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SHORT COMMUNICATION

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Gestational exposure to hydroxyprogesterone caproate suppresses reproductive potential in male rats

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Abstract Hydroxyprogesterone caproate was administered to pregnant rats at a dose level of 10 and 25 mg/kg body weight on 1st, 7th and 14th gestational day and the male pups (F1 generation) were allowed to grow for 90 days. The effect of gestational exposure to hydroxyprogesterone caproate on fertility was assessed by breeding F1 male rats with control female rats besides analyzing sperm quality and quantity in F1 male rats. The number of implantation sites and viable fetuses was significantly reduced in females mated with F1 males that were exposed to hydroxyprogesterone caproate during embryonic development. The decrease in sperm function was associated with a decrease in sperm motility, sperm viability and sperm count in F1 rats. The study clearly indicates that *in utero* exposure to hydroxyprogesterone caproate affects fertility in male rats.

Introduction

Hydroxyprogesterone caproate (trade name: Proluton), is one of the widely prescribed drugs to prevent threatened pregnancy and abnormal uterine bleeding in women. In the present study, the effect of gestational exposure to Hydroxyprogesterone on the fertility of progeny is investigated. Fertility has become an increasingly important issue over the past 50 years because data suggest that there has been a progressive increase in disorders of male and female reproduction and there is concern as to whether man-made compounds could have contributed to these changes. Recent epidemiological studies have shown decreased semen concentration, sperm motility with increased percentages of abnormal sperm and increased congenital abnormalities like hypospadiasis and cryptorchidism in many countries (Carlsen et al. 1992; Irvine, 1994; De Mouzon et al. 1996; Adami et al. 1996; Toppari et al. 1996; Paulozzi et al. 1997; Miller and Sharpe, 1998). These abnormalities have been attributed to deterioration in environment quality and increased exposure to xenoestrogens and antiandrogens. For example, exposure of rats to DDT an organochloride compound present ubiquitously is known to act as an antiandrogen and regulates at androgen receptor level (Kelce et al. 1995). Decrease in penis size was observed in alligators of lake Apopka (Florida, USA), where the concentration of DDT breakdown product p,p-DDE was found to be significantly high (Guillette et al. 1996). The children of diethylstilbestrol (DES) exposed women are suffering with increased reproductive abnormalities (Takasugi and Bern, 1988; Hines, 1992). It has been hypothesized that exposure to supra-normal levels of estrogen-like chemicals during embryonic development may interfere with mechanisms involved in the development of the male reproductive system and in determining sperm numbers (Sharpe and Skakkebaek, 1993).

In view of the deterioration of male reproduction in recent past, an elaborative programme was initiated to evaluate the gestational exposure to man-made chemicals on reproductive abnormalities in rats. In order to assess the reproductive potential of first generation, rats were selected as test organism since the pubertal stage can be reached with in 90 days and serves as good model for reproductive studies. We have reported earlier that *in utero* exposure to hydroxyprogesterone caproate caused significant decrease in the activity levels of steroidogenic enzymes in rats (Pushpalatha et al. 2003a) and decreased sperm count, sperm motility (Pushpalatha et al. 2004). The circulatory levels of testosterone, follicle stimulating hormone and luetinizing hormone in adult male rats exposed to hydroxyprogesterone during embryonic development was also significantly altered (Pushpalatha et al. 2004). Here we report the reproductive abnormalities in male progeny exposed to hydroxyprogesterone caproate *in utero*.

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Material and methods

Animals

Albino rats of Wistar strain were used in the experiments. They were raised in the animal house facility at the Department of Biotechnology, S.V. University, Tirupati, India. The rats were fed on pellet diet (Sai Durga agencies, Banglore) and water *ad libitum* and maintained under laboratory conditions (Temperature: 23 ± 1 °C; light: dark: 12:12 h; humidity 75%). The rats were housed in sterilized polypropylene cages (18 × 10 × 8″) lined with paddy husk.

Treatment of animals

Rats were allowed to mate and pregnant rats were isolated after identification using vaginal smear technique. The pregnant rats were divided in to three groups consisting of 10 animals in each group. The animals of group I, which served as controls, were treated same as the experimental group but received injections of a mixture of castor oil and benzyl benzoate (1:1.7) in 15 µl volume. The rats in groups II and group III were injected with 10 and 25 mg hydroxyprogesterone caproate/kg body weight respectively on 1st, 7th and 14th day of pregnancy. The hydroxyprogesterone doses selected in the present study were similar to the doses prescribed to humans (mg/kg body weight) and the drug was given to women during first trimester of pregnancy. The rats were allowed to deliver the pups and the pups were maintained on normal diet and water *ad libitum*. The F1 generation rats were sacrificed by cervical dislocation on 90th day after birth. Epididymis were quickly removed, weighed and used for sperm analysis. Epididymis index was determined by using the formula :

Epididymis index = weight of epididymid/weight of the rat × 100

Sperm analysis

Epididymal sperms were obtained by mincing cauda epididymis in normal saline. The sperms were counted using a Neubauer Chamber, as described by (Belsey et al. 1980). Motility of sperm was assayed microscopically (Belsey et al. 1980), within 5 min following their isolation from cauda epididymis at 37°C and the data were expressed as percent motility. The viability of the sperm was identified using 1% tryphan blue reagent (Talbot and Chacon, 1981). The function (HOS coiling) of the sperm was determined by exposing the sperms to hypoosmotic medium and observed for coiled tails under the microscope and the percent of coiling was estimated using the method described by (Jeyendran et al. 1992).

Fertility test and examination of fetuses

The number of copulation trials was determined by allowing each F1 generation male to mate with two females of same age group. Maximum number of trials to inseminate the females was 6 to 8 per male. During each mating period, daily vaginal smears were examined for the sperm. The presence of the sperm in the vaginal smear and/or a vaginal plug was considered evidence for successful mating. The day on which evidence of copulation identified was termed day zero of gestation. Some of the pregnant rats were laporatomized on 20th day of gestation and total number of implantations was counted and fetuses were removed by uterine opening. The number of live and resorbed fetuses (embryo) was recorded. Fertility index, pre- and postimplantation loss were calculated. The remaining pregnant rats were allowed to deliver and total number of pups, weight of pups and skeletal weight (Alizarin-S-staining method) of pups were determined.

Statistical analysis

The data were statistically analyzed by Analysis of Variance (ANOVA) followed by Dunnett's test for comparison with control, using SPSS 10.0.

Results

No mortality and no behavioral abnormalities were recorded in experimental rats indicating that the hydroxyprogesterone caproate does not show acute toxicity at the selected dose levels.

A significant decrease in epididymal index (−22.35), sperm viability (−23.91%), sperm count (−24%), sperm motility (−37.66%) and sperm coiling percentage (−28.19%) was observed in group II rats when compared with the control rats (Table 1). These changes were more pronounced in group III rats (Table 1).

Tables 2 and 3 summarize the data on the reproductive performance of prenatal hydroxyprogesterone caproate exposed rats. F1 generation male rats showed significant decrease in reproductive performance than the control rats. This effect is more pronounced in group III rats when compared to group II rats. There was a significant decrease in number of implantation sites (−55.35%); number of normal fetuses (−89.28%) in normal rats mated with F1 males from group III. The mean weight of embryos in females mated with F1 males from group III was also significantly ($p < 0.0001$) decreased on 20th day of pregnancy (Table 2). In addition, rats mated with F1 males from Group III gave birth to less number of live pups and the body weight and skeletal weight of the exposed pups was also significantly reduced (Table 3).

Table 1 Changes in epididymal index and sperm parameters in adult male rats exposed to hydroxyprogesterone caproate during gestation

Parameter	Control	Group II	Group III	ANOVA
Epididymedes	0.57 ± 0.01	0.44 ± 0.01	0.39 ± 0.005	F = 1151.10
		(22.35)	(31.88)	p < 0.0001
		p < 0.0001	p < 0.0001	df = 29
Sperm count (millions/ml)	50.00 ± 6.30	38.00 ± 2.30	27.00 ± 1.60	F = 83.50
		(24.00)	(46.00)	p < 0.0001
		p < 0.0001	p < 0.0001	df = 29
Sperm motility (%)	77.00 ± 2.60	48.00 ± 0.90	34.00 ± 1.70	F = 1379.50
		(37.66)	(55.84)	p < 0.0001
		p < 0.0001	p < 0.0001	df = 29
Sperm viability (%)	78.20 ± 5.26	59.50 ± 3.04	39.40 ± 2.30	F = 267.68
		(23.91)	(49.61)	p < 0.0001
		p < 0.0001	p < 0.0001	df = 29
Sperm physiological response (HOS coiling)(%)	72.42 ± 4.84	52.00 ± 5.16	31.14 ± 5.87	F = 151.24
		(28.19)	(57.00)	p < 0.0001
		p < 0.0001	p < 0.0001	df = 29

Values are mean ± SD of 10 individuals; values in parenthesis are percent decrease from control

Table 2 Effect of *in utero* exposure to hydroxyprogesterone caproate on reproductive potential of first generation male rats

Parameter	Control	Group II	Group III	ANOVA
No. of copulation trails	1.33 ± 0.51	3.66 ± 0.81	5.66 ± 1.03	F=71.26
		(+175.18)	(+325.56)	p < 0.0001
		p < 0.0001	p < 0.0001	df = 29
No. of implantation sites/female	11.20 ± 0.83	9.66 ± 1.21	5.00 ± 2.19	F = 44.98
		(−13.75)	(−55.35)	p < 0.0001
		p = 0.0029	p < 0.0001	df = 29
No. of resorption sites/female	–	2.00 ± 0.70	2.60 ± 1.86	
No. of embryos/female	11.20 ± 0.83	8.33 ± 0.51	2.80 ± 1.48	F = 174.20
		(−25.7)	(−75.0)	p < 0.0001
		p < 0.0001	p < 0.0001	df = 29
Weight of embryos (g)	7.60 ± 0.56	5.16 ± 0.24	4.58 ± 0.33	F = 160.49
		(−32.10)	(−39.73)	p < 0.0001
		p < 0.0001	p < 0.0001	df = 29
No. of normal fetuses/female	11.20 ± 0.83	7.66 ± 0.51	1.20 ± 0.70	F = 536.01
		(−31.60)	(−89.28)	p < 0.0001
		p < 0.0001	p < 0.0001	df = 29
No. of dead fetuses/female	–	1.7 ± 0.26	1.6 ± 0.54	

Values are mean ± S.D. of 10 individuals; values in parenthesis are percent change from control

Control: Control male rat × female rat

Group II: 10 mg/kg body wt. exposed male rat × female rat

Group III: 25 mg/kg body wt. exposed male rat × female rat

Table 3 Effect of *in utero* exposure to hydroxyprogesterone on reproductive output of F1 generation

Parameter	Control	Group II	Group III	ANOVA
Skeletal weight of embryos (mg)	99.78 ± 1.09	68.95 ± 0.73	62.60 ± 1.94	F = 2163.50
		(30.89)	(37.26)	p < 0.0001
		p < 0.0001	p < 0.0001	df = 29
No. of pups/female	12.33 ± 1.36	7.66 ± 0.51	4.58 ± 0.33	F = 205.89
		(31.60)	(59.10)	p < 0.0001
		p < 0.0001	p < 0.0001	df = 29
Weight of pups (g)	5.78 ± 0.41	5.50 ± 0.25	4.25 ± 0.07	F = 84.53
		(4.84)	(26.47)	p < 0.0001
		p = 0.0817	p < 0.0001	df = 29

Values are mean ± SD of 10 animals; values in the parentheses are % decrease from control

Control: Control male rat × female rat

Group II: 10 mg/kg body wt. exposed male rat × female rat

Group III: 25 mg/kg body wt. exposed male rat × female rat

Discussion

The significant reduction in epididymal index, sperm count, sperm motility, sperm viability and sperm function in hydroxyprogesterone caproate exposed rats may be due to sperm toxic effects. Exposure of hydroxyprogesterone to rats during embryonic development resulted in a decrease in fertility index (Meistrich, 1989). Exposed male rats were able to impregnate female but comparatively in lower number. The observed decrease in fertility in males has been attributed to a decrease in testosterone levels in circulation resulting in decrease in sperm quality and quantity. Earlier (Pushpalatha et al. 2004) reported significant decrease in serum testosterone levels in rats exposed to 25 mg hydroxyprogesterone caproate/kg body weight during embryonic development. Histopathological examination revealed severe effects on spermatogenic cells in the testes and degeneration of seminiferous tubules and lumen devoid of spermatozoa in rats exposed to hydroxyprogesterone transplacentally (Pushpalatha et al. 2003b).

Analysis of sperm from the rats exposed to hydroxyprogesterone caproate during embryonic development further revealed the decrease in epididymal sperm count and poor sperm motility (Pushpalatha et al. 2004). The sperm function as revealed by HOS test and viability by tryphan blue method also revealed deteriorated sperm quality. These testicular and spermatotoxic changes might be responsible for the observed poor reproductive performance by the group II and group III rats (Morales et al. 1988; Oehniger et al. 1988).

The data suggest that the implantation loss may at least partly be due to failure of fertilization due to poor sperm quality and lowered sperm number. The reported decreased testicular steroidogenesis (Pushpalatha et al. 2003a) and histopathological changes (Pushpalatha et al. 2003b) might also be responsible for the observed decrease in fertility index. Thus, the results of the present study indicate that hydroxyprogesterone caproate exposed during embryonic development will affect the reproductive potential of adult male rats. Experiments are in progress in our laboratory to study the recovery of suppressed male reproduction in hydroxyprogesterone caproate exposed rats after testosterone treatment.

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