Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 16
Sofia • 2010

Review articles

Importance of Androgens and Estrogens for Mammalian Spermatogenesis

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Normal proceeding of spermatogenesis required gonadotrophic (FSH and LH) and steroid (testosterone and estradiol) hormones during different developmental stages. The importance and mechanism of action of each hormone is demonstrated by the data from experimental models and transgenic animals lacking androgen or estrogen receptors, gonadotrophins and their receptors. Endocrine disrupters are estrogenic and/or anti-androgenic chemicals widely spread in the environment. Acting as agonist or antagonists of steroid receptors they interfere in hormonal balance having potentially hazardous effects on male reproductive function. Most of the studies in the literature concerning the fine steroid balance in regulation of spermatogenesis have investigated Sertoli and total germ cell population. The mechanism of estrogen action on different stages of male germ cell development is poorly investigated. The absence of information about this problem requires implementation of profound study on the mechanisms via which estrogens regulate particular phases of spermatogenesis (mitotic, meiotic and postmeiotic stages).

Key words: androgens, estrogens, spermatogenesis, endocrine disrupters.

Normal male fertility relies on normal spermatogenesis, process by which immature germ cells undergo division, differentiation and meiosis to give rise to haploid elongated spermatides and finally spermatozoa. These events occur in close association with somatic cells, namely Sertoli cells in the seminiferous epithelium that communicate with germ cells directly via Ligand /Receptor mediated interactions or via paracrine signallization. Germ cell development requires as expression and secretion of many Sertoli cell proteins in stage specific manner as well as regulation by steroid hormones (androgens and estrogens) [18]. The predominant sources of testosterone (T) are the Leydig cells dispersed in intesrtistium of the testis (together with fibroblasts, macrophages and leucocytes). Besides control within

the testis the full fertilizing potential of the released spermatozoa is also dependent on the progression and maturation of sperm trough the excurrent duct system and epididymis.

The hormones secreted in the testis are required for various functions in the body including maintenance of secondary sexual functions and feedback on the hypothalamus and the pituitary to control the secretion of the gonadotropins LH and FSH. It is well known that gonadotropins are major endocrine regulators of spermatogenesis [16, 19, 20, 29]. In response to GnRH front part of pituitary secrets LH and FSH that act on different target cells in the testis realizing particular functions in its endocrine regulation [20]. FSH targets the Sertoli cells to regulate spermatogenesis by stimulating the production of numerous growth factors. Leydig cells are main target of LH and they are primary involved in the secretion of androgens, notably T, as well other steroids including estrogen as end products obtained from the irreversible transformation of androgens by aromatase. The role of FSH and T is greatly investigated but there are still lots of confusions about the mechanisms of action of these hormones on their target cells in supporting of germ cell development. Quantitative studies on FSH- and FSH receptor knock-out mice demonstrated lower sperm count although mice are fertile [12]. Consequently while FSH is not essential for qualitative aspects of spermatogenesis the hormone is clearly essential for quantitative normal spermatogenesis. During postnatal life Sertoli cells and spermatogenesis are differently sensitive to FSH and T, connected with switch from mainly FSH dependent to day 20 to more T dependent after day 40 [20]. It has been shown that androgens alone stimulated all phases of germ cell development in hypogonadal mouse (*hpg*), which is congenitally deficient in GnRH and therefore LH and FSH [26] enhancing the necessity of androgens and its determination as survival factor for spermatogenesis. In adulthood T supported qualitative normal spermatogenesis in hypophisectomised rats. Moreover quantitative parameters were reached by T application after treatment with GnRHa or ethane dimethanesulphonate (EDS) in spite of lack or reduced FSH levels [20]. FSH or T alone increased Sertoli cell number in *hpg* mice and there is observed pronounced synergetic effect. FSH alone is able to maintain proliferation of spermatogonia whereas meiotic and postmeiotic differentiation is dependent on T and required synergy of both hormones [10]. Both FSH and T are considered as major survival factors for germ cells in the male as experimental deprivation of these hormones induced profound germ cell apoptosis [27]. To some extent preferential importance of T is suggested although the role of FSH should not be underestimated [2]. Androgens act on androgen receptors (AR) to control spermatogenesis. In the pubertal and adult testis AR is localized in interstitium - in Leydig and peritubular cells and in seminiferous tubules only in Sertoli cells but not in germ cells. In adult testis stage-specific expression is found in Sertoli cells with lowest level in late stages of spermatogenic cycle and highest in stage VII-VIII when spermatozoa are released into the lumen. This stage is considered as androgen-dependent stage at which androgens preferentially acts on spermatogenesis [3]. Importance of androgen signaling for male reproductive development and function is demonstrated by transgenic mice lacking AR. DeGendt and colleagues [6] have generated two types of AR knock-out animals — total knock-out in all target cells (ARKO) and selective knock-out in Sertoli cells in the testis (SCARKO). ARKO males have very small testes in abdominal position. Spermatogenesis is arrested at very early stage of germ cell differentiation and reproductive tract and external genitalia are not developed, so they are phenotypic female. SCARKO animals have testes with normal scrotal position but with reduced testis weight. Reproductive tract and external genitalia

are developed and spermatogenesis proceeds completion of meiosis and postmeiotic differentiation. Comparative analysis of two models demonstrated the autonomous role of Sertoli cells in classical genomic mechanism of androgen action in the male [28]. High level of intratesticular T is required for spermatogenesis normal in quantitative and qualitative manner. The plasma levels of T are also adequate to normal male sexual and reproductive function [20]. Circulating plasma T and its derivates dihydro-testosteron (DHT) and estradiol (E_2) realize feedback regulation of LH and FSH secretion. Normal proceeding of spermatogenesis required exact hormones during different developmental stages: 1) initiation and proceeding of first spermatogenic wave during puberty, 2) support of spermatogenesis in adulthood, 3) re-initiation of spermatogenesis after temporary disturbance or lost of germ cells [2].

Whereas the role of androgens, FSH and LH is incontestable for spermatogenesis the recent investigations in endocrine regulation of spermatogenesis show that estrogens (E) should be added to the group of hormones involved in this regulation. A new role of estrogens for male reproductive function was suggested. Discovery of the expression of estrogen receptors (ER) in testis, entire reproductive tract, several hypothalamic nuclei and pituitary supports the suggestion that E regulate the hypothalamus-pituitary-testis axis [23]. In order to exert their biological role estrogens interact with ER which in turn modulate the transcription of specific genes. Until 1996 only ER-α was discovered and than the novel ER-β was identified. It was shown that the ER-α and the ER-β are not always present in the same cells (or are present in different amounts) within the male genital tract [4]. ER like AR are members of the steroid/ thyroid hormone super-family of nuclear receptors, which share common structural architecture, and consist of six independent but interacting functional domains [1]. In addition to the classic genomic pathway (mediated by ER) estrogens can also induce extremely rapid response via nongenomic mechanism of action involving membrane associated ER (particular important in cardiovascular and neuronal tissues) [18]. Immunohistochemical studies for ER-α show that this protein is present in mouse undifferentiated gonad at day 10. In prenatal Leydig cells ER-α is expressed before existence of AR. These findings support the suggestion that estrogens may have a significant role very early in the gonadal differentiation process. Expression of ER-β in gonocytes, Sertoli and Leydig cells until birth was also observed. Around the time of birth the testis continues to express both ER subtypes and aromatase. In adult testis ER-α is restricted to Leydig cells whereas ER-β is widely distributed- confined to Leydig cells, peritubular cells, Sertoli cells and some populations of germ cells- spermatogonia, late primary spermatocites (pachytene) and round spermatides. This data support the hypothesis of direct estrogen action as on somatic cells (Sertoli cells, peritubular and Leydig cells) as well as on germ cells in the testis. The first definitive demonstration that estrogens were required for male fertility was the use of knock-out models for ER. Mice lacking functional ER-α (ERαKO) were infertile due to defect in efferent ductile development and function [11, 14] in this way spermatozoa can not reach their full fertilizing capacity. Conversely, in the ER-β knock-out mice (ERβKO) no abnormal development of germ cells has been observed and the male are fertile but it has been noted hyperplasia of the epithelium of seminal vesicles, bladder and prostatic gland [13]. Impaired fluid reabsorbtion in efferent ducts leads to accumulation of the fluid in the tubular lumen that in tern exerts pressure on seminiferous epithelium affecting spermatogenesis [8]. Dealing with the double knock-out mice (ERα/β) the phenotype is identical to that of ERαKO and the males are sterile [5]. Mice lacking a functional aromatase gene (aromatase knock-out, ArKO) are also

infertile. Evidence from several studies indicates that ER-α, ER-β, and aromatase are encoded by separate genes but are co-expressed with AR in the male reproductive tract [1]. Data of Ebling et al. [7] show that treatment for 70 days with estradiol induced full qualitatively normal spermatogenesis in *hpg* mice where T production is lacking and FSH levels were 1/3 from control value. These results clearly indicate that estrogens may play a role in spermatogenesis, via some stimulatory effects on FSH secretion in addition to direct effect via ER in the testis [19]. Quantitative studies of spermatogenesis by Atanassova et al. [2] showed that high doses of estrogens induced pronounced germ cell apoptosis and affect spermatogenesis by suppressing FSH and T whereas low doses of estrogens have mild stimulatory effect and suppressed apoptotic index [2]. All data provide strong evidence for an important role of estrogen in the regulation of the testis and male reproductive tract and hence for male fertility.

Several environmental contaminants are known to interfere at various stages of germ cell development interfering in the normal hormonal balance and thereby causing reduced sperm count. Endocrine disrupters are estrogenic and/or antiandrogenic chemicals widely spread in the environment that have potentially hazardous effects on male reproductive function resulting in infertility and erectile dysfunction. Endocrine disrupters are able to mimic natural hormones. They can inhibit the production and/or action of hormones and /or alter the normal regulatory function of the endocrine systems [25]. In this way this compounds disrupt the hormonal balance in particular estrogen / androgen balance by binding to hormone receptors during fetal and postnatal development and give rise to reproductive abnormalities persisting to adulthood. Besides reduced fertility and erectile disfunction endocrine disrupters can induce testicular and prostate cancers, abnormal sexual development, alterations in pituitary and thyroid gland functions, embryo/ fetal loss, bird defects, immune suppression, neurobehavioral disruption [25]. Rats exposed in utero to certain phthalates also exhibit disorders of sperm production (even in normal descended testes) and reduced fertility. These changes are probably related to the occurrence of dysgenetic areas and germ-cell-depleted (Sertoli cell-only) testes [9] and epididymal lesions. The disorders induced by phthalates are remarkably similar to testis dysgenesis syndrome (TDS) disorders in the human [9, 15]. These changes are therefore reflection of endocrine disruption, but the latter occurred secondary to the disgenesis [21]. Many environmental xenobiotic chemicals, such as polychlorinated biphenyls (PCBs), dichlordiphenyltricloroethane (DDT), dioxin, and some pesticides have estrogenic effects [24]. A large part of agricultural products (phytoestrogens), industrial chemicals and heavy metals impair normal reproductive function because of their widespread presence in the environment and their ability to accumulate and resist biodegradation. In addition many pharmacological and biological agents including radiation therapy affect male fertility disrupting hormonal balance. One of compounds, diethylstilbestrol (DES) was greatly investigated throughout the years because of its identifying as a transplacental carcinogen and its proven negative effect in both male and female offspring exposed prenatally to DES. It is a potent synthetic estrogen that for many years was thought to prevent complications of pregnancy and between the late 1940s and the early 1970s DES was prescribed for million women in USA [30] and Europe. The male offspring exposed prenatally to DES has an excess prevalence of reproductive abnormalities (criptorhydism, hypospadia, low sperm count, epididymal cysts) and infertility [30]. It was found that DES is associated also with many reproductive difficulties in young women whose mothers had been given this drug during pregnancy, like clear-cell adenocarcinoma of the vagina and cervix, inferti-

lity, miscarriage, preterm delivery and fetal/infant death. Much of our understanding of the fetal/ neonatal effects of DES has come from studies of animal models demonstrating that DES caused retardation of testis development and suppressed spermatogenesis acting on differentiation of germ cells via direct and indirect mechanisms [2]. McKinnell and colleagues [17] demonstrated that in rats treated neonatally with DES androgen receptor immunoexpression was virtually absent from all affected tissues including the testis and entire reproductive tract. Suppression of androgen production and action (expression of AR) is an integral part of the mechanism via which estrogen effect male reproduction [2]. Comparison of the effects in animal studies of administrating either an anti-androgen or a potent estrogen such as DES reveal remarkable similarities in the changes that are induced at birth (cryptorchidism, hypospadias, epididimal and/or prostate abnormalities) and in adulthood (small testes, low sperm count, testicular germ cell cancer) [22]. The similarity in phenotypic changes suggests that common pathways of action may be involved in at lest some of this changes. One possible explanation is that administration of anti-A may elevate endogenous E levels and that this might contribute to some of the adverse effects in addition to blockage of androgen action. Conversely, E administration might interfere with androgen production or action in addition to activating ER-mediated pathways. This both possibilities would fundamentally alter the androgen/estrogen balance by lowering androgen action and elevating estrogen action. All studies in the literature concerning the fine steroid balance in regulation of spermatogenesis have investigated Sertoli and total germ cell population. The mechanism of estrogen action on different stages of male germ cell development is poorly investigated. The absence of information about this problem requires implementation of profound study that would elucidate our understanding about the mechanisms via which estrogens regulate particular phases of spermatogenesis (mitotic, meiotic and postmeiotic stages). Such studies will contribute to evaluation of the importance of estrogen/androgen balance in functional maturation of germ and somatic cells in the testis and to discern direct and indirect mechanisms of estrogen action on different testicular cell populations.

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