Reduced Spermatogenesis in Rats Exposed Transplacentally to Hydroxyprogesterone

Tinnanooru Pushpalatha, Pamuru Ramachandra Reddy, Geddapara Trivikram and Pamanji Sreenivasula Reddy*

Department of Biotechnology, Sri Venkateswara University, Tirupati-517 502, India

Received June 12, 2003; accepted September 9, 2003

Summary  Prenatal exposure to hydroxyprogesterone can alter the spermatogenesis in adult rat. For the purpose of assessing the effects of prenatal exposure to hydroxyprogesterone on postnatal reproductive performance, rats were treated intraperitoneally with doses of hydroxyprogesterone (10, 25 mg/kg body weight) on 1st, 7th and 14th day of gestation. The reproductive performance of the male offspring was assessed after 90 d by determining the changes in testis weight and the number of spermatids. A significant ($p<0.001$) decrease in testicular weight and number of spermatids was observed in the experimental male rats. Testis of the experimental rats also exhibited symptoms of arrest of spermatogenesis. It is suggested that in utero exposure to supra-normal levels of hydroxyprogesterone affects male reproduction by altering the spermatogenesis.

Key words  Hydroxyprogesterone, Spermatogenesis, Prenatal exposure, Male reproduction, Rat.

More than five million pregnant women were given diethylstilbestrol (DES), a synthetic estrogen to prevent spontaneous abortions from 1948 until 1971, when its use for this purpose was banned (Palmlund et al. 1993). Daughters whose mother took diethylstilbestrol suffer reproductive organ dysfunctions, abnormal pregnancies and a reduction in fertility (Takasugi and Bern 1988, Hines 1992). As young adults these women also suffer increased rates of vaginal clear-cell adenocarcinomas (Herbst et al. 1971). It was found in the early 1960s, that treatment of neonatal mice with estrogenic steroids or diethylstilbestrol led to pathological changes in female reproductive tract, beginning with persistent vaginal stratification and comification and developing in to a variety of dysplastic and possibly neoplastic lesions. Estrogen is administered in the first few days after birth were large enough, to induce persisted changes lasted even after ovariectomy and thus, once induced, were estrogen-independent (Bern 1992). Long-term and permanent effects were detected in the adult as a result of exposure to diethylstilbestrol during development, which can occur without apparent birth defects in the neonate (Kavlock et al. 1996).

Though much information is available on female reproductive abnormalities due to in utero exposure to supra-normal concentrations of female hormone, not much information is available on male reproductive changes during the above conditions. An elaborative programme to evaluate the male reproductive changes in rats during in utero exposure to hydroxyprogesterone was undertaken in our laboratory. We have observed the inhibition of steroidogenic enzyme activity levels in the testis of rat exposed prenatally to hydroxyprogesterone (Pushpalatha et al. 2002). The present report is a part of the programme, examines, the alterations in spermatogenesis in the rat exposed in utero to hydroxyprogesterone.

Materials and methods

Wistar strain rats were obtained from Central Animal Facility, Department of Zoology, Sri

* Corresponding author, e-mail: reddy_1955@yahoo.co.in
Venkateswara University, Tirupati. The rats were maintained under a regulated light: dark (12:12 h) schedule and were provided food and water ad libitum. The rat feed was purchased from Kamadhenu agencies, Bangalore, India. Only adult male rats (90 day old) were used in the present study.

Hydroxyprogesterone (trade name: Proluton depot) was purchased from a local drug store. This drug is most commonly prescribed to control uterine bleeding and to protect threatened pregnancy in women. Hydroxyprogesterone (250 mg/1.0 ml) is available in an oily solution of caster oil IP and benzyl benzoate IP (1:1.7).

Rats were allowed to mate and the pregnant rats were divided into three groups of 10 animals each. The first group, which served as control, was treated the same as the experimental group but received injections of mixture of caster oil and benzyl benzoate (1:1.7) in 20 μl volume. Rats in groups 2 and 3 received intraperitoneal injections of 10 mg hydroxyprogesterone/kg body weight and 25 mg hydroxyprogesterone/kg body weight on 1st, 7th and 14th day of pregnancy. The male pups were maintained under controlled conditions, and the rats were weighed and decapitated 90 days after birth. The testes were immediately excised, blotted and weighed.

Testes were fixed in Bouin’s fixative immediately after isolation. After dehydration in alcoholic series and clearing in xylol, the tissues were embedded in paraffin wax. Sections of 5 μm thickness were made and stained with haematoxylin and eosin. Diameters of seminiferous tubule and lumen were measured with eye piece graticules that have been calibrated with a stage micrometer. Only tubules that appeared round were considered for tubular and lumen diameter measurements. The number of preleptotene primary spermatocytes (irregular nucleus with deeply stained masses of chromatin through out the nucleus), pachytene spermatocytes (large nucleus with thick stands of chromatin) and sertoli cells (large nucleus contained finely stippled chromatin uniformly distributed, with large nucleolus) were counted in 10 tubules in different areas of the sections. The data was analysed using one way ANOVA followed by Student-Newman-Keuls test to determine the level of significance.

Results

During the experimental period no mortalities were observed in control or in experimental rats. A significant (p<0.001) decrease in the testicular weight was observed in rats exposed in utero to hydroxyprogesterone as compared to the controls. The decrease was more in 25 mg injected rats when compared to 10 mg injected rats, where as significant increase in body weight was seen in experimental rats (Table 1). The diameter of seminiferous tubules and seminiferous tubular lumen significantly decreased in the experimental rats as compared to the control rats. A significant decrease in the number of preleptotene primary spermatocytes, pachytene spermatocytes and sertoli cells was also observed in prenatal hydroxyprogesterone exposed rats (Table 1).

Histological observations of the testis of the control rat consist of seminiferous tubules and inter-tubular elements. The seminiferous tubules show normal spermatogenesis with all cell types and well developed interstitial cells. Each seminiferous tubule shows the tubular wall with the outermost basement membrane. Resting on the basement membrane are the spermatogonia and the sertoli cells. Towards the lumen, the primary spermatocytes, secondary spermatocytes and spermatids adhere to the sertoli cells. Sperms are seen with heads embedded in the sertolicells and tails lying in the lumen (Fig. 1a). Transverse section of the testis of the rat exposed to 10 mg hydroxyprogesterone/kg body weight show symptoms of arrest of spermatogenesis. The epithelium, spermatogonia, spermatocytes and spermatids are affected in experimental rat testis (Fig. 1b). Transverse section of the testis of rat exposed to 25 mg hydroxyprogesterone/kg body weight shows complete arrest of spermatogenesis. The seminiferous tubules are disorganized. The epithelium, spermatogonia, spermatocytes and spermatids are severely damaged and degenerated. The tubules show necropsied spermatogenic cells and the lumen was empty of active sperms (Fig. 1c).
Discussion

Endogenous hormones exert effects throughout embryonic development, after birth, and into adult hood. Altered hormone levels in response to embryonic exposure to xenoestrogens have been reported in alligators (Crain et al. 1997) and rats (Cooke et al. 1998). However, the present study provides the first direct link between effects on spermatogenesis in rats exposed to supra-normal levels of hydroxyprogesterone prenatally.

The weight of the testis is largely dependent on the mass of differentiated spermatogenic cells and it has been used as a measure of spermatogenesis in rats (Schlappack et al. 1988). A positive correlation was observed between weight of testis and number of germ cells (Sinha Hikkim et al. 1989). The reduction in the testicular weights could be due to the germinal cell loss in hydroxyprogesterone-exposed rats due to inhibition of testosterone production. Earlier, we have observed a decrease in steroidogenic enzyme activity levels in rats exposed in utero to hydroxyprogesterone. (Pushpalatha et al. 2002).

The decrease in diameters of seminiferous tubules and tubular lumen and reduction in number of preleptotene primary spermatocytes, pachytene spermatocytes and sertoli cells and change in testicular architecture could be due to decreased intra-testicular concentration of testosterone due to decreased steroidogenesis. Suppression of testosterone production and intratesticular testosterone has been observed in rats exposed to hydroxyprogesterone prenatally (Authors’ unpublished data). A series of morphological and biochemical changes during the maturation of sperm head are reported to be under the influence of androgens (Sharpe et al. 1990). The decreased activities of 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase in the testis of hydroxyprogesterone-exposed rats (Pushpalatha et al. 2002) might be responsible for decreased testosterone levels in rats exposed in utero to hydroxyprogesterone.

In recent years, declines in reproductive performance in several vertebrate species, including humans, were attributed to exposure to estrogens or environmental estrogens during prenatal, neonatal or in adult stages. Attributed effects include demasculinization of reptiles (Gross and Guillette 1994), demasculinization and feminization of male salmonoids (Leatherland 1992), demasculinization of shore birds (Shugart 1980) and reduced sperm count in humans (Sharpe and

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Group 2</th>
<th>Group 3</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>225.4±15.32</td>
<td>250.7±18.31</td>
<td>274.6±14.57</td>
<td>23.215</td>
</tr>
<tr>
<td>(11.22)</td>
<td>(21.82)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testes weight</td>
<td>1.26±0.08</td>
<td>1.06±0.10</td>
<td>1.01±0.11</td>
<td>18.421</td>
</tr>
<tr>
<td>(g/100 g body wt)</td>
<td>(−15.87)</td>
<td>(−19.84)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seminiferous tubule diameter (μm)</td>
<td>267.8±13.14</td>
<td>229.5±12.32</td>
<td>196.4±10.37</td>
<td>88.667</td>
</tr>
<tr>
<td>(−14.3)</td>
<td>(−26.66)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumen diameter (μm)</td>
<td>112.6±11.63</td>
<td>94.8±8.6</td>
<td>79.6±8.8</td>
<td>28.551</td>
</tr>
<tr>
<td>(−15.8)</td>
<td>(−29.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preleptotene spermatocytes (cells/10 tubules)</td>
<td>244.5±14.3</td>
<td>193.6±11.4</td>
<td>180.4±9.87</td>
<td>79.583</td>
</tr>
<tr>
<td>(−20.81)</td>
<td>(−26.21)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pachytene spermatocytes</td>
<td>267.4±11.6</td>
<td>241.6±9.81</td>
<td>232.4±14.4</td>
<td>22.541</td>
</tr>
<tr>
<td>(cells/10 tubules)</td>
<td>(−9.64)</td>
<td>(−13.08)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sertoli cells</td>
<td>45.2±5.42</td>
<td>37.3±4.79</td>
<td>25.6±6.41</td>
<td>31.232</td>
</tr>
<tr>
<td>(cells/10 tubules)</td>
<td>(−17.47)</td>
<td>(−43.36)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±S.D. of 10 animals. Values in the parentheses are % change from control. Values are significantly different from control at *p<0.001.
Fig. 1.  
a) Transverse section of testis from control rats showing the presence of normal tubular structure with spermatogenic cells at different stages of development. Scale line = 50 μm. 
b) Transverse section of testis of rat exposed in utero to 10 mg hydroxyprogesterone/kg body weight showing symptoms of arrest of spermatogenesis with few spermatogonia, spermatocytes and spermatids. Scale line = 50 μm. 
c) Transverse section of testis of rat exposed in utero to 25 mg hydroxyprogesterone/kg body weight showing complete arrest of spermatogenesis with ruptured epithelium and very few spermatogonia, spermatocytes and spermatids. Scale line = 50 μm. ST: Seminiferous tubules. EP: Epithelium. SG: Spermatogonia. SM: Sperm. LU: Lumen.
Shakkeback 1993). The results of the present study provide the first evidence that prenatal exposure to hydroxyprogesterone will affect the spermatogenesis in rats. Further experiments which are needed to firmly establish the role of prenatal exposure to hydroxyprogesterone affecting the male reproduction in rats are in progress in this laboratory.

Acknowledgements

We thank Prof. K. V. S. Sarma, Department of Statistics, S. V. University for statistical analysis of data. We are grateful to University Grants Commission, New Delhi for financial assistance in the form of research grant [F.3-54/99(SR-II)] to PSR. We are grateful to Dr. Manjulatha Reddy for her valuable advice. Mr. Umasankar maintained the rat colony in the Department. The experiments were conducted in accordance to the regulations of Ethical Committee and comply with the laws of the Country.

References


