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Superovulation-Oocyte and Uterine Function

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과배란 - 난자 및 자궁기능

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요 약

외인성 성선자극 호르몬에 의한 과배란처리는 난모세포의 질, 수정, 배발달, 착상, 임신유지 등에 해로운 영향을 수반하는 광범위한 배란전후의 호르몬 분비이상을 야기한다. 최근 본 연구에서 흰쥐에서의 IGF-1이 착상전 및 자궁의 환경적 조절에 미치는 잠재적 역할을 확인하였다. 그 결과는 IGF-1이 아마도 탈락막의 조절과 배발달에 적합한 자궁환경유지에 중요한 역할을 수행한다는 것을 보여주었다. 과배란후 배의 손실과 착상의 실패는, 본 연구에서 관찰되었듯이 부분적인 자궁내 IGF-1의 작용저해에 기인하리라 사료된다. 또한 본 연구진들은 생쥐의 배에서 염색체 이상빈도에 대한 과배란을 유도하는 성선자극 호르몬의 영향에 대한 연구를 수행하였다. PMSG 5, 10 및 15 I.U.를 투여하여 과배란된 생쥐에서 생쥐의 수정란과 8-16 세포기 배의 염색체 분석을 실시하였다. 이수체(aneuploidy), 다배체(polyploidy) 또한 염색체의 구조적 이상은 4개군에서 관찰되었다. 그러나 다배체만이 과배란과 상관관계가 있었다. PMSG 10, 15 I.U. 처리군에서 다배체 발생율은 각각 2.9%와 10.5%였다. 더욱이 PMSG 용량에 따른 배의 다배체 발생빈도 사이에는 투여량에 따른 상관관계가 확인되었다($P<0.0001$). 과배란과 성선자극호르몬은 양의 상관관계가 있었으며 염색체와 과배란의 정도에는 투여량에 따른 상관관계가 있었다. 과배란된 난모세포의 발달 잠재력은 모축의 내분비적 환경뿐만 아니라 생존에 필요한 내인성 요인들 즉 유전적 상태 그리고 난자의 전체적 성숙등에 연관되어 있다고 사료된다.

I. INTRODUCTION

Superovulation technique has gained widespread use in laboratory and farm animals for the yield of multiple oocytes and embryos for research and commercial purposes and in humans in the treatment of varying types of female infertiliy. However, treatment with exogenous gonadotropins for the purpose of superovulation frequently results in impaired fertility. A number of investigators has ascribed the reduced fertility to abnormalities of failure in the process of fertilization, early loss or abnormal development of preimplatation embryos, implantation failure, and high fetal wastage. It appears that the detrimental effects and their associated mechanism(s) may be multifactorial, including genetic deficiencies in the superovulated oocyte /embryo and /or hostile factors in the maternal environment, such as insufficiency of progesterone or excessive estrogenic stimulation of progesterone or excessive estrogenic stimulation of the reproductive tract leading to asynchrony

between embronic and uterine development. However, the mechanism(s) involved in the reduced fertility after superovulation is notfully understood.

Early defects may occur in the process of follicular development and oocyte maturation before ovulation by hyperstimulation of ovarian tissues. These problems may relate to the prolonged action of exogenous gonadotropins resulting in excessive follicular steroid production. Pre- or periovulatory endocrine aspects, including the secretion of follicular steroids and endogenous gonadotropins, are believed to affect final maturation, subsequent fertilization, and developmental capability of superovulated oocytes. In this regard, a deviated course of the preovulatory follicular development and oocyte maturation in superovulated animals has been implicated as an important factor influencing the unpredictable and variable embryo viability. It is the purpose of this paper to review our recent experimental data of detrimental effects of superovulation associated with oocyte and uterine function(1~5).

Ⅱ. EXPERIMENTS

Using rat and mouse models, the experiments were carried out to understand the following objectives:

(a) To determine the effect of IGF-1 on preimplantation embryonic development *in vitro*.
(b) To examine alterations in the uterine IGF system following superovulation.
(c) To determine significance of the alterations in the uterine IGF system.
(d) To examine the ionic composition of the uterine luminal fluid following superovulation.
(e) To determine the role of IGF-1 in the uterine sensitization process for the decidual reaction.
(f) To determine whether superovulatory doses of PMSG affects embryo chromosomal complements in CD-1 mouse 8-to 16-cell stage embryos *in vivo*.
(g) To define the mechanisms that cause the chromosomal abnormalities after superovulation in CD-1 mouse zygote stage.

In addition, the incidence of chromosomal abnormality in unfertilized human oocytes following superovulation was estimated.

Ⅲ. SUMMARY AND CONCLUSIONS

1. The effect of IGF-1 in the preimplantation rat embryonic development

The study demonstrates a potential role for IGF-1 in the development of the preimplantation rate embryo. IGF-1 appears to promote morphological development of rat embryos to the blastocyst stage. The improvement in the development stage of embryos by IGF-1 appears to be accompanied by an improvment in viability of embryos, as determined by an increase in the number of live cells in the ICM, an increase in protein synthesis, and a greater rate of implantation and fetal development. The effects of hr-IGF-1 were observed at the concentrations at which IGF-1 has been shown to effect other cell types through the IGF-1 receptor.

These experimental observations support the hypothesis that IGF-1 may be involved in the maternal-to-fetal signalling mechanisms. This in turn may mediate synchronized development between the uterus and preimplantation embryos. Alternately, a well-regulated uterine IGF systerm may be required for synchronized development between the uterus and embryos. Therefore, factors that disturb the regulation of uterine IGF system may be detrimental to preim-

plantation embryonic development and subsequent postimplantation development in the rat.

2. The effect of superovulation on the uterine IGF system

In summary, treatment with a pharmacological dose (40IU) of PMSG creates superphysiological levels of estradiol-17β during the periovulatory period and sencondary estradiol-17β peak during the postovulatory period. Changes in the levels of estradiol-17β result in an alteration in the uterine IGF system which can be divided into two distinct phases. The first phase is observed in the first three days of pregnancy which is characterized by enhanced IGF-1 action. This may be the result of both increased IGF-1 levels and decreased IGFBP levels. The second phase is observed at the preimplantation period or at the time of implantation and is characterized by suppressed IGF-1 action. This may be caused by a reduction in the levels of IGF-1 and/or an increase in IGFBP levels. These changes may affect preimplantation embryonic development and subsequent implantation.

3. The effect of IGF-1 on the uterine microenvironment for preimplantation embryonic development

This study demonstrates a role for the uterine IGF system in increasing early embryonic loss following superovulatory treatment. It is likely that pharmacological dosages of exogenous gonadotropins create superphysiological levels of estradiol-17β in the circulation. Superphsiological levels of estradiol-17β enhance IGF-1 actions in the uterus by increasing IGF-1 levels and by decreasing IGFBP levels. Enhanced IGF-1 actions render a uterine environment hostile to preimplantation embryonic development. The alterations in electrolyte composition of the uterine luminal fluids may reflect, at least in part, changes in uterine microenvironment for preimplatation embryonic development following supervulation. These findings also suggest that the uterine IGF system is an important mediator of estrogen action in the regulation of uterine function.

Superovulatory treatment may change a volume of uterine luminal fluid. This may also adversely affect embryonic development. This detrimental affect of superovulation appears to not be mediated IGF-1. Apparently, IGF-1 is one of a number of autocrine and paracrine factors that regulate uterine function. Studies involving other growth factors and cytokines may provide further understanding on the mechanisms through which superovulatory treatment causes in uterine enviroment observed in this study.

4. The effect of IGF-1 on decidualization

This study demonstrates that IGF-1 may regulate decidualization at different levels such as decidual tissue formation and ALP activity. In particular, the uterine IGF systems appears to play an important role in the uterine sensitization process required for the decidual response. Enhanced and suppressed IGF-1 actions during the sensitization period may be detrimental to subsequent decidual tissue formation. Therefore, changes in the uterine IGF system during the preimplantation period following superovulation appears to have a significant effect on the decidual response. This may, at least in part, be responsible for the failure of implantation following superovulation in the rat.

The present study also demonstrates that GF and T_4 involved in the regulation of uterine ALP activity during the uterine sensitization period, in addition to the decidual tissue formation that has been demonstrated previously. However, IGF-1 was shown not to mediate the actions of

GH and T_4 on the decidual tissue formation and ALP activity. Instead, IGF-1 appears to regulate the decidualization process in the GH and T_4-dependent manner. The mechanisms by which GH and T_4 enable IGF-1 to regulate the decidualization process remains to be determined.

5. Investigation of effects of PMSG on chromosomal complement of CD-1 mouse embryos

Embryonic development, investigated extensively in mice following induced ovulation, has revealed that the occurrence of substantial mortality results during cleavage, at implanation, midpregnancy and parturition. The extent to which chromosome imbalances are related to embryonic mortality has not been explored. Observations here suggest that triploidy constitute an appreciable portion of preimplantation losses in CD-1 mice. The following are the final conclusions of this study.

(1) A positive dose-response relationship between PMSG dose and the incidence of polyploidy was detected in CD-1 mouse 8~16 cell stage embryos developed *in vivo*.

(2) Polyploidy, especially, triploidy, was derived from both digyny and diandry. The dose-response relationship between PMSG and the incidence of polyploidy may be caused by either suppression of meiotic division or alteration of the zona pellucida during oocyte maturation. These events may be related to asynchrony of oocyte maturation by exogenous gonadotropins.

(3) PMSG use for stimulated of ovulation has no effect on segregation of individual chromosome during the meiotic and mitotic division that would lead to aneuploidy in CD-1 mouse embryos.

6. Chromosome investigation of unferilized human oocytes after superovulation

The frequency of numerical chromosome abnormalities in metaphase II oocytes from 280 human unfertilized oocytes were determine. Aneuploidy was found to occur at a frequency of 22.8% in meiotically mature oocytes obtained from stimulated follicles during IVF procedures and did not vary with the dose of hMG or maternal age.

The observation of 16.8% of diploid oocytes suggests that blockage of the meiotic process at meiosis I level after superovulation may occur in the humans as it dose in the mouse.

Human triploid embryos account for up to about 15% of all first trimester spontaneous abortions. One percent of all fertilized oocytes are estimated to be polyploid *in vivo* (6) and 10% triploid after IVF is unknown. In the present study, the observation of diploid oocytes may provide evidence that some triploidies in IVF may be derived from fertilization of a diploid oocyte as the mouse study indicated. The high percentage of diploid may relate to the induction regiment used. Considering the asynchronous maturity of oocytes resulting from superovulation, it appeares that oocytes matured longer *in vivo* are less prone to show G1-PPC.

In conclusion, human unfertilized oocytes had an incidence of numerical chromosome abnormalities of 39.6% including 22.8% aneuploidy and 16.8% diploidy. This high frequency of chromosome abnormalities in unfertilized oocytes may explain the low pregnancy rate in IVF programs suggesting that natural selection against chromosome abnormalities may occur even prior to fertilization

Ⅳ. SUMMARY

Superovulation with exogenous gonadotropins creates a spectrum of pre or periovulatory hormonal changes with subsequent detrimental effects on oocyte quality, fertilization, embryo development, implantation and maintenance of pregnancy. Our recent study determined potential roles for insulin-like growth factor-1 (IGF-1) in uterine environment regulation and preimplantation in the rat. The evidence indicates that IGF-1 may play an important role in the maintenance of a receptive uterine environment for embryonic development and the regulation of decidualization. Embryonic loss and failure of implantations following superovulation may be partially attributed to disturbances in uterine IGF-1 action as observed in this study. We investigated the effects of superovulatory doses of gonadotropins on frequency of chromosomal abnormalities of mouse embryos. Chromosome analysis of mouse zygotes and 8- to 16-cell stage embryos from spontaneously ovulated, 5, 10, and 15 IU pregnant mare serum gonadotropin (PMSG) superovulated mice was carried out. Aneuploidy, polyploidy and structural chromosomal abnormalities were detected among the four groups. However, only polyploidy was correlated with superovulation. In 10 and 15 IU PMSG treated groups, the rate of polypoidy was 2.9% and 10.5%, respectively. Furthermore, there was a dose reponse relationship between the PMSG dose and the incidence of embryonic polyploidy ($P<0.0001$). Superovulation with gonadotropins showed positive correlations and a dose response relationship between chromosomal polyploidy and the degree of superovulation. The development potential of superovulated oocytes appears to be associated with intrinsic factors such as genetic condition and overall maturation of the ova, as well as maternal endocrine environment, upon with the ova depend for their survival.

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