Estrogen and Spermatogenesis*

LIZA O’DONNELL, KIRSTEN M. ROBERTSON, MARGARET E. JONES, AND EVAN R. SIMPSON

Prince Henry’s Institute of Medical Research (L.O., K.M.R., M.E.J., E.R.S.) and Department of Biochemistry (K.M.R.), Monash University, Clayton, 3168, Victoria, Australia

ABSTRACT

Although it has been known for many years that estrogen administration has deleterious effects on male fertility, data from transgenic mice deficient in estrogen receptors or aromatase point to an essential physiological role for estrogen in male fertility. This review summarizes the current knowledge on the localization of estrogen receptors and aromatase in the testis in an effort to understand the likely sites of estrogen action. The review also discusses the many studies that have used models employing the administration of estrogenic substances to show that male fertility is responsive to estrogen, thus providing a mechanism by which inappropriate exposure to estrogenic substances may cause adverse effects on spermatogenesis and male fertility. The reproductive phenotypes of mice deficient in estrogen receptors α and/or β and aromatase are also compared to evaluate the physiological role of estrogen in male fertility. The review focuses on the effects of estrogen administration or deprivation, primarily in rodents, on the hypothalamo-pituitary-testis axis, testicular function (including Leydig cell, Sertoli cell, and germ cell development and function), and in the development and function of the efferent ductules and epididymis. The requirement for estrogen in normal male sexual behavior is also reviewed, along with the somewhat limited data on the fertility of men who lack either the capacity to produce or respond to estrogen. This review highlights the ability of exogenous estrogen exposure to perturb spermatogenesis and male fertility, as well as the emerging physiological role of estrogens in male fertility, suggesting that, in this local context, estrogenic substances should also be considered “male hormones.”


I. Introduction

THE traditional view of estradiol as the “female” hormone and of testosterone as the “male” hormone has been challenged in recent years (1, 2). The increased interest in the role of estrogens in the male is largely due to the demonstration that male fertility is impaired in mice lacking estrogen receptor-α (ERα) (3–6), or aromatase (7–9), along with the discovery of a second estrogen receptor-β (ERβ) (10–13), which is widely expressed in the male reproductive tract (14).

Another important reason for the increased interest in the role of estrogen in male reproduction stems from various reports that exposure to estrogens in the environment may have a detrimental effect on male reproductive development and health and may be related to the reported decreases in sperm counts over the past 50 yr (15–17). Although controversy exists as to whether male fertility over the past five decades has truly declined (18), and whether the exposure of humans to the relatively low levels of estrogens in the environment would cause significant health problems (19), research into the effects of estrogens on the male reproductive system is clearly warranted (19, 20). In agreement with the notion that estrogen exposure during development may impair male fertility, it has been known for many years that estrogen administration to experimental animals during the neonatal period or adulthood can impair sperm production and maturation (21, 22).

Normal male fertility relies on normal spermatogenesis, i.e., the process by which immature spermatogonia within the testis divide and differentiate into the mature elongated spermatid form that is subsequently released from the seminiferous epithelium. The full fertilizing potential of the released spermatozoan is also dependent on the progression...
and maturation of sperm through the excurrent duct system and the epididymis.

While the administration of estrogens and xenoestrogens during fetal and neonatal development has been reported to be associated with a series of male reproductive disturbances, such as cryptorchidism, epididymal defects, impaired fertility, and an increased incidence of testicular cancer (e.g., Refs. 23–25 and references therein), an essential physiological role for estrogen in male fertility was not identified until the early 1990s. The infertility in mice lacking a functional ERα (ERαKO) was the first definitive demonstration that estrogen was required for male fertility (3–6). ERαKO mice are infertile primarily due to a defect in efferent ductule development and function (6, 26). Mice lacking a functional aromatase gene (aromatase knockout, ArKO) are also infertile; however, this appears to be primarily due to a specific defect in germ cell development (8). Thus lessons provided by mice transgenic for ERα and/or ERβ [i.e., ERαKO, ERβKO, and ERαβKO mice (see Ref. 27 for review)] as well as aromatase [ArKO mice (8, 9)], provide compelling evidence for a role for estrogen in spermatogenesis and male fertility.

Estrogen is clearly involved in numerous processes in the male, including bone turnover, behavior, and the cardiovascular system (see Ref. 2 for review). However, this review will focus on the effects of estrogen administration and deprivation on the production and function of spermatozoa. This review will address two important questions: 1) can exposure to estrogen or estrogenic substances interfere with spermatogenesis and male fertility? and 2) does estrogen have a physiological role in spermatogenesis and male fertility? Given that spermatogenesis relies on, among other things, the normal development and function of the hypothalamo-pituitary-testis axis, testicular cells (including Leydig cells, Sertoli cells, and germ cells), efferent ductules, and epididymis, the experimental evidence for the effects of, and/or a role for, estrogen in each of these systems will be presented. This review will also provide an overview of estrogen biosynthesis and action in general, and specifically in the testis. A brief overview of the phenotype of humans with mutations in ERs or aromatase will also be presented, as will an overview of a role for estrogen in sexual behavior, given that this is proving to be an important component of estrogen action in male fertility.

II. Overview of Spermatogenesis

Spermatogenesis is the process by which immature germ cells undergo division, differentiation, and meiosis to give rise to haploid elongated spermatids. This process takes place within the seminiferous tubules of the testis, in close association with the somatic cells of the seminiferous epithelium, the Sertoli cells (Fig. 1). When germ cell development is complete, the mature spermatids are released from the Sertoli cells into the tubule lumen, and proceed through the excurrent duct system, known as the rete testis, until they enter the epididymis via the efferent ducts (Fig. 1). During passage through the epididymis, the spermatids undergo a series of biochemical changes to become the motile spermatozoa capable of fertilization.

The testicular parenchyma, consisting of seminiferous tubules and interstitial tissue (Fig. 1), is enclosed by a capsule called the tunica. The interstitial tissue contains the blood and lymphatic vessels, which are essential for the movement of hormones and nutrients into, and out of, the testis. The most frequently encountered cell type in the interstitium is the Leydig cell (28), which is primarily involved in the se-

---

**Fig. 1.** Diagram of the testis and spermatogenesis. A, Diagram of the testis (T), rete testis (rt), efferent ducts (ed), and caput, corpus, and cauda epididymis. B, Cross-section through an adult rat testis, showing several seminiferous tubules (st) in various stages of development, and the interstitial space (it) which contains Leydig cells, blood and lymph vessels, and macrophages. C, High magnification of the seminiferous epithelium. A single Sertoli cell with a basally located nucleus (n) with a central nucleolus can be seen. The cytoplasm of the Sertoli cell surrounds germ cells at various stages of development. Spermatogonia (sg) are located closest to the base of the tubule, with spermatocytes (sc) above the spermatogonial layer. Round spermatids (rs) are visible above the spermatocyte layer and in this micrograph, elongated spermatids (est) are embedded within the Sertoli cell. The seminiferous epithelium resides on the basal lamina, which is made up of extracellular matrix and peritubular myoid cells.
cretion of androgens, notably testosterone, as well as other steroids including estrogen.

Within the seminiferous tubules, the Sertoli cells reside on a basement membrane, under which are the lymphatic endothelium and the peritubular myoid cells (29). The structure of the Sertoli cell is extremely complex, with numerous cup-shaped processes encompassing the various germ cell types (30) (Fig. 1). Developing germ cells form intimate associations with Sertoli cells, with multiple germ cell types in contact with one Sertoli cell. The various generations of germ cells, as described below, are not randomly distributed within the seminiferous epithelium, but are arranged in strictly defined cellular associations (31). It is the unique associations of these germ cells with Sertoli cells that constitute the cycle of the seminiferous epithelium, and each particular association of germ cells is referred to as a stage. The number of stages of spermatogenesis in a particular species is thus defined by the number of morphologically recognizable germ cell associations within the testis; in the mouse there are 12 stages, in the rat there are 14, and in the human there are 6 (32).

A. Germ cell development

Germ cell development involves a complicated series of events, and the various germ cell types can be distinguished on the basis of morphology and the differential expression of proteins.

Spermatogonia are present between Sertoli cells, close to the basement membrane of the tubule. They are the most immature germ cells in the testis, and include type A spermatogonia, intermediate spermatogonia (found only in rodents), and type B spermatogonia, the latter of which are considered to be committed to differentiation. The true stem cells of the germ cell population are considered to be a subset of the type A spermatogonial population, although their identity cannot be discerned on the basis of morphology (33). Spermatogonia undergo numerous mitoses to produce a large number of germ cells available for entry into meiosis. Thus, proliferation of the spermatogonial population provides the source for the millions of sperm that are produced per day (32).

After the last mitosis of type B spermatogonia, preleptotene primary spermatocytes are formed (32). These cells replicate their DNA and hence initiate meiosis (33). During the prophase of the first meiotic division, germ cells undergo morphological transitions that can be classified on the basis of nuclear size and morphology (34). In the zygotene phase, pairing of homologous chromosomes occurs, and cells with completely paired chromosomes are termed pachytene spermatocytes. After the pachytene phase, a brief diplotene phase follows in which the chromosome pairs partially separate, and the cells then undergo the first meiotic division to yield secondary spermatocytes. These cells quickly undergo the second meiotic division to yield the haploid sperm cell into the lumen of the seminiferous tubule (36).

B. Regulation

Germ cell development relies on a highly coordinated interaction with the Sertoli cell. Germ cells and Sertoli cells can communicate directly via ligand/receptor-mediated interactions or paracrine factors. The production and secretion of many Sertoli cell proteins involved in germ cell development occur in a stage-dependent manner (37), reflecting the ability of the Sertoli cell to adapt to the changing needs of the germ cell. For many years it was presumed that Sertoli cells were the major controlling factor in the timing of germ cell development; however, recent studies investigating rat-to-mouse spermatogonial transplantation clearly demonstrated that rat germ cells in contact with mouse Sertoli cells develop according to the kinetics of rat spermatogenesis, thus highlighting the role of germ cells in controlling their own fate (38).

As well as the production of spermatozoa, the testis is involved in the production of hormones that 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 the gonadotropins are the major endocrine regulators of spermatogenesis (39–41). LH targets the Leydig cell to stimulate the secretion of androgens, namely testosterone, which in turn acts on androgen receptors in the seminiferous epithelium to control spermatogenesis. FSH targets receptors within the Sertoli cell to regulate spermatogenesis by stimulating the production of numerous Sertoli cell factors. The roles of testosterone and FSH in the testis have been studied extensively, yet relatively little is known about how these hormones act within the Sertoli cell to stimulate and maintain spermatogenesis (39–41). Androgens alone have been shown to stimulate all phases of germ cell development in the hypogonadal (hpg) mouse, which is congenitally deficient in GnRH and therefore LH and FSH (42), highlighting the requirement of spermatogenesis for androgen. The question of whether FSH was essential for spermatogenesis in mice was answered by the generation of transgenic mice possessing targeted disruptions of the FSH receptor gene (43) or the FSH β-subunit gene (44). Males of both transgenic models are fertile and display all stages of germ cell development, as are the androgen-treated hpg mice (42), suggesting that FSH is not an absolute requirement for fertility. However, in both cases the testes are smaller, and less sperm are produced (42–44), due to the requirement for FSH during the neonatal period of Sertoli cell division (45, 46). More recent quantitative studies on FSH receptor knock-
out mice also demonstrated defects in sperm development, leading to the production of poor quality sperm (47). Thus while FSH is not essential for spermatogenesis, it is clearly essential for quantitatively normal spermatogenesis and fertility. In terms of the endocrine regulation of spermatogenesis by FSH, LH, and androgens, it is clear that the initiation and maintenance of quantitatively normal spermatogenesis and thus full fertility rely on the delicate balance of the hypothalamo-pituitary-testis axis. The focus of this review is the growing body of evidence that suggests that estrogen should be added to the list of hormones involved in the regulation of spermatogenesis.

III. Biosynthesis and Action of Estrogen

A. Estrogen biosynthesis

Estrogen biosynthesis is catalyzed by a microsomal member of the cytochrome P450 superfamily, namely aromatase cytochrome P450 (P450arom, the product of the CYP19 gene). The P450 gene superfamily is a very large one, containing more than 600 members in almost 100 families, of which cytochrome P450arom is the sole member of family 19. This heme protein is responsible for binding of the C19 androgenic steroid substrate and catalyzing the series of reactions leading to formation of the phenolic A ring characteristic of estrogens. The aromatase reaction employs 3 mol of oxygen and 3 mol of NADPH for every mole of steroid substrate and catalyzing the series of reactions leading to formation of the phenolic A ring characteristic of estrogens (49). The reducing equivalents for this reaction are supplied from NADPH via a ubiquitous microsomal flavoprotein, NADPH-cytochrome P450 reductase. In humans, a number of tissues have the capacity to express aromatase and hence synthesize estrogens. These include the ovaries and testes, the placenta and fetal (but not adult) liver, adipose tissue, chondrocytes and osteoblasts of bone, and numerous sites in the brain including several areas of the hypothalamus, limbic system, and cerebral cortex.

The human CYP19 gene was cloned some years ago (50–52), when it was shown that the coding region spans 9 exons beginning with exon II. Upstream of exon II are a number of alternative exons I which are spliced in the 5′-untranslated region of the transcript in a tissue-specific fashion (Fig. 2). The expression of the gene in the ovary and testis utilizes a proximal promoter, promoter II (53, 54), and thus transcripts in gonadal tissues contain sequence at their 5′-end that is immediately upstream of the translational start site. In contrast, placental transcripts contain at their 5′-end a distal exon, I.1, which is localized 100 kb upstream from the start of translation in exon II. This is because placental expression is driven by a powerful distal promoter upstream of exon I.1 (55). Transcripts in adipose tissue contain yet another distal exon located 20 kb downstream of exon I.1, exon I.4 (56), and a number of other untranslated exons have been characterized including one that is expressed in brain (57). Splicing of these untranslated exons to form the mature transcript occurs at a common 3′-splice junction that is upstream of the translational start site. This means that although transcripts in different tissues have different 5′-termini, the coding region and thus the protein expressed in these various tissue sites are always the same. However, the promoter regions upstream of each of the several untranslated first exons have different cohorts of response elements, and so regulation of aromatase expression in each tissue that synthesizes estrogens is different. Aromatase expression in the gonads is regulated by cAMP and gonadotropins due to an interaction of the gonadal promoter II with the transcription factors CREB (cAMP response element binding protein) and SP1 (58, 59). For comparison, the adipose promoter, I.4, is regulated by class I cytokines such as interleukin-6 (IL-6), interleukin-11 (IL-11), and oncostatin M, as well as by tumor necrosis factor-α (TNFa). The regulation of estrogen biosynthesis in each tissue site of expression is unique and has been reviewed previously (60).

B. Mechanisms of estrogen action

ERs were first characterized by Jensen and DeSombre in the 1970s (61). This led in turn to their cloning in the 1980s (62). Like aromatase, ERs are members of a large gene superfamily, in this case the ligand-activated nuclear receptor family, which also contains receptors for the other steroid hormones, thyroid hormone, vitamin D, retinoids, as well as a large group of so-called orphan receptors for whom no ligand was initially known, although in a growing number of cases, ligands have subsequently been found (63). For several years it was thought that only one form of nuclear ER existed. However, in 1996 a second form was reported in a number of species including rat, mouse, and human (10–13). This newly discovered receptor was termed ERβ, resulting in the classical ER being renamed ERα. The two receptors are not isoforms of each other, but rather are the products of distinct genes located on separate chromosomes.

The ER proteins are each composed of six functional domains labeled A–F, a signature characteristic of the entire superfamily (Fig. 3). The N′-terminal A/B domain is the least conserved among all members and demonstrates only 17% homology between the two ERs. It contains the activation function 1 (AF1) region, which is one of two regions critical for the transactivation function of the members of the re-
Receptor family. By contrast, the C domain is the most highly conserved region, being the DNA binding domain that contains the zinc-finger motifs. The E domain, or ligand-binding domain, is modestly conserved throughout the superfamily and confers ligand specificity on the members. Conservation of amino acid sequence in this region is 60% between the ERα and ERβ; however, each binds estradiol with about equal affinity, although the relative binding of other ligands differs substantially between them (64, 65). The E domain also contains the major dimerization surface of the receptors, and the second transactivation function, activation function 2 (AF-2), is also located in this region of the C'-terminus.

Transcription of the mouse ERα gene in vivo leads predominantly to a single transcript of approximately 6.3 kb, encoding a protein of 599 amino acids (66). The human ERα is slightly shorter at 595 amino acids (62). The existence of multiple promoter and regulatory regions in the 5'-untranslated region has been reported, as well as naturally occurring variants of the transcript in normal and malignant tissues, but as yet the existence of true protein products of these variants, as well as their significance, remains controversial (67). Initial studies of the rodent ERβ transcript indicated it was substantially shorter than the ERα, namely 485 amino acids (10, 11). This is largely due to a significantly shorter N'-terminal region. Indeed the existence of a functional AF-1 region in the ERβ has been questioned. However, open reading frames initiating upstream from that first described have now been discovered (68), suggesting the possibility that an ERβ protein of approximately 530 amino acids might also exist. Unlike the ERα gene, Northern blots of rodent tissues indicate the presence of multiple ERβ transcripts, and a number of variants have been described, including a conserved insertion of 18 amino acids in a C'-terminal region in the rat, mouse, and human, the deletion of one or more exons in these same species, and several isoforms in the extreme C'-terminus of the human transcript (69–71).

There is considerable tissue specificity in the expression of ERα and ERβ. Thus, ERα is the dominant species expressed in uterus, liver, adipose, skeletal muscle, pituitary, and hypothalamus, whereas ERβ is the major form in ovary and prostate, as well as other regions of the brain including the limbic system, cerebellum, and cerebral cortex (72, 73). The localization of ERα and ERβ in the testis and epididymis is discussed in Section IV.

The mechanisms of estrogen and estrogenic ligand action in the control of transcription have been recently reviewed elsewhere (74–78); however, the following will provide a brief overview of estrogen action on ER via classic pathways, ligand-independent ER activity, and nongenomic actions of estrogen.

The binding of ligand to ER and the events that lead to the regulation of target gene transcription are broadly similar among all steroid receptors, namely that they bind to response elements on the target gene and form a platform to which is recruited the complex of coactivators and transcription factors required for transcriptional activation. In general, estrogen action on ER involves ligand binding, dissociation of chaperone complexes and receptor phosphorylation; receptor dimerization; nuclear translocation; DNA binding and interaction with cofactors; and modulation of transcriptional activity.

Although ERα and ERβ bind estradiol with similar affinity, there is considerable selectivity of the different receptor subtypes in terms of affinities of various ligands (65). In particular, some phytoestrogens, such as genistein and coumestrol, have a significantly higher affinity for ERβ than ERα (64).

ER subtypes form homo- or heterodimers in vitro or in cells. In general terms, the ERα homodimer is more transcriptionally active than the ERβ homodimer in most systems (see Ref. 76 for review). The ERα/ERβ heterodimer was recently shown to have similar transcriptional activity to the ERα homodimer under hormone-saturating conditions, but is less active than the ERα homodimer at subsaturating estradiol concentrations (79).

The fact that there are distinct differences between the ER subtypes with regard to agonistic/antagonistic effects on transcription is now well known. For example, the ER agonistic activity of tamoxifen appears unique to ERα (see Ref. 76 for review). The distinct properties exhibited by ERα and ERβ have been exploited for therapeutic use by the development of selective ER modulators (SERMs) (see Refs. 74 and 78 for review); however, an understanding of the molecular events underlying SERM pharmacology has only recently begun to emerge (74, 78, 80). The molecular mechanisms underlying the differences in the functional characteristics of ERα and ERβ are well beyond the scope of this review, but include differences at the level of ligand binding/affinity, cofactor recruitment, and activity of the AF-1 and AF-2 domains (which are influenced by cell and promoter context) (see Refs. 74–76 for review). For example, the relative expression levels of the respective ER subtypes within a cell are key determinants of transcriptional activity in response to agonists and antagonists (79). Another way in which ER subtypes exhibit functional differences is in their ability to recruit coactivators and corepressors (74–76, 80, 81). Examples of coactivators of ERs are the steroid receptor coactivators (SRCs) (82) (see Ref. 83 for review). Often, coactivators such as SRC1 facilitate transcription via histone acetylation activity, which results in decondensation of chromatin and hence increases the ability of the transcriptional machinery to interact with the promoter (see Ref. 76 for review). In
contrast to coactivators, corepressors generally bind to ER in the absence of ligand or to antagonist-occupied ER and repress transcriptional activity. These frequently possess, or else activate, histone deacetylase activity. Examples of corepressors include nuclear receptor corepressor (NCoR) (84) and short heterodimer partner (SHP) (85, 86). The differences in transcriptional activation of ERα compared with ERβ by agonists and antagonists is due, at least in part, to differences in the ability of the ligand-receptor complex to recruit coactivators, which is related to the conformational changes induced in the helix H12 of the ligand binding cavity of each receptor by the agonist/antagonist (80). In summary, the ability of an ER subtype to stimulate transcription is dependent on promoter context, the nature of the ligand bound, the expression of coactivator and corepressor proteins, and the relative expression levels of ER subtypes in a given cell. It is also worthwhile to note that the ability of a ligand for the ER to influence transcription is determined by its structure, its affinity for ER subtypes, and the recruitment of coactivators and corepressors that is, in turn, dependent on cell and promoter context (see Ref. 78 for review).

ERs can also associate with target gene promoters in a manner that is not dependent on direct DNA binding, but involves the binding of ER to proteins within a preformed transcriptional complex. For example, ligand-activated ER can positively regulate transcription by associating with the activating protein 1 (AP1) transcription factor complex in target cells (see Ref. 77 for review). Similar interactions of ER with other transcriptional complexes exist (see Ref. 74 for review) indicating that ER can interact directly or indirectly with DNA to modulate transcription. ERα and ERβ exhibit different properties in both DNA binding-dependent and -independent mechanisms (see Ref. 76).

There is now also increasing evidence for ligand-independent transcriptional activation of ERs (87, 88). Epidermal growth factor (EGF)-induced phosphorylation of ERα results in ligand-independent transcriptional activity, a pathway that has been shown to have functional significance (87, 89, 90). Growth factor-induced phosphorylation of ER is dependent on the mitogen-activated protein kinase (MAPK) pathway (see Ref. 91 for review). MAPK phosphorylates the AF-1 region of ERα (92) and ERβ (93) and in the latter case has been shown to promote the recruitment of SRC1 in a ligand-independent manner (93). Other factors, such as cyclin D1, can activate ER-mediated transcription in the absence of estradiol (94, 95).

In addition to the classic genomic pathway of ligand-occupied ER interaction with target genes, estrogen can also induce extremely rapid (within seconds to minutes) increases in the concentration of calcium or cAMP second messengers (96, 97) in an apparently nongenomic mechanism of action (see Ref. 98 for review), presumably via receptors on the plasma membrane. Also, physiological concentrations of estradiol can induce a rapid release of nitric oxide in endothelial cells via membrane-bound receptors (99, 100). Nongenomic actions of estrogen appear to be of particular importance in cardiovascular (101) and neuronal (102) tissues. Evidence has been presented that in some systems estrogen appears to act on a membrane-associated ER that is immunologically related to ERα (103–107). ERβ transcripts can also produce membrane-associated forms (104). However, in other systems, nongenomic effects of estrogen do not appear to be via membrane-associated forms of ERα or ERβ (108–111). For example, nongenomic actions of estrogen on pancreatic β-cells appear to be via a membrane binding site with the pharmacological profile of the γ-adrenergic receptor (110). Therefore, the potential exists for estrogen action on plasma membrane receptors/complexes unrelated to ERα or ERβ.

In summary, ligand-bound ERs act as transcription factors and are capable of modulating target gene transcription by both DNA binding-dependent and -independent means. Molecular mechanisms of estrogen action are now known to be more complex than originally thought, with ligand-independent pathways that mediate cross-talk with growth factor signaling pathways, as well as rapid nongenomic actions. Understanding how estrogen will act within a cell is further complicated by the fact that ERα and ERβ differ in their functional characteristics and in their tissue localization. Thus, estrogenic ligand-dependent and/or ER-dependent regulation of target gene expression depends on the nature of the ligand, the relative levels of ER subtypes and coregulators, the molecular pathway of action and, in particular, the cell and promoter context.

IV. ERs, Aromatase, and Estrogen Production in the Testis

Since the 1930s, estrogen has been recognized to be synthesized in the male (112); however, the significance of finding this “female” hormone in the male was largely ignored as its role was considered to be of little importance to the functioning of the testis and in male fertility. In fact as late as the 1970s there was little knowledge regarding the cell types within the testes responsible for estrogen synthesis or action. In this section of the review, an overview of the localization of ERα, ERβ, and aromatase mRNA and protein in the testis from the fetal stage through to adulthood will be presented. The reader is also directed to other relevant reviews (113–116) for the discussion of ER and aromatase localization in the male reproductive tract.

A. ERs and aromatase in the fetal testis

ERs and aromatase are found at all stages of testicular development in the rodent. For more detailed information of male gonadal differentiation, see Refs. 117–119.

Immunohistochemical studies using antibodies that recognize ERα show that this protein is present in the mouse undifferentiated gonad at day 10 (120) and day 11.5 (121), suggesting that estrogen may have a role very early in the differentiation process. Leydig cells within the rodent fetal testis contain ERα until birth (120–125). In fact, ERs are expressed in the Leydig cells at a stage in development when the androgen receptor is not yet expressed (126), highlighting a role for estrogen at this stage (see Ref. 125). Although one study reports that ERα is present within the seminiferous tubules of the fetal testis (120), other studies do not (see Table 1). ERα is also present in the developing efferent ductules and epididymis (120, 121, 125).
### TABLE 1. Published studies investigating the localization of ERα, ERβ, and aromatase in the developing rodent testis, efferent ductules (e.d.), and epididymis

<table>
<thead>
<tr>
<th>Period</th>
<th>Organ/Cell Type</th>
<th>ERα</th>
<th>ERβ</th>
<th>Aromatase*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fetal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole testis</td>
<td>r (122)</td>
<td></td>
<td>r (122)</td>
<td>p (129, 131)</td>
</tr>
<tr>
<td>Leydig cells</td>
<td>p (120–125)</td>
<td></td>
<td>r (127)</td>
<td>p (136)</td>
</tr>
<tr>
<td>Sertoli cells</td>
<td>p (121, 123, 125)</td>
<td></td>
<td>r (127)</td>
<td>p (136)</td>
</tr>
<tr>
<td>Gonocytes</td>
<td>p (121, 123, 125)</td>
<td></td>
<td>r (127)</td>
<td>p (136)</td>
</tr>
<tr>
<td>Assoc. ducts</td>
<td>p (120, 121, 124, 125)</td>
<td></td>
<td>r (127)</td>
<td>p (136)</td>
</tr>
<tr>
<td><strong>Neonatal/pubertal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole testis</td>
<td>r (122, 180)</td>
<td></td>
<td>r (122, 180)</td>
<td>p (135–138)</td>
</tr>
<tr>
<td>Leydig cells</td>
<td>p (121–125)</td>
<td></td>
<td>r (127)</td>
<td>p (137)</td>
</tr>
<tr>
<td>Sertoli cells</td>
<td>p (121, 123–125)</td>
<td></td>
<td>r (127)</td>
<td>p (137)</td>
</tr>
<tr>
<td>Germ cells</td>
<td>p (121, 123–125)</td>
<td></td>
<td>r (127)</td>
<td>p (137)</td>
</tr>
<tr>
<td>Gerocytes/spermatogonia</td>
<td></td>
<td></td>
<td>r (127)</td>
<td>p (137)</td>
</tr>
<tr>
<td>Spermatocytes</td>
<td>p (121, 122, 124, 125, 134)</td>
<td></td>
<td>r (127)</td>
<td>p (137)</td>
</tr>
<tr>
<td>Epididymis</td>
<td>r (122) p (121, 122, 124, 125, 134)</td>
<td></td>
<td>r (127)</td>
<td>p (137)</td>
</tr>
<tr>
<td><strong>Adult</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole testis</td>
<td>r (73) p (124, 125, 142)</td>
<td></td>
<td>r (140)</td>
<td>r (73)</td>
</tr>
<tr>
<td>Leydig cells</td>
<td>r (142) p (124, 125, 142)</td>
<td></td>
<td>r (140)</td>
<td>r (73)</td>
</tr>
<tr>
<td>Sertoli cells</td>
<td>r (142) p (124, 125)</td>
<td></td>
<td>r (127)</td>
<td>r (73)</td>
</tr>
<tr>
<td>Germ cells</td>
<td>p (124, 125)</td>
<td></td>
<td>r (127)</td>
<td>r (73)</td>
</tr>
<tr>
<td>Spermatogonia</td>
<td>r (142)</td>
<td></td>
<td>r (127)</td>
<td>r (73)</td>
</tr>
<tr>
<td>Spermatocytes</td>
<td>r (127) p (123, 127)</td>
<td></td>
<td>r (127)</td>
<td>r (73)</td>
</tr>
<tr>
<td>Round spermatids</td>
<td>r (127) p (123, 127)</td>
<td></td>
<td>r (127)</td>
<td>r (73)</td>
</tr>
<tr>
<td>Elongated spermatids</td>
<td>r and p (142)</td>
<td></td>
<td>r (127)</td>
<td>r (73)</td>
</tr>
<tr>
<td>e.d.</td>
<td>r (159) p (125, 134, 159)</td>
<td></td>
<td>r (159)</td>
<td>r (73)</td>
</tr>
<tr>
<td>Epididymis</td>
<td>r (73, 159) p (124, 134, 159)</td>
<td></td>
<td>r (73)</td>
<td>r (73)</td>
</tr>
</tbody>
</table>

The demonstration of either protein (p), or mRNA (r) in each organ or cell type is indicated in the + column. The term "associated ducts" (assoc. ducts) includes the Wolffian ducts, which give rise to the epididymis and vas deference, as well as the developing fetal efferent ductules and epididymis. When a manuscript has specifically concluded that the molecule is not present in an organ or cell type, it is included in the – column. Studies that did not discriminate between ERα and ERβ are not included. Studies cited in Refs. 73, 121, 122, 140, and 150 were performed in mice, whereas all other studies used rat tissues. Neonatal/pubertal period represents the period from birth until approximately day 45.

* See Ref. 115 for a more complete review on aromatase localization in different species. p, Protein localization determined by immunocytochemistry, Western blot, or aromatase activity in purified isolated cells; r, mRNA localization determined by ribonuclease protection assay, RT-PCR, in situ hybridization, or Northern blot; assoc. ducts, associated ducts.
There is evidence for both ERβ mRNA and protein as early as day 16 of gestation in the gonocytes. Sertoli cells and Leydig cells (see Table 1), and this receptor subtype appears to remain in these cells until birth (122–124, 127). However, it is the gonocytes that express ERβ in higher abundance than the other testicular cells (123, 127), possibly suggesting a direct role in gonocyte maturation. ERβ is also present in rat associated ducts, which will eventually differentiate into the efferent ductules and adjoining epididymis (124, 125).

The fetal rat testis also has aromatase activity (128–130), which is first expressed by day 19 (129). The cell type responsible may be Sertoli cells, as estrogen production by fetal testes in culture is stimulated by FSH (131). Interestingly, this is close to the time when FSH receptors first appear on the Sertoli cells (132). There is also evidence that fetal Leydig cells have the capacity for LH-stimulated aromatization of androgens (133).

Thus the demonstration that rodent fetal testicular cells synthesize estrogen and express both ERα and -β suggests a role for estrogen in the development of the fetal male reproductive tract and in gonocyte differentiation.

### B. ERs and aromatase in the immature testis

Around the time of birth, the testis continues to express both ER isoforms and aromatase (Table 1). Most reports suggest that ERβ protein and mRNA, rather than ERα, appear to be localized to the rat seminiferous epithelium, in both Sertoli cells and developing germ cells (123, 127) (Table 1). In the mouse, the testis at this time also continues to abundantly express ERβ mRNA, with immunocytochemical studies localizing this ER to the germ cells (122).

ERα expression is restricted to the cells that lie outside of the seminiferous epithelium shortly after birth (121, 125), with ERα (121–125), along with ERβ (123, 127), continuing to be found in the Leydig cells of mice and rats during the neonatal period. ERα protein is also found in the rodent rete testis (125), efferent ductules (121, 125), and the epididymis (121, 124, 125), where it has remained since fetal development. In fact, ERα is expressed in much higher levels in the efferent ducts than the cauda epididymis (121, 125). ERβ is also found in the epididymis during development (122, 124, 134).

At this stage of development, basal aromatase activity is found in both the immature Leydig cells and Sertoli cells (135–137); however, activity is significantly induced by FSH in the Sertoli cells (135). In fact at this age, Sertoli cells were more active in producing estrogen than neonatal Leydig cells and adult Sertoli cells, suggesting that these cells are an important source of estrogen in the postnatal tests (137). At this age, germ cells have been reported not to contain detectable aromatase (138).

During days 10–26 in the immature rat, Leydig cells and Sertoli cells are dividing and undergoing functional maturation (see Ref. 139 for review). Again, ERα is absent from the seminiferous epithelium with only ERβ prominent in the epithelium of the immature rodent (Table 1). Specifically in the tubules of the rat, ERβ mRNA and protein are present in relatively low levels in spermatogonia (123, 127) and immature Sertoli cells (123, 127), and by day 21 ERβ is abundantly expressed in pachytene spermatocytes (123). Other germ cells do not show ERβ staining at this time (127). By day 12 in the mouse, ERβ is also specifically immunolocalized to the spermatocytes; however, its expression appears to decrease and was undetectable by day 26 (122). Again, rat and mouse Leydig cells express ERα at this time (122–125) (Table 1).

During the neonatal and pubertal period of development, ERα is prominent in the rete testis and efferent ductules (125, 134) and is present in the mouse epididymis (122). Recent studies on the immunoeexpression of ERs in the rat described the specific cellular and regional localization of ERα and ERβ in the developing rat efferent ductules, epididymis, and vas deferens and showed that the localization of ERα in the epididymis is confined to a relatively short window of development (134). ERβ is also present in the epididymis at this time (122, 134).

Aromatase appears to have an age-dependent pattern of expression. As the animal matures, the Leydig cells appear to significantly supplement estrogen production from the Sertoli cells (136), with Leydig cell basal aromatase activity increasing 3- to 4-fold (137) and now stimulated by LH (135). However, the Sertoli cells do continue to express aromatase during their maturation (135).

### C. ERs and aromatase in the adult testis

The presence of ERα, ERβ, and aromatase in the adult testis has been the subject of numerous recent studies. An intense effort has focused on the expression and localization of ER subtypes in the adult testis of mice (73, 140), rats (14, 123, 125, 127, 141, 142), primates (125, 143, 144), and humans (13, 145–147), primarily due to the fact that there has been a considerable amount of conflicting data.

Various evidence indicates that Leydig cells of rats and mice express ERα (125, 142, 146) (see Table 1); however, the localization of ERα in Leydig cells of primates and humans is more controversial; studies have shown immunostaining in some primate Leydig cells (125), and in human Leydig cells (147); however, other studies could not detect ERα immunostaining in Leydig cells from primates (144, 146) or humans (146, 147). In the adult mouse ERβ protein is expressed in the Leydig cells (146), but this does not seem to be the case in the adult rat (127, 142). Again, the localization of ERβ in Leydig cells from primates and humans is controversial with some studies showing no ERβ in Leydig cells from primates (143) or humans (13), whereas other studies showed immunoeexpression of ERβ in Leydig cells from two primate species (146) as well as humans (146–148).

By adulthood, rodent Leydig cells express a high level of aromatase (115, 138, 149–151), which is stimulated by LH (136, 152, 153) and steroids (153). In fact, aromatase activity is higher in the adult than at any other age (136) and is higher in the adult Leydig cells than in the Sertoli cells (149). The decrease in aromatase activity during Sertoli cell maturation into the adult form (see Ref. 154 for review) may be related to the control of Sertoli cell division and adult cell function (see Section V.E). The presence of aromatase in the Leydig cells of primates and humans is well established (see Ref. 115 for review).

Rat Sertoli cells contain both ERβ mRNA and protein from...
the fetus to adulthood (Table 1 and Section IV.A and B) (14, 123, 127, 141, 142). Immunohistochemical studies suggest that ERβ is not stage dependent in the rat (14, 123). In contrast to the rat, there are no reports of ERβ in mouse Sertoli cells (Table 1). Sertoli cells in primates (146) and humans contain ERβ (146–148) but not ERα (125, 146, 147).

There is now considerable evidence that germ cells contain both ERs and aromatase (see Fig. 4 for a summary of the proposed localization). In general, ERβ is the predominant, and potentially the only, ER in germ cells (see Table 1). One study in rats found immunoeexpression of ERα in spermatoocytes and spermatids (142); however, there are no other reports of ERα in rodent germ cells (see Table 1). ERα immunoeexpression in human germ cells within seminiferous...

![Fig. 4. Summary of the likely localization of ERα, ERβ, and aromatase in the adult testis.](https://academic.oup.com/edrv/article-abstract/22/3/289/2423875)
tubule fragments in culture has been suggested by one study (145), whereas other studies did not show ERα protein in primate or human germ cells (146, 147). ERβ is present in rat type A spermatogonia (127) as well as in intermediate and type B spermatogonia in some studies (123), but not others (127) and is also seen in spermatogonia in monkeys and humans (146, 148). ERβ is found in pachytene spermatocytes and round spermatids, but not elongating spermatids in rats (123, 127, 141) and in primates and humans (13, 143, 146, 148), although one study suggested ERβ in human elongating spermatids (145). However, one study in mice found ERβ only in elongated spermatids and not earlier germ cells (140) (see Table 1). Other studies in rats and humans found ERβ in Sertoli cells but not in germ cells (142, 147). A further complexity to the testicular localization of ERβ is that one study in mice could not detect ERβ mRNA at all in mouse testis by RNase protection assay (73). Therefore, considerable conflict in the literature exists in terms of ERβ localization in the testis (see Table 1 and below).

Aromatase mRNA and activity is found in germ cells from the pachytene spermatocyte stage in both rats and mice (149–151, 155), and aromatase remains in the germ cells as they mature into round spermatids (149–151, 155, 156) (see Fig. 4). Aromatase localization is observed to move from the Golgi apparatus to the cytoplasm during spermatid development (150). When round spermatids begin the morphological transformation into elongated spermatids, aromatase continues to be found in these cells (Table 1) and is immunolocalized to the flagella of the developing spermatid (150). Aromatase appears to be present in higher levels in mature spermatids of the rat than in earlier germ cells (149, 155). Aromatase mRNA and activity was higher in the germ cells of the mouse and rat when compared with Leydig cells (150, 155, 157), suggesting that the germ cells are an important source of estrogen in the testis.

When elongated spermatids are released from the epithelium, during the process of spermiogenesis, aromatase remains in the residual body that is subsequently phagocytosed by the Sertoli cell (150, 157). However, not all the cytoplasm is phagocytosed, and aromatase activity remains in the cytoplasmic droplet that is still attached to the flagellum as the sperm make their way through the epididymis (151, 158). Thus it appears as if mature sperm are able to synthesize their own estrogen, as they traverse the efferent ducts (116, 151, 158) (see Fig. 4). The ability to synthesize estrogen gradually decreases as the droplet slowly moves to the end of the tail during epididymal transit until it is finally lost (158). The demonstration of aromatase in sperm is important as it suggests that the sperm themselves could control the levels of estrogen present in the luminal fluid, directly modulating functions such as the reabsorption of fluid from the efferent ductules (116).

A very high level of expression of ERα is seen in the efferent ductules of the rat (125, 159). In fact, it has been found that it is the efferent ductules that possess the highest level of ERα immunostaining, relative to the testis, efferent ducts, and epididymis, throughout life (125). In addition, the efferent ductules appear to be the first male reproductive structure to express the ER in fetal development (160), suggesting a role for estrogen in the development of this tissue. ERα is present in the rat and mouse epididymis (73, 124, 159), although in low levels, while another report could not detect it in rats (125). Again, similar to earlier stages, both ERβ mRNA and protein are present from the efferent ducts to the epididymis of the mouse (73, 140) and the rat (124, 159). A very recent study of the localization of ERα and ERβ in the rat efferent ductules and epididymis showed that in adult rats ERα was strongly immunoreexpressed in the epithelial cells of the efferent ductules but was not found in the epididymis, whereas ERβ was found in epithelial cells and some periductal cells throughout the efferent ductules and epididymis (134). ERα is found in the nonciliated epithelial cells of the efferent ductules in primates and humans (146), although another study in humans did not observe ERα immunoreexpression in the efferent ductules (147). ERα was rarely detected in the epididymis of primates or humans (146, 147). In contrast, ERβ is found in both stromal and epithelial cells throughout the efferent ductules and epididymis in these species (146).

The studies reviewed in this section highlight the fact that there is considerable conflict in the literature regarding the localization of ERα and ERβ, particularly in the testis. The conflict usually arises when comparing studies utilizing immunohistochemical techniques. In the studies cited in Table 1 and above, there is considerable variation in the antibodies used. The antibodies have been raised against different regions of the ER molecules, and thus detect different epitopes (see Ref. 161 for a recent review on ERα and ERβ antibodies, including some of those used in the studies cited here). Most studies cited here have used antigen retrieval methods and have presented some evidence of antibody specificity, usually by preabsorption controls. Even though various studies used commercially available antibodies, it is clear that a range of commercial antibodies to ERs are not well characterized (see Ref. 161 for review). In addition, whether the antibody used detects ligand-occupied and/or inactive receptor, monomeric or dimerized receptors, an ER in heterodimer form, receptors bound to DNA, etc., is usually not known. A further complexity in the immunohistochemical studies cited in this review is that different methods of tissue fixation, antigen retrieval, antibody dilution, and antibody detection methods were used, all of which can affect the ability of an antibody to bind to its antigen. Thus at present it is almost impossible to reconcile the differences in the various reports on ERα and ERβ localization. Clarification of the exact localization of ER subtypes in the testis would be facilitated by the comparison of a panel of very well characterized antibodies, in which the exact nature of the protein detected is known.

In summary, the studies reviewed above suggest that the testis is capable of synthesizing and responding to estrogens throughout all stages of development. The localization of ERα, ERβ, and aromatase in the adult testis is summarized in Fig. 4 in an effort to bring together the literature in this area. The localization of ERα, ERβ, and aromatase demonstrates that estrogen action is likely to be important for Leydig cell, Sertoli cell, and germ cell development and function, as well as in the development and function of the efferent ductules and epididymis. In particular, germ cells are capable of local estrogen synthesis and response, via ERβ, sug-
suggesting that paracrine and intracrine actions of estrogens may be important in male germ cell development. The localization of aromatase in sperm in the testis, and as they traverse the efferent ductules and epididymis, together with the demonstration of high levels of ERα and ERβ in the efferent ductules, support the hypothesis that estrogen in sperm acts on ER in the efferent ductules (see Section V.B).

D. Estrogen production in the testis

The synthesis of estrogens in the testis has been reviewed extensively elsewhere (154, 162); therefore, only a few relevant details will be considered here. There is a high concentration of estrogen in rete testis fluid (163) and, in the rat, the concentration of estrogen in the caput epididymis is approximately 25 times the level measured in plasma (164). It is clear that the concentrations of estrogen in the testis and rete testis fluid far exceeds the concentration in male serum in various species (see Ref. 162 for review) thus suggesting a central role for estrogen in testicular and epididymal function.

In dissected testicular tissue from adult rats, the concentration of estrogen in interstitial tissue was 9 times higher than that in the seminiferous tubules (165). However, it is now becoming clear that the level of aromatase activity in germ cells of the adult rodent is equal to or higher than the aromatase activity in Leydig cells (149, 150) (see above), suggesting that while Leydig cells have previously been considered to be the primary source of estrogen in the testis (166), germ cells must now be considered to have an important role also (see Refs. 114–116 for review, and Section IV.C). Thus the source of the high concentration of estrogen in fluid leaving the testis may be due largely to the high levels of aromatase mRNA, protein, and activity in testicular germ cells and particularly in spermatids (149–151, 157, 158, 167, 168) (see Refs. 114–116 for review).

V. The Effects of Estrogen Administration or Deprivation on Spermatogenesis

Despite the abundance of published data on the response of the testis and spermatogenesis to either estrogen deprivation or estrogen treatment, the exact roles for estrogen in spermatogenesis remain uncertain. The confusion as to the involvement of estrogen in the initiation and maintenance of testicular function and spermatogenesis is likely due to the fact that estrogen action is important at numerous levels in male reproductive physiology including, but not limited to, effects on the hypothalamo-pituitary-testis axis, Leydig cells, Sertoli cells, germ cells, and epididymal function. Thus the broad range of effects that estrogen is likely to have in the male reproductive tract may complicate the interpretation of experimental findings. The following section discusses the effects of estrogen administration and deprivation on processes that are required for normal spermatogenesis and fertility.

A. Hypothalamo-pituitary-testis axis

As mentioned in Section II.B, the initiation and maintenance of spermatogenesis require the secretion of gonado-

tropins from the pituitary and thus is dependent on the balance of the hypothalamo-pituitary-testis axis. The negative feedback effect of testosterone on both the hypothalamus and the pituitary to regulate gonadotropin secretion is well known. In humans, the negative feedback of testosterone on the hypothalamo-pituitary axis to inhibit secretion of both LH and FSH is the basis for the current approach to male contraception (169). It is now becoming clear that a major component of the negative feedback action of androgens on gonadotropin secretion is mediated via aromatization to estrogen (170–173). In particular, studies in humans showed that administration of estradiol could further enhance gonadotropin suppression that was induced by a testosterone-based contraceptive (174), further indicating estrogen’s role as a negative feedback regulator of gonadotropin secretion. The demonstration of ERα and ERβ in the rodent hypothalamus and pituitary (73, 142, 175, 176), of ERβ in the monkey pituitary (143), together with the immunolocalization of aromatase in the brain and in particular the hypothalamus (177), indicates that estrogen has an important role in these tissues.

During pubertal development in the rodent, numerous studies have shown that neonatal exposure to either estrogens or estrogen-like compounds promotes changes in gonadotropin secretion (178–180) and can, in fact, alter the organization of the hypothalamo-pituitary-testis axis so that changes persist into adulthood (181). A single high dose of estradiol benzoate to 1-day-old male rats causes a reduction in both GnRH secretion and pituitary responsiveness to GnRH (182), as well as the profound suppression of circulating FSH, LH and, consequently, testosterone levels (180). Interestingly, recent studies administering low doses of estrogenic compounds during the neonatal period in rats could actually stimulate serum FSH levels during puberty, an effect that could not be explained by changes in inhibin B (178). Given that the appropriate concentrations of LH and FSH, as well as a tightly regulated onset of secretion during the neonatal and pubertal periods, is fundamental to whether normal spermatogenesis proceeds (183, 184), neonatal estrogen exposure can have important long-term effects on the hypothalamo-pituitary-testis axis and thus spermatogenesis. Interestingly, whether or not estrogen administration to juvenile mice will interfere with the hypothalamo-pituitary-gonadal axis appears to be strain-dependent (185), which could lead to confusion when interpreting the literature on the interaction between estrogen, the regulation of pituitary hormone production, and fertility.

In the adult, there are many examples of a role for estrogen in the regulation of gonadotropin secretion. Adult male rats given increasing doses of estradiol for 10 days showed significant decreases in circulating concentrations of FSH and LH, which leads to subsequent reductions in serum and testicular testosterone levels (186). Surprisingly, the authors of this study also noted a stimulatory effect of low doses of estradiol on FSH, as was demonstrated by studies in the neonate (178), and in adult hypogonadal (bgh) mice given reasonably physiological doses of estradiol (187), indicating that estrogen can participate in both negative and positive effects on the pituitary in the male. Some of the effects of estrogen on FSH secretion may be mediated by its ability to promote changes in the in vitro Sertoli cell production of...
inhibin B (188), which is an important mediator of FSH secretion in males (Ref. 189 and references therein). Interestingly, estrogen has also been shown to increase the expression of the ββ-subunit of inhibin B in breast cancer cells (190). Therefore, estrogen may play a role in the regulation of this peptide that is primarily involved in mediating pituitary gonadotropin secretion, but may also have other roles in the testis (see Ref. 191 for review).

While estrogen administration clearly causes decreases in circulating gonadotropin levels, the administration of aromatase inhibitors causes increases in serum LH and testosterone in adult dogs (192) and rats (193), and in serum testosterone and the responsiveness of Leydig cells to a bolus injection of LH in monkeys (194). An increase in the circulating concentration of FSH was also seen in rats treated with an aromatase inhibitor (193). Administration of an aromatase inhibitor to men causes increases in the circulating concentrations of LH, FSH, and testosterone (172, 195). An interesting study in which the effects of administration of an aromatase inhibitor was compared in normal vs. GnRH-treated hypogonadotropic-hypogonadal men demonstrated that estrogen acts at the hypothalamus to decrease GnRH pulse frequency and at the pituitary to decrease responsiveness to GnRH (173). Thus, in the human, it is clear that estrogen is an important regulator of the hypothalamo-pituitary-gonadal axis in both sexes.

Investigation into the role of estrogen in the control of the hypothalamic-pituitary-testis axis has been facilitated by transgenic mouse models (see Table 2). As would be predicted from aromatase inhibitor studies, male mice that lack a functional aromatase gene (ArKO) have increased levels of serum LH and testosterone yet normal levels of FSH (8, 196). Although ERα is present in the mouse hypothalamus and is the only ER in the mouse pituitary (73), male mice deficient in ERα (ERαKO) showed surprisingly little change in LH and FSH levels, although serum testosterone levels were higher (5). A later study with a larger number of animals showed that ERαKO males had a 2-fold increase in both circulating LH and in the pituitary content of LH β mRNA, but confirmed that ERαKO animals had no change in FSH (197). An elegant series of experiments performed by Lindzey and colleagues (197), and reviewed in detail elsewhere (27), suggested that estrogen is likely to be involved in facilitating the negative feedback effects of testosterone on the male mouse hypothalamic-pituitary-testis axis and further demonstrated that ERαKO animals may have had an increased sensitivity to androgens, suggesting an altered organization of the hypothalamic-pituitary-testis axis. The endocrine profile of the ERβKO has not been detailed; however, the fact that ERβKO males are fully fertile suggests no endocrine disruption (198).

Thus it is clear that either estrogen administration or deficiency can affect the development and/or the maintenance of the male hypothalamic-pituitary-testis axis. Given that spermatogenesis depends on the delicate balance of the hypothalamic-pituitary-testis axis (reviewed in Refs. 39–41), the role of estrogen in this balance is an important consideration in terms of the role of estrogen in spermatogenesis.

### B. Efferent ductules and epididymis

After the release of mature spermatids from the Sertoli cell during spermiation, spermatids via the seminiferous tubule fluid proceed into the rete testis. Arising from the rete testis are a series of tubules known as the efferent ductules, which connect the rete testis to the initial segment at the head of the epididymis (see Ref. 199 for review). The primary function of the efferent ductules is to resorb water, ions, and proteins, and various studies have shown that approximately 90% of rete testis fluid is resorbed within these ductules, so that spermatozoa become concentrated as they enter the epididymis, thereby ensuring that a large number of spermatozoa are released upon ejaculation (Refs. 162, 199, 200, and 201 and references therein). The passage of sperm through the initial segment, caput, corpus, and cauda epididymis is essential for the final maturation of sperm. The epididymal function is well known (204–207), studies in the 1970s showed that there is a high concentration of estrogen-binding sites in the immature and adult epididymis (208–210), suggesting a role for estrogens in sperm maturation and male reproduction. The concentration of estrogen leaving the testis is far higher than in the circulation (see Section IV.D and Ref. 162 for review), and estrogen receptors (ERα and ERβ) are present in the efferent ductules and epididymis (73, 125, 134, 140, 159, 160, 211–213) (see Section IV). In the rat efferent ductules, ERα is expressed at 3.5 times the level seen in uterus (159). The presence of abundant estrogen and ER in the efferent ductules and epididymis points to a role for estrogen in the regulation of these tissues and thus in modifying sperm maturation and function.

A study by McLachlan and colleagues in 1975 (24) showed that prenatal exposure of mice to diethylstilbestrol (DES) caused epididymal granulomas, suggesting an action of exogenous estrogen on epididymal development. Numerous studies since have shown that neonatal estrogen exposure causes an impairment of efferent ductule and epididymal development and function that can lead to deleterious effects on fertility in adulthood (e.g., Refs. 134 and 214–220). While at least part of these effects could be attributed to changes in the hypothalamic-pituitary-gonadal axis, a direct action on the epididymis to mediate these changes is likely given the high levels of ER expression.

Although the above studies indicate that estrogen administration can affect the development of efferent ductule and epididymal function and lead to an impairment of male reproductive function, the first major insights into the mechanism of estrogen action in the efferent ductules and epididymis, and the requirement for estrogen in male fertility, were gained from the ERαKO mouse. The ERαKO male...
mouse is infertile, due to disruptions to spermatogenesis, reduced epididymal sperm content, reduced sperm motility and fertilizing ability, as well as defects in reproductive behavior (4, 5, 221) (see Ref. 27 for review and Table 2 and Section V.G). Studies by Hess and colleagues (6) clearly demonstrated that dysfunction of the efferent ductules of the ERαKO mice contributed to the impairment of fertility. ERαKO testis weights initially increase during postpubertal testicular development, due to an accumulation of fluid in the lumen of the seminiferous tubules (5, 6), suggesting either an excess of fluid secreted by the testis and/or a failure of fluid resorption by the efferent ductules.

### Table 2. Comparison of reproductive phenotypes in ER and aromatase (cyp19) null male mice

<table>
<thead>
<tr>
<th></th>
<th>ERαKO</th>
<th>ERβKO†</th>
<th>ERαβKO</th>
<th>ArKO‡</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>H-P-G axis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum FSH</td>
<td>Normal (5, 197)</td>
<td>Not described</td>
<td>Not described</td>
<td>Normal (8)</td>
</tr>
<tr>
<td>Serum LH</td>
<td>Elevated (197)</td>
<td>Not described</td>
<td>Not described</td>
<td>Elevated (8, 196)</td>
</tr>
<tr>
<td>Serum T</td>
<td>Elevated (5, 197)</td>
<td>Not described</td>
<td>Not described</td>
<td>Elevated (8, 196)</td>
</tr>
<tr>
<td><strong>Testicular function</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>General observations</td>
<td>Germ cell loss is evident (5, 278). Seminiferous tubule lumen is dilated due to fluid build up from impaired efferent ductule function; appears to be the primary cause of spermatogenic failure (5, 6, 27, 291)</td>
<td>Histology of the testis appears normal (278)</td>
<td>Preliminary observations suggest that the testicular phenotype is similar to that of ERαKO, ie. disrupted fertility due to fluid build up (222)</td>
<td>Testicular histology normal at 14 wk (7–9, 196). There is an age-related disruption to spermiogenesis, but seminiferous tubule lumen volume is not increased (8). Some ArKOs show reduced seminiferous epithelial height (7)</td>
</tr>
<tr>
<td>Leydig cells</td>
<td>Not described</td>
<td>Not described</td>
<td>Not described</td>
<td>Hyperplasia/hypertrophy after 18 wk of age (8)</td>
</tr>
<tr>
<td>Sertoli cells</td>
<td>Seminiferous tubule fluid secretion decreased (6)</td>
<td>Not described</td>
<td>Not described</td>
<td>Number is normal (8)</td>
</tr>
<tr>
<td>Germ cells</td>
<td>Germ cells develop normally when transplanted into wildtype mice (292)</td>
<td>Not described</td>
<td>Reduced numbers of sperm in the epididymis (222)</td>
<td>Spermatid number reduced, round spermatids undergo apoptosis and/or show acrosomal dysgenesis after 18 wk of age (8)</td>
</tr>
<tr>
<td>Efferent ductules</td>
<td>Numerous developmental abnormalities and morphological changes; disturbance in resorptive function leads to testicular damage due to fluid build up (6, 26, 162, 223)</td>
<td>Not described</td>
<td>Not described, but similar phenotype to ERαKO mice suggests dysfunction (222)</td>
<td>Not described, but lack of enlarged seminiferous tubule lumen at any age suggests function is not markedly impaired (8)</td>
</tr>
<tr>
<td>Epididymis</td>
<td>Various morphological abnormalities (223)</td>
<td>Preliminary studies suggest some epithelial cells have abnormal morphology (162)</td>
<td>Not described</td>
<td>Not described</td>
</tr>
<tr>
<td>Sperm characteristics</td>
<td>Reduced numbers of sperm in the epididymis (5, 222). Reduced motility and fertilizing ability (5)</td>
<td>Epididymal sperm count normal (222)</td>
<td>Not described</td>
<td>Epididymal sperm content reduced at 1 yr of age, and motility decreased at 15 wk (unpublished data). Able to fertilize oocytes in vitro at 14 wk (7)</td>
</tr>
<tr>
<td>Sexual behavior</td>
<td>Normal motivation to mount females, reduction in intromissions but no ejaculations (221)</td>
<td>Normal (298)</td>
<td>Do not mount, and no intromissions or ejaculations (299)</td>
<td>Prolonged latency to mount and decreased number of mounts (7, 9)</td>
</tr>
<tr>
<td>Overall fertility</td>
<td>Infertile (5)</td>
<td>Fertile (198, 278)</td>
<td>Infertile (222, 278)</td>
<td>Initially fertile (196) but mice at 7 months of age sired no litters (8). Other lines show variable degrees of infertility (7, 9)</td>
</tr>
</tbody>
</table>

† There are no reported disruptions to spermatogenesis or fertility in male mice deficient in ERβ (198, 278).
‡ Three separate lines of ArKO mice have been generated in three different laboratories; one line from the laboratory of Simpson and colleagues (8, 196), one from Honda and colleagues (9), and one from Toda and colleagues (7).
the efferent ductules in the ERαKO mice was impaired (6). Interestingly, efferent ductules isolated from wild-type animals treated in vivo with the dual ER antagonist ICI 182,780, did not swell to the same extent as ERαKO tissues, suggesting that estrogen acting via ERβ may contribute to efferent ductule function (6). There have been no reports of efferent ductule and epididymal dysfunction in ERβKO mice that exhibit normal fertility (27, 198). However, more recent studies in which the effects of the dual ERα/ERβ antagonist ICI 182,780 on adult efferent ductule function in vivo were compared with the phenotype of ERαKO animals showed that both ICI-treated and ERαKO animals displayed similar disruptions to the rete testis and efferent ductules, suggesting that ERα, rather than ERβ, plays a major role in these tissues (26). The fact that ERαKO mice appear to have a similar phenotype to ERβKO mice (222) could be interpreted to mean that ERβ may not have a major role. However, detailed comparisons of the efferent ductule and epididymal morphology and function in ERαKO, ERβKO, and ERα/ERβ KO animals are required to confirm the involvement of ERβ in these tissues.

Recent studies on the morphology of the efferent ductules of the ERαKO mouse indicated a multitude of defects including decreases in efferent ductule epithelial cell height and number of cilia, and a loss of endocytotic apparatus, whereas examination of the epididymis also demonstrated abnormalities of epithelial cells and sperm granulomas (223). These observations highlight the importance of ERα in the adult function of the efferent ductules and epididymis but in particular point to a physiological role for estrogen in the development of these tissues. A second important study compared ER antagonist administration to postpubertal mice with ERαKO mice at a similar age to demonstrate that the development of blind-ending efferent tubules and an unusual growth of initial segment epithelial cells was a consequence of a congenital absence of estrogen action, while the dysfunction of efferent ductule epithelial cells is primarily due to a lack of estrogen action in the adult (26). Thus, the developmental abnormalities in efferent ductules and epididymal tissues in ERαKO mice demonstrate a physiological role for estrogen in the development of these tissues.

Although a congenital absence of ERα leads to permanent defects in efferent ductule development and function, so too does neonatal exposure to highly estrogenic compounds (134, 215, 216). Male rats exposed to a high dose of diethylstilbestrol (DES) during the first few days of life show dilatation of the rete testis and an accumulation of fluid in the testis during development (215, 216), eventually leading to a deleterious effect on the seminiferous tubules in adulthood (216). Fisher and colleagues (215) showed that this treatment causes a permanent reduction in the water channel protein aquaporin-1, which is found at high levels in the ERα-bearing nonciliated epithelial cells lining the efferent ductules. They suggested that the efferent ductule dysfunction induced by neonatal DES exposure is related to the absence of aquaporin-1, which may be required for fluid movement across the efferent ductule epithelium. Importantly, the effects of DES treatment were compared with neonatal treatment with a GnRH antagonist to show that the effect of DES on the development and function of the efferent ductules is via an estrogenic action, rather than decreases in LH, FSH, or androgens (215). Further studies showed that neonatal exposure to other estrogenic compounds has similar effects on the development and function of the efferent ductules, and the magnitude of the effect of each compound was comparable to its in vitro estrogenic activity (214). More recent studies show that neonatal exposure to DES caused various histological changes as well as changes in the cell- and region-specific expression of ERα protein in the efferent ductules as well as in the epididymis and vas deferens (134), highlighting the fact that the development of these tissues, and indeed the pattern of ERα expression, is a target for exogenous estrogen action. While neonatal estrogen exposure produces developmental defects in the efferent ductules and epididymis, short-term treatment of adult rats with estradiol benzoate also disturbs efferent ductule function (224).

It is perhaps surprising that a congenital absence of ERα and neonatal, or indeed adult, exposure to high levels of estrogens produce similar effects on the development and function of the efferent ductules. However, the fact that both models involving contrasting changes in estrogen during the neonatal period produce the same dysfunction in adulthood underscores the importance of estrogen during the development of these tissues. One proposition for the similar effects of neonatal estrogen exposure and deficiency is that inappropriate estrogen exposure may lead to down-regulation of ERs resulting in an estrogen deficiency syndrome similar to that seen in the ERα null mice. Such a proposition is supported by studies in the uterus, in which developmental exposure to DES clearly leads to decreases in ER levels in adulthood, and hence the uterus is permanently unresponsive to estrogen (225, 226). Similarly, studies in the testis suggest that neonatal DES exposure leads to down-regulation of ERα and androgen receptor, but an increase in ERβ (180), perhaps suggesting a permanent change in either estrogen responsiveness, or in estrogen-dependent gene expression. The proposition that ERs in the efferent ductules and epididymis are down-regulated by neonatal DES treatment, however, is not supported by immunolocalization studies in DES-treated rats. One study has shown that ER immunostaining intensity in the efferent ductules is increased by neonatal DES treatment (220), perhaps suggesting that the epithelial cells would become hypersensitive to the already highly potent agonistic action of DES. A second study showed that the morphological abnormalities in epididymal development induced by neonatal DES treatment were associated with changes in ERα, but not ERβ, immunoeexpression, such that ERα became more widespread in its expression pattern (134). Indeed, neonatal DES treatment leads to reduced androgen receptor expression in male rat reproductive tracts, an effect that was shown to be directly attributed to DES, rather than changes in androgen (227). This study showed that DES-induced changes in the efferent ductules and epididymis may be due, in part, to an altered androgen-estrogen balance (227).

Perhaps the observations on efferent ductule development and function in both the neonatal estrogen exposure and ERα null mouse models will be reconciled when a better understanding of the relative expression of the ER subtypes during the development of these tissues, and of the downstream
targets of estrogen action in these tissues, is gained. In vitro studies on the interactions of estrogenic ligands and ERs show clearly that the relative expression of both ERα and ERβ in a given cell type will determine the sensitivity of that cell to estrogen as well as determine how the cell responds to receptor agonists and antagonists (79).

A further complexity to the above account of the involvement of estrogen in efferent ductule and epididymal function is that the ArKO mouse did not show obvious fluid retention and disturbance of fluid resorption (8), as would be predicted by the ERαKO phenotype. This could be due to the fact that germ cell development is compromised in the ArKO (8), and therefore perhaps less fluid is being secreted by the seminiferous tubules in these animals. The demonstration that wild-type efferent ductules treated with a dual ERα and ERβ antagonist in vitro did not swell like ERαKO tubules (6) predicts that the lack of estrogen action on both ER subtypes would lead to a less pronounced effect on efferent ductule fluid resorption compared with ERα alone, and thus these observations could be extended to the interpretation of the ArKO phenotype. In vitro ER antagonist studies showed that while the effect on efferent ductules and rete testis was similar to ERαKO animals, most parameters measured showed a less profound effect in ER antagonist-treated animals compared with ERαKO (26). It is thus possible that the blockade of ERβ action along with ERα may cause less pronounced effects on the efferent ductules; however, the possibility that the less profound effects of the antagonist are due to incomplete blockade of estrogen action in these tissues cannot be ruled out. Also the difference between the ERαKO and the antagonist-treated mice (26) could also be related to the lack of ER during development compared with a lack of ER in adulthood. Since ArKO would have access to maternal estrogen during development, perhaps the lack of estrogen in adulthood would cause less profound effects on efferent ductule function than a lack of estrogen (or ERα) during development. An alternative explanation for the lack of obvious efferent ductule dysfunction in the ArKOS is that ERs in these tissues may be activated by ligand-independent means, or else that alternate ligands for these receptors could lead to continued fluid resorption by the efferent ductules (see Section V.G).

There are also studies to show that disruption of estrogen in adulthood can compromise efferent ductule and/or epididymal function and fertility. The administration of an aromatase inhibitor to adult male monkeys caused apparent defects in epididymal sperm maturation since sperm had compromised motility and condensation (194). Chronic aromatase inhibitor treatment in adult rats, however, did not compromise fertility, although a few rats showed evidence of a disturbance in efferent ductule fluid resorption (193). Interestingly, adult mice that had been treated for 35 days with an ER antagonist remained fertile, despite compromised efferent ductule function (26). While estrogen deficiency in the adult promotes efferent ductule and epididymal dysfunction, studies by Meistrich and colleagues (22) showed that estradiol benzoate given to adult mice for 50 days had negligible effects on sperm number in the testis but clearly reduced the sperm content of the epididymis and increased the time taken for sperm to transit through the epididymis. The authors demonstrated a decrease in sperm maturation and suggested that estradiol administration increases epididymal transport rate, with the result that sperm spend less time in the epididymis and cannot fully mature.

In summary, this section has highlighted the importance of the normal development and function of the efferent ductules and epididymis for spermatogenesis and fertility, and in particular the influence of estrogen on these tissues. Estrogen overexposure during neonatal development and in adulthood leads to changes in efferent ductule and epididymal tissues that can produce permanent defects in their function and hence have deleterious effects on male fertility. Importantly, studies from ERα null mice demonstrate that estrogen plays a crucial role in the development and normal functioning of these tissues that has profound implications for male fertility.

C. Testicular descent

Testicular descent is an important aspect of male sexual development, and fetal Leydig cells produce factors, notably androgens, that are necessary for this process. The relocation of the testes from the urogenital ridge to the inguinal abdominal wall (transabdominal descent) and the subsequent migration of the testis into the scrotum (inguinoscrotal descent) is a hormonally regulated process (see Ref. 228 for review).

The undescended testes and resultant infertility in mice lacking functional androgen receptors or type 2 5α-reductase underscores the importance of androgens in testicular descent (228–231); however, it is well known that in utero exposure of male fetuses to high levels of estrogens can also interfere with this process. For example, the treatment of pregnant women with DES is associated with cryptorchidism of the male offspring (25).

Animal models have been used to study estrogen-induced cryptorchidism, and several studies have suggested that estrogen exposure may interfere with the fetal hypothalamic-pituitary-testis axis, leading to an inhibition of fetal Leydig cell androgen production, thus interfering with testicular descent (see Ref. 113 for review). However, ERs are present in fetal Leydig cells of the rat (see Section IV) and thus a direct action of estrogen on these cells is possible. Insight into the mechanism by which in utero estrogen exposure causes cryptorchidism comes from recent studies on the insulin-3 (InsI3) gene. Targeted disruption of this gene, which is specifically expressed in fetal Leydig cells, causes bilateral cryptorchidism (232, 233). Since both in utero exposure to estrogen and lack of a functional InsI3 gene causes cryptorchidism, it is possible that InsI3 plays a role in estrogen-induced cryptorchidism. Indeed, two recent studies have clearly demonstrated that in utero exposure to 17β-estradiol or DES causes a specific down-regulation of InsI3 transcription in fetal Leydig cells (234, 235). Steroidogenic factor-1 (SF-1) is an important transcriptional activator of InsI3 in fetal Leydig cells (236), and therefore estrogen may down-regulate InsI3 transcription via SF-1. While one study has shown that in utero estrogen exposure decreases testicular SF-1 expression (237), other studies have shown that the estrogen-induced decrease in InsI3 was not coincident with a decrease in SF-1 (234, 235),...
and therefore the exact mechanism by which estrogen regulates fetal Leydig cell Insl3 transcription is unclear. Thus an important consequence of estrogen overexposure during the prenatal period is a decrease in transcription of Insl3 in fetal Leydig cells, leading to cryptorchidism and deleterious effects on fertility. Given that cryptorchidism is also associated with an increased risk of testicular cancer (228), inappropriate estrogen exposure is an important consideration for the etiology of this disease. Indeed, overexpression of aromatase in a mouse model has been shown to result in testicular Leydig cell tumors (238).

Estrogen deprivation during fetal development is also associated with problems with testicular descent. Although ERαKO mice have descended testes, defects in cremaster muscle development was noted in these animals, indicating a role for ERα in some aspects of male reproductive tract development and testicular descent (239). A male patient deficient in ERα had bilaterally descended testes (240). Aromatase-deficient mice also do not appear to have defects in testicular descent (8, 196); however, it is likely that these mice are subjected to maternal estrogens in utero. There are no reports of undescended testes in three male patients identified with aromatase deficiency (241–243); however, preliminary reports from our laboratory of a recently identified aromatase-deficient man indicate that this patient is cryptorchid (our unpublished data).

D. Leydig cells

Leydig cells first appear in the testis during day 15 of embryonic development in the rat (see Ref. 244 for review). These fetal Leydig cells secrete high concentrations of androgens that are required for Wolffian duct development and subsequent male sexual development. The fetal Leydig cells present at birth are not progenitors of the adult Leydig cell population; rather they remain present in low numbers in the mature testis, presumably in a quiescent state (245). During the prepubertal period, there is a rapid growth of Leydig cells, which arise from mesenchymal precursor cells, while after about day 28 in the rat, morphologically recognizable Leydig cells divide to produce the adult Leydig cell population (246) (see Ref. 139 for review). Leydig cells through pre- and postnatal development differ in their morphology as well as function (see Refs. 28, 139, and 247 for review). In the adult, perhaps the most notable function of the Leydig cell is to produce androgens that are necessary for spermatogenesis and the maintenance of secondary sexual functions. The following section will briefly review the evidence for a role for estrogen in Leydig cell function. The reader is also directed to another relevant review (113).

The proliferation of precursor and adult-type Leydig cells during a defined period of pubertal development is important for the establishment of the adult complement of Leydig cells. Estrogen appears to play an inhibitory role in this process and therefore may be important in controlling the steroidogenic capacity of the adult testis. Neonatal estrogen exposure can interfere with Leydig cell development and proliferation during puberty (see Ref. 113 for review). Using ethane dimethanesulfonate (EDS) to cause the destruction of Leydig cells as a model to study Leydig cell regeneration and development, estrogen was shown to block Leydig cell regeneration, probably via the inhibition of the rapid phase of Leydig cell proliferation (248). The fact that estrogen treatment between days 5–16 after EDS treatment was most effective at inhibiting Leydig cell regeneration suggests that estrogen can act on precursor Leydig cells (248). It has been suggested that, while LH is clearly important in regulating Leydig cell development, locally produced factors also appear to be involved (249, 250). These studies, together with the fact that more mature Leydig cells have higher levels of aromatase activity (251), have led to the hypothesis that mature Leydig cells may produce estrogens that inhibit precursor Leydig cell development (248). Consistent with this hypothesis is the fact that precursor Leydig cells have 20 times the level of ER mRNA than do mature Leydig cells (252). Also of interest is the fact that neonatal estrogen exposure, at a time when Leydig cells are developing, results in permanently decreased serum testosterone levels in the adult rat, despite no decreases in LH, suggesting that inappropriate exposure to estrogens during Leydig cell development can cause permanent changes to Leydig cell function (181). Taken together, these studies lend support to the hypothesis that estrogen may act in a paracrine fashion in the testis to control Leydig cell development (see Ref. 113 for review).

In addition to the potential role for estrogen in controlling Leydig cell development, there is also evidence for estrogen acting as a paracrine factor in the control of adult Leydig cell steroidogenesis. Although LH is the primary driving force for testosterone production, the theory that intratesticular factors are required to modulate Leydig cell steroidogenesis, such as by mediating LH responsiveness, does have merit (see Ref. 253 for review). Estrogen has been shown to inhibit Leydig cell steroidogenic enzymes that are required for testosterone biosynthesis. For example, estrogen has been shown to inhibit P450 17α-hydroxylase/C17,20 lyase activity in both neonatal (254, 255) and postpubertal (256) testes. DES can decrease testosterone production in adult rats in the absence of changes in LH (257). Estrogens and xenoestrogens have also been shown to inhibit androgen production by testicular tissue from the Atlantic croaker amphibian, and this effect appears to be predominantly via a nongenomic mechanism (258). In humans, estrogen can directly inhibit testicular steroidogenesis, and at least part of this action may be by altering Leydig cell LH receptors (259). Indeed, there is a suggestion that estrogen may be involved in mediating Leydig cell responsiveness to LH (260) (see Ref. 113 for review), which may partially explain the reported increase in Leydig cell responsiveness to LH in monkeys given an aromatase inhibitor (194). Given that estrogen appears to have an inhibitory action on adult Leydig cell steroidogenesis, it is interesting to note that androgens, produced in high concentrations by adult Leydig cells, have been shown to regulate Leydig cell production of estrogen sulfotransferase, which is an enzyme involved in the sulfurylation and inactivation of estrogens (261). Finally, it should be mentioned that ArKO mice show evidence of Leydig cell hyperplasia/hypertrophy after 18 weeks of age (8); however, this is likely to be a consequence of the elevated LH levels in these animals. Thus, normal Leydig cell function and development are
important for male sexual development, testicular steroidogenesis during puberty and adulthood, and hence normal fertility. The demonstration of estrogen modulation of, and action at, each stage of Leydig cell development suggests that estrogen exposure could have important consequences for Leydig cell function and hence for male fertility.

**E. Sertoli cells**

The proliferation of Sertoli cells occurs from day 16 of fetal life in the rat and reaches a maximum 2 days before birth (262). Approximately 1 million Sertoli cells are present in the rat testis at birth and, with the continued proliferative activity of these cells, albeit at a declining rate (262), the numbers increase to a maximum of around 40 million at day 15 of postnatal life (263). After postnatal day 15, proliferation ceases (262), differentiation commences (see Ref. 139 for review), and the number of the Sertoli cells in the testis remains stable throughout adulthood (263) (see Ref. 264 for review). It is well known that this period of Sertoli cell proliferation, and the postproliferative differentiation and maturation of these cells, is essential for the full spermatogenic potential of the adult. This is perhaps best illustrated by experiments showing changes in the testicular size and spermatogenic potential of the adult after the alteration during puberty of factors that either potentiate Sertoli cell proliferation (265, 266) or interfere with or delay their maturation (179, 267).

In the following section, a role for estrogen in the control of Sertoli cell proliferation and differentiation will be discussed, and therefore it is pertinent to briefly review the hormonal control of these processes. Sertoli cell proliferation is likely controlled by numerous factors, including pituitary hormones and intratesticular factors (see Ref. 264 for review). The importance of FSH in this phase of development was demonstrated by the administration of human recombinant FSH to rats during the neonatal period, resulting in an increased number of Sertoli cells and an increased spermatogenic potential of the adult (266), and by the fact that neonatal administration of human recombinant FSH to hpg mice also stimulated Sertoli cell numbers (46). While FSH is thus a mitogenic factor for neonatal Sertoli cells (see Ref. 264 for review), thyroid hormone is thought to inhibit Sertoli cell division but promote differentiation. This knowledge comes from the observation that transient neonatal hypothyroidism results in enlarged testes and enhanced production of sperm (268). The mechanism behind this observation is now known to be via a direct action of thyroid hormone on Sertoli cells to inhibit Sertoli cell proliferation and stimulate differentiation (265, 269). Thyroid hormone acts directly on its receptor, which is expressed at high levels in proliferating Sertoli cells, and then declines toward the end of the proliferative period (270). Thus it is clear that endocrine signals control Sertoli cell division and differentiation.

Sertoli cells produce considerable amounts of estrogen during the period of division, leading to the suggestion that estrogen is involved in this process. Aromatase activity is highest in Sertoli cells from prepubertal rats, declines as Sertoli cells mature, and is hormonally regulated, principally by FSH (see Ref. 154 for review). Indeed, the FSH-induced aromatase activity and the measurement of estradiol produced by primary cultures of Sertoli cells from immature rats is the basis of a bioassay for FSH (271). ERs are present in Sertoli cells throughout development and appear to be primarily of the β-form (123, 127, 142, 143) (see Section IV). Dorrington and colleagues (154, 272) have proposed that estradiol, along with FSH, may be a mitogen for Sertoli cell division and have demonstrated that in granulosa cells of the ovary, estrogen induced TGFβ, which in turn stimulated DNA synthesis. This suggests that in these somatic cells of the gonads, FSH may induce aromatase activity and hence estrogen production, which stimulates TGFβ, which then, along with FSH, promotes cell division (272). The fact that similar control mechanisms exist in Sertoli cells led these authors to hypothesize that estrogen may participate in the FSH-mediated mitogenic activity on Sertoli cells via induction of TGFβ (154, 272).

Confirmation of whether estrogen has an action on Sertoli cell division may be facilitated by ER and aromatase null mice models. There are no descriptions of Sertoli cell numbers in ERαKO, ERβKO, or ERαβKO animals. Sertoli cell numbers in ArKO mice were not different from wild-type animals in our initial published studies (8) although very recent preliminary data on larger numbers of animals suggest an increase in Sertoli cell numbers in ArKO animals (our unpublished data), thus lending support to the hypothesis that estrogen may act to control the neonatal period of Sertoli cell division and differentiation.

Estrogen may also be involved in the postproliferative period of Sertoli cell maturation. Although studying the direct effects of exogenous estrogen treatment on Sertoli cells is complicated by disruptions to circulating hormones (see Section V.A), some studies have shown a specific action of estrogen in Sertoli cell development. Sharpe and colleagues (179) compared the effect of neonatal exposure to DES to a GnRH antagonist administered for the same period to dissect out the specific actions of inappropriate estrogen exposure during days 2–12 of life in the rat. These studies showed that while DES and GnRH antagonist decreased FSH levels and Sertoli cell numbers, DES caused a more profound delay of Sertoli cell maturation, as evidenced by the immunolocalization of Sertoli cell proteins, and permanent defects in spermatogenesis and testicular histology in adulthood (179), suggesting that exogenous estrogen has an inhibitory role in Sertoli cell maturation.

The theories put forward by Sharpe et al. (179), Dorrington and Khan (154), and others that estrogen has a stimulatory effect on Sertoli cell division yet a negative effect on Sertoli cell differentiation and development is supported by various observations: 1) the demonstration that estrogen production is high in proliferating Sertoli cells yet is lower in Sertoli cells with a more differentiated morphology (135, 134, 273); 2) toward the end of the period of Sertoli cell proliferation, FSH-induced aromatase activity starts to decline, partly due to a decreased Sertoli cell responsiveness to FSH (see Refs. 154 and 272 for review); 3) coincident with the fall in estrogen production and Sertoli cell mitotic activity, TGFβ, which may stimulate Sertoli cell mitosis in response to estrogen, also declines (154); 4) thyroid hormone, which stimulates Sertoli cell differentiation (265, 269), decreases aromatase activity in prepubertal Sertoli cells (274); 5) the capacity of Leydig cells
to produce testosterone increases from about day 14 post-partum (247), and androgen has been shown to inhibit Sertoli cell aromatase activity either directly (275) or via androgen-mediated effects on peritubular cells (276), suggesting that androgens from the maturing Leydig cells may participate in the down-regulation of aromatase during the switch from Sertoli cell division to differentiation; 6) various growth factors produced by the Sertoli cell can control Sertoli cell estrogen production and responsiveness to FSH and could therefore be involved in switching off aromatase during the period between division and differentiation (154); 7) germ cells, which are starting to develop during the switch between Sertoli cell division and differentiation, decrease Sertoli cell aromatase activity (277). Finally, perhaps the most interesting observation that supports an inhibitory role of estrogen in Sertoli cell differentiation is the fact that cells with the morphological features of a differentiated Sertoli cell are found in the ovaries of mice lacking both functional ERα and ERβ (ERαβKO mice) (222, 278) as well as in ovaries from ArKO mice (279). This observation suggests that the removal of estrogen or estrogen-induced factors is required for granulosa cell survival and differentiation, and the loss of estrogen results in an environment in which Sertoli cells are able to differentiate, presumably in this case from granulosa cells.

Finally, it is of interest to note that estrogen is thought to have a role in regulating the expression of the cell adhesion molecule neural cadherin (NCad) in the immature mouse testis (280) and in cultured mouse Sertoli cells (281). Given that NCad is thought to be important for cell-cell interactions in the testis, particularly between germ cells and Sertoli cells (282, 283), this may be one way in which estrogen is involved in establishing and maintaining the seminiferous epithelium.

In summary, the normal proliferation, differentiation, and functional maturation of Sertoli cells is essential for the initiation of spermatogenesis and the full spermatogenic potential of the adult. Estrogen administration studies show that Sertoli cell proliferation and function can be affected by exogenous estrogens and estrogen-like substances, leading to permanent defects in reproductive function in adulthood.

**F. Germ cells**

Germ cell development involves a series of mitotic and meiotic divisions and differentiation from the immature spermatogonial into the specialized elongated spermatid. The development of germ cells is well known to be dependent on the action of FSH and testosterone on the Sertoli cell, and both of these hormones have been shown to prevent germ cell apoptosis as well as to potentiate division and/or differentiation (see Refs. 39–41 for review). In addition to the well documented hormonal control of spermatogenesis by androgens and FSH, evidence for a direct role for estrogen in mediating germ cell proliferation, viability, and function is now emerging.

While it is known that neonatal estrogen exposure leads to permanent defects in germ cell development in adult rats (179), it is possible that this is due to permanent defects in Sertoli cell function rather than an effect on germ cells themselves. A direct action of estrogen on germ cells, however, is entirely possible given the localization of aromatase and ERs during various stages of germ cell development (see Section IV). Therefore, a paracrine action of estrogen from the Sertoli cells on the germ cells is possible, as is an action of estrogen that is produced within germ cells (see Fig. 4). Although the actions of estrogen on Leydig and Sertoli cells appear to be mainly inhibitory (see Sections V.D and E), there is accumulating evidence that estrogen has a predominantly stimulatory effect on germ cells.

Studies in which estrogen was administered to rats during the neonatal period between days 5 and 11 showed that the numbers of undifferentiated and differentiating type A spermatogonia were increased at day 15 of life (284). Although these studies suggest a stimulatory role for estrogen in spermatogonial division, it is difficult to conclude whether this effect is direct, or via perturbation of the hormonal signals from the pituitary (see Section V.A). However, in vivo and *in vitro* studies in Japanese eels suggest a direct stimulatory effect of estrogen on these cells, since spermatogonial renewal in this species was stimulated by estrogen but blocked by the estrogen antagonist tamoxifen (285). Studies using rat gonocytes (or prespermatogonial cells) in culture clearly demonstrated an effect of estrogen in stimulating gonocyte proliferation (286). The proliferation of these spermatogonial precursor cells, as evidenced by 5-bromo-2’-deoxyuridine (BrDU) incorporation, was stimulated by a 1 μM dose of estradiol but not by higher doses, and the stimulatory effect was blocked by the ER antagonist ICI 164,384. The effect of estradiol was mirrored by that of platelet-derived growth factor (PDGF) and, since the addition of estradiol and PDGF in combination did not have additive effects, the authors postulated that estradiol and PDGF acted via a similar mechanism to stimulate gonocyte proliferation (286). Given that aromatase activity in the Sertoli cells is high during the neonatal period when gonocytes are proliferating and differentiating into spermatogonia, and that gonocytes and differentiating spermatogonia during the early neonatal period have been shown to contain ERβ (122, 123, 127) (see Section IV), a direct action of estrogen in stimulating precursor germ cell mitosis is possible.

Recent evidence for a direct role for estrogen in preventing germ cell apoptosis was gained from studies using human adult seminiferous tubules cultured *in vitro* (145). When seminiferous tubules were cultured in the absence of serum and survival factors, spermatocyte and spermatid apoptosis was induced. The apoptosis of these cells could be prevented by low doses of estradiol, or higher doses of dihydrotestosterone, suggesting that estradiol is a potent inhibitor of germ cell apoptosis. The effect of estradiol was rapid, within 4 h, leading the authors to speculate that at least part of the effect may be mediated by a nongenomic action of estrogen; however, the authors used immunocytochemistry to show that ERs were present within these cells (145).

The fact that round spermatid apoptosis has been shown to occur in the seminiferous tubules of older ArKO mice (8) also highlights a role for estrogen in acting as a spermatid survival factor. In these mice, the numbers of spermatogonia and spermatocytes did not differ compared with wild-type animals; however, significant decreases in round and elongated spermatid numbers were seen after 18 weeks of age. *In situ* detection of apoptotic cells suggested that numerous
round spermatids undergo apoptosis, leading to decreases in the spermatid numbers (8). In agreement with this finding, adult monkeys treated with an aromatase inhibitor showed a decrease in the conversion of round to elongated spermatids as evidenced by flow cytometry, and a decrease in sperm output from the testis, also suggesting that estrogen is important for spermatid differentiation (194, 287). The fact that aromatase deficiency leads to defects in spermatid differentiation is also supported by earlier studies in which a granulosa cell-secreted factor, purified to homogeneity and shown to have aromatase-inhibitory activity, induced round spermatid degeneration and a decrease in mature spermatids in adult male rats (288, 289).

A surprising finding in ArKO mice was that round spermatids that did not undergo apoptosis early in spermiogenesis had acrosomal dysgenesis (8). The acrosome is a vesicle associated with the postmeiotic spermatid nucleus and contains a number of hydrolytic enzymes that are required for the sperm’s penetration of the zona pellucida of the ovum. The acrosome arises from the Golgi complex and, during acrosome biogenesis, proacrosomal granules in the Golgi coalesce to form a single large vesicle that becomes closely associated with the spermatid nucleus in the early stages of spermiogenesis (see Ref. 139 for review). The acrosomal vesicle gradually spreads out and flattens over the nucleus, in a process that involves anterograde and retrograde vesicular trafficking between the Golgi and the developing acrosome (290). The observation of abnormal acrosome development in the ArKO mouse suggests that acrosome biogenesis could be an estrogen-dependent process. This hypothesis is supported by the immunolocalization of high levels of aromatase in spermatids of the developing spermatid (150), as well as the presence of ERβ in spermatids (see Section IV and Table 1).

The stimulatory effect of estrogen on spermatogenesis was recently investigated in detail by the administration of estradiol to hpg mice (187), which lack FSH and LH due to a congenital deficiency of GnRH. The testes of the postpubertal hpg mouse are underdeveloped with spermatogenesis arrested at the early stages of germ cell development; however, the administration of estradiol-filled SILASTIC implants to these mice for a period of 70 days was able to induce all stages of spermiogenesis (see Table 2).

The stimulatory effect of estrogen on spermatogenesis was recently investigated in detail by the administration of estradiol to hpg mice (187), which lack FSH and LH due to a congenital deficiency of GnRH. The testes of the postpubertal hpg mouse are underdeveloped with spermatogenesis arrested at the early stages of germ cell development; however, the administration of estradiol-filled SILASTIC implants to these mice for a period of 70 days was able to induce all stages of spermiogenesis (see Table 2). This somewhat surprising finding strongly suggests that estrogen is capable of inducing spermatogenesis. Although the literature reviewed above suggests that estrogen can have a direct action on germ cells, the estrogenic induction of spermatogenesis in the hpg mouse may also have been due to an indirect effect via the stimulation of low levels of FSH. The relatively physiological levels of estrogen present in these mice after 35–70 days of treatment were associated with a significant stimulation of FSH, presumably by a direct action at the pituitary. Although the levels of FSH were approximately one-third of the levels in wild-type animals, an effect of these low levels of circulating FSH on stimulating germ cell development cannot be ruled out (187). Despite the obvious caution needed when studying the hormonal regulation of spermatogenesis in a mouse congenitally deficient in all gonadotrophic stimulus, the induction of spermatogenesis by estrogen in this model provides evidence that estrogen can stimulate male germ cell development.

Recent studies have suggested that estrogen is involved in the function of mature spermatozoa. The incubation of human spermatozoa in the presence of estrogen is known to stimulate various sperm functions including motility and lactate production (see Ref. 98 for review). Evidence has been presented that the stimulatory actions of estrogen on human spermatozoa are via a membrane-associated ER, which is a 29-kDa protein that is recognized by an antibody to the ligand-binding domain of the human genomic ERα (107). The action of estradiol on this receptor is apparently nongenomic, as the effect was rapid (within minutes) and involved a rapid influx of calcium. Estradiol action via this receptor causes changes in tyrosine phosphorylation of various proteins and inhibited the nongenomic actions of progesterone such as the progesterone-induced acrosome reaction (107).

Although the above studies suggest a role for estrogen in germ cell development, it should be pointed out that recent reports show that the administration of a dual ERα/ERβ antagonist ICI 182,780 to wild-type mice for 35 days did not produce observable changes in the morphology of the seminiferous epithelium, apart from the expected distention of the rete testis (26). However, the ability of the ICI antagonist to cross the blood-testis barrier and enter the adluminal compartment of the seminiferous epithelium locally block estrogen action has not been demonstrated, nor has the ability of the antagonist to fully block the high levels of estrogen that are present in the testis.

Therefore, the studies presented above provide evidence of a stimulatory role for estrogen in germ cell development including spermatogonial division, germ cell viability and differentiation, acrosome biogenesis, and function of spermatozoa.

G. Comparison of the spermatogenic phenotype of mice with targeted disruptions of ERs or aromatase

Previously published studies have described the fertility and/or testicular phenotypes of the ERαKO (5, 6, 27, 278, 291), ERβKO (198, 278), ERαβKO (222, 278), and ArKO (7–9, 196) mice. Although the phenotypes of these animals have been mentioned above, a more direct comparison between the phenotypes of animals in which aromatase or the ERs are inactivated is of interest when reviewing the evidence for a role for estrogen in spermatogenesis (see Table 2). Although spermatogenesis is clearly disrupted in the ERαKO mouse (5), the lack of germ cell development and the reduction in mature spermatids in the epididymis can be primarily attributed to compromised fluid resorption due to defective efferent ductule function (5, 6, 27, 223) (see Section V.B). Efferent ductule ligation experiments show that the seminiferous epithelium in the ERαKO secretes significantly less fluid than wild-type animals (6), and this could be attributed to either fluid build-up causing Sertoli cell dysfunction or a direct effect due to the lack of ERαKO in the testis. It seems likely that the spermatogenic phenotype in the ERαKO mice is primarily due to an indirect effect via efferent ductule dysfunction, rather than a direct effect on spermat-
ogogenesis (6, 27). Studies by Mahato and colleagues (292) showed that the infertility in the ERαKO mice is not due to a defect within the ERα null germ cells themselves, since transplantation of ERαKO germ cells to wild-type mice depleted of germ cells demonstrated that ERα-deficient germ cells can develop in an environment in which aromatase and ERs are present. The fact that mouse germ cells do not require ERα for development is perhaps not surprising because mouse germ cells do not appear to contain ERα, nor do germ cells in other species (see Table 1 and Section IV).

The lack of any apparent spermatogenic phenotype in mice lacking a functional ERβ (198, 278), however, is surprising, given the fact that ERβ appears to be the only ER present in the germ cells, and the predominant, if only, subtype in the Sertoli cell (see Section IV). Indeed in most species, ERβ appears to be the most predominant and more widely expressed ER in the testis. The absence of spermatogenic disruption in these mice is not readily explicable, but could perhaps be due, in part, to a compensation by ERα, since ERα expression and localization in ERβKO testes have not been explored. In the efferent ductules of the ERαKO mice, ERβ has been shown to have a different subcellular localization compared to wild-type tissues (140). Thus, perhaps there is a permanent change in the localization of ERα in the ERβKO testis. The fact that germ cells from ERαKO mice can develop normally when transplanted in wild-type mice (292), together with the fact that ERβKO animals do not show direct disruptions to spermatogenesis (see Ref. 27 for review; see Table 2), raises questions as to the role of ERs in germ cells. However, the potential action of estrogen in germ cells via nonclassical receptors cannot be ruled out. As discussed above, nongenomic actions of estrogen have been implicated in Leydig cell function (258), germ cell viability (145), and sperm function (107). The nongenomic action of estrogen on plasma membrane-associated receptors has been documented in various reproductive systems (see Ref. 98 for review, and Section III.B). The estrogen-dependent initiation of this nongenomic pathway can include a rapid increase in cAMP (e.g., Ref. 293), or intracellular calcium (e.g., Ref. 107), or the MAPK pathway (e.g., Ref. 294). Consequently, it is possible that estrogens have actions independent of ERα and ERβ, and thus the potential for estrogen acting in the testes of mice with inactivated ERα and/or β cannot be discounted.

To date, three laboratories have generated ArKO mice (7–9). The transgenic lines from each of the three laboratories all show defects in sexual behavior (see Table 2); however, fertility in these mice is variable (see Ref. 7 for a direct comparison of the three phenotypes). All mice show normal spermatogenesis at 14 weeks of age (7–9). One line of ArKO mice shows progressive disruptions to spermatogenesis until by 1 yr of age, all animals show evidence of spermatogenic disruption (8). In contrast, ArKO mice generated recently in another laboratory show no disruptions to spermatogenesis at 14 weeks to 10 months of age, although there is a significant reduction in seminiferous epithelial height in these animals (7). Testicular histology in older mice from a third line of ArKO mice has not been described (9). The reason for the heterogeneity in the phenotypes of different ArKO mice is unclear. Diet can provide variable levels of phytoestrogens that may contribute to the heterogeneity. Another source of variation could be in the extent of in utero exposure of male ArKO pups to estrogen from the maternal circulation as well as that produced by wild-type and heterozygous littermates. Also pups are presumably exposed to maternal estrogens during the lactational period, which may also be affected by the phytoestrogen content of the mother’s diet. Thus variations in estrogen exposure of ArKO males in utero, before weaning, and during adulthood are likely to contribute to the variable spermatogenic phenotype.

In our laboratory, we noticed a decrease in fertility of ArKO mice after the age of 18 weeks (8), and this was related to the decrease in the numbers of round and elongated spermatids in the testes, despite no changes in earlier germ cell numbers. The fact that round spermatids undergo apoptosis and/or have disruptions to acrosome biogenesis in the ArKO mice (8) and contain both aromatase and ERβ (see Section IV) suggests local estrogen action in these cells. A surprising finding in these ArKO mice was that the spermatogenic phenotype was age dependent, developing only after 18 weeks of age, leading to the speculation that dietary phytoestrogens could allow the maintenance of spermatogenesis in younger animals (8). Whether the disruptions to spermatogenesis have an earlier onset and are more profound in ArKO animals raised on a phytoestrogen-free diet, and thus whether dietary estrogens could have a modulatory role in the testis of ArKO mice, is currently under examination in our laboratory.

A second unexpected finding in our ArKO mice (8) and in other ArKO mice (7, 9) was the absence of seminiferous tubule dysfunction related to dysfunction of the efferent ductules as is seen in the ERαKO (6, 26, 27, 223, 291) (see Table 2). Thus while spermatogenesis is disrupted in ArKO mice (8), this does not seem to be primarily related to failure of the efferent ductules to resorb fluid, since no significant increase in either testis weight or seminiferous tubule luminal volume was seen (8). It is possible that efferent ductule morphology and function could be impaired in the ArKO, yet there may be reduced fluid secretion by the seminiferous tubules and thus fluid buildup may not be obvious. A second proposition is that ArKO animals, which will be exposed to maternal estrogens in utero, may have normal development of the efferent ductules, which allows these tissues to function normally in adulthood.

The lack of an effect on efferent ductules and the late onset phenotype in the ArKOs (8) leads to the speculation that there is ligand-independent activation of ERs within the ArKO reproductive tract. There is evidence for ligand (estrogen)-independent activation of ERs in various systems, e.g., by cyclin D1 (94, 95) or by growth factors (see Ref. 87 for review; see Section III.B). An alternative explanation to account for these observations is that, despite the absence of aromatase products in ArKO animals (196), there is the potential for other endogenous estrogenic ligands to activate ER-mediated transcription. In vitro relative binding affinity studies show that ERα and ERβ can interact with a variety of non-aromatase-derived ligands such as 5-androstene-3β,17β-diol and 5α-androstane-3β,17β-diol (65). If ERs in ArKO mice do interact with alternate ligands such as those derived from androgens, presumably there would be a gradual disappearance of such ligand(s) in the testis after pubertal...
Finally, it is also worthwhile to note that recent reports on the infertility of mice deficient in vitamin D receptors indicate that the testes of these mice have disrupted estrogen biosynthesis due to a reduction in aromatase expression (295). While the authors report that the spermatogenic phenotype is similar to both the ERαKO and ArKO phenotypes, further analysis of this phenotype is required to ascertain whether the observed reduction in sperm count and disruption to seminiferous epithelial morphology are due to a direct effect on germ cell development, such as in the ArKO, and/or due to dysfunction of the efferent ductules, such as in the ERαKO.

Studies on the spermatogenic phenotypes in ER (reviewed in Ref. 27) and aromatase null mice clearly provide evidence that estrogen and ERs are required for spermatogenesis and normal male fertility (see Table 2). Studies in the ERα null mouse have shown that one of the most important estrogen, or at least, ERα-regulated events is the development and function of the efferent ductules, and that a lack of ERα in this tissue has profound implications for male fertility. The observations in ArKO mice suggest that aromatase and estrogen are important for germ cell development. However, since ERβ is apparently the only ER present in germ cells, and ERβKO mice are fully fertile, it is unclear how estrogen is acting in germ cells. Observations in isolated germ cells have provided evidence for plasma-membrane ER and/or non-genomic actions of estrogen; a mechanism that may not be revealed in ERβKO mice. Discrepancies between the ERαKO, ERαβKO, and ArKO phenotypes (see Table 2) perhaps suggest the presence of novel genes encoding ERs and/or aromatase and/or ligand-independent activation of ERs. Thus, while the studies in ER and aromatase null mice indicate that ERα is essential for normal male fertility, as is aromatase, the discrepancies between the phenotypes demonstrate that a more comprehensive understanding of estrogen action in the testis is required.

VI. Estrogen and Spermatogenesis in Humans

Clearly the primary evidence demonstrating a pivotal role for estrogen in spermatogenesis has been derived from animal models, as reviewed above, and knockout models, such as the ERαKO, ERβKO, ERαβKO, and ArKO mice, have been especially useful in elucidating the spermatogenic consequences of removing the global and local actions of estrogen. Naturally occurring mutations in humans, which render them devoid of estrogen or resistant to its actions, can similarly provide us with information specific to the role of estrogen in human spermatogenesis. Such mutations have been extremely rare, and indeed were once thought to be lethal. Only a single case has been reported for a mutation in the ER (240), whereas two cases have been described resulting from aromatase deficiency in men (242, 243) and one in a male infant (241). It is valuable for the purposes of this article, however, to review the salient features of the reproductive phenotypes of these men, to better understand the direct and indirect impact estrogen has upon human spermatogenesis and fertility.

The first aromatase-deficient adult male presented with scant estradiol (<7 pg/ml) and elevated testosterone, 5α-dihydrotestosterone, androstenedione, FSH, and LH levels (243). Semen analysis was not performed on this patient and at the time of reporting, he was a virgin; hence, no conclusions can be drawn about the effect of his aromatase deficiency upon his fertility. Morishima and colleagues (243) report that the volume of both testes was greater than 25 ml and that the consistency of the testes was normal. At 24 yr of age, the patient exhibited Tanner stage 5 pubic hair and genital development. Behaviorally, he was heterosexually oriented and reported a normal pattern of nocturnal emissions and ejaculations. The second aromatase-deficient male studied also had undetectable levels of estradiol; however, he had normal serum concentrations of testosterone and androstenedione, with only slightly elevated levels of FSH and LH (242). Semen analyses of this patient revealed a more than 20-fold decrease in sperm count and all sperm were immotile. A testicular biopsy showed hypospermatogenesis and germ cell arrest, mainly at the level of primary spermatocytes. Consequently, this man was infertile, one of the symptoms for which he initially sought therapy. Treatment with human menopausal and chorionic gonadotropins, estradiol, or testosterone, did not restore the sperm count (242). Testis volume was subnormal in this male, the volume of each testis being only 8 ml at 29 yr of age, although other sexual parameters of pubertal development were normal. His sexual identity and psychosexual orientation were heterosexual. It should be noted that azoospermia and infertility were also reported in a brother of this man who did not have an aromatase deficiency; therefore, the infertility of this estrogen-deficient male may not be related to a lack of estrogen (242).

In the single reported case of an adult male with a mutation in the ERα gene, a premature stop codon resulted from the replacement of cytosine with thymine at codon 157, and thus the translated receptor would lack the DNA binding and ligand binding domains and be functionally inert (240). Like the aromatase-deficient males, gonadotropin levels in this man were elevated, and circulating testosterone was normal [similar to the patient described by Carani and colleagues (242)], but serum estrogens were elevated (119 pg/ml). This male experienced normal onset of puberty and was normally masculinized, each testis with a volume of 20–25 ml. Semen analysis revealed a normal sperm density (25×10⁶/ml) but with a decreased sperm viability of only 18% (normal = 50%). Treatment with high-dose ethinyl estradiol did not restore the hormone profile of this patient to within normal parameters, despite a 10-fold increase in the serum free estradiol concentration (240). Behaviorally, this male expressed no gender identity disorder and was heterosexually oriented, and his sexual function was apparently normal.

Evidently, few conclusions about the action(s) of estrogen on human spermatogenesis may be drawn from this small number of examples. The available data, although scant, certainly imply that estrogen is required for human spermatogenesis to proceed normally, and for full male fertility.

Other, nonreproductive similarities exist between the case of male estrogen insensitivity and those described for aromatase-deficient men. These include significant skeletal development leading to the onset of spermatogenic disruption (8).
aberrations, such as osteopenia, osteoporosis, increased bone turnover, and delayed or absent epiphyseal fusion, and compromised circulating lipid levels and insulin resistance. While beyond the scope of the current paper, these facets of the phenotypes were reviewed by Faustini-Fustini et al. (296) and Grumbach and Auchus (297).

VII. Estrogen and Sexual Behavior

We have reviewed the data that demonstrate that estrogen has the capacity to have an impact upon male fertility in terms of direct and indirect roles on the development and maintenance of spermatogenesis and reproductive function. Evidence is now accumulating that this impact extends to male sexual behavior, and that the absence of estrogen impairs male fertility at the level of sexual behavior (see Table 2). While an analysis of the estrogen control of behavior clearly extends beyond this review, a brief overview is warranted.

Perturbations have been reported in the sexual behavior of ER null mice, disturbances that by themselves (to the exclusion of the spermatogenic aberrations) would render the mice either infertile or with diminished fertility (see Ref. 27 for review; see Table 2). EReKO mice, while motivated to engage in sexual activity in terms of numbers of mounts, display a severely reduced number of intromissions and ejaculations (221). In contrast, sexual behavior of EβKO male mice is reportedly not perturbed (299), suggesting that Eα is the receptor required to transduce normal sexual behavior in males (27). Sexual behavior has recently been reported as being completely abolished in EReKO mice (299), highlighting the fact that estrogen action is required for normal sexual behavior. Of particular interest is the fact that EαβKO mice do not mount receptive females, whereas EReKO and EβKO mice display normal mounting behavior (see Table 2), indicating that expression of either ER is sufficient for mounting behavior and suggesting that Eβ plays a role in maintaining sexual behavior in the brain, but that lack of Eα in EβKO mice can be compensated for by ERe (299). The information from ER null mice complements evidence from Honda and colleagues (9), Toda and colleagues (7), and our own laboratory (our unpublished data) (see Table 2), that ArKO mice display disturbed sexual behavior, either not mounting receptive females at all, or taking longer to mount and mounting less often than wild-type control males.

A single study has been performed to assess the impact of estrogen on human male sexual behavior (300). By restoring estrogen to the second aromatase-deficient patient that was described in the literature, significant modifications of this man’s sexual behavior occurred. Although originally reported as having a normal libido (242), the administration of estradiol to this patient increased libido, frequency of sexual intercourse, masturbation, and erotic fantasies (300). Thus the evidence for an involvement of estrogen in sexual behavior gained from mouse models may have parallels in the human.

VIII. Summary

Spermatogenesis involves the proliferation and differentiation of germ cells into mature spermatids. After release from the seminiferous epithelium, sperm proceed through the efferent ductules and epididymis, a process that is important for sperm motility and fertilizing ability. Spermatogenesis has been known for many years to be regulated by FSH and androgens; however, emerging evidence from animal models, including transgenic mice, suggests that estrogen should be added to the list of hormones important for spermatogenesis.

That estrogen can influence testicular and epididymal function is not unexpected, given the evidence presented that estrogen biosynthesis, via the aromatase enzyme, and action on its receptors (α and/or β), occurs in these tissues. Estrogen is produced by the testis from the fetal period throughout adulthood and, similarly, Eα and β are found in the testis at all ages. While some cells express both ERA and β, such as the Leydig cells, the cells in the seminiferous epithelium appear to predominantly contain ERβ. Nongenomic actions of estrogen can also occur in the testis, although whether this involves the “classic” ERs remains uncertain.

Evidence has been presented that estrogens act at multiple levels to control, or interfere with, spermatogenesis. Estrogen is clearly involved in the negative feedback effects of testosterone on the brain to control pituitary gonadotropin secretion, and hence an absence of, or inappropriate exposure to, estrogens leads to disturbances in the delicate balance of the hypothalamo-pituitary-testis axis in both mice and men. In view of the fact that the development and spermatogenic potential of the testis is reliant upon this axis, such disturbances are likely to have a deleterious effect on spermatogenesis and fertility.

We and others (162) have reviewed the evidence for an essential role for estrogen in the development and maintenance of the efferent ductules and epididymis. An absence of ERA causes defects in efferent ductule development, resulting in disturbed function, particularly in terms of fluid reabsorption, causing a reduced number of sperm to enter the epididymis. A consequence of the disturbed fluid dynamics is a buildup of fluid in the testis, resulting in seminiferous epithelial damage and impaired germ cell development. The fact that overexposure to estrogens during neonatal development can also produce similar defects in efferent ductule function highlights the need for a tightly coordinated series of estrogen-dependent events in this tissue.

There is compelling evidence for a role for estrogen in testicular function. Notable roles for estrogen in Leydig cells include the coordinated regulation of progenitor Leydig cells into immature and adult forms. Estrogen also plays a role in testicular descent, at least in part, by the regulation of fetal Leydig cell gene expression. Sertoli cells are influenced by estrogen overexposure, which is probably related to the fact that estrogen appears to play an inhibitory role, acting as an internal control mechanism in Sertoli cell proliferation, development, and function. A compelling body of evidence has also been presented that estrogen has a functional role in germ cells. Germ cells contain ERs as well as aromatase, and thus it is possible that estrogen acts in an intracrine manner
in these cells, to control viability/apoptosis and, potentially, acrosome biogenesis. In addition, nongenomic actions of estrogen on sperm function can now be considered along with the well documented nongenomic actions of progesterone (98).

The models of gene disruption and estrogen administration studies reviewed in this manuscript and the deleterious effects of such situations on male fertility have answered many questions as to the role of, and sites of action for, estrogen in several aspects of male fertility, from testicular function to sexual behavior. However, a large amount of conflicting data also exist, such as the comparison of the estrogen in several aspects of male fertility, from testicular biogenesis. In addition, nongenomic actions of progesterone (98).

Mice deficient in ERβ-gene structure, chromosomal localization, and expression pattern. J Clin Endocrinol Metab 82:4258–4265


Handelsman DJ 2000 Myth and methodology in the evaluation of human sperm output. Int J Androl 23[Suppl 2]:50–53


Steinberger E, Dukett GE 1965 Effect of estrogen or testosterone on initiation and maintenance of spermatogenesis in the rat. Endocrinology 76:1184–1189


Couse JF, Korach KS 1999 Estrogen receptor null mice: what have we learned and where will they lead us? Endocr Rev 20:358–417


Hess RA 1990 Quantitative and qualitative characteristics of the stages and transitions in the cycle of the rat seminiferous epithelium; light microscopic observations of perfusion-fixed and plastic-embedded testes. Biol Reprod 43:525–542


53. Jenkins C, Michael D, Mahendroo M, Simpson E 1993 Exon-specific northern analysis and rapid amplification of cDNA ends (RACE) reveal that the proximal promoter II (P II) is responsible for aromatase cytochrome P450 expression in human ovary. Mol Cell Endocrinol 95:1361–1367.


73. Couse JF, Lindzey J, Grandien K, Gustafsson JA, Korach KS 1997 Tissue distribution and quantitative analysis of estrogen receptor-α (ERα) and estrogen receptor-β (ERβ) messenger ribonucleic acid in the wild-type and ERα-knockout mouse. Endocrinology 138:4613–4621.


82. Onate SA, Tsai SY, Tsai MJ, O’Malley BW 1995 Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. Science 270:1354–1357
100. Russell KS, Haynes MP, Sinha D, Clerisme E, Bender JR 2000 Human vascular endothelial cells contain membrane binding sites for estradiol, which mediate rapid intracellular signaling. Proc Natl Acad Sci USA 97:5930–5935
113. Abney TO 1999 The potential roles of estrogens in regulating Leydig cell development and function: a review. Steroids 64: 610–617
121. Axelrod SM, Katzenellenbogen BS 1993 Stimulation of estrogen receptor-mediated transcription and alteration in the phosphorylation state of the rat uterine estrogen receptor by estrogen, cyclic adenosine monophosphate, and insulin-like growth factor-I. Mol Endocrinol 7:743–752
137. Rosenfeld CS, Ganjam VK, Taylor JA, Yuan X, Stiehr JR, Hardy MP, Lubahn DB 1998 Transcription and translation of estrogen receptor-β in the male reproductive tract of estrogen receptor-α knockout and wild-type mice. Endocrinology 139:2982–2987
158. Pavao M, Taish AM 2001 Estrogen receptor antibodies: specificity and utility in detection, localization and analyses of estrogen receptor α and β. Steroids 66:1–16


Tezón JA, Blaquier JA 1983 Androgens control androgen-binding sites in rat epididymis. Endocrinology 113:1025–1030

Danzo BJ, Eller BC 1979 The presence of a cytoplasmic estrogen receptor in sexually mature rabbit epididymides: comparison with the estrogen receptor in immature rabbit epididymal cytosol. Endocrinology 105:1128–1134


Downloaded from https://academic.oup.com/edrv/article-abstract/22/3/289/2423875 by guest on 20 January 2018


282. Hess RA, Cooke PS, Bunick D, Kirby JD 1993 Adult testicular enlargement induced by neonatal hypothyroidism is accompanied by increased Sertoli and germ cell numbers. Endocrinology 132:2607–2613


