Estrogen-Induced Congenital Hypospadias in Developing Rat Embryos

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Abstract

Hypospadias is a defect of the urethra and penis in the male. In this research the incidence of the male hypospadias induced by estrogen (estradiol) administration was investigated during embryonic development. Three groups of pregnant rats were exposed to 0.15mg/kg estradiol valerate via a single intramuscular injection on one of the following gestational days: 12, 14 or 16 and named as experimental groups 1,2 and 3 respectively. All the pregnant rats were sacrificed on the last day of pregnancy (day 20). The fetuses collected and counted and the external genitalas examined for detection of hypospadias as well as other malformations.

The data showed that in addition to the significant reduction in the size of the litters in experimental groups, the incidence of the male hypospadias is high, especially in the group 1. Furthermore, most of the fetuses had severe malformations including anencephalia, exencephalia, agnatha, syndactylia and tetradactylia.

Key words: Estrogen, teratology, hypospadias

Introduction

It is well known that estrogens are required for the normal maturation of the female. Estradiol is the major class of estrogens which is produced by the ovaries. In nonconception cycles, ovarian estradiol is synthesized from the androgenic precursors, in granoluza cells of developing follicles. However, during conceptional cycle, the placenta is another source for estrogens production [1]. Estrogens not only have been used extensively for replacement therapy in estrogen-deficient, but also a large number of oral contraceptives contain estrogens or progestines (or both) [7].

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Many published reports indicate the adverse effects associated with the use of estrogens or oral contraceptives. In non-pregnant subjects, estrogen therapy gives rise to a number of side effects which are related to their estrogenic and their general metabolic effects [3].

In relation to this subject, there are many scientific papers that indicate several millions pregnant women around the world with the synthetic estrogen diethylstilbestrol therapy led to an increase in incidence of testicular cancer, hypospadias, and cryptorchidism among their male newborns. These abnormalities, probably arise during fetal development [4].Also a number of papers have appeared reporting the occurrence of adenocarcinoma of the vagina among the young women whose mothers were treated with large doses of diethylestilbestrol in their early pregnancies [2,4].

The similarity between these effects and the adverse changes in male reproductive development and function raised the question of whether these changes are attributable to altered exposures to estrogenic and other endocrine-disrupting agents during fetal development.

Materials and Methods

1-Animals

Ten-week-old virgin female Wistar albino rats (supplied by Hesarak Institute of Serum Production, Karaj, Iran) weighing 120-150grams were used. The animals received standard diet and water *ad libitum* and were housed in air conditioned animal room at 24 ± 2°C with artificial light from 6 AM to 6 PM. The females were mated overnight and examined the next morning for the detection of a vaginal plug. The day of plug detection was called day zero of pregnancy.

2-The experimental procedure

After mating, the pregnant rats were classified randomly into control and experimental groups. Because the period of organogenesis in the rat is during the early days of the second half of pregnancy, exposure of pregnant rats to

estrogen was made on 12th, 14th or 16th day of gestation. The pregnant rats were given a single intramuscular injection of 0.15mg/kg estradiol (Aburaihan Co., Iran) in gluteal region, in one of the above days.

All pregnant rats were kept in animal house up to the last day of pregnancy and fed with standard diet. On the last day of pregnancy (20th day) they were sacrificed for examination of uterine contents. Fetuses were removed from the uterine hornes and inspected for external abnormalities. All fetuses were fixed in formalin (10%) and examined under stereomicroscope.

3. Statistical analyses

Results are expressed as means ±SEM. Statistical analyses were performed using ANOVA with the Student's *t*-test in order to asses the significance of differences between individual means.

Results

1. The abortive effects of the hormone:

Early hormonal administration during the second half of the pregnancy revealed significant reduction in the number of each pregnancy (Table 1).

Table 1: ***The mean number of fetuses in experimental and control groups***

Hormonal exposure day (after mating)	Mean number of fetuses in the last day of pregnancy
12	6.43(±2.64) * n=8
14	7.86(±2.91) ** n=7
16	8.00(±3.92) *** n=7
control	11.63(±1.85) n=6

**P<0.001compare with the control group(Student's t-test)*
*** P<0.01*
**** P<0.05*
n: number of dams in each group

2. The teratogenic effects of the hormone:

In examination of the remaining fetuses in the experimental group, three widespread anomalies such as exencephalia, microphthalmia, anencephalia, amelia, focomelia, agnatha defect (undeveloped jaw), syndactylia and tetradactylia were observed. The internal reproductive system showed no difference between experimental and control fetuses while the examination of the external reproductive system in the male fetuses, revealed a remarkable defect in the urogenital perineum, which is called hypospadias. Hypospadias is a congenital anomaly in which the urethra opens on the ventral surface of the penis or in the perineum.

Discussion

Although the female hypospadias is a rare congenital abnormality but the incidence of male hypospadias is more frequent. Hypospadias is a urethral and penile deformity which might occurs during male genital development. Some papers indicate that the occurrence of hypospadias is secondary to more serious disorders of sexual differentiation, which is probably an androgen-related genital anomaly [6-9]. However, it is suggested that, although hypospadias has no known single etiology but it has been linked to androgen insensitivity caused by mutations of the androgen receptor gene [8]. On the other hand few investigations have been reported that male pseudohermaphroditism may be due to steroid 5 alpha-reductase 2 deficiency [5].

The present research revealed a marked sensitivity to teratogenic action of estradiol. In rats, treatment with a single dose of injectional estradiol (0.15mg/kg), during the period of organogenesis (days 10-15 of pregnancy), was teratogenic to produce various types of anomalies. This period may be a critical period in the developmental and differentiation processes. This developmental period of rat embryos coincides with the 5th and 6th weeks of the human development.

The results obtained from the present investigation show a different probable reason for the etiology of hypospadias and indicate that estradiol administration (during the second half of pregnancy) can disrupt the normal development of genital organs. The teratogenicity of estradiol in rats was characterized by a high incidence of severe defects, among few detectable systems. Administration of hormone during the second half of pregnancy reduces the number of fetuses which indicates that the earlier the exposure to hormone the smaller the litter size (Table 2).

Table 2: ***Distribution of the incidence of hypospadias in different experimental groups***

Hormonal exposure day (after mating)	No.of dams	No. of fetuses male & female	No. of male fetuses with hypospadias
12	8	27/30	17
14	7	28/28	7
16	7	19/28	3

The results of the analysis of the number of fetuses indicate a significant difference between the number of fetuses in the experimental and the control groups (Table1). Also, if the number of fetuses in the experi-

mental groups is compared with the control group (Students' *t*-test) significant differences will be met.

In conclusion, the results obtained show that with a single injection of estradiol during the critical periods of organogenesis the chance of anomalies such as hypospadias may increase. Thus, this may be a serious caution to women who are using contraceptive pills after conception.

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References

1. *Hadley ME: Endocrinology, Second ed., Prentice-Hall international Inc., 1988, p:455.*
2. *Hendrickx AG, Korte R, Leuschner F, et al.: Embryotoxicity of sex steriod hormone combinations in nonhuman primates I-Norethisterone acetate + ethinylestradiol and progesterone + estradioi. Teratology 1987, 35:119-127.*
3. *Greespan FS , Baxter JD: Basic and clinical endocrinology, fourth ed., Prentice-Hall international Inc., 1994, pp:424-426.*
4. *Jensen TK, Toppari J, Keiding N, et al.: Do environmental estrogens contribute to the decline in male reproductive health? Clin Chem 1995; 41:1896-901.*
5. *Mendonca BB, Inacio M, Costa EM: Male pseudohermaphroditism due to steroid 5 alpha - reductase 2 deficiency: diagnosis, psychological evaluation and management. Med Baltimore 1996; 75:64-76.*
6. *Sandberg DE, Meyer-Bhlburg HF, Yager TJ, et al: Gender development in boys born with hypospadias.. Psychoneuroendocrinology 1995; 20: 693-709.*
7. *Sridaran R.: Ovarian esteroid production in rats treated with gonadotropin-releasing hormone during early pregnancy. J Steroid Biochem 1987; 26:1-6.*
8. *Sutherland RW, Wiener JS, Hicks JP: Androgen receptor gene mutations are rarely associated with isolated penile hypospadias. J Urol 1996; 156:828-831.*
9. *Tong SY, Donaldson K, Hutson JM: When is hypospadias not hypospadias? Med J Aust 1996; 164: 153-4.*