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Estrogen-Induced Abnormalities in Rat Germ Cell Development during Puberty

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The present study aimed to characterize the estrogen effect on different stages of germ cell development in tandem with Sertoli cell support to defined germ cell types. Neonatal treatment with diethilstilboestrol (DES) or GnRH-antagonist (GnRHa) exerted similar negative effects on spermatogonia (Sg), whereas spermatocytes (Sc) were more affected by DES compared to GnRHa. Among the spermatogonia, more differentiated types intermediate (In) and B-Sg underwent more pronounced changes than A-Sg. In the population of spermatocytes, leptotene and zygotene stages were most sensitive to hormonal manipulation and the effect of DES was more severe than that of GnRHa. DES caused retardation of testis development at puberty and suppressed spermatogenesis acting on differentiation of Sg, initiation and proceeding of meiosis via direct and indirect mechanisms. Differential effect of DES and GnRHa on Sg and Sc and their subtypes demonstrated differential sensitivity of mitotic and more advanced meiotic stages of spermatogoenesis to neonatal hormonal disbalance.

Key words: estrogens, androgens, spermatogenesis, Sertoli cells, testis.

Introduction

Exposure to estrogens during neonatal life is reported to cause delayed development of the testis and permanent impairment of spermatogenesis in adulthood that adversely affects total germ cell (GC) and Sertoli cell (SC) populations [2]. Neonatal administrations of estrogens suppressed FSH production at the time when this hormone is essential for initiation of spermatogenesis at puberty. For that reason the negative effect of estrogens were attributed to suppression of gonadotropin secretion during the treatment that results in inhibition of testosterone (T) production by Leydig cells, as well [1].

The effect of estrogens, in particular DES, on different steps of germ cell differentiation was not investigated. In this respect **the aim** of the present study was to characterize the estrogen effect on different stages of germ cell development in tandem with Sertoli cell support to defined germ cell types that would reveal their differential sensitivity to DES. These data would elucidate our understanding about the mechanisms via which estrogens regulate particular phases of spermatogenesis and to evaluate the importance of estrogens, androgens and gonadotrophins.

Material and Methods

We used experimental model for manipulation of neonatal hormonal environment by treatment with DES-10 μg or DES-0.1 μg (2-12 day); GnRH antagonist -10mg/kg (2 and 6 day); co-administration of 10 μg DES and 200 μg T-propionate. In situ detection of germ cell apoptosis by TUNEL method [5] and subsequent 121-point counting using clock-face sampling of 25 fields (3025 p) [2] were applied. We estimated absolute nuclear volume (ANV) of SC, total GC (TGC), spermatogonia (A-Sg, In and B-Sg) and spermatocytes including preleptotene (Pl), leptotene and zygotene (L+Z) and pachytene (Ph), as well as the ratios of GC/SC. Comparison of the different parameters of the various treatment groups was made using Student's t- test.

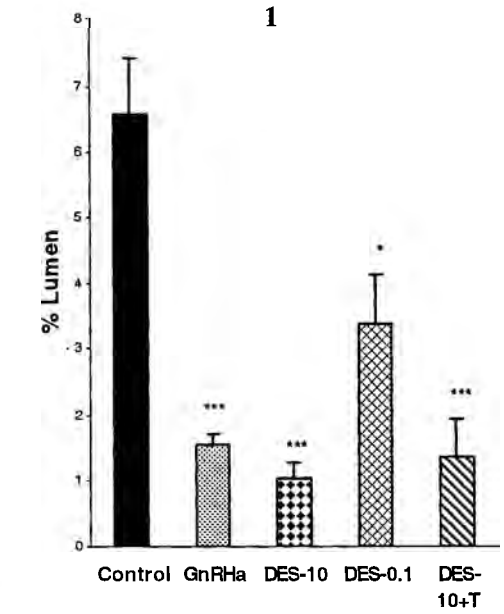
Results and Discussion

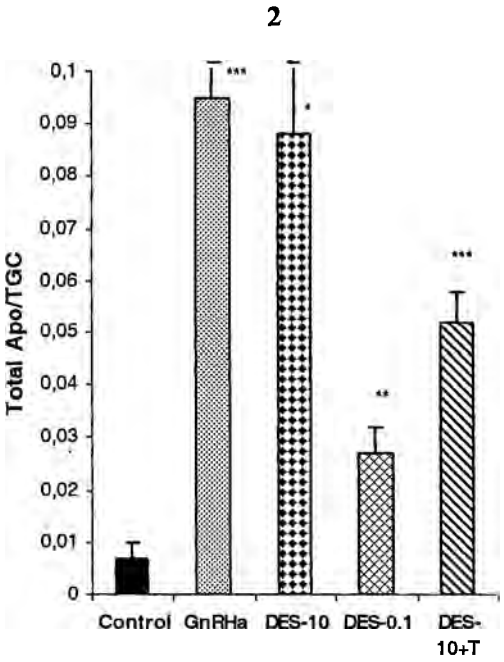
On day 18 in the control group GC development proceeds to the late pachytene stage of meiotic prophase-I. The lumen was observed in most seminiferous tubules. There were seen single apoptotic cells.

Both the neonatal administration of DES-10 or GnRHa induced pronounced structural changes involving elevation of GC apoptosis and retardation of lumen formation (Figs. 1, 2). Quantitative analysis revealed that both treatments caused 3-fold reduction of spermatogonial ANV than control whereas ANV of Sc was decreased 10 times by DES-10 and 6 times by GnRHa (Fig. 3). Differences between mean values of DES-10 and GnRHa were significant that implied the direct estrogen action on meiotic germ cells. Co-administration of DES-10 with T partially prevented negative estrogen effects on Sg and Sc. There was a milder effect of 100-fold lower dose of DES-0.1 on the investigated parameters of spermatogenesis. The similarities in the action of high levels of estrogen and those induced by GnRHa (both treatments inhibit T-production by Leydig cells; [4]) indicate that gonadotrophin suppression is involved in indirect mechanism of action of DES.

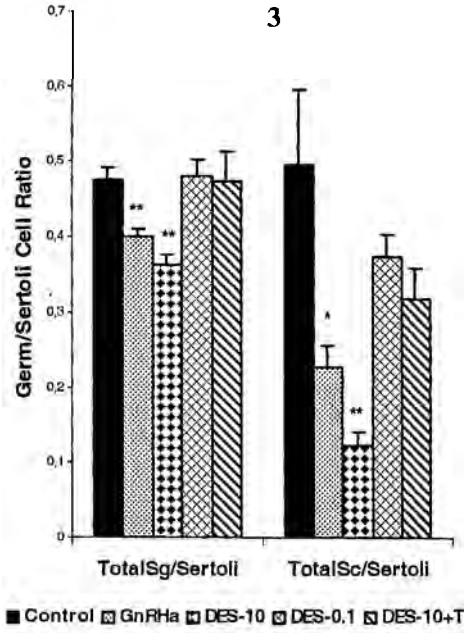
The function of SCs to support GCs, known as efficiency of spermatogenesis, was evaluated by estimation of ANV of GCs per unit SC ANV (Fig. 4). The ratio of TGC/ SC decreased in larger extent in DES-10 (50 % reduction than control) compared to GnRHa group (30%). The differences between mean values of DES-10 and GnRHa were significant. The SC supporting capacity to Sg and Sc was affected by treatment with DES-10 or GnRHa. The direct estrogen action is evident at ratio Sc/ SC but not Sg/SC, similarly to ANV of Sc and Sg. Spermatogenic efficiency remained in a normal range in experimental group of DES-0.1 and DES-10+T. The ratio Sg/SC is unaffected and that of Sc/SC is lower by 25-35% but not significantly different compared to control. These data suggest direct action of low estrogen levels on GCs (Sg and Sc) rather than indirect mechanism via SCs and their supporting function. Sertoli and germ cells were reported to express ER-β [3] and the direct adverse effect of high estrogen levels on functional maturation of SCs was demonstrated by Sharpe et al. [5].

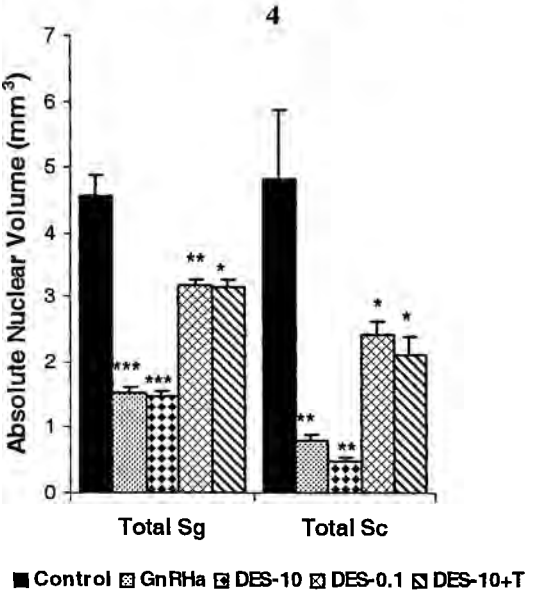
The differential sensitivity of different types Sg and Sc to neonatal hormonal manipulation was shown in Fig. 5 and 6. Exposure to DES-10 or GnRHa caused more pronounced changes in advanced types of spermatogonia — ANV of types In+B-Sg were reduced by 80% than control whereas that of A-Sg decreased non significantly by 35%. Among the Sc, L-Z stages were most sensitive to different hormonal treatments and the effect of DES was significantly more severe than those of GnRHa (13-fold and 8-fold decrease of ANV respectively). In support of this sug-





Figs. **1-4**. Quantification of spermatogenesis on day 18 of control and neonatally treated rats with GnRHa, DES-10 μg, DES-0.1 μg or co-administration of DES-10 μg and 200 μg T including Lumenal per cent volume (Fig. **1**); Apoptotic index (Fig. **2**); Absolute Nuclear Volume (mm^3) of GC types (Fig. **3**); Absolute Nuclear Volume of GC types per unit SC nuclear volume (Fig. **4**). Data represent mean value ± SE (* $p< 0.05$; ** $p< 0.01$; *** $p< 0.001$). TGC- Total Germ cells





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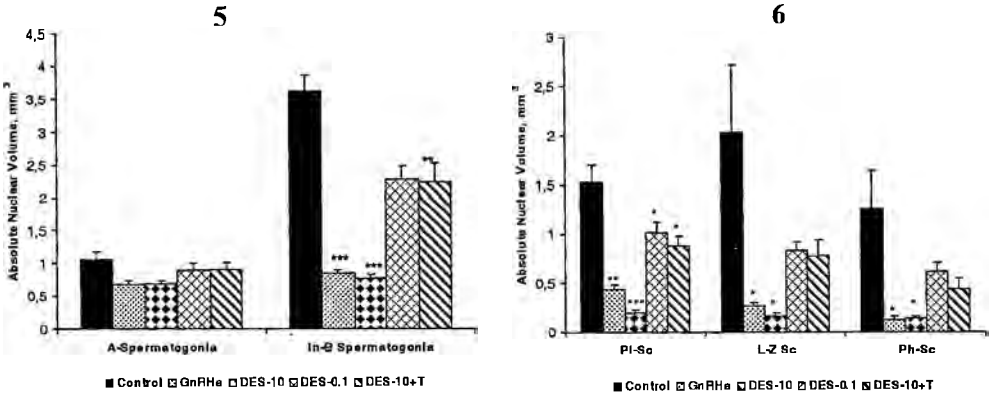

Figs. 5-6. Quantification of Absolute Nuclear Volume (mm^3) of subtypes of spermatogonia (Fig. 5) and spermatocytes (Fig. 6) on day 18 of control and neonatally treated rats with GnRHa, DES-10 μg, DES-0.1 μg or co-administration of DES-10 μg and 200 μg T. Data represent mean value ± SE (* $p< 0.05$; ** $p< 0.01$; *** $p< 0.001$)

gestion are the data for combined treatment of DES-10+T. There was a lesser restoration effect of T-therapy toward L-Z stages (40% of control value) compared to that of Pl-Sc (60% of control value).

Conclusion

DES and GnRHa exerted similar negative effects on Sg, whereas Sc were more effected by DES compared to GnRHa. Among the Sg, more differentiated types In+B-Sg underwent more pronounced changes than A-Sg. In the population of Sc, L-Z stages were most sensitive to hormonal disbalance and the effect of DES was more severe than those of GnRHa. DES caused retardation of testis development at puberty and suppressed spermatogenesis acting on differentiation of Sg, initiation and proceeding of meiosis via direct and indirect mechanisms. Differential effect of DES and GnRHa on Sg and Sc and their subtypes demonstrated differential sensitivity of mitotic and meiotic stages of spermatogoenesis to neonatal hormonal manipulation.

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