will face a different set of issues when counseling and working with patients using known donors. Most people using a known sperm donor are single women, same-sex couples and married couples where the husband is infertile but there is a desire to continue the male "line." Known-donor inseminations often have fewer requirements for mandatory laboratory testing but involve more legal issues.

There are some advantages with a known sperm donor. It allows the couple to know in greater detail the donor's medical, physical and psychological background. Using a known donor allows the couple to keep in close contact with the biological father on a regular basis. The sperm does not have to be frozen and/or quarantined. It is less expensive, since most known donors are typically not paid.

Disadvantages of using a known sperm donor include legal issues, the risk of infection and relationship issues. Other important issues the nurse may need to discuss with the single patient or couple and known donor are safe sex practices, screening for sexually transmitted diseases (STDs) and genetic anomalies, time commitments and, most importantly, parental-claim issues. Seeking advice from a licensed clinical social worker and legal representative is strongly recommended.

Nurse-assisted insemination

Prior to the actual insemination, the nurse or nursing assistant will discuss what will take place in the treatment room. The nurse will encourage the patient to have her partner, another family member or a friend present to provide support. It is important to document when there is a family member or friend in the room during the insemination. In the treatment room the patient should undress from the waist down and

sit on the examination table. Her partner or support person should sit on an accompanying chair at the head of the table. Both the nurse and the physician will verify and identify the sperm specimen (whether it is known or anonymous) with both patient and partner. The nurse will document that the specimen has been checked appropriately, and document the name and relationship of family members or other persons present. The physician will discuss the result of that day's semen analysis which has been performed on the prepared specimen. While waiting for the procedure to begin the nurse can answer any questions the couple may have. After the procedure is completed the nurse will instruct couples on their activities for the next 24 hours, whether to have sex and when, when to start luteal-phase medications if ordered by the physician, when to have a pregnancy test, and hyperstimulation instructions, if needed.

Nurse-performed insemination

In some clinics, where it is legal to do so, the nurse will perform the insemination. The procedure will be the same as when it is carried out by the physician. In the case of a nurse-performed insemination, many of the nurse's tasks will be performed by an assistant, who remains in the room during the procedure. In both physician- and nurse-performed inseminations, but more often in the latter, the husband or partner may be allowed to depress the syringe after the catheter is in place, to give a greater feeling of participation in the conception process.

Conclusion

The nurse plays an integral role in assisting patients through the entire treatment cycle. Support and compassion provided by the nurse are key to a positive experience for the patient and a successful outcome.

Further reading

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Chapter 14

Complications of ovulation induction I: high-order multiple births, miscarriage, ectopic pregnancy, congenital anomalies, ovarian cancer

Richard P. Dickey

> Parallel to the great progress achieved during the last two decades in the therapeutic induction of ovulation was an increasing incidence of the two main complications associated with this kind of treatment, namely, ovarian hyperstimulation syndrome (OHSS) and multiple pregnancies.
>
> Schenker *et al.* 1981 [1]

Multiple births

All multiple births are associated with significant neonatal, maternal and family morbidity, but this is particularly so for triplet and higher-order multiple births (HOMB) [2–4]. Ovulation induction (OI), outside of in-vitro fertilization (IVF), is estimated to be responsible for 20% of twins, 40% of triplets and 70% of infants live born in quadruplet and higher-order deliveries in Western nations [5–7]. On average 10% of births resulting from clomiphene (CC) will be twins, and less than 1% of births will be triplets and HOMB. These figures increase to 20% and 8% respectively for births resulting from gonadotropins. For younger patients who are high responders, the incidence of triplet and higher-order pregnancies (HOMP) can be 20% [8,9]. Nearly all HOMB due to OI with gonadotropins in controlled ovarian hyperstimulation (COH) protocols might have been prevented by use of lower doses of gonadotropins, with very little reduction in the number of patients able to conceive. The majority of women who require OI will be able to become pregnant within 3–6 cycles of single or double follicular development using oral drugs or low-dose gonadotropins, with a 10% chance of twins and less than a 1% chance of HOMB.

The average cost of prenatal care, delivery and neonatal care through the first six weeks of life is 4–5 times higher for a twin pregnancy and 10 times higher for a triplet pregnancy than for a singleton pregnancy [10]. Most of the increased cost is due to prematurity and the need for increased hospital stay. Fortunately, many women can carry twins and deliver near term without danger. For those women who cannot carry, or who do not desire twins for other reasons, but require ovulation induction in order to become pregnant, IVF with single embryo transfer (SET) is the only solution.

Patient factors that increase the risk of neonatal and maternal morbidity in multiple pregnancy

Patient characteristics that increase the possibility of adverse neonatal outcome (prematurity, intrauterine growth restriction [IUGR]) and adverse maternal outcome (pre-eclampsia, placenta previa, abruption, cesarean section) are the same for singleton pregnancy and multiple pregnancy, only more frequent and more severe in the latter. Adverse maternal outcomes increase by 20–50% in triplet and quadruplet pregnancies compared to twin pregnancies, and cesarean sections increase 300–500% [11]. Although the adverse effects of multiple pregnancy in babies and mothers can be reduced by early recognition, prenatal care, nutrition and leave from work, whether or not infertility patients with one or more risk factors should start OI or continue an OI cycle when more than one

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preovulatory follicle is present demands careful consideration. The risk to the patient, and lifelong risk to her babies, must be weighed against the risk associated with IVF-SET. When the cause of infertility is male factor, IUI in unstimulated cycles should be the first line of treatment [12] .

Patient and cycle factors that increase the risk of multiple pregnancy

Patient and cycle factors associated with an increased incidence of HOMP in CC-IUI and COH-IUI are depicted in Tables 7.1 and 8.4 respectively. These are age, number of preovulatory follicles ≥ 10 mm, estradiol level, number of previous treatment cycles and use of donor sperm [8]. The necessity of counting all follicles ≥ 10 mm cannot be overemphasized [8,9]. In a prospective observational study of 4,062 COH-IUI cycles, three or more follicles ≥ 10 mm were present in 100% of triplet and higher-order pregnancies [8]. The presence of three or more follicles on ultrasound (US) was unable to predict triplets or more in 15% of cycles when follicles ≥ 12 mm were counted, 45% of cycles when follicles ≥ 14 mm were counted, 72% of cycles when follicles ≥ 16 mm were counted and 92% of cycles when follicles ≥ 18 mm were counted (Table 14.1). Failure to count follicles smaller than 16 mm is the principal reason why attempts to prevent HOMP and HOMB by cancelling cycles when three or more preovulatory follicles are present has been unsuccessful.

The risk of HOMP is particularly high for young patients [8]. For women aged less than 32, the incidence of three or more gestational sacs was 20.5%, and of four or more sacs was 9.5%, when there were seven or more follicles ≥ 10 mm (Table 14.2). The incidence of three or more sacs was 5.9%, and of four or more sacs was 2.0%, when there were 3–6 follicles ≥ 10 mm. For women aged 32–37 the incidence of three or more sacs was 12.1%, and of four or more sacs was 2.7%, when there were seven or more follicles. There were no pregnancies with three or more sacs after age 32 when there were 3–6 follicles, and none, irrespective of follicle number, for ages ≥ 38. With one exception, all pregnancies with four or more sacs occurred in patients with estradiol levels ≥ 1,000 pg/mL.

The incidence of triplet and higher-order gestations was highest in the first two cycles of COH-IUI when there were seven or more follicles ≥ 10 mm. None occurred after the first cycle in patients with 3–6 follicles (Table 14.3) [8]. However, this may have been due to the small number of pregnancies. The pregnancy rate decreased sharply after both the second and third cycles for patients with seven or more follicles, and after the third cycle for patients with 2–6 follicles. By contrast, pregnancy rates, although only half as high, remained constant through four cycles for patients with one or two follicles.

Table 14.1. Follicle size and the ability to predict triplet and higher-order pregnancies

Follicle size	Percentage of triplet pregnancies	
	< 3 Follicles	≥ 3 Follicles
≥ 10 mm	0%	100%
≥ 12 mm	15%	85%
≥ 14 mm	45%	55%
≥ 16 mm	72%	18%
≥ 18 mm	92%	8%

Adapted from Dickey *et al*. 2005 [8].

Techniques to reduce high-order multiple births

Limiting pregnancies from OI to singletons or twins can only be accomplished if there are no more than one or two preovulatory follicles ≥ 10 or 12 mm, irrespective of whether CC, other oral medications or gonadotropins are used. Techniques to reduce multiple births due to OI are: (1) use of oral drugs so that multiple follicular development is modulated by negative feedback of estrogen, and use of low-dose gonadotropin, or pulsatile GnRH; (2) techniques used after multiple follicles develop such as cancellation, conversion to IVF and aspiration of supernumerary follicles; and (3) selective reduction after pregnancy is diagnosed (Table 14.4) [13].

Use of clomiphene citrate (CC) or tamoxifen (TMX) initially

CC or TMX combined with IUI should be the first-line treatment for women with anovulation and unexplained infertility, after correction of any endocrine disorders, and a trial of IUI alone for at least three cycles in couples with strictly male-factor infertility [13]. In a prospective observational study of 3,381 CC-IUI cycles, twin and triplet gestational sacs averaged 8.9% and 1.6% respectively through six cycles, with no triplet gestations after the second cycle [14].

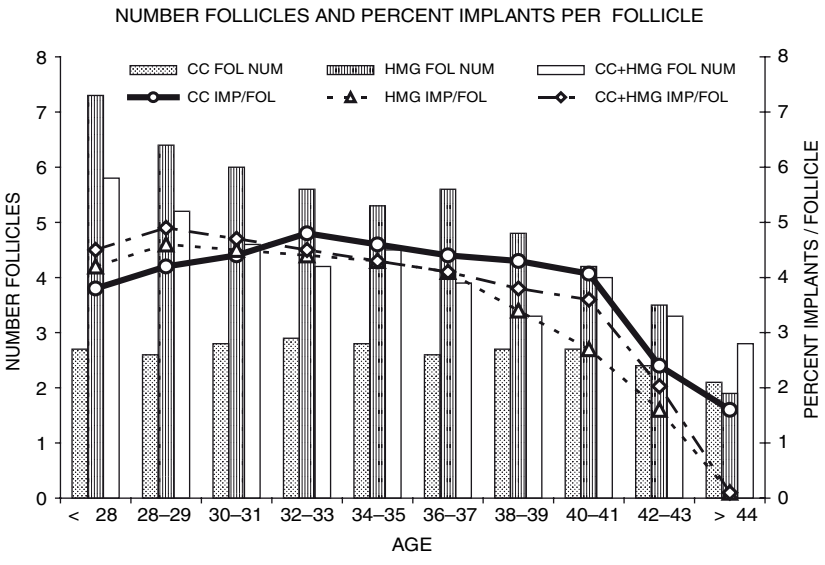

Figure 14.1 Relationship of number of follicles ≥ 12 mm to implantation rate per follicle in CC-IUI, hMG/FSH-IUI and CC+hMG/FSH-IUI cycles; patients aged < 43 without tubal factor. Adapted from Dickey *et al.* 2001 [15].

CC-IUI results in fewer twins and HOMP than COH-IUI because it stimulates growth of fewer follicles, not because gonadotropins induce growth of better oocytes [15]. The implantation rate per preovulatory follicle ≥ 12 mm is the same for CC as it is for gonadotropins (Fig. 14.1) [15]. However, 21% of CC cycles are monofollicular and 53% of cycles have no more than two follicles ≥ 12 mm, compared with gonadotropin cycles, of which 8% are monofollicular and 20% have no more than two follicles [15]. Although sufficiently large studies are yet to be reported, multiple pregnancy rates with TMX are believed to be half as high as with CC, due to the growth of fewer preovulatory follicles. While pregnancy rates per CC-IUI cycle are low – 10% for one follicle and 14% for two follicles ≥ 15 mm (Table 7.1) – conceptions continue to occur through 4–6 cycles for most indications and through three cycles for couples with very poor sperm quality or when the patient's age is over 42 (Fig. 7.5B,C) [14].

Switching to COH-IUI after three cycles of CC-IUI without pregnancy (the algorithm)

Switching to COH-IUI after 3–6 unsuccessful cycles of CC-IUI has been the standard algorithm for treatment of anovulatory and unexplained infertility in Europe and the United States for more than 40 years. HOMP rates after switching to COH-IUI were inversely related to the number of previous CC-IUI cycles (0 = 8.9%, 1 = 7.5%, 2 = 5.7%, ≥ 3 = 0/37 [< 2.7%]) in a retrospective study (Table 14.5) [16]. After switching to COH-IUI, the average pregnancy rate through the first three cycles and the incidence of twin implantations was unaffected by previous treatment with up to four cycles of CC-IUI. However, after four or more unsuccessful CC-IUI cycles, the COH-IUI pregnancy rate was less than 4%. IUI must be performed in the CC cycles for there to be a reduced incidence of HOMP in COH-IUI cycles, because failure to become pregnant may have been due to cervical or male factor. The observation in this study that pregnancy rates average 4% over three cycles when COH-IUI is used after four or more failed cycles of CC-IUI suggests that IVF instead of COH-IUI is indicated in patients who fail to become pregnant after multiple cycles of CC-IUI.

CC followed by gonadotropins in the same cycle

Sequential use of CC followed by human menopausal gonadotropin (hMG) in the same cycle (CC+hMG) was first reported in 1966. It was intended to reduce the amount and duration of COH used in OI and to increase pregnancy rates over CC alone. It was also anticipated that it would reduce multiple pregnancies compared to COH. The higher pregnancy rates for CC+hMG-IUI compared to CC-IUI were indeed realized; however, twin and HOMP rates were as high as for COH-IUI [15].

Minimal doses of gonadotropins

Use of minimal doses of gonadotropins before IUI results in pregnancy rates per cycle and twin and HOMP rates comparable to CC-IUI. In studies that included

Table 14.2. Pregnancy rates per cycle and multiple rates per pregnancy: relation to age, number of follicles ≥ 10 mm and estradiol pg/mL

Age	1–2 Follicles ≥ 10 mm					3–6 Follicles ≥ 10 mm						
	Cycles	Preg	2 Sacs	Births	Twins	Cycles	Preg	2 Sacs	3 Sacs	≥ 4 Sacs	Births	Twins
	#	%	%	%	%	#	%	%	%	%	%	%
<32	223	14.3	6.2	12.1	7.4	450	22.7	17.6	4.0	2.0	18.7	16.7
E_2 <1000	86	14.5	3.7	12.4	4.3	298	22.1	19.7	4.5	2.3	18.1	20.4
E_2 >1000	37	13.5	20	8.1	33.3	152	23.7	13.9	2.8	2.8	19.7	10
32–37	248	11.7	24.1	9.7	16.7	508	18.9	20.8	5.2	0.0	14.4	17.6
E_2 <1000	204	10.8	22.7	8.8	11.1	352	18.5	23.1	3.1	0.0	13.9	14.3
E_2 >1000	44	15.9	28.6	13.6	33.3	156	19.9	16.1	9.7	0.0	15.4	25
38–43	171	6.4	9.1	4.1	14.3	201	14.9	16.7	0.0	0.0	9.4	5.3
E_2 <1000	156	6.7	11.1	3.2	20	184	12.5	17.4	0.0	0.0	7.6	7.1
E_2 >1000	15	13.3	0	13.3	0	61	16	9.1	0.0	0.0	8.2	0

Patients without tubal factor, endometriosis with tubal involvement or poor sperm quality.
E_2, estradiol measured by chemiluminescence (CHL): 1000 pg/mL by CHL = 820 pg/mL by RIA = 1224 pg/mL by monoclonal antibody.
Adapted from Dickey *et al*. 2005 [8].

a minimum of 500 patients and minimal stimulation with initial doses of 37.5–75 IU of follicle-stimulating hormone (FSH) or hMG, totaling 8,370 cycles, clinical pregnancy rates ranged from 10% to 20% per cycle, twin pregnancies ranged from 6% to 15% and HOMP from 0% to 1.3% (Table 14.6) [17–21]. In all of the five studies, protocols were followed that required cancellation for excessive numbers of follicles. In a study not included in Table 14.6, the pregnancy rate and multiple pregnancy rate were the same for starting doses of either 52.5 IU or 75 IU hMG, but 13.5% of cycles that started with 75 IU had to be cancelled because of excessive numbers of follicles, compared to none started with 52.5 IU [22]. When minimal doses of gonadotropin are used, monofollicular and dual follicular cycles occur more often than with COH, but OI can be continued for 4–6 cycles with little decrease in pregnancy rates per cycle (Fig. 8.1D). The impression that pregnancies do not occur, or decrease markedly, after the third gonadotropin cycle is true only for COH-IUI, and even then it is mainly true for patients who develop large numbers of preovulatory follicles [8].

Starting gonadotropin stimulation on cycle day 7 or after selection of a dominant follicle

Delaying the start of gonadotropin stimulation until a dominant follicle (≥ 10 mm) is seen on ultrasound results in fewer preovulatory follicles ≥ 15 mm [23]. In normally cycling women a dominant follicle ordinarily forms by cycle day 7.

Pulsatile GnRH

Pulsatile gonadotropin-releasing hormone (GnRH) administered subcutaneously or intravenously results in greater than 27% pregnancy rates per cycle and multiple birth rates of 5–8%, primarily of twins [24]. The need for more frequent administration compared to gonadotropin, the lack of consistent availability in some countries and the lack of a clear advantage over minimal gonadotropin stimulation, in pregnancy and multiple pregnancy rates, has kept GnRH from being more frequently used in clinical practice.

Using hMG instead of FSH, and addition of hCG or rLH during the late proliferative phase

Addition of low-dose hCG (50–200 IU/day) in FSH and hMG regimens, after a dominant follicle has been selected, has been shown to impede development of smaller follicles [25]. However, larger randomized studies are needed to establish whether use of hMG rather than recombinant FSH (rFSH) or highly purified urinary FSH (uFSH), and addition of recombinant luteinizing hormone (rLH) or hCG in the late proliferative stage of OI, will reduce multiple

(E_2), in gonadotropin IUI cycles 1–3

		≥ 7 Follicles ≥ 10 mm								
Trips	≥ Quads	Cycles	Preg	2 Sacs	3 Sacs	≥ 4 Sacs	Births	Twins	Trips	≥ Quads
%	%	#	%	%	%	%	%	%	%	%
1.2	1.2	344	24.4	20.5	10.7	9.5	20.3	15.7	12.9	5.7
1.8	0.0	43	18.2	3.8	23.1	0.0	13.4	10.5	15.8	0.0
0.0	3.3	201	28.8	28.1	5.2	13.7	25.4	17.6	11.8	7.8
2.7	0.0	272	27.2	27	9.5	2.7	22	26.7	3.3	3.3
2.0	0.0	106	24.5	23.1	3.8	0.0	21.7	17.4	0.0	0.0
4.2	0.0	166	28.9	29.2	16.7	2.0	22.3	32.4	5.4	5.4
0.0	0.0	84	16.7	0	0.0	0.0	10.7	0	0.0	0.0
0.0	0.0	30	20	0	0.0	0.0	10	0	0.0	0.0
0.0	0.0	54	14.8	0	0.0	0.0	11.1	0	0.0	0.0

pregnancies without adversely affecting overall pregnancy rates [13].

Cancellation of cycles for excessive numbers of follicles or high estradiol concentration

Withholding hCG and advising couples to abstain from unprotected intercourse when excessive numbers of follicles or high estradiol concentrations are present is the most widely used technique for reducing HOMP in OI cycles. Cancelling cycles in order to prevent multiple pregnancy is only completely effective if all preovulatory follicles ≥ 10 mm are counted [8,9]. In large retrospective studies, it has been estimated that cancelling cycles because of excessive numbers of follicles or estradiol levels could reduce HOMP by half, but would result in cancellation of approximately 50% of cycles [9,26]. Patients aged 38 or over, and those who fail to become pregnant in the first two COH-IUI cycles, have a reduced risk of HOMP (Tables 14.2, 14.3). It is possible that higher doses of gonadotropin can be safely used in these patients without risk of HOMP. If this is confirmed in other studies, cycles would not need to be cancelled for excessive numbers of follicles in these patients, but might still be cancelled to avoid the risk of OHSS.

Coasting

Coasting, or delaying hCG administration and discontinuing gonadotropin stimulation until estradiol levels fall, is used in IVF cycles to prevent hyperstimulation. However, lower pregnancy rates are the usual result. Likewise, when coasting is used in OI cycles to reduce the incidence of HOMP, pregnancy rates have been poor and the average twin rate may still be 50% [27]. The effect of coasting has been studied extensively in IVF cycles [28]. Estradiol levels continue to rise for one day after cessation of hMG or FSH, they then level off on the second day and fall precipitously on the third day. Coasting for four or more days markedly decreases pregnancy and implantation rates in IVF cycles. Reducing the dose of hCG from 10,000 to 5,000 IU also results in a marked decrease in pregnancy rates, but without a corresponding decrease in the incidence or severity of OHSS [28].

Aspiration of supernumerary follicles

Only one prospective study has been reported where aspiration of supernumerary follicles was performed before administrating hCG in 100 or more cycles [29]. In that study, aspiration was used when there were four or more follicles ≥ 14 mm in 45% of 571 COH-IUI cycles. Four percent of cycles were cancelled without aspiration because of excessive number of follicles. The twin and triplet implantation rates were 8% and 1.7% respectively. Aspiration of supernumerary follicles offers an alternative to cancelling cycles. However, cost and effectiveness remain to be defined in larger trials. No other studies consisting of more than just a few cases have been reported since 1998.

Table 14.3. Pregnancy rates per cycle and multiple rates per pregnancy: relation to cycle number and number of follicles ≥ 10 mm, in gonadotropin IUI cycles

1–2 Follicles ≥ 10 mm				3–6 Follicles ≥ 10 mm					≥ 7 Follicles ≥ 10 mm				
Cycle	Cycles	Preg	2 Sacs	Cycles	Preg	2 Sacs	3 Sacs	≥ 4 Sacs	Cycles	Preg	2 Sacs	3 Sacs	≥ 4 Sacs
	#	%	%	#	%	%	%	%	#	%	%	%	%
One	309	14.6	15.5	612	20.8	19.7	6.3	1.6	363	27.5	24.0	10.0	5.0
Two	124	9.7	8.3	270	19.2	17.3	0.0	0.0	181	24.9	22.2	13.3	11.1
Three	38	10.5	25.0	106	17.0	11.1	0.0	0.0	72	16.7	33.3	0.0	0.0
Four	28	10.7	0.0	83	6.0	0.0	0.0	0.0	44	2.3	0.0	0	0.0

Patients aged < 38 without tubal factor, or poor sperm quality.
≥ 3 sacs per pregnancy: cycle 1–2 vs. cycles 3–6 when ≥ 7 follicles $P < 0.025$.; cycle 1 vs. cycle 2 when 3–6 follicles $P < 0.04$.
Table not previously published (adapted from Dickey *et al*. 2005 [8]).

Switching to IVF

Switching to IVF is an alternative to cancelling cycles in order to avoid HOMP due to OI. However the unanticipated additional cost to the infertile couple, and the requirement for an ongoing IVF program, limit the use of this method of avoiding HOMP away from IVF centers.

Table 14.4. Techniques used to reduce the incidence of multiple births (HOMB) due to ovarian stimulation

(1)	**Minimal stimulation in initial cycles**
	Use of clomiphene (CC) for 4 cycles before initiating hMG (Algorithm)
	Use of tamoxifen or aromatase inhibitor (letrozole) instead of CC[a]
	Sequential regimen (CC for 5 days before hMG/FSH) (ineffective)
	Minimal stimulation with < 75 IU hMG or FSH
	Start stimulation on cycle day 7 or after selection of a dominant follicle (> 10 mm)[a]
	Pulsatile GnRH
	Using hMG instead of FSH, addition of hCG or rLH during the late proliferative phase[a]
(2)	**Cancellation of cycles for excessive number of follicles or high estrogen concentration**
	Coasting (ineffective)
	Aspiration of supernumerary follicles[a]
	Conversion to in-vitro fertilization
(3)	**Selective reduction**

[a] Additional studies required

Multifetal pregnancy reduction

Multifetal pregnancy reduction (MFPR) from quadruplet and higher-order gestations to twins significantly decreases the risk of delivery before 28 weeks and associated neonatal and developmental morbidity, as well as risk to the mother [30]. However, twins reduced from quadruplet and higher-order gestations often deliver earlier and are more likely to have restricted fetal growth or intrauterine growth restriction (IUGR) than unreduced twins [31]. Loss of the remaining embryos or fetuses has been reported to occur in 8–16% of MFPR cases, but appears to decrease with increased operator experience [30]. Whether triplet gestations benefit from MFPR to twins is less certain [32]. Reduction of twins to singletons for non-genetic reasons has been followed by an unusually high incidence of adverse outcomes [33].

A decision for MFPR should be delayed as long as possible, to allow time for spontaneous reduction. Spontaneous reduction of one or more gestational sacs or embryos occurs before the twelfth week of gestation in 36% of twin, 53% of triplet and 65% of quadruplet pregnancies [34].

Other complications of ovulation induction

(For OHSS and ovarian torsion see Chapter 15.)

Miscarriage

Reports that CC causes an increase in spontaneous abortion arose from mostly small inadequately

Table 14.5. Relationship of number of previous cycles of CC-IUI to pregnancy and multiple pregnancy rates during the first three COH-IUI cycles

Number of CC-IUI cycles	Cycles	Pregnancies per cycle[a]			Multiple gestation per pregnancy			
		#	%	OR (95% CI)	2	%	≥ 3	%
0	1459	318	21.8	1.00	61	19.2	28	8.8
1	408	80	19.1	0.88 (0.66–1.15)	15	18.8	6	7.5
2	268	53	19.8	0.83 (0.60–1.16)	10	18.9	3	5.7
3	130	25	19.2	0.83 (0.53–1.31)	5	20.0	0	0.0
4	57	11	19.3	0.84 (0.43–1.64)	2	18.2	0	0.0
5	23	1	4.3	0.16 (0.02–1.19)	0	0.0	0	0.0
6–12	32	1	3.1	0.11 (0.02–0.83)	0	0.0	0	0.0

[a] Pregnancy rate per cycle: cycles 1–4 (19.6%) vs. cycles ≥ 5 (3.6%), $P = 0.006$, chi square.
Adapted from Dickey *et al.* [16].

Table 14.6. Pregnancy outcomes in patients treated with low-dose gonadotropins: results of five published studies that included a minimum of 500 patients and a total of 8,370 cycles

Reference	Balasch *et al.* 1996 [17]	Papageorgiou *et al.* 2004 [18]	Alsina *et al.* 2003 [19]	Ragni *et al.* 2006 [20]	Wang *et al.* 2003 [21]
Type of study	Prospective	Retrospective	Prospective	Retrospective	Retrospective
Patients	WHO II CC failures	Mixed	WHO II	Mixed	Mixed
Patients (cycles)	234 (534)	1256 (3219)	343 (945)	621 (1259)	824 (2413)
Initial stimulation	75 IU FSH	50 or 75 rFSH	50 IU FSH	50 IU rFSH GnRH antag.	37.5 IU FSH
Criteria for hCG	1 follicle ≥17 mm	1 follicle ≥ 15 mm	1 follicle ≥ 16 mm	1 follicle ≥ 18 mm	N/S
Criteria to cancel	4 ≥ 14 mm	3 ≥ 15 mm	4 ≥ 16 mm	3 ≥ 15 mm 5 ≥ 11 mm	3 ≥ 16 mm
Cancelled (%)	5.2%	1.1%	13.5%	10.5%	21.5%
Clinical pregnancy rate per cycle (%)	17.4%	10.4%	14.4%	10.3%	20.1%
Twins	15%	8.1%	5.9%	9.5%	7.4%
≥ 3 Sacs (%)	0	0.3%	0	0	1.3%
Birth rate per cycle (%)	15.5%	8.3%	N/S	7.8%	N/S
Twins (%)	N/S	10.2%	N/S	10.1%	N/S
≥ Triplets (%)	0	0.03%	N/S	0	N/S

N/S, Not stated.
Adapted from Dickey 2009 [13].

controlled studies without a comparable group of infertile women not taking CC. In two large prospective studies, the incidence of abortion following CC was similar to spontaneous conceptions and less than following hMG. In the first, a five-year multi-hospital study that compared outcomes in 186 hMG pregnancies, 1,034 CC pregnancies and 29,900 spontaneous conceptions, miscarriage rates were 19.4% for hMG, 14.8% for CC and 13.9% for spontaneous ovulation cycles [35]. In the second study, from a single

infertility center, combined biochemical and clinical abortion rates were 36.4% in 107 hMG or FSH pregnancies, 23.7% in 1,738 CC pregnancies and 20.4% in 3,471 spontaneous pregnancies [36].

Ectopic pregnancy

In a retrospective analysis of 2,086 spontaneous and 1,391 CC pregnancies following IUI or coitus, 3.0% of spontaneous pregnancies and 3.4% of CC pregnancies were ectopic when endometriosis was present. When there was no endometriosis or tubal disease, 1.4% of spontaneous pregnancies and 0.8% of CC pregnancies were ectopic [37].

Congenital anomalies

In a total of 2,369 births following CC administration reported to the manufacturer, the incidence of birth defects was 2.4%, compared to a 2.7% incidence in the population as a whole [38]. There have been no substantive reports of increased anomalies following use of hMG or FSH.

Ovarian cancer

A 1992 meta-analysis suggested a 2.8-fold increased risk of invasive epithelial ovarian cancer in infertile women who had used CC without becoming pregnant compared to fertile untreated women [39]. A subsequent cohort study concluded that the increase in ovarian cancer after OI was limited to women who took drugs for more than 12 cycles without becoming pregnant [40]. A pooled analysis of results from eight case–control studies of nulliparous infertile women found that OI was associated with a 2.4-fold increase in borderline serous ovarian tumors and a 1.6-fold increase in invasive cancers [41]. Most of the cancers were discovered within six months from the start of OI. These risks should be balanced against a reported 2.4-fold increased risk of ovarian cancer in women with a history of polycystic ovary syndrome (PCOS), compared to controls [42].

Conclusion

The most common and most significant potential complication of OI is the occurrence of multiple pregnancies, with their serious implications for both mothers, prenatally and intrapartum, and their babies. The incidence of multiple pregnancies following OI can be minimized by careful selection of the appropriate drug and dose of the drug for the individual patient, and by careful monitoring of the response. Strategies for achieving this have been described in Chapters 7 and 8, on the administration of the oral and injectable medications used for OI, in which the importance of safety has been emphasized. OHSS is another of the main complications of OI, and this is addressed in Chapter 15. The incidence of miscarriage, congenital anomalies and ectopic pregnancies does not appear to be affected by the drugs used for IO. Always of concern to practitioners and their patients is the reported increase in the incidence of ovarian cancers following the use of drugs for OI. Patients must be fully informed of the facts and reassured that the real chance of their developing ovarian cancer, although it may be increased, is very small indeed.

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Chapter 15

Complications of ovulation induction II: ovarian hyperstimulation syndrome, ovarian torsion

Botros R. M. B. Rizk

Introduction

Ovarian hyperstimulation syndrome (OHSS) is characterized by bilateral, multiple follicular and thecalutein ovarian cysts and an acute shift in body fluid distribution resulting in ascites Fig. 15.1. OHSS in patients undergoing controlled ovarian hyperstimulation has been observed to occur in two distinct forms, early onset and late onset, with possibly different predisposing factors. Early OHSS presents 3–7 days after the ovulatory dose of human chorionic gonadotropin (hCG), whereas late OHSS presents 12–17 days after hCG. Early OHSS relates to an "excessive" preovulatory response to stimulation, whereas late-onset OHSS depends on the occurrence of pregnancy, is more likely to be severe and is only poorly related to preovulatory events. Rizk and Smitz, in an analytical study of the factors that influence the incidence of OHSS, found a wide variation among different centers [1]. This is partly because of different definitions of the grades of severity and partly because of the adoption of different criteria for its prevention. The incidence of OHSS has been estimated at 20–33% for mild cases, 3–6% for moderate cases and between 0.1% and 2% for severe cases [2–4].

Overview of OHSS classifications

There has been no unanimity in classifying OHSS, and divergent classifications have made comparisons between studies difficult [3]. Aboulghar and Mansour reviewed the classifications used for OHSS over the last four decades and included as many as six categories of severity [5]. The most recent classification was introduced in 1999 by Rizk and Aboulghar [6]. They classified the syndrome into only two categories, moderate and severe, with the purpose of categorizing patients into more defined clinical groups that correlate with the prognosis of the syndrome. The new classification can be correlated with the treatment protocol and prognosis.

The mild degree of OHSS, used by most previous authors, was omitted from the new classification, as this degree occurs in the majority of cases of ovarian stimulation for the assisted reproductive technologies (ART) and does not require special treatment. The great majority of cases of OHSS present with symptoms belonging to the category of moderate OHSS. In addition to the presence of ascites, visible on ultrasound, the patient's complaints are usually limited to mild abdominal pain and distension, and hematological and biochemical parameters are normal.

Moderate OHSS

- A single grade: patients may experience discomfort, pain, nausea, abdominal distension; no clinical evidence of ascites, but ultrasonic evidence of ascites and enlarged ovaries, normal hematological and biological profiles. This grade of OHSS can be treated on an outpatient basis with extreme vigilance.

Severe OHSS

- Grade A: dyspnea, oliguria, nausea, vomiting, diarrhea, abdominal pain; clinical evidence of ascites plus marked distension of the abdomen or hydrothorax; ultrasound scan shows large ovaries and marked ascites; normal biochemical profiles. Can be treated as an inpatient or outpatient, depending on the physician's comfort, the patient's compliance and medical facilities.

Manual of Intrauterine Insemination and Ovulation Induction, ed. Richard P. Dickey, Peter R. Brinsden and Roman Pyrzak. Published by Cambridge University Press.

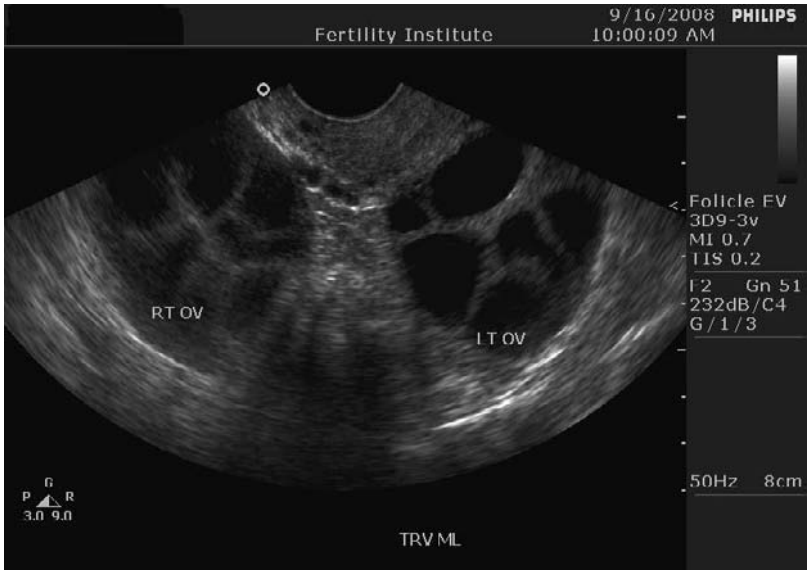

Figure 15.1 Bilateral ovarian enlargement in patient with mild–moderate OHSS. Fertility Institute of New Orleans 2008.

- Grade B: all symptoms of grade A, plus massive tension ascites, markedly enlarged ovaries, severe dyspnea and marked oliguria; biochemical changes in the form of increased hematocrit, elevated serum creatinine and liver dysfunction. Should be treated in an inpatient hospital setting with expert supervision.
- Grade C: OHSS complicated by respiratory distress syndrome, renal shut-down or venous thrombosis. This is a critical condition and must be treated in an intensive-care setting.

Characterization of patients at risk of severe OHSS

Several studies have attempted to collect and analyze data in order to characterize the patient population at risk for OHSS and to define risk factors for developing this syndrome [7]. More recently, the epidemiology of OHSS has been studied in large series by three groups of investigators from Belgium, Israel and Egypt with the same two objectives [3,8,9]. Patient factors found to increase the risk of OHSS include younger age, low body mass index (BMI), previous history of OHSS, and WHO II anovulation, especially polycystic ovary syndrome (PCOS) [10]. OHSS occurred equally in primary and secondary infertility, and was not increased in patients with hyperinsulinism or insulin resistance [11].

Treatment factors that influence the incidence of OHSS

Iatrogenic factors that increase the risk of OHSS include the use of gonadotropins in long protocols, and clomiphene for 10 days instead of five days. The number of days that gonadotropins are administered is apparently more important than the dose or type, whether urinary-derived human menopausal gonadotropin (hMG) and follicle-stimulating hormone (FSH) or recombinant FSH [7]. OHSS is somewhat more frequent when gonadotropin-releasing hormone (GnRH) agonists and antagonists, instead of gonadotropin alone, are used; but the difference is most likely to be due to the increased number of days of stimulation in GnRH agonist cycles, compared to non-GnRH cycles. The duration of OHSS is longer and its expression is more severe when pregnancy ensues, especially when there are multiple conceptuses [7].

Prevention of ovarian hyperstimulation syndrome

Rizk in 1993 suggested "Ten Commandments" for the prevention of OHSS [12]. These consisted of identifying patients at risk, using treatment other than gonadotropins for PCOS patients, such as metformin and ovarian diathermy, and the use of low doses and GnRH antagonists when gonadotropins were necessary. A second "Ten Commandments" addressed the

secondary prevention of OHSS and included withholding or delaying hCG, follicular aspiration, switching to in-vitro fertilization (IVF) with cryopreservation of all embryos, and progesterone for luteal-phase support [7]. Many of these measures have been already described as methods to reduce high-order multiple births (HOMB) in Chapter 14. Other measures unique to the prevention of OHSS are: use of GnRH antagonists instead of agonists to prevent premature luteinizing hormone (LH) surge, decrease in the dose of hCG, use of LH or GnRH agonist in place of hCG for triggering ovulation, administration of albumin, use of glucocorticoids and administration of dopaminergic drugs.

GnRH antagonist as an alternative to the long agonist protocol

In a recent Cochrane review, the efficacy of GnRH antagonist was compared to the long agonist protocol in assisted conception [13]. In comparison with the long GnRH-agonist protocol, there was no statistically significant reduction in the occurrence of severe OHSS (RR 0.50, OR 0.79, 95% CI 0.22–1.18); however, there were significantly fewer pregnancies with GnRH antagonist (OR 0.79, 95% CI 0.63–0.99). In a review which compared the two GnRH antagonists cetrorelix and ganirelix to the long GnRH-agonist protocol, a significant reduction of OHSS was observed in cetrorelix studies (OR 0.2, 95% CI 0.10–0.54) but not for ganirelix (OR 1.13, 95% CI 0.24–5.31) [14]. The pregnancy rate in the cetrorelix studies was not significantly different from that achieved with the long GnRH-agonist protocol (OR 0.91, 95% CI 0.68–1.22). The pregnancy rate in the ganirelix protocols was significantly lower than that in the long GnRH-agonist protocol (OR 0.76, 95% CI 0.59–0.98). The final word has not been said in relation to the development of OHSS in GnRH-antagonist cycles; further studies will clarify this situation.

Cancelling cycles to avoid OHSS

Withholding hCG is the most common method used to prevent OHSS in patients predicted to be at high risk of developing OHSS [15]. Estradiol criteria for withholding hCG to prevent OHSS are similar to those for preventing HOMB, except for a wider range of 800–4,000 pg/mL. Nearly all authorities recommend withholding hCG and advising couples to abstain from coitus when estradiol is ≥ 4,000 pg/mL. Most believe the OHSS risk is increased when estradiol is ≥ 3,000 pg/mL. Equal or more important criteria than estradiol levels for cancelling cycles are the number of 8–10 mm follicles that may have acquired LH receptors. These recommendations are only valid for estrogen concentrations measured by radioimmunoassay (RIA). Estradiol concentration results measured by chemiluminescence (CHL) will be approximately 1.22 times greater, and estradiol measured by monoclonal antibody will be approximately 1.50 times greater than when measured by RIA. Therefore, the critical levels of 3,000 and 4,000 pg/mL for RIA are equal to 3,660 and 4,880 pg/mL for CHL, and 4,500 and 6,000 pg/mL for monoclonal antibody.

Coasting or delaying hCG administration

Coasting, or delaying hCG administration to prevent OHSS, is similar to coasting to avoid multiple pregnancy, with the possible exception of the estradiol criteria. In gonadotropin cycles, coasting should begin (no more FSH should be administered) when the estradiol concentration is ≥ 1,500 pg/mL if the lead follicle is less than 15 mm. Estradiol concentration ordinarily doubles every two days, and lead-follicle diameter increases 2 mm per day once lead follicles have grown to 8–10 mm and have acquired LH receptors. Estradiol levels ordinarily continue to rise for one additional day, and volume of the dominant follicle continues to increase for four days after FSH administration is discontinued [16]. The duration of coasting should be limited to less than four days to avoid the decreases in implantation and pregnancy rates that occur after longer periods of coasting. Pregnancy rates also decrease if estrogen concentration falls by 60% or more during coasting. Withholding administration of hCG until the estradiol level drops below 2,500–3,000 pg/mL has been deemed to be effective in lowering the risk of OHSS, without significantly reducing the possibility of pregnancy [7].

Decrease in hCG dosage, use of alternatives to hCG to trigger ovulation

There are few clinical trials published regarding the impact of the dose of hCG on the occurrence of OHSS [17], but there is a general consensus among clinicians that the dose of hCG should be halved when estradiol levels are ≥ 3,000 pg/mL. Alternatives to hCG to trigger ovulation include GnRH agonists, native GnRH and recombinant LH. In IVF cycles there is no evidence of a reduction in the incidence of OHSS, and the percentage of ongoing pregnancies is reduced, when ovulation has been triggered by other than urinary or recombinant hCG [7].

Intravenous albumin, hydroxyethyl starch solution

Experience in subjects with different forms of third-space fluid accumulation has shown that albumin is efficacious in preventing and correcting hemodynamic instability. However, a series of publications for and against the efficacy of albumin in preventing OHSS has been published with contradictory results. A multifactorial role of albumin in the prevention of OHSS has been proposed. First, it acts to sequester vasoactive substances released from the corpora lutea. Secondly, albumin also serves to sequester any additional substances which may have been synthesized as a result of OHSS. Finally, the oncotic properties of albumin serve to maintain intravascular volume and prevent the ensuing effects of hypovolemia, ascites and hemoconcentration. However, a Cochrane review found albumin not to be effective in preventing OHSS [18].

Hydroxyethyl starch (HES) is a synthetic colloid, a glycogen-like polysaccharide derived from amylopectin. It has been used as an effective volume expander and is available in several types of molecular weight with different chemical properties. Several small studies have suggested a beneficial effect of HES in decreasing OHSS, indicating that HES should be further investigated [8].

Glucocorticoid administration

The pathophysiology of OHSS suggests the involvement of inflammatory mechanisms during the development of the fluid leakage associated with OHSS. Therefore, investigators hypothesized that glucocorticoids could possibly prevent OHSS in patients at high risk. However, Rizk and others found no protective effect of intravenous glucocorticoids [2].

Dopamine agonists in the prevention of OHSS

Vascular endothelial growth factor (VEGF) secreted by the hyperstimulated ovary acting via the VEGF receptor 2 is a major cause of OHSS, and its specific inhibition prevents increased vascular permeability. Dopamine receptor 2 (Dp-r2) agonists, used in the treatment of human hyperprolactinemia, inhibit VEGFR-2-dependent vascular permeability (VP) and angiogenesis when administered at high doses in animals. The symptoms of OHSS have been successfully prevented with the dopamine agonist bromocriptine, and more recently with the Dp-r2 agonist cabergoline (Dostinex) 0.5 mg daily for eight days beginning on the day of hCG administration [7,19].

Treatment of ovarian hyperstimulation syndrome

The clinical course of OHSS depends on its severity, whether complications already have occurred, and the presence or absence of pregnancy (Fig. 15.2). The clinical management involves dealing with electrolytic imbalance, neurohormonal and hemodynamic changes, pulmonary manifestations, liver dysfunction, hypoglobulinemia, febrile morbidity, thromboembolic phenomena, neurological manifestations and adnexal torsion [7,8,20]. The general management will be adapted according to the levels of severity. Specific approaches, such as paracentesis and pleural puncture, should be carefully performed when necessary. Medical management requires familiarity with the disease. Many of the problems that occur happen as a result of lack of realization by internists and medical specialists that the syndrome is different from similarly presenting medical syndromes. A better understanding of the underlying pathophysiological mechanisms will help to refine the management.

Outpatient management for moderate OHSS

Based on the classification of Rizk and Aboulghar [6], moderate OHSS will be followed up by regular telephone calls, at least daily, and twice-weekly office visits. The assessment at the office includes pelvic ultrasound, complete blood count, liver function tests and coagulation profile. The patient should be instructed to report to the hospital if she develops dyspnea, if the volume of urine is diminished, or upon development of any unusual symptoms such as leg swelling, excessive abdominal distension, dizziness, numbness and neurological problems.

Outpatient management of severe OHSS

The question of whether severe OHSS should be managed on an outpatient basis depends on the classification and definition of severity, comfort of the physician, and compliance and reliability of the patient. OHSS grade A is treated by aspiration of ascitic fluid, administration of intravenous fluids and evaluating all biochemical parameters on an outpatient basis.

In-hospital management of severe OHSS

Patients with severe OHSS grade B and C are admitted to hospital for treatment. Indications for hospitalization

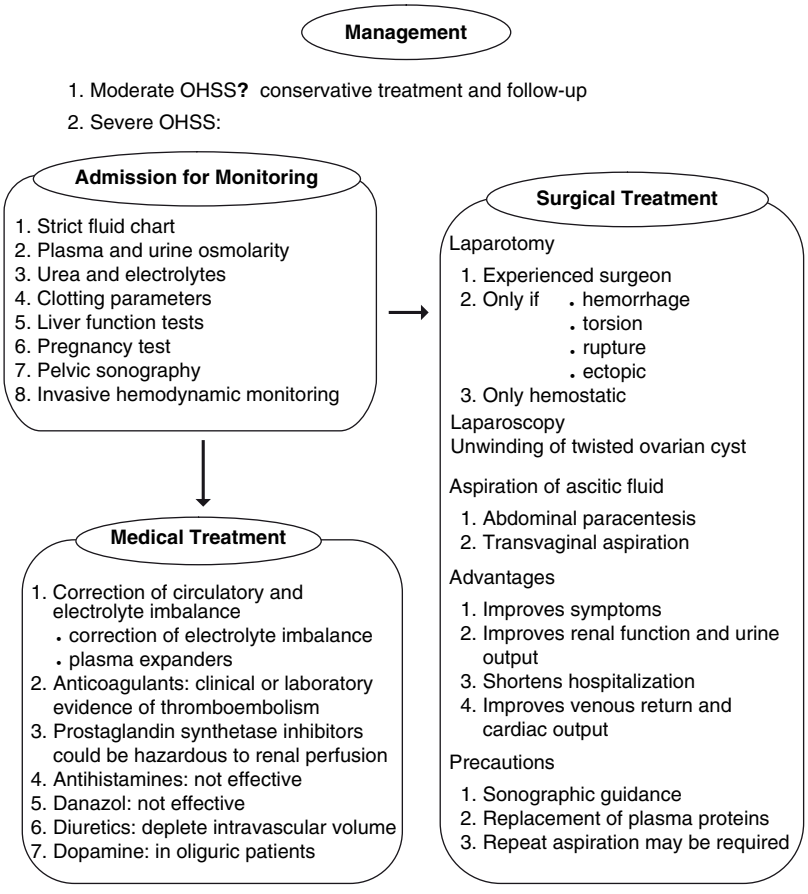

Figure 15.2 Management of OHSS. Adapted from Rizk 2006 [7].

are shown in Table 15.1. Hospitalization should be considered if one or more of these symptoms or signs are present [21]. Great caution is required in all grades, because complications can occur suddenly.

Clinical and biochemical monitoring in hospital

The patient's general condition requires regular assessment, with documentation of vital signs, together with daily weight and abdominal girth measurement. Strict fluid balance recording is needed, particularly of urine output.

Biochemical monitoring should include serum and electrolytes, renal and liver function tests, a coagulation profile and blood count. Serum and urinary osmolarity and urinary electrolyte estimation may be required as the severity of the disease process increases. Respiratory compromise and/or significant deterioration of renal function require evaluation of blood gases and acid–base balance. The frequency of these investigations will depend on the severity of the condition.

Ultrasonographic examination provides accurate assessment of ovarian size and the presence or absence of ascites, as well as pleural or pericardial effusions. Ultrasound will also help in the diagnosis of intra- or extrauterine pregnancy, as well as multiple or heterotrophic pregnancy. Chest x-ray will provide information on the presence of hydrothorax. Assay of β-hCG will help to diagnose pregnancy as early as possible.

Invasive hemodynamic monitoring (central venous pressure and pulmonary artery pressures) may be needed under certain circumstances, when volume expanders are being employed.

Medical treatment

Circulatory volume correction

The main line of treatment is correction of the circulatory volume and the electrolyte imbalance. Every effort should be directed towards restoring a normal intravascular volume and preserving adequate renal function. Fluid replacement should begin with intravenous crystalloid fluids at 125–150 mL per hour. Normal

Table 15.1. Indications for hospitalization in OHSS

Severe abdominal pain or peritoneal signs
Intractable nausea and vomiting that prevents ingestion of food and adequate fluids
Severe oliguria or anuria
Tense ascites
Dyspnea or tachypnea
Hypotension (relative to baseline), dizziness or syncope
Severe electrolyte imbalance (hyponatremia, hyperkalemia)

Adapted from Rizk 2006 [7].

saline and Ringer's lactate solution have been used successfully. Plasma colloid expanders may be used if necessary. One concern with using plasma expanders is that the beneficial effect is transitory before their redistribution into the extravascular space, further exacerbating ascites formation. Albumin has been utilized at 200 mL of 25% albumin solution. Dextran, mannitol, fresh frozen plasma, and hydryoxyethyl starch (HES) have also been used. HES has the advantage of a non-biologic origin and high molecular weight (200–1,000 kDa vs. 69 kDa for albumin). HES 6% and 10% have been used successfully, but larger prospective randomized controlled studies are needed [7].

Electrolyte replacement

Appropriate solutions will correct electrolyte imbalances. If hyperkalemia is significant, a cation exchange resin may be needed. Sodium and water restriction have been suggested, but other investigators found no change in the patient's weight, abdominal circumference or peripheral edema when sodium and water were restricted. Therefore, salt and water restriction are not widely advocated [7].

Anticoagulants

Anticoagulant therapy is indicated if there is clinical evidence of thromboembolic complications or laboratory evidence of hypercoagulability. Venous thrombosis is the most common serious complication of OHSS. Prophylactic treatment with heparin should be used whenever there is a high risk of thromboembolism [8]. In cases of severe OHSS, the following situations are recognized as indicating an increased risk of thromboembolism: immobilization, compression of pelvic vessels by large ovaries or ascites, pregnancy coagulation abnormalities and hyperestrogenemia. Prevention using mobilization and antithrombosis stockings are insufficient, as thrombosis may occur at all localizations and may be systemic in nature.

Anticoagulant prophylaxis

Prophylaxis with heparin remains debatable, since there are no randomized studies proving its efficacy in preventing thromboembolic complications during severe OHSS. In some clinical scenarios, thromboembolism still occurs despite giving heparin [8]. Despite these reservations, Rizk recommends giving heparin or a low molecular weight heparin such as enoxaparin for patients with severe OHSS [7]. The incidence of deep-vein thrombosis is markedly increased in patients with Leiden factor V mutation, one of the thrombophilias [22]. Others are protein C and S deficiency, and antithrombin III deficiency. Leiden factor V mutation occurs in 4% of northern European women. Patients should be questioned about a history of personal or familial thrombosis, and, if positive, should be tested for Leiden factor V mutation. If OHSS occurs, patients with Leiden factor V mutation should be placed on prophylactic heparin.

Duration of anticoagulation

The duration of anticoagulant administration is debatable. Some investigators have reported late thrombosis up to 20 weeks post-transfer, and many investigators are in favor of maintaining heparin therapy for many weeks [7]. The severity of OHSS must be separated from the risk of thromboembolism because intrinsic coagulopathy may trigger thrombosis, even in moderate cases. However, those who have followed a more liberal policy for prophylaxis have had to deal with operating on ruptured ectopic pregnancies in anticoagulated patients. Therefore, thromboembolism will remain a more difficult complication to prevent and may complicate the outcome.

Antibiotics

Infections are not uncommon in the setting of OHSS treatment, because of frequent catheterizations, venepuncture, transvaginal aspiration of ascitic fluid and pleural drainage. Furthermore, hypoglobulinemia is present in severe cases. Preoperative antibiotic prophylaxis is highly recommended. Some authors have also suggested the administration of immunoglobulins; however, this intervention still awaits further evaluation [8].

Diuretics

Diuretic therapy without prior volume expansion may prove detrimental, since it causes further contraction

of the intravascular volume, thereby worsening hypotension and its sequelae. Diuretics will increase blood viscosity and increase the risk of venous thrombosis. Diuretic use should be restricted to the management of pulmonary congestion.

Dopamine

Dopamine used in oliguric patients with severe OHSS results in significant improvement in renal function [23,24]. Dopamine produces its renal effect by increasing renal blood flow and glomerular filtration rate. Dopamine therapy should be given cautiously and under strict observation. In one report, intravenous dopamine 4.32 mg/kg per 24 hours beginning within 10 hours of admission was administered to seven patients hospitalized with severe OHSS following gonadotropin stimulation for IVF or gamete intrafallopian transfer (GIFT) [23]. Additional treatment consisted of: bed rest, restriction of fluid intake to 500 mL/day, daily monitoring of urine output and measurement of abdominal girth and weight. Biochemical and hematological clotting factors were measured daily in the pregnant women. Serum hCG was measured every two days, and the patients were given a protein- and salt-rich diet in order to increase the oncotic and osmotic blood pressure. Dopamine treatment was continued until there was complete resolution of ascites. In the five patients who were pregnant, dopamine treatment was required for 9–22 days. The duration of treatment was related to the magnitude of the increase of hCG, with the longest (18–22 days) in patients with triplets, the shortest (9–10 days) in patients with a singleton pregnancy, and intermediate (14 days) for patients with twins. In the two non-pregnant women, dopamine was only required for seven days. In another report, a 750 mg tablet of docarpamine was taken orally every eight hours by 27 patients, hospitalized because of OHSS and refractory to the initial therapy with intravenous albumin [24]. Clinical symptoms associated with ascites were gradually improved; there were no major adverse effects.

Indomethacin

Indomethacin has been investigated as an inhibitor of prostaglandin synthesis, which might play a role in the pathophysiology of OHSS. In clinical practice, no clinical improvement in ascites formation followed use of indomethacin in severe OHSS patients. Furthermore, oliguria and renal failure have been attributed to indomethacin in cases of OHSS in the absence of hypovolemia, by interfering with the renal perfusion. Finally, there is a theoretical risk to the fetus when indomethacin is used in significant amounts in early pregnancy [7].

Aspiration of ascitic fluid and pleural effusion in severe OHSS

The presence of ascites is the hallmark of OHSS, and symptoms resulting from ascites are the most common reason for hospitalization. Aspiration is not indicated in every patient. Paracentesis by the transabdominal or transvaginal route is indicated for severe abdominal pain, pulmonary compromise as demonstrated by pulsoximetry, tachypnea and renal compromise as demonstrated by oliguria and increased creatinine concentration. Paracentesis constitutes the single most important treatment modality in life-threatening OHSS not controlled by medical therapy.

Abdominal paracentesis

Paracentesis is followed by increased urinary output shortly after the procedure, with a concomitant decrease in the patient's weight, leg edema and abdominal circumference. The creatinine clearance rate is also increased following the procedure. Paracentesis offers temporary relief of respiratory and abdominal distress, but, since the fluid tends to recur, some patients need repeated paracentesis and drainage of effusions before spontaneous improvement occurs. The amount of fluid aspirated can range between 200 and 4,000 mL. The risk of injury to the ovaries can be minimized by ultrasonographic guidance. Monitoring of plasma proteins is essential, and human serum albumin should be infused whenever necessary. Percutaneous placement of a pigtail catheter is a safe and effective treatment modality for severe OHSS that may represent an alternative to multiple vaginal or abdominal paracentesis [7].

Transvaginal ultrasound-guided aspiration

Transvaginal ultrasound-guided aspiration is an effective and safe procedure. Injury to the ovary is easily avoided by puncture under ultrasonic visualization. No anesthesia is required for the procedure, and better drainage of the ascetic fluid is accomplished because the pouch of Douglas is the most dependent part.

Autotransfusion of ascitic fluid

Transvaginal aspiration of ascitic fluid and autotransfusion of the aspirated fluid has been used in treatment of severe OHSS. The procedure is simple, safe and

straightforward, and shows a striking physiological success in correcting the maldistribution of fluid and proteins, without the use of heterogeneous biological material. However, autotransfusion of ascitic fluid is not recommended, because of the possible reinjection of cytokines into the circulation.

Pleurocentesis and treatment of pulmonary complications

Evaluation and treatment of patients with severe OHSS complaining of dyspnea includes physical examination, chest ultrasound and x-ray and arterial blood gases. It is essential to evaluate accurately any pulmonary complications that may result in hypoxia. If a pulmonary embolus is suspected, a computed tomography (CT) scan or ventilation perfusion scan should be performed. Pulmonary comprise should be treated with oxygen supplementation. Thoracocentesis may be necessary for patients with significant hydrothorax. However, a dramatic improvement in the clinical status may occur after paracentesis.

Adult respiratory distress syndrome (ARDS)

ARDS is encountered after fluid overload. The importance of a strict fluid input/output balance in patients with moderate complications of OHSS is stressed. Optimum management may require admission to an intensive-care unit. ARDS subsides after 3–6 days with fluid restriction, forced diuresis and dopamine therapy [8].

Pericardiocentesis

Pericardial effusion is life-threatening. It occurs rarely, but if it does, drainage by specialists may be necessary [8,20].

Surgical treatment

Anesthetic considerations in OHSS patients

Surgery is infrequently needed, but if it is required there are several aspects which are important for the anesthesiologist. Careful positioning of patients during surgery is important, as the Trendelenburg position may further compromise the residual pulmonary functional capacity. Establishment of access lines may be necessary in patients with contracted vascular volume. Drainage of pleural effusions may assist in improving pulmonary status.

Surgery for ruptured cysts

Laparotomy, in general, should be avoided in OHSS. If deemed necessary, in cases of hemorrhagic ovarian cysts, it should be performed by an experienced gynecologist, and only hemostatic measures should be undertaken, so as to preserve the ovaries.

Ovarian torsion

Ovarian torsion is an infrequent complication of ovulation induction which, if unrecognized and untreated, results in the loss of one or both ovaries. Presenting symptoms are severe unilateral adnexal pain in a patient with enlarged ovaries due to ovarian stimulation or with multiple pregnancy. Sonography with Doppler flow analysis can be diagnostic, but a finding of apparently normal blood flow does not rule out ovarian torsion. In a review of 80 cases of ovarian torsion at a single hospital, Doppler flow sonography was performed preoperatively in 90% of cases, but demonstrated compromised blood flow in only 29% of those scanned [25]. Oophorectomy was performed in 39 cases (48%). Seventeen (21%) patients had ovarian torsion following OI (11 after OI for IVF, 3 after OI with gonadotropins for IUI, 3 after clomiphene); of these only 1 (6%) required oophorectomy. Twenty-four patients were pregnant at the time the diagnosis was made, 10 spontaneously and 14 following OI. The mean gestational age was 10.4 weeks (range 4–28) at the time of ovarian torsion. Although the adnexa usually appear dark, hemorrhagic and ischemic, they can be saved if the diagnosis is made soon enough by simply unwinding the torsion, often as a laparoscopic procedure.

Surgery for ectopic pregnancy associated with OHSS

The association between OHSS and ectopic pregnancy is not commonly encountered. Diagnosis of tubal pregnancy by vaginal ultrasound examination at this stage is not always possible. The presence of large ovaries filling the pelvis makes ultrasound scanning of other structures difficult. Fluid in the pouch of Douglas has no diagnostic significance in the presence of ascites.

Other surgery

Mesenteric resection after massive arterial infarction has been reported. Rarely, vascular surgery is required to treat thromboses that are complicated by recurrent emboli or resistant to medical intervention. Posterolateral thoracotomy and subclavian arteriotomy and thromboarterectomy by the Fogarty technique have

been reported. Inferior vena cava interruption to prevent massive thromboembolism has also been used [7].

Pregnancy termination

Pregnancy termination, performed in extreme cases, has been reported to improve the clinical picture of neurological, hematological and vascular complications [7].

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Chapter 16

The psychological issues of intrauterine insemination

Rebecca J. Trimble

Introduction

It is important for the physician to recognize the psychological issues involved in fertility treatment. The emotions and attitudes of the couple can be powerful, conflictual and problematic in making decisions regarding the treatment options facing them. The physician must be aware of and understand the patients' feelings/beliefs in order to guide the couple in a direction that will provide a positive outcome.

Intrauterine insemination using husband/partner sperm

A recommendation of intrauterine insemination (IUI) can be difficult for the couple to accept, even if the husband's or partner's sperm is to be used. This relatively low level of intervention may be recommended after a complete fertility evaluation and several months of a couple trying unsuccessfully on their own to achieve a pregnancy. The medical reason for recommending IUI may relate to poor fertility status of the male sperm or poor fertility status of the female reproductive environment, or both.

If the medical diagnosis of infertility rests with the man, he may experience feelings of guilt and doubt about his potency in not being able to impregnate his partner through normal sexual intercourse. Such a diagnosis may affect his sense of masculinity. He may experience feelings of anger because he cannot achieve something, without medical intervention, that should be a natural expression of physical intimacy. The woman may also feel anger and/or resentment toward her partner if the problem lies with his sperm.

If IUI is recommended because of a condition in the woman, she may experience feelings of guilt and inadequacy, and a general lowering of self-esteem. A woman may feel that her body is defective and has let her down. She, too, may resent the fact that a baby cannot be produced naturally in a romantic or intimate setting. Her partner may feel either generalized anger about their problem or anger toward her for not being able to conceive. The sexual relationship of the couple may also be negatively affected as the effort to become pregnant becomes more and more like work. However, the sexual issues for the couple do generally resolve after fertility treatment ends.

If the physician is informed about, and is sensitive to the emotional/psychological issues that may arise in treatment, he or she will be better able to identify and openly discuss them with the couple. If indicated, they should be referred for professional counseling, preferably to someone who specializes in fertility work, and/or to a spiritual counselor, if religious reasons are getting in the way of accepting insemination as an option for achieving pregnancy.

Intrauterine insemination using donor sperm

The psychological issues described previously regarding IUI using husband/partner sperm also apply to couples who must consider using donor sperm to conceive. In addition, there are other more complex emotional/psychological issues that must be addressed when donor sperm is required.

When a couple is told they must use donor sperm in order to get pregnant, it is usually a severe emotional blow. The inability to produce a child who is genetically half his and half hers is a very difficult concept for many infertile couples to accept. From an emotional standpoint, the first order of business is to grieve the loss of the biological child. As in any grieving process, the stages may include initial denial or disbelief, "bargaining" with God, deep sadness, intense anger, and finally

Manual of Intrauterine Insemination and Ovulation Induction, ed. Richard P. Dickey, Peter R. Brinsden and Roman Pyrzak. Published by Cambridge University Press.

acceptance. The length of time for resolution varies with each couple, but the grief should be dealt with before moving forward to select a donor. As a general rule, three months should be allowed for resolution of the grief associated with the loss of hope for a biological child. This time may vary depending on the couple and how slowly or quickly they feel ready to consider other options for family creation.

The notion of giving birth to a child who does not look like its putative father or a blend of father and mother can result in deep sadness for the couple. The fantasized child is gone. For a man, it can stir feelings of severe inadequacy, both generally and sexually. Because fertility in men has mistakenly been associated with virility historically, there has been a code of secrecy about the use of donor sperm to protect the male image. Fortunately, through better education and information, some of these myths have been broken, and now many couples are showing a willingness to be more open and honest, no longer ashamed about their infertility. The use of both donor sperm and ovum for purposes of family creation is growing and becoming more widely accepted.

Many sperm banks will provide the couple with a list of anonymous donors with profiles, including basic information such as ethnic background, eye color, hair color, complexion, height, weight, religion, educational pursuits, blood type, hobbies, interests etc. Usually couples wish to find a match that is as close as possible in physical characteristics so that the offspring will resemble a blend of both parents. If they find a match they are comfortable with, they may also choose to reserve extra sperm straws for future attempts to have full siblings.

Some couples may not feel comfortable proceeding with the use of a sperm donor. They may feel it gives the mother an unfair advantage in a relationship with the child for him/her to be genetically connected to the mother but not to the father. The man might feel left out, and this could lead to a sense of emotional distancing from his partner. The woman could feel an inordinate sense of emotional power in the marriage, especially if the marital relationship is already competitive in nature. Some couples turn to adoption as their choice for family creation because they feel on a more equal footing in their relationship with the child. Others may decide upon childfree living if adoption is not an option. The literature has suggested that, if genetic continuity is the primary goal, then use of a donor may not be the best choice. On the other hand, if parenting is the primary life goal, use of a donor may be a very good choice for family creation. For the woman, being able to experience pregnancy and birth, and having the option to breastfeed, are considered significant benefits of donor insemination. For many couples, having a baby by donor insemination results in a wonderful outcome to a challenging fertility issue. It is wise, however, for physicians to recommend pretreatment counseling to explore the complex emotional issues surrounding use of a donor for family creation.

Use of an unknown donor

In many ways, using an anonymous donor is less complicated than using a known donor. Complex family or interpersonal dynamics may be more easily avoided. However, use of an anonymous donor has its own complexities. If a medical issue arises with the child, pertinent information may not be available. When the child is older, he/she will likely be curious about his/her genetic origins, just as most adopted children are. This places the burden on the parents to decide whether they want their child to be able to obtain information about his/her genetic father when he/she gets older, or if they wish the genetic parent's identity to be anonymous and, therefore, completely protected and unavailable to them and/or the child.

Making that decision is even more complex with the internet and the wealth of information it makes available. There are now several internet donor registries for children born of sperm donation. These websites are intended to assist offspring searching for biological fathers or half-siblings, parents looking for medical information, and donors willing to provide information to offspring who seek it. Just as there are registries for connecting adopted offspring and biological parents, registries are also arising to assist those born of anonymous sperm donation.

There are also now sperm banks whose donors agree to allow their identities to be divulged to their offspring at the age of 18, if the child so wishes. Whatever decisions a couple makes about secrecy versus disclosure and anonymous donor versus known donor, the couple should consider not only their own wishes, but also what will be in the best interest of their child at different stages of his or her life.

It is important to remember that curiosity about one's genetic origins is natural. A child will ask questions about his/her genetic history as he/she is growing up; therefore, a parent can expect to be in a position at some point of either telling the truth or lying (by

omission or commission) to the child. If at the age of 30 or 40 the adult offspring of a donor inadvertently finds out that "Dad" is not his or her biological father, it may feel as if the parents have been engaged in a lifelong deception. This can be a crushing blow, and the trust in the parent–child relationship may be severely damaged.

If the relationship between parent and donor child is healthy and strong, and if the child should meet the genetic father at an appropriate age, the primary bond will likely remain with the parents who raised him/her (Daddy and Mommy). Once the genetic curiosity is satisfied, there may be no need for a continuing relationship with the genetic parent. If a relationship does develop, it will more likely resemble that of an acquaintance, a friend or a relative. More and more donor parents are able to understand their child's innate curiosity about his/her genetic origins, and are not threatened by the possibility that questions may arise as the child grows older.

Most of the psychological literature regarding donor sperm recommends that the parents consider telling the truth to a child about his/her genetic origins, just as we tell the truth to children who are adopted. As in adoption, the earlier the age and the more simple and loving "the story," the easier it is for the child to accept this information about him/herself. The older the child, the more shocking the information, and the more difficult it is to deal with and emotionally integrate. Adolescence is a particularly difficult time to share such powerful information, because developmentally the child is already in a state of "healthy rebellion." The developmental task of adolescence is separation/individuation, or separating emotionally from one's parents in order to form an individual identity.

In family systems theory, secrecy is referred to as "the burden of secrecy." Secrecy tends to denote shame and generate dysfunction. Parents should not communicate overtly or covertly to an offspring that use of donor sperm is anything shameful. Rather they should strive to help the child understand how proud they are of him/her and how grateful they are to the donor for his willingness to help them in fulfilling their dream to have the child. In the final analysis, however, each couple must make their own decision about secrecy/disclosure, taking into account their beliefs and the beliefs of the family and culture in which they live.

Use of a known donor

Use of a donor who is known to the couple (such as a brother or a friend) often results in more complex emotional/psychological issues for the couple. It is possible for uncomfortable sexual thoughts/fantasies to arise regarding the genetic combination of father/mother, particularly if the experience is perceived by parties involved more emotionally than scientifically. It can be quite difficult to look across the table at Christmas dinner and see one's child who looks exactly like Uncle Jimmy, the sperm donor, rather than "Daddy." And, if it is a family secret, a great deal of anxiety can surround the matter. The more open and honest a couple is, the easier it is to find acceptance and move proudly forward with the beautiful child Uncle Jimmy helped Daddy and Mommy create. Of course, each couple must make decisions about secrecy/disclosure, depending upon their own feelings and family dynamics. Unfortunately, some families can be very negative and judgmental about such matters. What will be in the best interest of the child should always be the guiding concern. It is universally acknowledged that if anyone outside the couple is told about the use of a donor to conceive, the child must be told in order to protect him/her from the possibility of hearing such powerful information from someone other than the parents. When a known donor is being considered for family creation, it is highly recommended that all parties involved meet with a counselor as a part of the decision-making process, to explore their feelings and discuss the complicated issues that may arise from this experience.

The insemination procedure

After a donor is chosen and it is time to prepare for the insemination cycle, the physician will prescribe the protocol (medications, ultrasounds, blood work etc.) that in his/her medical opinion will optimize the chances for pregnancy. The time frame leading up to the insemination can be quite stressful on the couple in regard to medical demands, juggling work and social schedules, dealing with family, health insurance, financial concerns and more. Couples often experience a great deal of anxiety leading up to the procedure, and even more while waiting for the results of the pregnancy test.

If husband/partner sperm is used, it can be difficult and embarrassing to produce a specimen "on demand" at the medical facility. There may be times when the man cannot produce under pressure and has to do so at home and bring the specimen in prior to the procedure.

If donor sperm is used, the man may experience feelings of sadness and being left out. The woman may

feel anxious and strange as she prepares to be inseminated with another man's sperm.

In the treatment room, the woman is usually accompanied for the insemination by her partner. Once the sperm specimen is ready and she is in position for the procedure, the physician may invite the partner to participate, and after placing the catheter, allow him to push the sperm directly into the uterus. This participation may help him feel more a part of the experience and give the couple a sense that they are accomplishing this together. Following the procedure, the woman may be asked to remain on the examination table for a brief period. Then, the couple is sent home to wait for the pregnancy test date. Depending on other fertility concerns, the woman may be told to resume normal activity, or in some cases bed rest may be recommended.

The pregnancy test

A positive pregnancy test generally produces a response of both jubilation and relief on the part of the couple. If the pregnancy is strong and continues, for the couple who used IUI with husband/partner sperm it is like a dream come true, and generally the fact that IUI was required to conceive becomes insignificant as the excitement about the pregnancy and anticipation of the birth of a biological child becomes the focus of attention.

When donor sperm is used, the feelings about having used a donor tend to wax and wane over the course of the nine-month pregnancy. Donor concerns tend to diminish for some couples and become more prevalent for others as the excitement and anticipation of the birth of the baby draws near. Most often, the excitement of birth and family creation ultimately overshadows the donor issue. The donor experience is typically accepted and integrated into the life of the couple by the time of delivery. Despite curiosity about what the baby will look like, once there is a healthy baby to love, most other concerns tend to fade.

When the donor is anonymous, the feelings of the couple are different than when a known donor is used. With use of a known donor, the issues of family or friendship connections may become more paramount before or after birth. Although a couple can try to anticipate how they might feel about having a baby by a known donor, they can never know for sure until the baby is born. If psychological/emotional issues become more troubling for a couple during the pregnancy, delivery or postpartum periods, the physician should recommend professional counseling.

If the pregnancy test is negative, tremendous sadness on the part of the couple about the failure of the procedure may be the immediate and predominant emotional reaction. After the initial disappointment is addressed, however, the couple should meet with the physician to review the cycle and discuss recommendations for future treatment. Sometimes it is advisable for the couple to take a break between cycles to recover from the stress and disappointment of a previous cycle. Pacing is an important part of coping with a prolonged fertility treatment experience. Patience and understanding on the part of the physician are of paramount importance in helping the couple cope with the highs and lows of treatment.

Single women and intrauterine insemination

In modern society many women are pursuing advanced education and careers, postponing decisions regarding marriage and children. Women in their thirties and forties who have not married (whether by choice or through lack of opportunity) are having to face the fact that female fertility wanes significantly after age 35. A single woman may decide to try to have a biological child before her fertility time line runs out. IUI using anonymous sperm donor is a viable option to consider, and may be sought through a local fertility clinic. Or a woman may ask a male friend for a sperm specimen and attempt a self-insemination procedure at home.

From a legal perspective, it is safer to use an anonymous sperm donor, which allows the single mother to have unchallenged parental rights and prevents potential legal complications by a known party's involvement. For some single women, having a child by anonymous donor can prove to be a very gratifying method of family creation. However, it is a difficult job to raise a child as a single parent and, in considering this decision, certain reality factors must be addressed. It is important that a woman be financially able to provide for the child, and that the woman have a good support network of family and/or friends to help with the child when assistance is needed. It is likewise important for a single mother to make certain there are positive male influences in the child's life, regardless of the child's gender. A US-based organization called Single Mothers by Choice offers support and information to women who are considering having a child as a single parent.

The lesbian couple and intrauterine insemination

Lesbian couples are also approaching fertility clinics requesting IUI using anonymous sperm donation to create a family. Gays and lesbians are adopting and fostering more often, and lesbian women are choosing to have biological children with their supportive partners. As gratifying as having a child may be to a lesbian couple, there may also be difficult legal ramifications. In most states of the USA the birth mother is the legal parent and the lesbian partner may have no legal rights to the child. If at a future time the couple should separate, the child and the non-biological parent could be denied a continuing relationship by the biological parent. Some states, however, allow for second-parent adoptions, where the non-biological same-sex parent is allowed to adopt the child.

Many of the non-traditional methods of family creation previously described are so new that we do not yet have a great deal of information available about how these children fare emotionally and psychologically as adults in the modern world. The one universal truth that we do know, however, is that children who grow up in a loving, supportive home environment tend to grow into happier, healthier adults, regardless of the circumstances of their birth.

Conclusions

IUI, using partner or donor sperm, can offer a relatively low-level, less invasive and less expensive form of fertility treatment. The result of a healthy pregnancy through IUI and the joys of birth and family creation can put an end to the previously heartbreaking struggle of infertility. Countless thousands of families worldwide have benefited from IUI. Although there are potential emotional and psychological complications, they can be minimized by couples being offered and (hopefully) accepting proper professional counseling before embarking on treatment and, if needed, while a child is growing up.

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ACCESS (Australia's National Infertility Network): www.access.org.au.

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Infertility Network (United Kingdom): www.infertilitynetworkuk.com.

Infertility Resources in Europe: www.fertilityeurope.eu.

International Consumer Support for Infertility: www.icsi.ws.

RESOLVE: The National Infertility Association (United States): www.resolve.org.

Single Mothers by Choice (United States): www.singlemothersbychoice.com.

Chapter 17

Ethical, legal and religious considerations of artificial insemination

Kathryn Venturatos Lorio

Introduction

As assisted means of reproduction have enabled childless couples and individuals to enjoy parenthood, they have also raised ethical, legal and religious questions. The physician facilitating the creation of these new families must be aware of the issues raised and the potential areas of controversy that might affect the patient's decision of whether and how to undergo infertility treatment.

Terminology

As one explores the subject of intrauterine insemination (IUI), one should be aware of the various terms that may be used to explain the same procedure. Although "intrauterine insemination" is the modern term for the insertion of sperm into a women's reproductive tract by a means other than sexual intercourse, the term "artificial insemination" is commonly used in court opinions, legislation and religious documents to describe the same procedure. Other terms that are also used to convey the technique are "assisted insemination" and "alternative insemination" [1]. Thus, when researching the legal or religious treatment of the procedure, one must be prepared to approach the subject using various terms.

Guidelines, regulations and commissions

Various sources of guidance or regulation are available [2]. Partially due to the controversial aspects of the questions involved, the method of dealing with the issues may vary from state to state and country to country. Guidelines, rather than statutes, provide the overseeing of assisted reproduction in many nations [3:S9–12]. Self-regulation by physicians or other professionals often provides recommended standards. In the United States, which lacks comprehensive national legislation regarding assisted reproduction, such guidelines are provided in the form of committee reports, opinions or bulletins issued by professional organizations such as the American Society for Reproductive Medicine (ASRM) or the Society of Assisted Reproductive Technology (SART) [4]. Noncompliance with the standards may be discovered through on-site inspections, and sanctions may result in suspension or cancellation of programs from the organization or denial of reimbursements when certain policies are not followed [4]. Among the advantages of this type of regulation is that those making the policies are generally among the most well-informed and current in their field. Additionally, this approach offers a flexibility and opportunity to change at frequent intervals when circumstances warrant a new approach. Also, due to the expertise involved in drafting the policies, they are often more exact and precise in their methodology. For example, the ASRM recommends that both anonymous donors and donors known to the receiving patient undergo the same screening processes, whereas the Food and Drug Administration (FDA) requires such screening only for anonymous sperm donors [5].

Another way to establish policies for use of assisted means of reproduction is to create interdisciplinary boards or commissions charged with the task of establishing norms. Examples include the Warnock Committee in the United Kingdom, the Waller Commission in the state of Victoria, Australia, and the Law Reform Commission of Canada. Due to its open nature and the input of various constituencies, this approach lends itself to consensus, and frequently results in the adoption of legislation.

A more limited approach is one of administrative regulation, often dealing with issues of licensing and public health. Generally not applicable to private disputes, this avenue lends itself more to issues of consumer protection and narrow government intervention. One such regulation is the 1992 Fertility Clinic

Manual of Intrauterine Insemination and Ovulation Induction, ed. Richard P. Dickey, Peter R. Brinsden and Roman Pyrzak. Published by Cambridge University Press.

Success and Certification Act in the United States [6], which requires that clinics supply information regarding success rates, but limits sanctions against the noncompliant to being listed as not reporting or removal from membership in professional organizations.

Some countries have been quite thorough in their dealings with these issues, having adopted comprehensive legislation covering issues such as accessibility of assisted reproduction to patients, health and record-keeping requirements, recognition of filiation between the intended parent and the child, and prohibitions and sanctions for performing certain procedures. In contrast, other jurisdictions merely provide indirect control by granting or withholding funding, insurance coverage or protective legislation to participants [2].

Although the degree of legislation varies, at least 59 countries, including the United States and the United Kingdom, have statutes governing the use of assisted reproductive technologies. In other nations, guidelines provide the source of regulation (Table 17.1) [3].

Once laws or guidelines are set in motion, many nations provide for surveying adherence to the norms through the establishment of a licensing body. Adherence to statutes and guidelines is conducted through the submission of periodic reports and/or on-site inspection. Sanctions for non-compliance include withdrawal of licensing, fines, imprisonment, and possibly closure of the facility [3:S11–12].

Countries with licensing bodies include Austria, Canada, France, Germany, Greece, Hungary, Israel, Korea, the Netherlands, Norway, Russia, Saudi Arabia, Slovenia, Spain, Sweden, Switzerland, Taiwan, Tunisia, Turkey, the United Kingdom and Vietnam. Countries with licensing bodies that administer the process through periodic reporting include Austria, France, Germany, Greece, Hungary, Israel, Korea, the Netherlands, Norway, Russia, Slovenia, Spain, Sweden, Switzerland, Taiwan, Tunisia, Turkey and Vietnam. Periodic reports are also administered in Belgium, Bulgaria, Denmark, Hong Kong, Italy and Latvia.

On-site inspections are conducted in Greece, Hong Kong, Hungary, Korea, the Netherlands, New Zealand, Russia, Slovenia, Switzerland, Taiwan and the United Kingdom [3:S11–12].

Access

Access to assisted reproduction varies from locale to locale. In some countries there are no particular requirements to use assisted reproductive technology. In others, only married couples may avail themselves of the treatments. Some countries allow only those in a stable or committed relationship, with varying definitions of such commitment. Varying from having no requirement to requiring marriage or a stable relationship as a prerequisite to access to assisted reproductive technologies, most countries favor some sort of commitment between the parties seeking to avail themselves of the technologies (Table 17.1) [3:S17–18].

Nations with no requirements include Australia, Belgium, Brazil, Bulgaria, Canada, Korea, Latvia, Mexico, the Netherlands, New Zealand, South Africa, Spain, Thailand, the United Kingdom and the United States [3:S18].

Countries in which being married is required by statute in order to gain access to the technology include Hong Kong, Taiwan, Tunisia and Turkey; in China, Egypt, Japan, Lithuania, Morocco, the Philippines and Singapore this prerequisite is stipulated in guidelines [3:S18].

Countries with statutes requiring either a married or stable relationship include Austria, the Czech Republic, Denmark, France, Germany, Greece, Hungary, Israel, Italy, Norway, Russia, Slovenia, Sweden and Switzerland; in Argentina, Chile, Croatia, India and Ireland this prerequisite is stipulated in guidelines [3:S18].

Some countries require couples wishing to undergo fertility treatments to first demonstrate their infertility or the probability that, without such aid, genetic diseases would be inherited by their offspring [7–11]. France allows the use of medically assisted procreation on the request of a parent "in order to remedy infertility, the pathological nature of which has been medically ascertained or in order to avoid the transmission to the child of a particularly serious disease" [7]. Iceland's artificial fertilization law provides that "artificial insemination with donor sperm may only be carried out if the fertility of the male partner is impaired, if he has a serious hereditary disease, or if there are other medical reasons" [8]. Portuguese law on medically assisted procreation allows for the use of medically assisted procreation "in the case of infertility or in order to treat a serious disease or prevent the risk of transmission of genetic, infectious, or other diseases" [9]. In Russia, donor sperm may be used in cases of "infertility, ejaculatory and sexual disorders or unfavorable medico-genetic prognosis for men and absence of sexual partner for women" [10].

Many of the patients seeking IUI today are single women. In nations with no restrictions on recipients,

Table 17.1. Intrauterine insemination laws in various nations

Country	Governance					Requirements for couples	National health plan	Posthumous conception		
	Statute or law	Guidelines	None	Licensing body	Penalties for non-compliance			Allowed	Not allowed or not used	No data or not mentioned
Argentina		x				Stable relationship			x	
Australia		x				No requirements. Permitted for single women and lesbian couples	Yes	x		
Austria	x			x	Fines	Marriage or stable relationship	Partial	x		
Belgium	x					No requirements. Permitted for single women & lesbian couples	Complete	x		
Brazil		x				No requirements. Permitted for single women & lesbian couples				x
Bulgaria	x					No requirements	Partial	x		
Canada	x			x	Fines, imprisonment or both	No requirements. Permitted for single women & lesbian couples				x
Chile		x				Stable relationship. Permitted for single women				x
China		x				Marriage			x	
Colombia			x			Stable relationship				x
Croatia		x				Marriage or stable relationship	Yes			x
Czech Republic	x					Marriage or stable relationship	Partial			x
Denmark	x				Fines	Marriage or stable relationship	Partial		x	
Ecuador			x			Stable relationship			x	

Table 17.1. *Continued*

Country	Governance					Requirements for couples	National health plan	Posthumous conception		
	Statute or law	Guidelines	None	Licensing body	Penalties for non-compliance			Allowed	Not allowed or not used	No data or not mentioned
Egypt		x				Marriage			x	
France	x			x	Same as previous law	Marriage or stable relationship	Complete		x	
Germany	x			x		Marriage or stable relationship	Partial		x	
Greece	x			x	Criminal, civil penalties including fines, and imprisonment	Marriage or stable relationship. Permitted for single women	Complete	x		
Hong Kong	x				HRTO § 39	Marriage			x	
Hungary	x		x	x	Possible withdrawal of license	Marriage or stable relationship. Permitted for single women	Partial			x
India		x				Marriage or stable relationship. Permitted for single women		x		
Ireland		x				Stable relationship				x
Israel	x			x		Marriage or stable relationship. Permitted for single women and lesbian couples		x		
Italy	x				€600, 000 fine, cancellation of license	Stable relationship			x	
Japan		x				Marriage	Yes		x	
Jordan			x			Marriage			x	
Korea	x			x	Cancellation of license, suspension of business. Fine, imprisonment	No requirements			x	

Latvia	x						No requirements. Permitted for single women					x	
Lithuania	x						Marriage					x	
Malaysia				x			Marriage				x		
Mexico	x						No requirements					x	
Morocco			x				Marriage				x		
Netherlands	x				x	Loss of license	No requirements. Permitted for single women and lesbian couples		x				
New Zealand	x					Penalties dependent on acts	No requirements. Permitted for single women and lesbian couples		x				
Norway	x				x	Fine, imprisonment, withdrawal of license	Stable relationship				x		
Peru				x			Permitted for single women				x		
Philippines			x				Marriage				x		
Portugal				x			Stable relationship	Partial				x	
Romania				x			Not an issue					x	
Russia	x				x	Withdrawal of license	Marriage or stable relationship. Permitted for single women					x	
Saudi Arabia	x				x	No response	No information					x	
Singapore			x				Marriage				x		
Slovenia	x				x	€209 to 20, 820 fine	Stable relationship				x		
South Africa			x				No requirements. Permitted for single women and lesbian couples		x				
Spain	x				x	Detailed in Statute	No requirements. Permitted for single women.		x				

Table 17.1. *Continued*

Country	Governance					Requirements for couples	National health plan	Posthumous conception		
	Statute or law	Guidelines	None	Licensing body	Penalties for non-compliance			Allowed	Not allowed or not used	No data or not mentioned
Sweden	x			x	Loss of license to perform IVF	Stable relationship	Complete		x	
Taiwan	x			x	Suspension of license for ART	Marriage			x	
Thailand		x				No requirements				x
Tunisia	x			x	Imprisonment, fine	Marriage	Partial		x	
Turkey	x			x	Closure of center	Marriage	Partial			x
United Kingdom	x			x	Depends on act. Center could be closed	No requirements. Permitted for single women and lesbian couples	Partial	x		
Uruguay			x			Stable relationship			x	
United States		x				No requirements. Permitted for single women and lesbian couples				x
Venezuela			x			Not an issue			x	
Vietnam	x			x	Fines	Marriage. Permitted for single women				x

Compiled from tables originally published in Jones HW, Cohen J, eds. International Federation of Fertility Societies surveillance 07: a survey of the current status of assisted reproductive technology procedures around the world. *Fertil Steril* 2007; **87** (4, Suppl 1): S1–67 [3]. With permission.

access is restricted only through custom, clinical preference or practicality due to the denial of insurance coverage in some instances. In the United States, there are no laws prohibiting single women from using these services, and although the Ethics Committee of the ASRM recommends that programs treat all requests for services equally without regard to marital status or sexual orientation, many insurance companies have refused elective coverage [12]. Hospitals (pursuant to the Hill–Burton Act, 42 U.S.C. § 291), clinics and individual physicians in the United States who refuse to perform IUI in single women risk costly and time-consuming legal challenges [13–15]. In 1992, the Canadian Royal Commission reported that in a survey of practitioners 72% refused insemination treatments to lesbians [16]. In Canada, where no requirements are set by legislation, a decision by the British Columbia Supreme Court in 1996 finding a doctor's refusal to treat lesbians to be unlawful discrimination based on sexual orientation helped to change a pattern of many practitioners refusing insemination to lesbians. In nations with requirements as to recipients, the results vary. Some, including Chile, Greece, Hungary, India, Israel, Peru, Russia and Vietnam, allow single women to be treated; others, even those recognizing registered partners, do not permit access to lesbians. These restrictions are one of the reasons for fertility tourism, as couples travel to Belgium, Finland, Greece, Spain and the United States, where treatment is accessible [3:S17].

Even where participants are not barred directly by statute from using assisted reproductive technologies, the practicalities of funding may present roadblocks. Although most nations do not provide coverage, in other countries statutes provide for complete or partial coverage of permitted services through national health plans. These include Belgium, France, Greece, Israel, Slovenia and Sweden. Partial coverage is offered in Austria, Bulgaria, the Czech Republic, Denmark, Germany, Hungary, Italy, the Netherlands, New Zealand, Norway, Spain, Tunisia, Turkey and the United Kingdom [3:S15]. In the United States, there is no national health plan. However, fourteen states have legislation requiring health insurance companies to either cover or offer coverage for infertility diagnosis and treatment [17].

Intrauterine insemination with husband's sperm

IUI using husband's sperm raises the fewest legal, moral and ethical considerations, since the procreation takes place within the family unit. However, even that method raises religious objections due to the "unnatural" nature of the procedure. Although early cases raised questions as to whether conception of a child by husband insemination constituted consummation of the marriage [18], issues of filiation are not raised since the husband is the biological father of the child. Possible issues of consent to such insemination by the husband have arisen in situations where the sperm is extracted from an ill man, and have also arisen in the context of a husband contesting the payment of support obligations [19]. Also, even where a husband initially consented to such insemination and his sperm has been cryopreserved, the issue of how long his consent remains effective may arise. With the introduction of intracytoplasmic sperm injection (ICSI), requests for IUI by the husband have reduced, making this procedure among the least likely to raise legal dilemmas.

Use of donors

More difficult questions are raised when the husband's sperm is not used in the insemination, either because of a fertility problem or because use of the husband's sperm may increase the chance of the resulting child having a particular genetically transmitted disease. In Costa Rica, use of donor sperm is prohibited by a constitutional amendment [20]. In some nations, the use of donor sperm is prohibited by laws or guidelines [3:S29–30]. In others, mostly for protection of resulting offspring, there are restrictions on donors. For example, age restrictions have been imposed in a number of countries, such as Russia, which requires donors to be between the ages of 20 and 40 years [10], and India, where donors must be between the ages of 21 and 45 [3:S28]. Countries and jurisdictions prohibiting by statute the use of donor sperm for other than in-vitro fertilization (IVF) include Hong Kong, Italy, Slovenia, Tunisia and Turkey; in Croatia, Egypt, Lithuania, Morocco and the Philippines, the prohibition is provided in guidelines [3:S28–30].

Screening of donors

Most jurisdictions where donor sperm may be used require screening of donors and/or sperm. Among the most common requirement is that, in order to protect against the transmission of the human immunodeficiency virus (HIV), syphilis and hepatitis, sperm must be cryopreserved and quarantined for six months and then tested prior to insemination [10,21]. In Spain, donors must undergo a health assessment that analyzes

their phenotypic and psychological characteristics and includes an assessment as to whether the donor suffers from any genetic, hereditary or infectious diseases that could be transmitted to offspring [22]. The responsibility of verifying proper screening may be on the clinic or medical facility at which the insemination takes place [22]. Due to concerns of incest, many countries put limits on the number of children that may be born of the same donor. Taiwan prohibits a man with a previous history of donation to donate sperm to achieve a live birth. Austria imposes a limit of three couples per donor [3:S28]. In France, gametes from the same donor may not be used to deliberately result in the birth of more than five children [23]. Spain allows a maximum of six children to be born from the gametes of the same donor [22]. In Russia, the birth of 20 children from the same donor per 800,000 residents is considered sufficient to discontinue the use of the specific donor in that region [10].

Record keeping

Due to the mandate on reporting success rates in the United States, in order to be certified, clinics must meet the standards set by the Centers for Disease Control and Prevention (CDC). In order to help clinics meet those standards, the Practice Committees of SART and ASRM have published guidelines to assist clinics in ensuring that they have proper personnel with necessary expertise and that record-keeping procedures be established regarding the identity of participants, outcome of procedures and dispositions of gametes [24]. Additionally, the guidelines suggest the full documentation in the patient's medical record of the communication by the physician of the risks, benefits and alternative procedures, including alternatives that would result in the patient not undergoing treatment at the physician's facility. Accompanying this documentation should be the written informed consent of the patient prior to the performance of any medical procedures.

Payment of donors

A question that is answered differently in various nations is whether or not the donor of sperm may be paid for the sperm. In most nations, donors are not paid. In Germany, for example, payment is prohibited with criminal penalties [25]. In Spain [22], Finland [26] and France [27,28], legislation mandates that the donation of gametes be non-remunerative. Legislation passed in Canada in 2004 also prohibits the purchase or sale of gametes [3:S33]. Since gamete donation has traditionally been viewed as a gift from one couple to another, France also requires the consent of the donor's spouse to the donation [29]. Minimal expenses, such as for travel, are permitted in South Africa, whereas in the United States, Japan and Russia, payment is usually made, albeit minimal in the case of Russia [30].

Donor anonymity and informing the offspring

Donor anonymity also varies from nation to nation. Traditionally, donation had been anonymous to protect the physician, donor and intended parents. That is still the case in many nations where anonymity of the donor is protected [3:S33–6]. However, due to genetic and identity concerns for the child, many nations, concerned about psychological and medical problems of the offspring [31], have altered their positions on anonymity [3:S33–6]. The United Kingdom, which originally provided for anonymity in the Human Fertilisation and Embryology Act in 1990, reversed its position in 2006 in an effort to afford protection to the child. The Act now requires that information on their gamete donors should be available to the resulting children when they reach the age of 18 [3:S33]. The Czech Republic requires that healthcare institutions providing assisted reproductive technology maintain anonymity of the donor and infertile couple, but mandates release of health information to the child upon the request of a member of the infertile couple prior to the procedure, a legal representative of the child born of the procedure, or the adult child him- or herself [32]. Many other countries also require, by statute, guidelines or custom, that such information be available on request to offspring at a certain age (generally 18) [3:S33–6]. Other countries allow for the actual identity of the donor to be revealed. Although the United States lacks legislation on the subject, there has been a trend favoring the revealing of identity and information about donors to resulting offspring and offering a dual-track system where patients can choose donors who have either elected anonymity or wish to be known [33].

Countries in which anonymity is protected by statute include Denmark, France, Israel, Latvia, Switzerland, Taiwan, Turkey and Vietnam; those in which it is protected by guidelines include Argentina, Lithuania, the Philippines and Singapore; and it is protected by custom in others, including Ecuador, Peru, Uruguay and Venezuela. Countries in which

non-identifying information about the donor is provided on request (where recruitment is limited to donors who are willing to identify themselves to offspring at maturity), by statute, include Canada, Germany, Greece, Hong Kong, Hungary, the Netherlands, New Zealand, Norway, Slovenia, Spain, Sweden and the United Kingdom; and by guidelines Australia and South Africa. Countries that require the identity of the donor to be revealed include, by statute, Austria, Germany, the Netherlands, New Zealand, Norway, Spain (if life-threatening), Sweden, Switzerland and the United Kingdom; by guidelines, Australia (where gamete donors are required to consent to the release of such information) and South Africa [3:S34–5].

Related to the question of anonymity is the issue of whether offspring should be informed of the method by which they were conceived. Although this is ultimately a decision of the parents, the trend is toward more openness concerning the subject, and physicians should be prepared for inquiries from donor offspring [34]. The Ethics Committee of the ASRM recommends that written policies be adopted by physicians and clinics to deal with such inquiries [35]. Obviously, this is related to the issue of proper maintenance of records concerning donors. It is also recommended that these concerns be raised with patients in advance and agreements be reached as to the release of donor information in the future.

Family donations

More issues are raised when family members are selected as donors. In addition to the advantage of maintaining a genetic connection between the intended parent and the child, family donations also offer the benefit of reducing costs. However, such donations offer their own set of complications, raising issues of filiation and potential psychological problems if the donor and child have frequent social contact. Although generally regarded as ethically acceptable, the appearance of incestuous connections must be avoided. Accordingly, consanguineous donations from first-degree relatives are considered unacceptable. In any case, it is advised that family members undergo counseling and that each engage the advice of independent legal counsel to avoid conflicts of interest [36].

Filiation of the offspring

When sperm is provided by a donor to a married woman, the filiation of the child may be at issue. Early cases considered a child conceived with donor sperm to be an illegitimate product of an adulterous relationship, as exemplified by the Canadian case of *Orford* v. *Orford* [37], and the later Illinois case of *Doornbos* v. *Doornbos*, which had a similar result even though the husband had consented to the procedure [38]. These early cases have been replaced by the more enlightened theory that since there is no sexual intercourse between the donor and the woman in the process of IUI, no adultery has taken place [39] and any child born of such a procedure to a married woman with the husband's consent is deemed the legitimate child of the husband and wife [40]. Legislation in most countries now provides that the husband or partner of the mother be considered the father of any child conceived through intrauterine donor insemination with the husband or partner's consent [22,41–43]. In the United States, where each state may have different laws, most states that have statutes addressing the subject, provide the same [44]. Likewise, the Uniform Parentage Act, a model act adopted by many states, has similar provisions [45].

In addition to the relationship of the child to the husband or partner, there remains the question of the relationship of the donor to the child, both as to his obligations to the child and his rights to a place in the child's life. Countries that have statutes on the subject generally release the donor from any rights or responsibilities to the child [42,46–48]. The same is true of the treatment in the Uniform Parentage Act and individual states in the United States [44,45]. About half of the states have adopted the 1973 version of the Uniform Parentage Act, which absolves donors of legal parenthood provided the recipient is married and the semen is provided to a licensed physician for use in artificial insemination. The 2000 version of the Act, which has been adopted by Washington and Texas, does not contain the marriage and physician requirements [49] .

Surrogacy

Quite controversial is the subject of traditional surrogacy, involving the IUI of the sperm of the male partner wishing to parent into a third-party surrogate. In such a case, the surrogate, who would be the biological mother of any child conceived and born of the insemination, agrees to surrender the child to the man who supplied the sperm and his partner. This form of surrogate motherhood has met with great disapproval, as it is viewed as abandonment by the mother, and baby selling in instances where the surrogate receives a payment.

With the advent of IVF and embryo transfer, gestational surrogacy, wherein the surrogate carries an embryo that does not possess her genetic material, raises other legal questions. A medical reason for gestational surrogacy is indicated when the woman who provides the egg is unable to carry a pregnancy to term and her husband is fertile. It is also possible that either the egg, the sperm or both come from donors and the embryo has no genetic material from the couple who pays for the IVF procedure and gestational surrogacy. The subject of gestational surrogacy is beyond the scope of this book, but it will be addressed briefly since some of the discussion pertains to both types of surrogacy.

The issue of payment to the surrogate has raised questions of "human dignity, the instrumentalization of the human body, potential exploitation of vulnerable women and inappropriate inducement (coercion) of women," leading the European Society of Human Reproduction and Embryology (ESHRE) Task Force on Ethics and Law to conclude that "altruistic surrogacy is the only acceptable form" [50]. The Task Force further advised that the only acceptable payments would be those for medical expenses not reimbursed by insurance, and other pregnancy-related expenses, as well as actual (as opposed to potential) income lost. Additional ethical concerns relate to the relinquishment of autonomy by the surrogate and issues of informed consent by the surrogate, her family (including her partner) and the commissioning parents.

The law generally presumes that the husband of a mother is the father of any children born or conceived during the marriage. Thus arrangements must be made for a married surrogate's husband to disavow any relationship to the child. Additionally, the surrogate mother must surrender the child for adoption to the commissioning parents. The question of whether a woman should be able to contract, prior to pregnancy, for the surrender of her child poses serious public policy concerns. Most nations frown upon such arrangements. As early as 1984 the United Kingdom's Warnock Report recommended that surrogacy arrangements be discouraged. The Surrogacy Arrangement Act of 1985, as amended by the Human Fertilisation and Embryology Act of 1990, was based partially on this conclusion. The Act makes it illegal to advertise for surrogacy and restricts payment to "reasonable expenses," while not recognizing surrogacy arrangements as binding contracts [51]. Disapproval of surrogacy by legislation by various nations may range from not recognizing the efficacy of the contract [9,22] to banning the procedure completely [52,53]. Russia, however, both allows for the use of donor gametes and embryos in surrogacy programs and permits a small remuneration for the surrogate's services, as well as actual expenses incurred [10]. There is no uniform legislation on surrogacy in the United States [54]. Some state statutes ban payment of surrogates. Other states void the contracts. A small minority of states recognize surrogacy agreements as valid, and provide for judicial preauthorization.

States with surrogacy laws include Alabama, Arizona, Arkansas, the District of Columbia, Florida, Illinois, Indiana, Iowa, Kentucky, Louisiana, Michigan, Nebraska, Nevada, New Hampshire, New York, North Dakota, Tennessee, Utah, Virginia, Washington, West Virginia, Wisconsin and Wyoming [54]. Payment of surrogates is banned in Arizona, the District of Columbia, Florida, Kentucky, Michigan, Nevada, New Hampshire, New York, Utah, Virginia and Washington [54]. Contracts for surrogacy are void in Arizona, the District of Columbia, Indiana, Kentucky, Louisiana, Michigan, Nebraska, New York, North Dakota, Utah, Virginia and Washington. States that recognize surrogacy agreements as valid and provide for judicial preauthorization include Illinois, New Hampshire, Tennessee and Virginia [54].

Posthumous conception

Another controversial practice is that of posthumous reproduction, posing a conflict between two ethical principles, personal autonomy of the persons desiring to reproduce and beneficence as affecting the welfare of the child [55]. National laws on this subject vary. The ESHRE Task Force on Ethics and Law finds posthumous use of sperm by couples to be acceptable if there is prior written consent by the deceased partner [56], as does the Ethics Committee of the ASRM in the case of cancer patients [57]. Many nations are not as approving. In Greece, postmortem reproduction is permitted with court approval only if the deceased spouse or partner had suffered from a disease that either could affect fertility or endanger his life and had also consented in a notarized document to the postmortem fertilization [11]. Spain recognizes no legal relationship between a deceased man and a child subsequently born of his sperm unless the reproductive material was in the mother's uterus at the time of the man's death [22]. Similarly, France [7] and Switzerland [52] require that both members of a couple be alive at the time of any assisted reproduction. Denmark requires the destruction of sperm on the donor's death [58].

In the United States, there is no prohibition on the use of the sperm of a deceased man. However, cases have arisen as to whether or not the resulting children may be recognized as the decedent's. Many of these cases have arisen in the context of claims on behalf of the children to their biological father's insurance benefits. Results have been mixed [59–64]. The Uniform Parentage Act was amended in 2002 to allow for the posthumously conceived child of a deceased parent to be recognized as the child of the deceased parent if "the deceased spouse consented in a record that if assisted reproduction were to occur after death, the deceased individual would be a parent of the child" [65]. This provision was adopted by Colorado [66], Delaware [67], Texas [68] and Washington [69]. Two states – Louisiana [70] and California [71] – have explicitly recognized such children for purposes of inheritance under specific circumstances.

Religious perspectives

In addition to the legal restraints on the use of IUI, one must also consider the religious teachings that influence patients as they seek assistance with infertility. It is important to realize, however, that nations with the same religion may have vastly different laws due to the influence of history, politics and cultural differences. Although the Catholic perspective is manifest in the assisted reproduction legislation in some traditionally Catholic nations (such as Italy) [72], religion and legislation do not always correspond so closely. Other nations with large Catholic populations (such as Spain) are much more open to assisted reproduction [22]. An even larger discrepancy may be observed in Greece, where the vast majority of citizens are Eastern Orthodox and the recent legislation on medically assisted reproduction is much more liberal than the tenets espoused by the Church [73].

Christian

Catholic

Within the Christian sect, views on assisted reproduction vary considerably. The most conservative view is that of the Roman Catholic Church. The papal instruction *Donum Vitae* (Respect for Human Life) issued on February 22, 1987 [74] was authored by then Cardinal Ratzinger (Pope Benedict XVI) after wide consultation by episcopates. The instruction bases its judgment on two "fundamental" criteria: "the life of the human being called into existence and the special nature of the transmission of human life in marriage." Heterologous insemination is seen as violating the rights of the child, depriving him of his "filial relationship with his parental origins and can hinder the maturing of his personal identity." It is also condemned as depriving "conjugal fruitfulness of its unity and integrity." Also not morally justified is the artificial insemination of unmarried women, including widows. Surrogate motherhood is viewed in the same light.

Homologous artificial insemination is also considered unacceptable, as it severs the "inseparable connection, willed by God and unable to be broken by man on his own initiative, between the two meanings of the conjugal act: the unitive meaning and the procreative meaning" [74]. Just as the use of contraception separates these two aspects of marriage, so too does homologous artificial insemination. Thus, obtaining sperm through masturbation is not approved. However, if the gathering of sperm is done in such a way that the "technical means is not a substitute for the conjugal act but serves to facilitate and to help so that the act attains its natural purpose," the procedure is considered acceptable [74]. The instruction clearly states that "marriage does not confer upon the spouses the right to have a child, but only the right to perform those natural acts which are per se ordered to procreation" [74]. In speaking to the question of legislation, the instruction states that it is "part of the duty of the public authority to ensure that the civil law is regulated according to the fundamental norms of the moral law in matters concerning human rights, human life and the institution of the family" [74].

Eastern Orthodox

The Eastern Orthodox Church makes a clear distinction between homologous insemination and heterologous insemination. Even though semen may be procured by masturbation for the purposes of homologous insemination, since the purpose is for procreation, "there seems to be no serious objection on moral grounds" [75]. However, the use of a third party in the procreation process, whether for heterologous insemination or surrogacy, is strictly disapproved of. Also, posthumous conception is viewed as unacceptable, as is the insemination of single women, since "to honor the 'sacredness' of life requires that the transmission of life be accomplished by the 'one flesh' relationship of two persons joined in a 'monogamous, heterosexual, blessed conjugal union'" [75].

Protestant

Protestant denominations have a liberal attitude toward assisted reproduction [76]. Most denominations

accept IUI and IVF with spouse gametes and no embryo wastage; however, some find IVF with donor gametes and surrogacy objectionable. Christian Scientists oppose the use of drugs and surgical procedures, but not artificial insemination. Seventh Day Adventists oppose donor insemination but not the use of drugs, and do not condone surgery that might require blood transfusion.

Jewish

The Jewish tradition emphasizes the Biblical commandment to "be fruitful and multiply" [77]. Celibacy is not condoned [78]. Although ejaculation should be vaginally contained to avoid *hash'-chatat zerah*, or destruction of the seed, a majority of halakhists who interpret the Jewish code of conduct of *Halakha* conclude that homologous insemination is permissible, although it is recommended that the manner of collecting sperm should be discussed with a halakhic authority prior to treatment [79]. It is noteworthy that the objection is not related to the unnatural nature of the act, but to the possibility of wasting seed.

The issue of heterologous insemination has raised more controversy. Although some rabbis condemn it as morally reprehensible, most authorities do not view it as full-blown adultery (*mamzerut*) under Jewish law [79]. A major concern is the possibility of incest. One rabbi offered the controversial suggestion that the incest problem could be avoided by the use of non-Jewish sperm. Indeed, in Israel many fertility clinics tout the availability of non-Jewish sperm as a special feature of the facility. Despite the moral objections raised to donor insemination, most authorities have not declared a ban on the use of non-Jewish donor sperm. The use of "traditional" surrogacy has not been sanctioned, leading some scholars to conclude that it is implicitly forbidden [80].

Islam

Viewed as an "encompassing" religion, Islam's instructions or *sharia* apply to many areas of human activity. The primary sources of the *sharia* are the *Qur'an*, containing the word of God as revealed to the prophet Mohammed, the *sunna* and *hadith*, which is a collection of traditions of the prophet "authenticated" by jurists, and *ijma*, the unanimous views of Islamic scholars and *qias*, which uses intelligent reasoning and analogy to deal with issues not mentioned in the *Qur'an* or *hadith* [81]. In the absence of guidance from the *Qur'an* or *hadith*, which are regarded as not open to interpretation, Islamic scholars use a process known as *ijtihad* to interpret proper behavior, and the conclusions are issued as *fatwas*.

The Grand Sheikh of Al-Azhar issued the first *fatwa* on assisted reproduction for Sunni Muslims, who constitute a majority of the Muslim world, on March 23, 1980 [81]. The *fatwa* concludes that, although intercourse is the basic way for sperm to impregnate as per Allah's will, if pregnancy cannot be achieved in this manner due to illness, then insemination of a woman with her husband's sperm is permissible. However, this is allowable only during the existence of a marriage; thus the possibility of posthumous conception is precluded. Donated sperm may not be used, as it "confuses" origins and is viewed as adulterous. Similarly, adoption is forbidden for reasons dealing with family origin; thus, the concept of surrogacy is also unacceptable.

In the 1990s, Ayatollah Ali Hoseyni Khamenei, successor to Ayatollah Khomeini, and spiritual leader of many Shi'ite Muslims, issued a *fatwa* permitting sperm donation [81]. However, in 2003, the Iranian parliament passed a law forbidding sperm donation, which resulted in a reversal of Ayatollah Khamenei's original *fatwa*. Egg donation, in contrast, was deemed permissible since, under Shi'ite Islam law, the husband could enter a temporary *muta* marriage with the egg donor, thus keeping the procedure within the marriage of the three involved parties [81]. Shi'ite authorities are not in accord on this issue.

Confucianism

One writer, theorizing as to the acceptability of donor insemination in China, posits that a traditional Chinese in a Confucian society, faced with sex without reproduction or reproduction without sex, would pick the latter. However, because of the reluctance of Chinese men to donate sperm, thinking that sperm contain the indispensable *jing* or *yuan qi* necessary for good health and life, the medical community is concerned about the possibility of incest due to the scarcity of donors [82]. Surrogate motherhood remains controversial. Some scholars promote a ban on surrogate motherhood that threatens the adoptive family due to its lack of confidentiality. Commercial surrogacy has been banned in the Hong Kong Special Administrative Region of China and proscribed by the Chinese Ministry of Health [82].

Buddhism and Shinto

With no dogma on the subject of assisted reproduction in the Buddhist and Shinto traditions, the opinions among priests are inconclusive, and are suspected to reflect those of their followers. In Japan, where 85% of the population claims links to both traditions [83], a Special Committee of the Health Science Council issued their report on ideal reproductive treatment using donor sperm, eggs and embryos in December 2000 [84]. The report permits donor insemination for married couples with an infertility problem, but prohibits either form of surrogate motherhood.

Hinduism

Hindu *dharma* or duty places an emphasis on family formation. Although infertility is viewed as part of the *karmic* cycle of life and perhaps a result of misdeeds, it may also be viewed as a "challenge to a higher calling," leading the Hindu couple to seek a remedy that is somewhat non-traditional [85].

Conclusion

Ethics, public policy and religious perspectives will undoubtedly affect the attitudes patients bring with them as they consult a physician on matters as intimate as the creation of a family. Additionally, despite sincere requests from patients, physicians must operate within the legal framework of the jurisdiction in which they are practicing. Thus, in addition to medical expertise, sensitivity to ethical, legal and religious landscapes may only serve to enhance the service rendered by physicians.

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Index