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# Experimental Treatment of Placental Insufficiency in Animal Model (by IGF-1)

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**Abstract** Introduction: Fetal growth restriction occurs in up to 10 % of pregnancies, and is one of the major causes of infant mortality. The primary cause of fetal growth restriction is placental insufficiency. We suggest that insulin-like growth factor-1(IGF-1) is effective in improving placental blood circulation and corrects fetal weight deficits in animal models. Materials and Methods: The experiment was conducted in 18 pregnant Wistar rats. The animals were divided into 3 groups (intact group- laboratory rats without PI receiving placebo ; control group- laboratory rats with PI receiving placebo; experimental group- laboratory rats with PI model receiving IGF-1 via subcutaneous injections. This study was carried out in strict accordance with the recommendations in the Guide of European convention for the Protection of Vertebral Animals. The protocol was approved by the Intercollegiate Committee on the Ethics of Animal Experiments (Association of Medical universities and universities for Pharmacy & Pharmacology: Permit Number: 11-10). All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering. Results: All rats without PI had spontaneous labor by the end of the pregnancy. In intact group, average number of newborn rats were 9,5±1,96 and the average weight of one newborn rats was 7,38±0,095 grams. In the control group all rats delivered hypotrophic fetuses. Number of newborns in broods and their body weight did not differ significantly in the experimental animal group comparing with intact group. The mean number of newborn rats in the control group was 10,0±1,05 with the mean body mass 7,44±0,138г. Offspring's number was 25 % higher in animals treated with IGF-1 compared with the intact group ($p \leq 0,05$). Discussion: Our method gave valid and reliable information proving subcutaneus insulin growth factor-1 administration reduced fetal growth restriction associated with placental insufficiency. Insulin growth factor-1 acts in response to such signals as nutrients, oxygen and hormones via the IGF receptors, and the insulin receptor. Deletion of IGF-1 gene leads to reduced birth weight.

***Keywords:*** *placental insufficiency, insulin growth factor-1, fetal growth restriction, placental blood circulation, animal models, placentome, uterine artery coagulation*

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## 1. Introduction

Fetal growth restriction occurs in up to 10 % of pregnancies, and is one of the major causes of infant mortality and morbidity. The primary cause of fetal growth restriction, when not attributable to structural or genetic defects of the fetus is placental insufficiency (PI).

In turn, PI often complicates pregnancy duration. While wide range of factors underlies this condition, nevertheless PI occurs when the placenta either does not develop properly or because it has been damaged.

PI is an answer of placenta for all sorts of unfavorable influence and fetal growth is reliant upon the proper placental growth and function. Maternal vascular pathology usually results in PI [4,5].

In vivo studies of the human placenta are difficult since this method is associated with risks to both the mother and fetus. Therefore, all we know about placenta function is a result of ultrasound investigation. Animal models reproduce human condition and allow us to study more thoroughly some aspects of pregnancy such as trophoblast development, placentation and placental transport to improve our understanding and facilitate development of effective medications [7,8].

Unfortunately, although different kinds of medicines have been proposed to manage PI no one turns out to be evidently effective to treat or prevent this condition. However, understanding mechanisms underlying PI and early recognition of such condition could help us to manage PI which seems to be most effective in the early stages of the disease [1,3]. Medications are not usually helpful if any abnormal changes have already developed in placenta and when they are accompanied with fetal growth restriction.

Clinical and experimental research suggested that the improved uteroplacental and fetoplacental blood circulation could prevent inappropriate fetal growth [2].

There are numerous growth factors, which regulate body processes by participating in cells growth, differentiation, haemopoesis, cells interaction, and angiogenesis and by increasing penetrability of placental vessels. Previous studies demonstrated that insulin-like growth factor-1 (IGF-1) might be effective for improving placental blood circulation and corrects fetal weight deficits in animal models [14].

IGF-1 - is a single chained polypeptide and one of the most essential growth factor. IGF-1 and growth hormone (GH) interacts with insulin to modulate its control of carbohydrate metabolism. These hormones do not act independently on growth process [3].

IGF-1 accelerates proteins biosynthesis and slows down their destruction in the placenta. IGF-1 also has the same structure as proinsulin. IGF-1 is an important fetal growth regulator. When fetal growth restriction develops concentration of this hormone decreases dramatically [3,4].

The suggestion was that IGF-1 treatment in experimental model of PI could prevent or minimize its development. In order to prove the hypothesis we created experimental PI model on rats and try to treat it by IGF-1.

# 2. Materials and Methods

The experiment was conducted in 18 pregnant Wistar rats. Their initial body mass varied between 200-250 grams. The rats had unlimited access to water and food and were under vivarium conditions. This study was carried out in strict accordance with the recommendations in the Guide of European convention for the Protection of Vertebral Animals. The protocol was approved by the Intercollegiate Committee on the Ethics of Animal Experiments (Association of Medical universities and universities for Pharmacy & Pharmacology: Permit Number: 11-10). All pregnant rats were divided into 3 groups.

**1 group (intact)** - 6 pregnant laboratory Wistar rats without PI receiving placebo (water was injected)

**2 group (control group)** - 6 pregnant laboratory rats with PI receiving placebo (water was injected)

**3 group (experimental group)** - 6 pregnant laboratory rats with PI model receiving IGF-1 via subcutaneous injections.

Female rats were placed with male rats during estrus-proestrus phase in ratio 1:1. The first day of pregnancy was considered if a mucus plug was detected in the vagina. After the rats got pregnant they were placed apart under standard conditions.

**Experimental model of PI:** On the 10-th day of pregnancy the laparotomy with bilateral uterine artery coagulation was performed to induce PI.

## 2.1. Surgery Stages

Under aseptic conditions and general anesthesia (solution of Zoletil 0,05 ml and solution of Rometar 0,04 ml and Atropini 0,1 %-0,02 ml) a lower midline incision for laparotomy was performed which is represented in Figure 1, Figure 2. Marked cyanosis in Figure 3 is a result of ischemia associated with relative blood deficiency (Figure 3).

We reconstructed all abdominal layers with uninterrupted vicryl suture and applied iodium on the restored skin afterwards.

**IGF treatment:** IGF-1 was administered subcutaneously in dose 0,8 mcg/kg animal weight one time per day for 7 days beginning on the 12-th day of pregnancy. The same placebo (water in the same volume) dose was given subcutaneously to the intact and control groups of animals.

**Outcome points:** The severity of PI and the effectiveness of IGF-1 were evaluated by the number of newborn rats in the broods and by newborn rats' body mass directly after their birth.

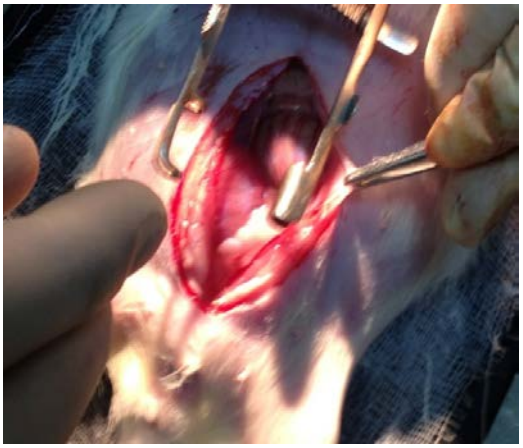

**Figure 1.** Laparotomy

*Under aseptic conditions and general anesthesia (solution of zoletil 0,05 ml and solution of rometar 0,04 ml and atropini 0,1 %-0,02 ml) a lower midline incision for laparotomy was performed.*

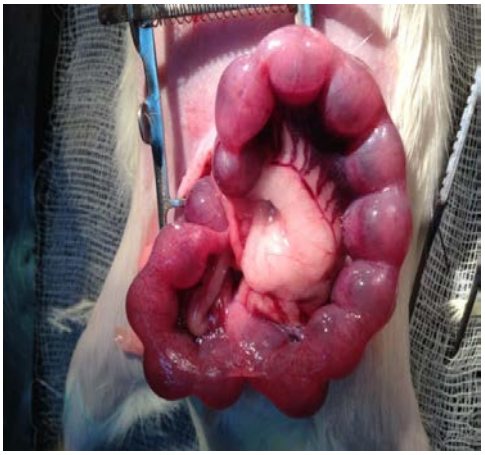

**Figure 2**. Pregnant bicornuate uterus before uterine artery coagulation

*Comparing to Figure 3 there is no visible cyanosis*

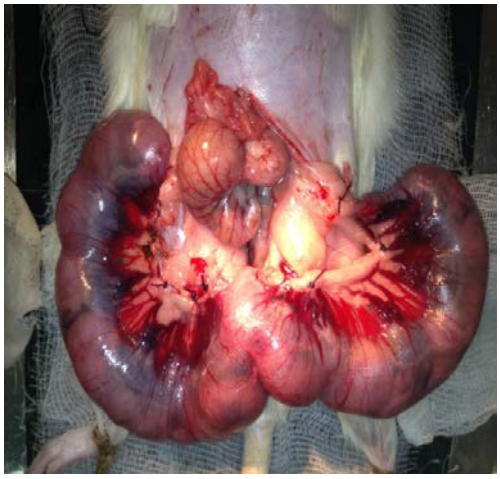

**Figure 3**. Uterine ischemia after uterine artery coagulation

*Marked cyanosis in Figure 3 is a result of ischemia associated with relative blood deficiency*

## 3. Results

It was demonstrated that all rats without PI had spontaneous labor by the end of the pregnancy which lasted for 22-25 days. In the 1-st (intact) group, average number of newborn rats in the broods were 9,5±1,96 and the average weight of one newborn rat was 7,38±0,095 grams. (Table 1).

In the 2-nd group all rats except one with experimentally induced PI receiving placebo delivered termly hypotrophic fetuses. One of the rat had premature labor on the 16 th day of gestation delivering 5 preterm newborns. Body mass of the 5 premature young rats was 0.94±0,1. Other 5 rats had spontaneous labor at term on the 22-25-th day of gestation. The mean number of newborn rats in broods was 8,0±1,97 and the mean body mass was 6,77±0,11 grams. It was revealed almost a 9% of body mass reduction comparing with rats in intact group.

**Table1. The effect of a 7 day subcutaneous IGF-1 administration in a dose of 0,8mkg/kg on the body mass and number of newborn rats**

| Parameter \ Group | Intact | Control (PI+placebo) | Experiment (PI+ IGF-1) |
|---|---|---|---|
| Mean number of newborn rats in the brood | 9,5 ± 1,96 | 8,0±1,97 | 10,0±1,05** |
| Number of broods | 6 | 5 | 6 |
| Mean body mass of a newborn rat | 7,38 ± 0,095 | 6,73±0,1 10* | 7,44±0,138 |
| Total number of newborn rats in the group | 57 | 40 | 60 |

Note:
* - significant differences with intact group ($p \leq 0,05$)
** - significant differences with control group ($p \leq 0,05$).

Number of newborns in broods and their body weight did not differ significantly in the third animal group (with experimentally induced PI who received IGF-1) comparing with intact group. The mean number of newborn rats in the 3-rd group was 10,0±1,05 with the mean body mass 7,44±0,138r. Interestingly, that offspring's number was 25 % higher in animals treated with IGF-1 compared with the 2-nd group ($p \leq 0,05$).

## 4. Discussions

In earlier studies, PI was induced by experimental decrease of uterine blood inflow by uterine artery embolization in sheeps. IGF-1 effectively prevented the development of fetal growth restriction in sheep fetuses [3,4,6]. One of the beneficial effects of using IGF-1 was the increase in its concentration in fetal plasma and in amniotic fluid. Other researchers showed the same results [5,8,9,10,11].

The analysis of the different routes of IGF-1 administration demonstrated that the most effective way to prevent PI was an intravenous or intraamniotic injection of IGF-1. Nevertheless, all parenteral routes of IGF-1 administration leaded to the improvement of fetal growth rate even after uterine artery embolization [4,6,8,9,11].

Thereby, in big agricultural animals IGF-1 showed beneficial effects in experimentally induced PI. However, small laboratory animals research is the most essential part for studying the pharmacological effects of any promising agent. That is why we studied IGF-1 clinical effectiveness when administered subcutaneously in rat model of PI.

To induce PI uterine vessels werecoagulated in pregnant rats.

The practicalities of testing IGF-1 in animal models of PI present numerous challenges, both applied and regulatory. The data showed that uterine artery coagulation in pregnant female rats leaded to severe fetal hypotrophy due to PI.

When laboratory animal research is conducted very small dose of such an expensive substance as IGF-1 is required.

Our method gave valid and reliable information proving subcutaneus IGF -1 administration reduced fetal growth restriction associated with PI. IGFs act in response to such signals as nutrients, oxygen and hormones via the IGF receptors, and the insulin receptor (InsR). Deletion of IGF1 gene leads to reduced birth weight [15]. Conversely, in sheep models elevated levels of plasma IGF-1 are associated with increased maternal plasma glucose concentrations and enhanced fetal growth [16].

In accordance with other references [5,8,10] we suggest that IGF-1 mediated correction of PI was due to the following factors:

1. Activation of angiogenesis and consequently the development of natural bypasses instead of impaired vessels
2. Improvement of vascular permeability in placenta
3. Influence on anabolic metabolism
4. Growth stimulation together with growth hormone
5. Enchanted GLUT isoform transporter expression and relocalization to the cell membrane.
6. IGF-1 regulates substrate transport and hormone secretion and influences maternal substrate availability or placental nutrient uptake and transport [16]
7. IGF-1 stimulates amino acid uptake in cultured trophoblast cells [17].

The first described effects of IGF-1 are related to uteraplacental and fetal placental blood flow. In fact in our laboratory animal research IGF-1 has already showed its therapeutic effect on the 7th day of administration whereas

in bigger farm animals research longer treatment was required (almost one month). Apparently it depends on rodent physiology.

Previous studies in ruminants indicate that adverse intrauterine conditions determine changes in placental morphology. These changes are associated with an increased proportion of type A and B, and fewer type C- and D placentomes during late gestation in sheep [18]. Placentomes are the areas of the endometrium where trophoblasts of the chorion attach. Placentomes are divided into the maternal and fetal parts. Each part is represented by connective tissues, capillaries, epithelial cells (maternal portion only) and trophoblasts (fetal portion only). After implantation the placentomes grow dramatically and in 80 days of gestation reach maximum weight. During the second half of gestation placentomes attenuate bringing fetal and maternal capillaries closer so that nutrient transfer increases [16].

Placentomes are divided into four types (A-D). They have different structure but apparently their function might be the same [19]. During gestation A and B placentomes prevail over C and D placentome types which are more common for multiple pregnancies or late gestation. Placentome types C and D are also larger and more everted comparing to placentomes A and B. Previous studies have suggested that due to the fact that placentomes evert placental nutrient transfer to the fetus increases throughout gestation (in sheep the number of placentomes C and D rises at 125–135 days of gestation) [20]. The presence of IGF-1 binding proteins (IGFBP-1-3) in placentomes suggests that IGF-1 affects physiological processes in these structures and consequently improves their function.

## 5. Conclusion

1. In the experimental model we induced PI by uterine artery coagulation in pregnant rats. Such model is helpful to study the set of processes taking place during this condition.

2. Subcutaneous IGF-1 administration in a dose of 0,8mkg/kg for 7 days prevented fetal hypotrophy in rats with PI.

3. The therapeutic benefit of IGF-1 is associated with its ability to improve uteraplacental and fetal placental blood flow, to stimulate growth and influence on anabolic metabolism.

4. IGF -1 treatment involves the regulation of multiple different glucose transporters in the placenta and maintains fetal carbohydrate supply which is vital for appropriate fetal growth.

5. IGF-1 interacts with IGF-1 binding proteins in placentomes which results into the increase of nutrient transfer.

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