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Estrogens and male reproduction

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ABSTRACT

The role of estrogen on male reproductive function has become clearer in the last decade. During these years the study of the effect of testosterone, estrogen or an aromatase inhibitor in hypogonadal men provided a first evidence of the effects of estrogens in the regulation of gonadotropin secretion. At the same time, the development of a line of transgenic male mice lacking estrogen receptor α, estrogen receptor ß or aromatase gene provided further evidence about the role of estrogens not only in the regulation of gonadotropin secretion, but also on the effects of estrogens on testicular function and development. A confirmation of these actions of estrogens came from the observation of naturally occurring mutations of the estrogen receptor and of the aromatase gene in human males. Based on these data it has been demonstrated that estrogens are major regulators of gonadotropin secretion acting both at pituitary and hypotalamic level. The presence in the human reproductive structures of estrogen receptor α, estrogen receptor ß and the aromatase enzyme indicates the existence of receptor α, estrogen receptor ß or aromatase estrogen actions at this level. Anyway, the precise role of estrogens in testicular development and function and on the regulation of human spermatogenesis has not yet been precisely clarified.

Key words *: estrogen, aromatase, gonadotropin feedback, ERKO, ArKO*

I. INTRODUCTION

The establishment of estrogen role in male reproduction is a very recent acquisition in endocrinology [27].

The study of transgenic mice lacking functional estrogen receptors or functional aromatase enzyme permitted to shed new light into the role of estrogens on male reproduction [6]. Again, the discovery of human mutations in both the estrogen receptor alpha [28] and the aromatase gene [8] gave the opportunity to compare the role of estrogens on human male reproductive function to that of animal models.

Recently, in vitro, in vivo and immunoistochemical studies, together with animal and human models of estrogen deficiency, provided preliminary data useful for elucidating also the mechanism of estrogen actions on male reproductive cells [1, 19, 23].

II. ESTROGEN ACTIONS

In males estrogens derive from circulating androgens via aromatization by the aromatase enzyme (Ar). Estrogen actions are mediated by the binding to specific nuclear receptors (ERs), which are transcription factors that regulate the expression of target genes after hormone binding [12]. Two subtypes of ERs have been described: estrogen receptor α (ERα) and estrogen receptor ß (ERß). Estrogens exert their actions on the cell through a nuclear interaction after binding their receptors. A rapid, nongenomic action of estrogens, mediated through cell-surface recep-

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tors, which are not believed to act via a transcriptional mechanism [12] does exist, accounting for some estrogen actions which need to be clarified in detail.

III. DISTRIBUTION OF ESTROGEN RECEPTORS (ERs) AND AROMATASE (Ar) IN THE MALE REPRODUCTIVE SYSTEM

ERs and Ar are widely expressed in the male reproductive tract in both animals and humans. The demonstration at this level of the presence of both the Aromatase enzyme and ERs implies that estrogen biosynthesis does occur in the male reproductive tract and that both locally produced and circulating estrogens may interact with ERs in an intracrine/paracrine and/or endocrine fashion [1, 6, 8, 12, 19, 22, 23, 28]. Thus, estrogen action on male reproductive tract is strongly supported since male reproductive structures are able to produce and to respond to estrogens [1, 6, 8, 12, 19, 22, 23, 28].

1. ERs and Ar in the rodent testis.

During prenatal life, ER alpha is abundantly expressed in the developing ductules and epididymis, but it's not well established if it's present within the seminiferous tubules. In adult rat and mouse, ER alpha is expressed in the Leydig cells, but not in Sertoli cells. It seems that ER alpha is also expressed in adult rodent germ cells even though it has to be confirmed by further studies [6, 22].

ER beta is found very early in the gonocytes, Sertoli cells and Leydig cells, showing the gonocytes the higher expression. ER beta is widely expressed by the rat seminiferous epithelium (Sertoli cells and germ cells) as well as by Leydig cells, efferent ductules and epididymis. ER beta seems to be the only ER in germ cells: it is found in pachytene spermatocytes, round spermatids, and perhaps in elongated spermatids. ER beta is expressed in Leydig, Sertoli and germ cells in adult mice. In the male reproductive tract, ER beta distribution is inversely related to that of ER alpha, showing a progressive increase from proximal to distal ducts. ER alpha has a functional role in the mouse rete testis and efferent ductules as demonstrated in αERKO mice and in normal mice treated with an estrogen receptor antagonist [18].

Ar is expressed in both Leydig and Sertoli cells in the rodent fetal testis, but not in gonocytes and immature structure of seminal tract. Ar is expressed by the Sertoli cells at the time when FSH receptor is expressed too; fetal Leydig cells have also the ability to produce estrogens in response to LH, but aromatase from this cell-type is expressed to a lesser degree in fetal and neonatal rats. Germ cells do not express aromatase during prenatal life, a phenomenon which will become of great importance only in the adult rodent testes. By adulthood, rodent Leydig cells show a higher aromatase activity with respect to every other age and in comparison to Sertoli cells.

Aromatase too is expressed at a high level in germ cells throughout all stages of maturation and its expression appears to be higher as the immature germ cell becomes a mature spermatid [22].

It is not well established whether aromatase is expressed in the seminal tract of adult rodents.

2. ERs and Ar in the human testis

Both ERs have been found in human testis and reproductive tract. In the male fetus ER beta expression is higher than ER alpha, being the latter absent or expressed at very low levels. Particularly in the human fetus ER beta is present in the seminiferous epithelium (Sertoli cells and few germ cells) and in the epididymis, while ER alpha is undetectable in these structures, thus suggesting a major role of ER beta during prenatal development and function of male reproductive structures [30].

In adult men, ER alpha is expressed only in Leydig cells, while ER beta has been found in the efferent ducts. No ERs have been detected in the epididymis. Besides ER beta was documented in both Leydig and Sertoli cells [6, 22].

Ar expression in the human testis involves both somatic and germ cells from pachytene spermatocytes through elongated spermatids. Ar is also expressed in both human Leydig and Sertoli cells. Recently it has been demonstrated the presence of aromatase gene not only in immature germ cells but also in completely mature human spermatozoa [5, 16, 22].

IV. ROLE OF ESTROGENS ON MALE REPRODUCTION

Our knowledge of the role of estrogens in male reproductive system has been significantly improved by the creation of knock-out transgenic mice models lacking functional ER alpha, ER beta or Aromatase gene (αERKO, ßERKO, ArKO mice respectively).

Adult, sexually mature, αERKO mice are infertile even though the development of the male reproductive tract is unaffected [6]. The adult histological picture of αERKO male mice testes is characterized by atrophic and degenerating seminiferous epithelium together with dilated tubules and a dilation of the rete testis. Damage of testicular tissue becomes evident at about 40-60 days, when the tubules are completely dilated with a corresponding significant increase of testicular volume and when the seminiferous epithelium becomes atrophic [6, 15]. A severe impair-

ment in tubular fluid absorption in the efferent ducts was demonstrated to be the cause of infertility in αERKO male mice; this probably derives from the interaction of estrogens to ER alpha which causes a progressive swelling of the seminiferous tubules as a consequence of tubular dilatation. This process results in a severe impairment of spermatogenesis, coupled with testicular atrophy [6, 15].

ArKO mice are initially fully fertile [11], but fertility decreases with advancing age [24] and conversely ßERKO mice are fully fertile and phenotypically normal also during adolthood [17]. Histology of the testes of one year old ArKo mice shows a disruption of spermatogenesis at early spermatogenetic stage, without significant changes in the volume of the seminiferous tubules lumen, together with Leydig cells hyperplasia [22, 24]. The mechanism involved in the development of infertility is different in ArKO male mice since the early arrest of spermatogenesis suggests a failure of germ cells differentiation probably caused by the lack of estrogens in the testicular environment.

Recently, the developement of the αßERKO mice, in which both alpha and beta estrogen receptor are disrupted, showed a male phenotype very close to that of αERKO mice with infertility and dilated seminiferous tubules. From the data shown it seems that functional alpha estrogen receptor, but not beta, is needed for the development and maintenance of normal fertility in male mice even though beta receptor is widely expressed in the rat didimis [6, 22].

Some naturally occurred mutations in the estrogen receptor or in the aromatase gene in human males helped us understanding the role of estrogens in the regulation of the hypothalamo-pituitary-gonadal axis in the human male [26] (Table 1).

Data obtained from men affected by congenital estrogen deficiency have provided conflicting and confused results concerning fertility rate (Table 1). The only man with estrogen resistance discovered up to now, a human equivalent of the ERKO mice, had normal testis volume and normal sperm count with slightly reduced motility [28]. The four adult male patients affected by congenital aromatase deficiency showed a variable degree of impaired spermatogenesis [3, 4, 14; 20, 21, 26]. The patient described by Carani et al., showed both a severely reduced sperm count and an impairment of sperm viability with germ cell arrest at the level of primary spermatocytes [4]. In a new patient a complete germ cell arrest was documented by the biopsy of the testis even though semen analysis was not performed because of the patient's religious beliefs. In this patient a surgically treated bilateral cryptorchidism has been documented at the medical history [20]. Data concerning the patient described by Morishima et al. are lacking since sperm was not analyzed [3, 21]. Another patient with congenital aromatase deficiency showed oligoasthenozoospermia. However in this patient another genetic disorder cannot be rouled out since his brother who had a normal aromatase enzyme expression had the same alterations in semen analysis [14]. Of interest, it should be remarked that a clear cause-effect relationship between infertility and aromatase deficiency is not demonstrable also in the patient studied by Carani et al., since also one of his brothers was infertile despite the absence of mutations in the aromatase gene [4].

The variable degree of fertility impairment in men with congenital estrogen deficiency (Table 1) does not permit a conclusive demonstration of a direct relationship between estrogen deficiency and infertility, even though a possible role of estrogen on human spermatogenesis is strongly suggested. Recently, in fact, the administration of aromatase inhibitors to infertile men with an impaired testosterone to estradiol ratio, with high estradiol levels, resulted in an improvement of the spermatic parameters [23]. Thus these data together with the demonstration of aromatase activity in human ejaculated spermatozoa [1] suggest a role for estrogen also in human male reproduction.

V. ESTROGEN AND THE REGULATION OF GONADOTROPIN SECRETION IN THE MALE

Studies performed on normal and GnRH deficient men showed that testolactone administration prevented the testosterone-induced LH suppression in normal men [9]. These same effects appeared evident in GnRH-deficient men whose gonadotropin secretion had been normalized by pulsatile GnRH infusion [9]. This raised the possibility of a suppressive effect of estradiol on LH secretion at least at the pituitary level [9, 10].

The administration of dihydrotestosterone, a nonaromatizable androgen, in normal men, did not induce a reduction in gonadotropin secretion [2].

Estrogen have also been showed to decrease GnRH pulse frequency at hypotalamic level and to reduce GnRH responsiveness at the pituitary level [13]. Since no ERs, at least of the alpha subtype, have been found in GnRH secreting neurons [29], the issue of the precise mechanism of action of estrogen in the regulation of the hypotalamo-pituitary-gonadal axis in the male still remains open.

The study of men with aromatase deficiency shows that estrogens, rather than androgens are the principal regulators of gonadotropin feedback, acting both on basal gonadotropin secretion (Table 1) and on gonadotropin responsiveness to GnRH [14, 20, 21, 25].

In a young patient affected by congenital aromatase defi-

Table 1 : Reproductive phenotypes of men with congenital estrogen deficiency.

	ESTROGEN RESISTANCE	AROMATASE DEFICIENCY
LH	Increased [28]	1) **Increased** [21] 2) **Normal-increased** [4] 3) **Normal** [20] 4) **Normal** [14]
FSH	Increased [28]	1) **Increased** [21] 2) **Increased** [4] 3) **Increased** [20] 4) **Increased** [14]
Testosterone	Normal [28]	1) **Increased** [21] 2) **Normal** [4] 3) **Low-normal** [20] 4) **Increased** [14]
Estradiol	Normal [28]	1) **Undetectable** [21] 2) **Undetectable** [4] 3) **Undetectable** [20] 4) **Undetectable** [14]
Semen Analysis	Sperm count : $25x10^6$/mL Viability : 18% [28]	1) **Not performed** [21] 2) **Sperm count : $1x10^6$/mL** **Viability : 0%** [4] 3) **Not performed** [20] 4) **Sperm count : $17.4x10^6$/mL** **Viability : 55%** [14]
Gender Identity	Male [28]	**Male** [6, 8, 27, 28]
Sexual Orientation	Heterosexual [28]	**Heterosexual** [6, 8, 27, 28]

ciency no alterations were found in gonadotropin secretion [7]. This finding rises the possibility that the role of estrogens on the hypotalamo-pituitary-testicular axis becomes relevant in a later stage of life than infancy [8, 25, 26].

VI. CONCLUSIONS

In conclusion, estrogens seem to play an important role in the regulation of male reproductive function. Estrogens are major regulators of gonadotropin secretion not only at the pituitary level but also at the hypotalamic level. Their actions on the regulation of testicular function and spermatogenesis can be hypothesized by the presence of both estrogen receptors and the aromatase gene in the testis at various levels, but the precise role of estrogen in the regulation of spermatogenesis is still unclear. Further studies are needed to completely elucidate the role of estrogens on male reproductive function and their possible clinical implications.

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