

The Abortional and the Teratogenical Effects of 17-beta Estradiol Valerate Administration during Embryonic Development in Rats

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ABSTRACT

Among the female sexual hormones, the estrogen and progesterone are of significant importance. These hormones are prescribed for the treatment of certain female genital system disorders as well as used in the synthesis of contraceptive drugs. It has been shown that administration of the above hormones during early pregnancy may produce abortion in early gestational days. In the present investigation, we have studied the possible abortional and teratogenical effects of a single intramuscular injection of 0.15 mg/kg esteradiol valerate during the first or second half of pregnancy, in rats. The data show that; (1) The abortional effects of administration of esteradiol during the first half of pregnancy, in comparison with the control group, is significant. Although the injection of this hormone during the second half of pregnancy does not produce total abortion but there is a reduction in the number of fetuses. Therefore, it may be said that with injection of a single dose of hormone during the first half of pregnancy (from day zero to day 10) the chance of abortion is high (in human embryos, this period is about the first four weeks of development). (2) Administration of esteradiol may also produce severe fetal abnormalities such as anencephalia, exencephalia, microphthalmia. In addition to the above abnormalities, in the male fetuses, there is also a remarkable defect in the urogenital system.

Keywords: esteradiol, contraceptive, teratogen, teratology, embryonic development

INTRODUCTION

It is well established that esteradiol and progesterone modulate gonadotropin-releasing hormone (GnRH)-induced luteinizing hormone (LH) secretion from cultured rat pituitary cells. Short-term esteradiol and long-term progesterone treatment exert inhibition, while short-term progesterone and long-term esteradiol treatment lead to enhancement of GnRH-stimulated LH secretion [1]. Both positive and negative regulation of LH, by GnRH and the gonadal steroids progesterone and esteradiol 17 beta respectively, are well documented in literature's [2, 3]. Esteradiol is the major estrogen produced by the ovaries [2, 4]. In non-conception cycles ovarian esteradiol is synthesized, from the androgenic precursors, in granulosa cells of developing follicles [2, 3]. However in conception cycle, the placenta is another source for estrogen production [2-4].

In the pregnant rat, luteinizing hormone stimulates the ovarian production of testosterone which is aromatized to esteradiol [2-4]. Esteradiol promotes progesterone synthesis by the ovary [2-4]. To determine if the administration of GnRH disrupts pregnancy by suppressing ovarian steroid production Sridaran (1987) treated rats on days 7-12 of pregnancy with GnRH or GnRH agonist. The results indicated that the abortifacient effects of GnRH administration in rats is not due to its effect on the uterus but to its suppressive effects on ovarian progesterone secretion [5, 6]. In earlier study four women in their early pregnancy were treated by intravenous injections of superactive luteinizing hormone-releasing hormone (LHRH) agonist in a dose of 50-125 micrograms for a period of four days. No abortifacient effects of these large doses of the LHRH was found [7]. Also in a trial the ability of esteradiol 17 beta cyclopentylpropionate (ECP) and prostaglandin F2 alpha (PGF2 alpha) to induce abortion during early gestation in pregnant feedlot heifers were compared. It was found that

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although PGF2 alpha is a more effective compound to induce abortion but ECP can also induce abortion [8]. In an *in vitro* fertilization program it is shown that a decline in sex steroids; hCG, esteradiol and progesterone, may well negatively influence endometrial development during the periimplantation pregnancy [9].

Serial measurements of serum progesterone, esteradiol, hCG and human placental lactogen (hPL) in 33 women experiencing early pregnancy failure and 72 healthy women having uncomplicated pregnancies show that steroid production by corpus luteum were similar in both groups up to 6 weeks gestation but thereafter placental steroidogenesis was not evident in those women in whom spontaneous pregnancy losses occurred. Placental production of the two protein hormones hCG and hPL did take place and whereas the circulating levels were not as high as in normal pregnancies, levels did usually increase before clinical evidence of miscarriage occurred [10]. Sex steroidal hormone treatments may produce congenital anomalies and embryotoxicity. Recent studies on the embryotoxic effects of a combination of norethisterone acetate (NEA) and ethinyl esteradiol (EE) treatment on macaques and baboons indicated an increased embryoletality and the highest dose of NEA + EE is also maternally toxic [11].

Since it is possible for a pregnant woman to continue her contraceptives, even though with unknown pregnancy, or in the case of illegal termination of undesired pregnancy to use large doses of estrogen, the aims of this investigation were to answer the following questions: Dose the administration of the esteradiol during pregnancy produce abortion? If yes, what is the critical period(s) of the effects of esteradiol? and If abortion does not occur, what is the probable teratogenical effects of esteradiol?

MATERIALS AND METHODS

Animals. Ten week old virgin female rats (supplied from Hesarak Karaj Institute of Serum Production) weighing 120-150 g were used. The animals received standard diet and water *ad libitum* and were housed in air-conditioned animal room at $24 \pm 2^\circ \text{C}$ with artificial light from 6 AM to 6 PM. The females were mated overnight and examined the next morning for the presence of a vaginal plug.

The day of plug detection was called day zero of pregnancy.

Hormone exposure. To determine the effective dose of the injected hormone, it was necessary to find out the suitable effective administrating dosage. To do this, it was decided to use the "try and error" principle. The procedure was started with a single injection of 0.8 mg/kg esteradiol, by using Hamilton syringe in one of the following days of gestation; 0, 2, 4, 6, 8, or 10 (first half of pregnancy) and 12, 14, or 16 (second half of pregnancy). The results show that, with the above dose, there is no chance to have any fetuses. Therefore, the injected dose was gradually reduced to 0.15 mg/kg.

The experimental procedure. After mating, the pregnant rats were classified randomly into control and experimental groups (Table 1). The experimental groups 1 and 2 further subdivided into different subgroups. The number of animals in each subgroup was six to eight. The pregnant rats in each group received only a single intramuscular injection of hormone in certain gestational days. All pregnant rats were kept in animal house up to last day of pregnancy and fed with standard diet. On the last day of pregnancy (20th day) the pregnant rats were sacrificed for examination of uterine contents. Fetuses were removed from the uterine horn and inspected for external abnormalities. All fetuses were fixed in formalin (10%) and examined for a visceral abnormalities by stereomicroscope.

Statistical analyses. Statistical analyses were performed using ANOVA and the Students' *t*-test in order to assess the significance differences between individual means.

RESULTS

The abortional effects of the hormone. In general the abortional effects of the administration of the esteradiol during the first half of pregnancy is obvious. In administration of hormone during the second half of pregnancy the number of fetuses per pregnancy was reduced in the groups which received hormone earlier. Furthermore, the fetuses had severe anomalies. The results obtained from the experimental group 1 show that the abortional effects of a single injection of hormone in any day

Table 1. The characteristic of experimental and control groups.

Exp. group 1	Injection of 0.15 mg/kg hormone during the first half of pregnancy (the gestational day 0, 2, 4, 6, 8 or 10)
Exp. group 2	Injection of 0.15 mg/kg hormone during the second half of pregnancy (the gestational day 12, 14 or 16)
Control group	Injection of normal saline (equal volume)

Table 2. The summary data.

Hormonal exposure day (after mating)	Mean number of fetuses in the last day of pregnancy	Uterus symptoms
0	Zero	
2	Zero	
4	Zero	Mild inflammation
6	Zero	Inflammation
8	Zero	Inflammation with hemorrhage
10	Zero	Inflammation with signs of aborted fetuses
12	60.43 ($\pm$ 0.93)*** n = 8	
14	7.86 ($\pm$ 1.09)** n = 7	
16	8.00 ($\pm$ 1.48)* n = 7	
Control group	11.63 ($\pm$ 0.65) n = 8	

Values represent mean (SEM), * $p < 0.05$, $p < 0.001$ compared with the control group (Students' *t*-test), n = number of pregnant animals in each group.

during the first half of pregnancy is obvious so that there was not any fetuses (all fetuses were aborted, Table 2). In this group examination of the uterus showed inflammation and hemorrhage. The results obtained from the experimental group 2 show that in administration of hormone during the second half of pregnancy, some fetuses were aborted and some of them were not (Table 2).

The teratogenic effects of the hormone. In examination of the remaining fetuses in the experimental group 2 a widespread anomalies such as exencephalia, microphthalmia, or non-developmental eyes, anencephalia, amelia, focomelia, agnatha defect (undeveloped jaw), sindactylia and tetradactylia and in the male fetuses, a remarkable defect in the urogenital system were seen.

DISCUSSION

In more endocrine glands the hormonal output is controlled by a negative feedback mechanism. The increased level of the female sex hormones (estrogen or progesterone) may induce a negative

feedback on the production of the hypothalamic gonadotropin releasing hormones (GnRH) and hypophysial gonadotropins (FSH and LH) [2]. It is obvious that for a successful pregnancy a normal level of the sex hormones is required [2].

The results obtained from the present investigation reveal that a single injection of 0.15 mg/kg hormone (during the first half of pregnancy) may induces abortion of all fetuses. In this case, the uterus of the injected rats were hypertrophied, inflamed and the signs of the abortion, autolysis and absorption of the remaining parts of the fetuses were clear. Earlier it was shown that esteradiol 17 beta cyclopentyl-propionate can induce abortion [8]. These results also revealed a marked sensitivity to teratogenic action of esteradiol. Recently it is shown that there is an association between oral contraceptives use after conception and the risk of congenital urinary tract anomalies in offspring [11]. In rats, treatment with a single dose of injectional esteradiol (0.15 mg/kg) during the period of organogenesis (days 10-15 of pregnancy) was sufficient to produce various types of anomalies. This period may be a critical period in the developmental and differentional processes, especially in the nervous system. The teratogenicity

of estradiol in rats was characterized by a high incidence of severe defects in the central nervous system. Hendrickx *et al* (1987) showed that with the administration of EE and NEA, in macaques monkeys and baboons, the fetal mortality is increased [12]. This developmental period of rat embryos is about the first four weeks of human development [13, 14]. Although administration of hormone in the second half of pregnancy does not cause total abortion but the reduction of the number of fetuses may be accounted for abortion of some fetuses. As shown in table 2, in the experimental groups which received hormone in the 12, 14 or 16th days of pregnancy the mean number of fetuses is low as compared with control group. The results of the analysis of variances (ANOVA) of the number of fetuses indicate to a significant differences between the number of fetuses in experimental (12, 14 or 16th day of pregnancy groups) and control group ($p = 0.01$). Also, if the number of fetuses in experimental groups be compare with control group (Students' *t*-test) the results show significant differences (Table 2).

It is possible that the occurrence of abortion in the first half of pregnancy may be due to the effects of exogenous hormone. The exogenous hormone may cause disturbance in the process of implantation and primary growth of the fetuses. Such effects may be inserted through the short or long circuit feedback mechanisms. The results of these mechanisms may be the reduction of the plasma level of the ovarian or placental hormones. Since during the second half of the pregnancy the fetuses are stabilized in the uterus and the placenta is the main source of the sex hormones, the abortional effects of the injected hormone may be replaced by the embryotoxic effects. The results presented in Table 2 indicate the above comments. When the pregnant rats pass the critical period the teratogenical effects and the disturbances of exogenous factors (in this case estradiol) will gradually reduce.

In general it may be said that disturbance in the cellular reaction between diencephalon and surface ectoderm or disturbance in the process of differentiation of prosencephalon to telencephalon and diencephalon may be produce some severe abnormalities such as anencephalia, exencephalia and microphthalmia. It is obvious that the fetuses which have severe abnormalities could not continue the process of development and normally rejected.

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REFERENCES

1. Ortmann, O., Johannsen, K., Knuppen, R. and Emons, G. (1992) Acute effects of estradiol and progesterone on melittin and gonadotropin- releasing hormone-induced LH secretion. *J. Endocrinology* 132: 251-259.
2. Dorrington, J.H. and Armstrong, D.T. (1979) Effects of FSH on gonadal functions. *Rec. Prog. Horm. Res.* 35: 301-342.
3. Leung, P.C.K. and Armstrong, D.T. (1980) Interactions of steroids and gonadotropins in the control of steroidogenesis in the ovarian follicle. *Ann. Rev. Physiol.* 42: 71-82.
4. Hillier, S.G. (1981) Regulation of follicular oestrogen biosynthesis: a survey of current concepts. *J. Endocrinol.* 89: 3p-18p.
5. Sridaran, R., Ghose, M., and Mahes, V. B. (1988) Inhibitory effects of a gonadotropin-releasing hormone agonist on the luteal synthesis of progesterone, estradiol receptors and prolactin surges during early pregnancy. *Endocrinology* 123: 1740-1746.
6. Sridaran, R. (1987) Ovarian steroid production in rats treated with gonadotropin-releasing hormone during early pregnancy. *J. Steroid-Biochem.* 26: 1-6.
7. Skarin, G., Nillius, S. J. and Wide, L. (1982) Failure to induce early abortion by huge doses of a superactive luteinizing hormone- releasing hormone agonist in women. *Contraception* 26: 457-463.
8. Refsal, K. R., and Seguin, B. E. (1981) Estradiol- 17 beta cyclopentylpropionate and prostaglandin F2 for induction of abortion during the first trimester of pregnancy in feedlot heifers. *J. Am. Vet. Med. Assoc.* 179: 701-703.
9. Hutchinson-Williams, K. A., Lunenfeld, B., Diamond, M. P., Lavy, G., Boyers, S. P., and DeCherney, A. H. (1989) Human chorionic gonadotropin, estradiol, and progesterone profiles in conception and nonconception cycles in an *in vitro* fertilization program. *Fertil. Steril.* 52: 441-445.
10. Whittaker, P. G., Stewart, M. O., Taylor, A. and Lind, T. (1989) Some endocrinological events associated with early pregnancy failure. *Br. J. Obstet. Gynaecol.* 96: 1207-1214.
11. Li, D., Daling, J. R., Mueller, B. A., Hickok, D. E., Fantel, A.G. and Weiss, N. S. (1995) Oral contraceptive use after conception in relation to the risk of congenital (urinal) tract anomalies. *Teratology* 51: 30-36.

12. Hendrickx, A. G., Korte, R., Leuschner, F., Neumann, B. W., Prahalada, S., Poggel, A., Binkerd, P. E., and Gunzel, P. (1987) Embryotoxicity of sex steroid hormone combinations in non-human primates I-Norethisterone acetate + ethinylestradiol and progesterone + estradiol. *Teratology* 35: 119-127.
13. Kaufman, M. H., (1992) *The atlas of mouse development*, p7, Academic press.