Histol Histopath (1994) 9: 59-63

Histology and Histopathology

Effect of estrogenization in the first day of life on the reproductive system in male rats

A. Limanowski, B. Miśkowiak and B. Otulakowski
Department of Histology and Embryology and Department of Anatomy,
K. Marcinkowski University of Medical Sciences, Poznań, Poland

Summary. The aim of the present report was to investigate dynamics of morphological and functional changes in the reproductive system of male rats between 20th and 84th day of life, injected neonatally with a single dose of stilbestrol. Marked reduction in relative weights of testes and accessory sexual glands was demonstrated in various periods of life. This was associated with inhibition of spermatogenesis at the stage of primary spermatocytes and with morphological as well as functional alterations in epididymis, seminal vesicles and ventral prostate. In the serum, high levels of LH and lowered testosterone levels were demonstrated.

Key words: Neonatal estrogenization, Reproductive male system, LH, Testosterone

Introduction

Recent studies on differentiation of hypothalamic sexual centres have confirmed the dominating role of androgens in induction of hypothalamus defeminization (Sakuma, 1984). In either sex, hypothalamus always remains potentially feminine (Arnold and Breedlove, 1985). Our previous studies and those of other authors have shown that administration of sex hormones to male and female rats in the first day of life results in profound alterations in their reproductive system which are observed when animals reach maturity (Gorski and Wagner, 1965; Gorski, 1968; Dalterio et al., 1985; Limanowski et al., 1987, 1990a,b; Saez et al., 1991). In the newborn rats, the first minutes after birth were shown to represent particularly sensitive period to defeminizing action of androgens (Corbier, 1985). In male rats, appropriate concentration of testosterone during this periods leads to masculine differentiation of hypothalamus. Administration of estrogens to male rats and mice within the perinatal period leads to profund changes in the reproductive system after the animals reach maturity, including inhibition of spermatogenesis, structural and functional changes of Leydig cells with subsequent atrophy of seminal vesicles and inhibition of their secretory function (Limanowski, 1969, 1978; Aguilar et al., 1984; Dalterio et al., 1985). The changes are accompanied by a disturbed secretion of gonadotropin, sex hormones and LHRH (Elkind-Hirsh et al., 1984; Watts and Fink, 1984; Handa et al., 1985).

The present investigation was undertaken to examine the course of changes occurring in the reproductive system of the rat at various time intervals after estrogen administration, from the first day of life till the time when maturity is reached.

Offprint requests to: Dr. Bogdan Miśowiak, Department of Histology and Embryology, K. Marcinkowski University of Medical Sciences 6 Świecicki St., PL-60-781 Poznań, Poland

Materials and methods

The studies were performed using male rats of Wistar strain. The animals were given an s.c. injection of 1 mg Stilbestrol propionate (Polfa, Poland) in the first day of life. At 20, 28, 35, 45, 59, 65, 74 and 84 days of life experimental rats and control (untreated rats of the same age) were sacrificed in ether anesthesia by exsanguination from the left ventricle of the heart. Body weight, weights of gonads and of accessory sexual glands were recorded. Serum LH and testosterone levels were estimated by RIA techniques. The estimations were performed in the RIA Lab of the Department of Endocrinology of our University, using antibodies and ^{125}I-labelled antigens of their own make.

The results were subjected to statistical analysis using Duncan's test. Gonads, epididymis, seminal vesicles and ventral prostate were fixed in Bouin's solution, their sections were stained by the H-E method and examined under the light microscope.

Results

The relative weights of gonads and of accessory glands are shown in Table 1. A marked decrease in

Table 1. Relative weights of testis, epididymis, seminal vesicles and ventral prostate of control and neonatal estrogenized rats. Results expressed as means ± SD. n= 6.

	DAY OF LIVE							
	20	28	35	45	59	67	74	84
Testes (mg/100g BW)								
Control	683±96	671±34	1192±108	681±185	1349±23	1265±23	1008±28	1027±43
Estrogenized	195±19*	239±22*	230±23*	155±5**	423±32**	422±40**	462±15*	244±66**
Epididymis (mg/100g BW)								
Control	106±12	139±9	243±30	191±30	399±18	436±18	779±13	786±22
Estrogenized	53±3	61±5*	123±4*	90±9*	129±8**	83±6**	82±3**	78±5**
Seminal vesicles (mg/100g BW)								
Control	22±4	33±3	35±6	18±5	235±17	206±4	331±17	307±21
Estrogenized	47±3	47±6	93±8	53±2	54±10**	26±2**	32±3**	21±2**
Ventral prostate (mg/100g BW)								
Control	16±2	41±3	42±17	22±6	164±8	131±6	364±7	364±7
Estrogenized	23±2	27±3	13±2	14±1	66±3*	32±6**	28±2**	30±1**

Significantly different from control: *p< 0.05, **p< 0.01.

Table 2. LH and testosterone blood plasma level of control and neonatal estrogenized rats. Results expressed as means ± SD. n= 6.

	DAY OF LIVE							
	20	28	35	45	59	67	74	84
LH (ng/ml)								
Control	30±3	78±3	50±2	24±2	30±1	32±2	51±1	62±1
Estrogenized	38±1	83±7	85±9*	86±8*	96±6*	100±11*	83±6	217±23*
Testosterone (ng/ml)								
Control	0.38±0.05	0.75±0.05*	0.86±0.06*	1.04±0.11*	1.14±0.17	0.83±0.06*	1.04±0.07*	1.22±0.11*
Estrogenized	0.25±0.04	0.18±0.03	0.23±0.05	0.14±0.03	0.53±0.01	0.22±0.03	0.69±0.12	0.59±0.08

Significantly different from control: *p< 0.05.

Fig. 1. A. Testis of the control 45-day-old rat. Normal spermatogenesis is seen. **B.** Testis of the 45-day-old rat estrogenized on the first day of life. Inhibition of spermatogenesis is demonstrated. H & E. x 200

relative weights of gonads and of epididymis was evident in all studied age groups of rats treated with estrogens in the first day of life. Seminal vesicles demonstrated a decrease in their relative weight beginning on the 59th day and ventral prostate-beginning at 28th day of life. In estrogen-treated adult rats, when compared to the control group, the differences were marked and significant beginning on the 59th day of the rat's life.

Serum concentration of LH and testosterone in the experimental and control groups are listed in Table 2. Beginning on the 35th day of the rat's life, a significant increase in serum LH was noted, when compared to the control group, which was most pronounced in 84-day-old rats. This was accompanied by a decreased testosterone level, beginning on the 28th day of the rat's life.

Inhibition of spermatogenesis was observed starting on the 35th day of age which, at day 59, when the rats reached maturity, involved most evidently the stage of primary spermatocytes while the Leydig cells exhibited a fibroblast-like appearance. Similar alterations persisted in estrogen-treated rats examined in later life (Figs. 1A,B, 2A,B). Until day 35 after estrogenization, changes

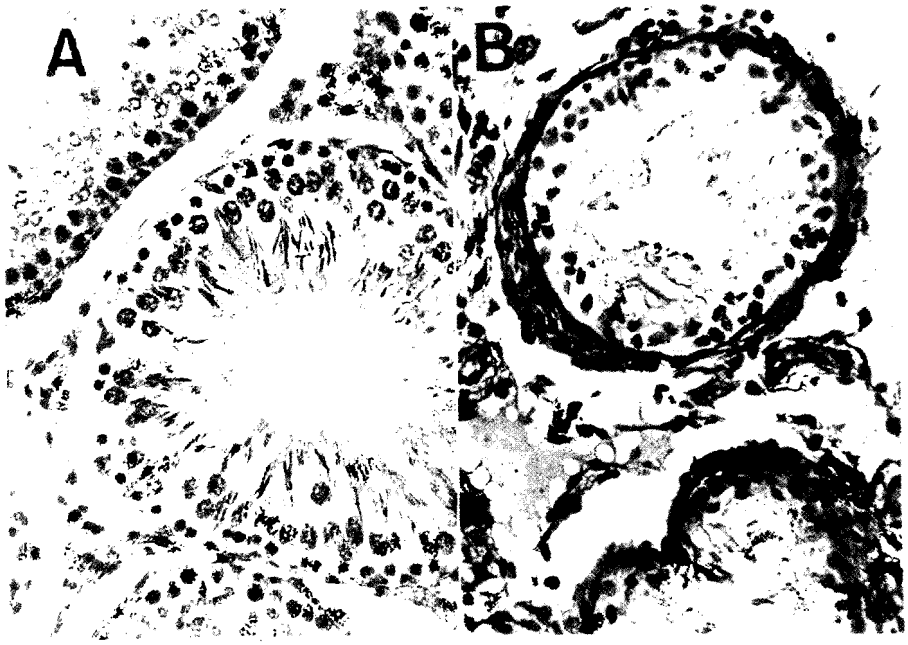

Fig. 2. A. Testis of the adult 84 days old, control rat. Normal spermatogenesis and normal Leydig cells are seen. **B.** Testis of the adult 84 days old rat treated in the first day of life with estrogen. Inhibiton of spermatogenesis, dedifferentiation of Leydig cells into cells resembling fibroblasts and thickening of basal lamina of seminiferous tubules are seen. H & E. x 200

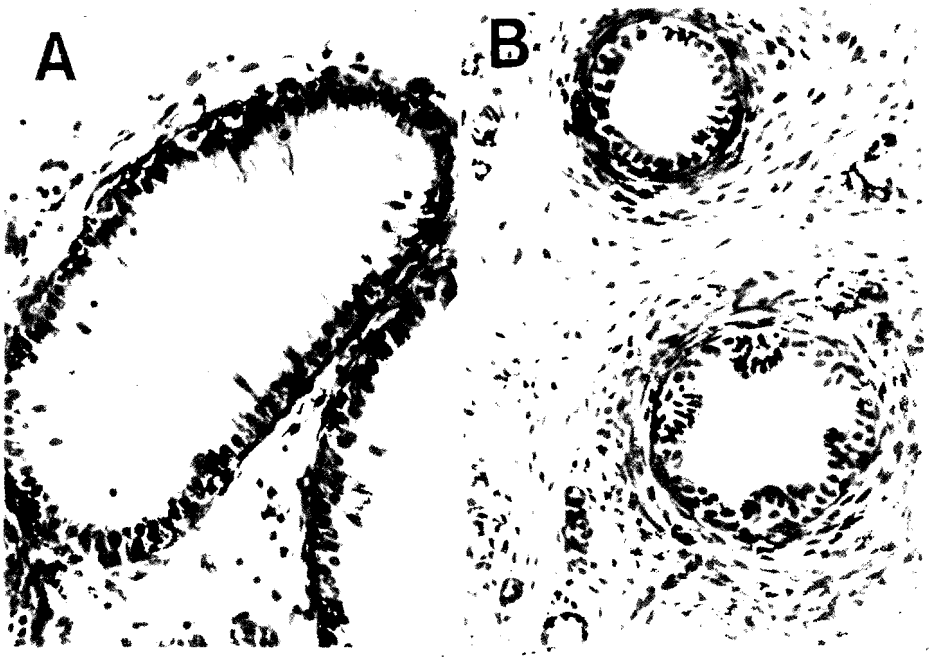

Fig. 3. A. Epididymis of adult control rat. **B.** Epididymis of adult rat estrogenized on the first day of life. Proliferation of connective and smooth muscle tisues are observed. H & E. x 200

in the accessory glands were limited to hyperplasia of connective and muscle tissues (Figs. 3A,B). In later periods, profound degenerative changes were additionally seen in glandular epithelium of all glands, especially in seminal vesicles. In adult rats the glandular epithelium was metaplastically transformed into multilayer planar epithelium, which was cornified and showed no signs of secretory functions (Fig. 4B). Control conditions have been demonstrated in Fig. 4A.

Discussion

The observed reduction in the relative weight of gonads and of accessory glands treated with estrogens in the first day of life is in accord with reports by other authors (Aguilar et al., 1984; Dalterio et al., 1985). In the testis, spermatogenesis was arrested at the level of primary spermatocytes, with concomitant transformations of Leydig cells into fibroblast-like cells. Studies of recent years have shown that, apart from the principal spermatogenesis steering mechanism involving gonadotropins, the process is additionally regulated by intercellular control mechanism operative in the testis. Sertoli cells, Leydig cells, components of seminiferous epithelium and of tunica propria secrete a number of factors such as, EGF, TGF alpha and beta, FGF and IGF-I which mediate reciprocal interactions of the cells. Sertoli cells, for example, secrete approximately 80 factors which affect, among others, components of seminiferous epithelium. Leydig cells carry receptors for EGF and TGF alpha, which modulate steroid synthesis in the cells. The above data allow us to interpret changes in testes of rats treated with estrogens in the first day of their life as resulting from, among others, disturbed interactions between cellular components of the testis.

The accessory glands and seminal vesicles in particular have shown marked atrophic changes in the glandular epithelium. In animals treated with estrogens in the first day of their life, the hyperplasia of the connective and muscle tissues in ventral prostate has been particularly striking. Mawhinney and Neubauer (1979) described a stimulatory effect of estrogens on growth and functions of prostate in various animal species. The decrease in serum testosterone level, observed in our investigations in neonatally estrogen-treated rats, has corresponded with the morphological appearance of Leydig cells and this in turn has influenced morphology and functional status of the accessory glands and seminal vesicles in particular. Increase in serum LH concentration in rats which were treated with stilbestrol in the first day of life suggests that Leydig cells have been damaged under the influence of stilbestrol administration. This in turn has resulted to a certain extent in a kind of pharmacological castration. Aguilar et al. (1984) have reported, on the other hand, a decrease in serum LH level in rats treated with estrogens in the first and fifth days of life.

The changes in the reproductive system of rats perinatally-treated with estrogens, which have been described above and which have been noted by us as well as by other investigators, support the view on exceptional sensitivity of hypothalamic sexual centres which, under influence of gonadal androgens, differentiate securing the male pattern of Gn-RH secretion (Gorski and Wagner, 1965). Corbier (1985) has shown that the critical timing of the effect involves the hour 0, i.e. the birth period. Recent studies of Reisert and Pilgrim (1991), and Pilgrim and Reisert (1992) have shown that sexual dimorphism of cerebral structures involves not just the hypothalamic sexual centres - the

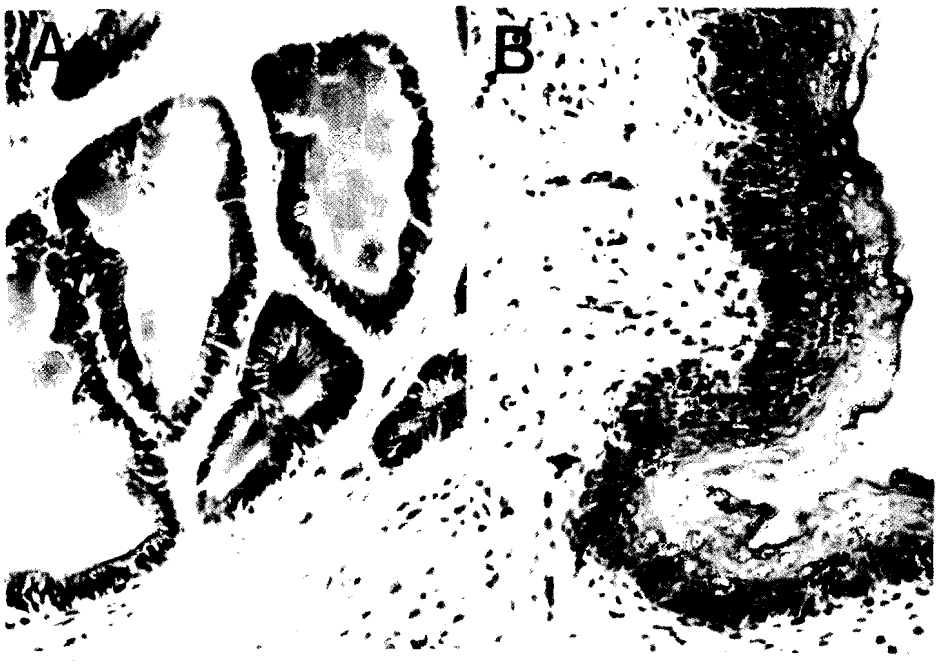

Fig. 4. A. Seminal vesicle of adult control rat. **B.** Seminal vesicle of adult rat treated with estrogen on the first day of life. Evident transformation of secretory epithelium into multilayer desquamating epithelium without secretory function is seen. H & E. x 200

sexually dimorphic nuclei preopric area, but also the limbic system, amygdala, stria terminalis, organization of the cerebral cortex, arrangement of neurocytes including the monoaminoergic neurons, the course of nervous fibres and concentration of neurotransmitters. Also, the role of other internal and external factors with affinity to the nervous system cannot be excluded. In our own studies (Miśkowiak et al., 1993) we have demonstrated inhibitory effect of MSG (monosodium glutamate) on the male rat reproductive system which appears when the neurotoxin is administered perinatally in high doses. In another report (Limanowski et al., 1991) we have shown synergistic effect of melatonin on the reproductive system of male rat treated with estrogens on the first day of life.

Summing up the presented literature data and our own results, we may conclude that a single dose of estrogens administered in the critical perinatal period of life disturbs sexual differentiation of male rat brain and injures gonad components, as reflected by the permanent alterations in structure and function of the reproductive system described by us, evident when the rats reach maturity.

References

Aguilar E.,Tejero A., Vaction M.D. and Fernandez Galaz C. (1984). Dissotiation of luteinizing hormone and follicle-stimulating hormone control mechanism in male and female rats by neonatal administration of estradiol benzoate or testosterone propionate. Horm. Res. 9, 108-116.

Arnold A.P. and Breedlove S.M. (1985). Organizational and activational effects of sex steroid on brain and behavior: a reanalysis. Horm. Behav. 19, 469-498.

Corbier P. (1985). Sexual differentiation of positive feedback: effect of hour of castration at birth on estradiol induced luteinizing hormone secretion in immature male rats. Endocrinology 116, 142-147.

Dalterio S., Bartke A., Steger R. and Mayfield D. (1985). Neonatal exposure to DES in BALB-c male mice: effects on pituitary-gonadal function. Biochem. Behav. 22, 1019-1024.

Elkind-Hirsch K., King J.C., Geroll A.A. and Leeman S.E. (1984). Neonatal estrogen affect preoptic/anterior hyptohalamic LHRH differently in adult male and female rats. Neuroendocrinology 38, 68-74.

Gorski R.A. and Wagner J.W. (1965). Gonadal activity and sexual differentiation of the hypothalamus. Endocrinology 76, 226-239.

Gorski R.A. (1968). Influence of age on the response to perinatal administration of a low dose of androgen. Endocrinology 82, 1001-1004.

Handa R.J., Corbier P., Shryna J.E., Schoomnaker J.N. and Gorski R.A. (1985). Differential effects of perinatal steroid environment on three dimorphic parameters of the rat brain. Biol. Reprod. 32, 855-870.

Limanowski A. (1969). The influence of single dose of stilbestrol dipropionate, LH and testosterone on the structure and function of the seminal vesicles in rats. Folia Morphol. 28, 326-332.

Limanowski A. (1978). Age dependent effects of hormonal stimulation of gonad of male rat estrogenized on the first day of life. Endokrynol. Pol. 29, 257-268.

Limanowski A., Miśkowiak B., Filipiak B. and Kozak W. (1987). Early stimulation with testosterone of sexual system of male rat treated with a single dose of stilboestrol on the first day of life. Endokrynol. Pol. 38, 467-476.

Limanowski A., Filipiak B. and Miśkowiak B. (1990a). Effect of testosterone administration during perinatal period on the reproductive system of female rats. Endokrynol. Pol. 41, 41-47.

Limanowski A., Miśkowiak B. and Filipiak B. (1990b). Perinatal effects of sex hormones on the reproductive system in male and female rats. Endokrynol. Pol. 41, 49-55.

Limanowski A., Otulakowski B. and Miśkowiak B. (1991). Effect of melatonin on reproductive system in male rat, treated with estrogen in the first day of life. Folia Histochem. Cytobiol. 29, 71-74.

Mawhinney M.G. and Neubauer B.B. (1979). Actions of estrogen in male. Invest. Urol. 16, 409-420.

Miśkowiak B., Limanowski A. and Partyka M. (1993). Effect of neonatal monosodium glutamate (MSG) administration on reproductive system of male rat. Endokyrnol. Pol. (in press).

Pilgrim Ch. and Reisert I. (1992). Differences between male and female brains - developmental mechanism and implications. Hormon. Metabol. Res. 24, 353-359.

Reisert I. and Pilgrim Ch. (1991). Sexual differentiation of monoaminergic neurons-genetic or epigenetic? Trends Neurosci. 14, 468-473.

Sakuma Y. (1984). Influences of neonatal gonadectomy or androgen exposure on the sexual differentiation of the rat ventromedial hypothalamus. J. Physiol. (London) 349, 273-286.

Saez J.M., Avallet O., Lejeune H. and Chaletain P.G. (1991). Cell-cell communication in the testis. Horm. Res. 36, 104-115.

Watts A.G. and Fink G. (1984). Pulsatile luteinizing hormone release and inhibitory effect of estradiol-17-beta in gonadectomized male and female rats: effects of neonatal androgen or exposure to constant light. Endocrinology 115, 2251-2260.

Accepted August 13, 1993