The production of fertile spermatozoa and eggs is the most important starting point for successful reproduction of mammals, including human. A number of statistical and scientific studies indicate an increasing frequency of infertility in human, leading to social and economical problems (ESHERE task force on ethics and law et al., 2009). To resolve the causes of the infertility and to understand fertilizing mechanisms better, many countries of the world have been supporting tremendous research funds in the reproduction field. For example, in South Korea, the national research foundation (NRF) has supported about 20 research grants with over one million dollars in total during years of 2006 and 2008 (Figure 1). Governmental support in S. Korea in reproduction research field has been greatly increased every year (Figure 1), and a total of 27 researches, including individual and group research projects, with nearly $2.6 million have been granted at year of 2009. Even though continuous scientific efforts have made remarkable progresses for treatment of human infertility, we have not completely understood detailed mechanisms causing the infertility, and a number of important issues remain to be answered for dealing of human infertility.

It is generally believed that a male factor is the contributing cause in 40% to 60% of human infertility (Schlegel, 2009). Thus, it is very important to understand how male fertility is maintained and is regulated during the life span. There is no doubt that male hormones, testosterone and its metabolites, play a central role on control of the male reproduction. However, an increasing number of scientific evidence has revealed that male fertility is greatly influenced by other intrinsic and/or extrinsic factors, including estrogens, as well (Sharpe, 1998; Hess et al., 2001; Sierens et al., 2005). The functions of estrogen in the male reproductive tract have recently caught a large attention because estrogen has been considered as a representative female hormone for a long time. Indeed, many recent studies have revealed that...
estrogens are naturally present and synthesized in the male reproductive tract (Sharpe, 1998; Hess et al., 2001; Hess, 2003). Moreover, other researches have demonstrated that many environmental toxicants perform estrogenic effects in vertebrates and thereby affect the male fertility (Bretweld et al., 2007; Meistrich and Shetty, 2008). Therefore, examination of a functional role of estrogen in the male reproductive tract would provide better understanding about mechanism of male reproduction and causes of male infertility, so thus leading to improved treatment of human sterility originated from the male.

In this review, a role of estrogen in the efferent ductules of the male reproductive tract will be mainly discussed. However, readers who are interested in function of estrogen in the testis are encouraged to refer to a number of published review articles (Sharpe, 1998; Sierens et al., 2005).

**BIOSYNTHESIS AND ACTIONS OF ESTROGENS**

Like in most of steroid hormone-producing tissues, the testis utilizes cholesterol as a precursor for production of androgens and estrogens (Figure 2). A conversion of cholesterol into testosterone involves in a serial action of steroidogenic enzymes, including cytochrome P450 side chain cleavage, steroidogenic acute regulator, 17β-hydroxysteroid dehydrogenase (HSD), and 3β-HSD (Figure 2). Even though testosterone itself is a biologically active steroid hormone, testosterone can be further metabolized into dihydrotestosterone (DHT) by 5α-reductases or into estrogens by cytochrome P450 aromatase (P450 aromatase). Testosterone and DHT bind with androgen receptor (AR) and estrogens bind with estrogen receptor α (ERα) and/or ERβ to perform their biological functions.

**Figure 1.** Research funding status in reproductive research field granted from national research foundation (NRF) of S. Korea between years of 2006 and 2008. Numbers above the bars indicate numbers of research projects supported by NRF at each year. At year of 2008, a number of research projects include 2 group projects which the amount of research funding has not been added into a total. One US dollar is equivalent to 1,200 Korean won.

**Figure 2.** Steroidogenic pathway of estrogen biosynthesis. The cholesterol, a common precursor of steroid hormone, is converted into testosterone by sequential actions of a number of steroidogenic enzymes. Testosterone undergoes a further modification either into dihydrotestosterone (DHT) by 5α-reductases or into estrogens by cytochrome P450 side chain cleavage, steroidogenic acute regulator, 17β-hydroxysteroid dehydrogenase (HSD), and 3β-HSD (Figure 2). Even though testosterone itself is a biologically active steroid hormone, testosterone can be further metabolized into dihydrotestosterone (DHT) by 5α-reductases or into estrogens by cytochrome P450 aromatase (P450 aromatase). Testosterone and DHT bind with androgen receptor (AR) and estrogens bind with estrogen receptor α (ERα) and/or ERβ to perform their biological functions. StAR: steroidogenic acute regulator; SCC: cytochrome P450 side chain cleavage; 17β-HSD: 17β-hydroxysteroid dehydrogenase; and 3β-HSD: 3β-hydroxysteroid dehydrogenase.
into dihydrotestosterone (DHT), a potent androgen, or 17β-estradiol (E2) by the action of 5α-reductases or cytochrome P450 aromatase (P450Arom), respectively (Figure 2). The synthesis of testosterone from cholesterol precursor in the testis takes place in the Leydig cells, under an influence of lutenizing hormone (LH) secreted from anterior pituitary (Robaire and Hermo, 1988). These steroid hormones can bind with their respective receptors, androgen receptor (AR) and estrogen receptor (ER), which act as ligand-dependent transcriptional activators (Nilsson et al., 2001). There are two forms of ERs, ERα and ERβ, that could act as homo- and/or hetero-dimer to exert their biological functions by regulating expression of target genes (Nilsson et al., 2001). A detailed explanation about action of steroid hormone and receptor would be acquired from other review articles (Nilsson and Gustafsson, 2000; Nilsson et al., 2001).

**STRUCTURE OF THE EFFERENT DUCTULES**

The male reproductive tract is consisted of the testis and excurrent ducts. The excurrent ducts are further divided into the efferent ductules (EDs), epididymis, and vas deferens (Figure 3). The epididymis is separated into 4 different regions, initial segment (IS), caput epididymis, corpus epididymis, and caudal epididymis, depending upon their histological and functional characteristics (Figure 3A) (Ilio and Hess, 1994). The EDs is located between the rete testis of the testis and the IS of the epididymis, and is covered by the epididymal fat. The EDs is divided into proximal, common, and distal regions, based on their morphological and structural features (Figure 3B) (Ilio and Hess, 1994). The EDs is made of a series of tubules, having a lumen inside enclosed with a single layer of columnar or pseudostratified columnar epithelial cells (Hamilton, 1975; Jones and Jurd, 1987) (Figure 3C). The epithelial layer of the EDs is surrounded by a thin sheet of smooth muscle, and the space among tubules of the EDs is filled with connective tissues (Figure 3C). The epithelia of the EDs are composed of two different cell types, ciliated and nonciliated cells. The ciliated cell is characterized with numerous true cilia on the apical side facing the lumen (Figure 3D and E). In addition, the nucleus of the ciliated cell is frequently displaced in apical cytoplasm (Figure 3D), and reduced frequencies of endocytotic vesicles and lysosomal granules are found in the ciliated cell (Figure 3E). The nonciliated cell is the major cell type present in the epithelial layer of the EDs. The nonciliated cell has a number of unique features distinguishable from the ciliated cells, for examples, numerous endocytotic vesicles at apical cytoplasm, lysosomal granules in various sizes, microvilli at apical face, and a nucleus located at basal region (Figure 3D and E). Both of the ciliated and nonciliated cells are attached to neighboring cells by a junctional complex composed of three different components, including the segmented or incomplete tight junction between adjacent nonciliated cells (Suzuki and Nagano, 1978). Such leaky

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**Figure 3.** Structure of the male reproductive tract and the efferent ductules. A) A gross anatomical picture of the mouse male reproductive tract. The efferent ductules (EDs) is located between the testis (T) and the initial segment (IS). Caput: caput epididymis; Corpus: corpus epididymis; Cauda: caudal epididymis; and Vas: vas deferens. B) An enlarged picture of the EDs. The EDs is connected to the rete testis (RT) of the testis. The EDs is divided into proximal (P), conus (C), and distal (D) regions. C) Histological picture of the EDs. The EDs is consisted of a layer of the epithelium (Ep), underlined by a thin smooth muscle (SM) layer. Lu: lumen and CT: connective tissues. D) An enlarged H and E staining picture of the EDs. Ciliated cells have cilia (Ci) protruding into the lumen. Nonciliated cells possess numerous lysosomal granules (Ly) in the cytoplasm. N: nucleus. E) Light photomicrographs of the EDs. Nonciliated cells have microvilli (Mv) at its apical side. Numerous endocytotic vesicles (Ev) in variable sizes are seen in the cytoplasm of the nonciliated cells. Ciliated cells have cilia (Ci) at the apical face. N: nucleus.
type of junctional complex indicates that the epithelium of the EDs may be permeable for bulk fluid movement (Pudney and Fawcett, 1984).

**FUNCTIONS OF THE E-effereHT DUCTULES**

It is generally accepted that the developmental origin of the EDs in rodents is the proximal mesonephric tubules (Linder, 1971; Marshall et al., 1979; Takeuchi, 1992). As an embryonic homology with the kidney proximal tubules, the EDs shares common physiological functions with the kidney (Hinton and Turner, 1988). The EDs plays a number of important functions for male reproduction. The first function of the EDs is the transport of sperm and fluid from the testis to the head of the epididymis. Even though the main force of directing the flow and spermatzoa from the testis toward the epididymis has not been clearly established, it is considered that a combination of forces generated from the testis and the EDs would drive the fluid flow through the ductules (Mason and Shaver, 1952; MacMillan and Harrison, 1955; Winet, 1980). Next, the EDs is capable of reabsorbing most of the testicular fluid entering from the rete testis. It has been found that more than 90% of the fluids secreted from the testis is reabsorbed in the rat EDs (Levine and Marsh, 1971; Jones and Jurd, 1987). The underling mechanism for reabsorption in the EDs remains to be established. Many researchers have proposed that endocytosis is the main mechanism of moving the fluid across the epithelium (Hoffer et al., 1973; Flickinger et al., 1978; Goyal et al., 1988). On the other hand, others have suggested that fluid movement through the epithelium of the EDs may be coupled with the active transport of electrolytes (Hamilton, 1975; Jones and Jurd, 1987). Other functions of the EDs are production and secretion of proteins and hormones into the lumen. A number of histochemical and morphological studies have demonstrated that the EDs possesses steroidogenic enzymes, such as 3β- and 17β-hydroxysteroid dehydrogenase (Tingari, 1973) and steroid hormone receptors, including androgen and estrogen receptors (Hess et al., 1997b; Cooke et al., 1991). Recently, we have found that the EDs expresses the transcript of P450Arom in the EDs, which converts androgen into estrogen (Kim et al., 2008). Thus, these findings suggest that the EDs has an endocrine activity being capable to synthesize steroid hormones. In addition to functions of the EDs described above, the EDs can remove abnormal spermatzoa by phagocytosis (Hoffer and Hamilton, 1974).

**SOURCES OF ESTROGEN IN THE MALE REPRODUCTIVE TRACT**

A significant quantity of estrogen is present in the semen and testicular fluid of several mammalian species (Hess, 2000). As mentioned in earlier part, the testis of the male reproductive tract possesses P450Arom enzyme complex and thus is able to synthesize estrogens. Expression and localization of P450Arom in the testis are summarized in Table 1. During the fetal period, the presence of P450Arom is found in the Leydug and Sertoli cells in the testis (Table 1). In the immature testis, P450Arom activity is also detected in both the Leydig and Sertoli cells, while the Sertoli cells have significantly higher P450Arom activity than the Leydig cells (Rommerts et al., 1982; Tsai-Morris et al., 1985). Our recent study showed the immunolocalization of P450Arom not only in the Leydig cells but also in the developing germ cells of the immature mouse testis (Lee et al., 2008). The presence of P450Arom in the adult testis has been well determined by numerous researches. Unlike in the immature testis, the Leydig cells have higher P450Arom activity than the Sertoli cells (Tsai-Morris et al., 1985). Expression and activity of P450Arom are also found in germ cells in the mature testis (Hess, 2000; Rago et al., 2003; Lee et al., 2008). Interestingly, human and rat spermatzoa traveling through the EDs and epididymal ducts also have P450Arom activity (Nitta et al., 1993; Rago et al., 2003). These findings suggest that sperms released from the testis would serve as a mobile source of estrogen present in the rete testis and epididymal fluid (Hess et al., 1995). Even though it is widely believed that the testis is the major source of estrogen present in the testicular fluid and semen, recent studies have suggested that the EDs and epididymis would be additional estrogen-producing sites in the male reproductive tract. Our recent study has demonstrated the presence of P450Arom transcript in the EDs and the epididymis (Kim et al., 2008). Especially, it is notable that the transcript of P450Arom in the EDs is only detected

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Fetal</th>
<th>Neonatal</th>
<th>Prepuberal</th>
<th>Adult</th>
</tr>
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<tbody>
<tr>
<td>Testis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leydig cells</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
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<tr>
<td>Sertoli cells</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Germ cells</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Efferent ductules</td>
<td>nd</td>
<td>+</td>
<td>-</td>
<td></td>
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<tr>
<td>Epididymis</td>
<td></td>
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</tr>
<tr>
<td>Epithelial cells</td>
<td>nd</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Spermatozoa</td>
<td>np</td>
<td>np</td>
<td>+</td>
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</tr>
</tbody>
</table>

1 Spermatozoa present in epididymal ducts.
nd = Not determined. np = Not present at the developmental period.
during neonatal and prepubertal period, not at adult period, while the epithelia of the epididymis express P450Arom mRNA during an entire postnatal period (Kim et al., 2008). Carpino et al. (2004) have also detected an immunolocalization of P450Arom in the epithelia of human EDs and proximal epididymis. Together, it is clearly indicated that estrogen present in the male reproductive tract is synthesized and secreted from a number of cell types. In addition, the findings of the presence of P450Arom in the male reproductive tract imply the potential role of estrogen on regulation of functions of the testis and the excurrent ducts, being briefly discussed in the next part of this review.

**FUNCTIONS OF ESTROGEN IN THE EFFERENT DUCTULES**

A role of estrogen in the EDs has been largely resolved by examination of ER knockout mice. The ERα gene knockout mouse (αERKO) was generated by Lubahn et al. (1993). It has been found that αERKO male is infertile, and its testis becomes degenerated as early as 20 days of age but appears normal until the puberty, followed by atrophic by 150 days of age (Eddy et al., 1996; Hess et al., 1997a). A concentration of spermatozoa in the epididymis of αERKO mouse is significantly decreased, compared with that of wild-type mouse (Eddy et al., 1996). Histological evaluation of the testis of αERKO mouse showed numbers of abnormalities, including dilated rete testis and seminiferous tubular lumen, a thin epithelial layer in seminiferous tubule, and reduced rete testis epithelial height (Lee et al., 2000; 2008; 2009). Another considerable morphological aberrant in the male reproductive tract is observed in the EDs of the αERKO mouse. The EDs in the adult αERKO mouse has an extensively dilated lumen, a very thin epithelial layer, poorly organized microvilli in nonciliated cells, and reduced number of cilia in ciliated cells (Hess et al., 1997a; Lee et al., 2000; 2009). In addition, the EDs of the αERKO mouse has much less endocytotic apparatus and lysosomal granules, which are predominantly found in the apical region of the nonciliated cell of the wild-type mouse (Figure 3) (Morales and Hermo, 1983; Hermo and Morales, 1984; Ilio and Hess, 1994). Moreover, treatment of the adult wild-type mouse with a pure anti-estrogen (ICI 182, 780) results in morphological and histological changes close to those observed in the EDs of the αERKO mouse (Lee et al., 2000). In recent studies, we have showed that most morphological abnormal features found in the EDs of the adult αERKO mouse are recognizable as early as 10 days of age (Lee et al., 2008; 2009). Thus, it is conclusive that morphological defects observed in the ED of αERKO mouse is the result of a concurrent response to the lack of functional ERα during early postnatal developmental period and adulthood (Lee et al., 2000; 2009).

The EDs reabsors more than 90% of the rete testis fluid, so thus increasing concentration of spermatozoa as they enter the head of the epididymis (Levine and Marsh, 1971; Jones and Jurd, 1987). This function of the EDs is important to ensure a proper maturation of spermatozoa in the epididymis and a large number of sperm released upon ejaculation (Robaire and Hermo, 1988; Chulow et al., 1994; Chan et al., 1995). An underlined mechanism of the

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**Table 2. Summary of the presence of estrogen receptor α (ERα) and ERβ in the efferent ductules**

<table>
<thead>
<tr>
<th></th>
<th>Fetal</th>
<th>Perinatal</th>
<th>Adult</th>
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</thead>
<tbody>
<tr>
<td>ERα</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelium</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Connective tissue</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ERβ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelium</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Connective tissue</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Data from Hess et al. (1995)¹, Lee et al. (2008)², and Sierens et al. (2005)³
testicular fluid reabsorption in the EDs has been largely unknown until estrogen involves in regulation of fluid movement in the EDs (Hess et al., 1997a). The in vitro ligation of the wild-type EDs results in a rapid removal of the luminal fluid of the EDs, while the EDs of αERKO mouse fails to do so (Hess et al., 1997a). Even, the lumen of the αERKO EDs becomes expended, indicating the fluid movement from the basal side to the luminal side through the epithelium of the EDs (Hess et al., 1997a). The fluid absorption occurring in the EDs of the wild-type mouse is blocked by treatment with ICI 182,780 (Hess et al., 1997a). This study was the first evidence demonstrating a functional role of estrogen in the male reproductive tract (Hess et al., 1997a), and additional researches have demonstrated an importance of estrogen on functional regulation of the EDs (Hansen et al., 1997; Sharp, 1998). However, a molecular mechanism how estrogen regulates the fluid reabsorption in the EDs remained to be answered. As briefly discussed in the earlier part of this article, the fluid movement through the epithelium of the EDs would occur in a passive way through a leaky epithelium and/or in an active way coupled with active ion transport by the epithelium (Hamilton, 1975; Jones and Jurd, 1987; Spring, 1999). In addition, the presence of both ERα and ERβ in the EDs is described in the earlier section of this review. One of our studies has revealed that estrogen involves in the control of fluid reabsorption in the EDs by differential regulation of ion transporter’s expression through ERα and ERβ (Lee et al., 2001). Comparison of gene expression of various ion transporters in the EDs of wild-type and αERKO mice showed that estrogen modulates ERα-mediated gene expression of ion producer and/or transporters coupled with Na+ movement across the epithelium of the EDs, such as carbonyl reductase II and Na+-K+-ATPase (Lee et al., 2001). In addition, treatment of αERKO mouse with ICI 182,780 to block the effect of ERβ in the EDs demonstrated that gene expression of Cl− movement-coupled ion transporters, for example cystic fibrosis transmembrane regulator and a down-regulated in adenoma (DRA), is ERβ-mediated (Lee et al., 2001). Moreover, our recent study has demonstrated that aberrant gene expression of ion transporters in the EDs of the adult αERKO mouse is detectable as early as at 10 days of postnatal age (Lee et al., 2008). Together, these researches provide evidence, in which the presence of a functional ERα is required to maintain and regulate the function of the EDs during the prepubertal developmental and postpubertal period. Others have demonstrated dramatic expressional reduction of aquaporin (AQP)-1 and -9 in the EDs of the αERKO mouse, possibly relating with significant decreases of sperm concentration and motility in the αERKO mouse (Ruz et al., 2006). Therefore, examinations with the EDs of the αERKO mouse strongly suggest that the presence of the ERα is essential for retaining normal function of the EDs.

It is noteworthy that P450Arom gene knockout (ARKO) male mouse, which is not capable to synthesize estrogen, is fertile until 1 year of age and has normal structure of the EDs (Toda et al., 2008). In addition, ERβ knockout (βERKO) male mouse is also fertile and does not show apparent morphological abnormality in the EDs (Krege et al., 1998; Antal et al., 2008). Moreover, the EDs of the double knockout mouse of ERα and ERβ has morphological aberrant features similar with those of αERKO mouse (Couse et al., 1999). Thus, it is believed that ERα, rather than ERβ, plays more predominant role in maintenance of morphology and physiology of the EDs. A detailed molecular mechanism in which estrogen regulates the fluid reabsorption in the EDs remains to be established. Further studies will provide us specific information about a role of estrogen in regulation of the physiological function of the EDs.

**EFFECTS OF ENVIRONMENTAL TOXICANTS ON THE EFFERENT DUCTULES**

There are a number of environmental chemicals which influence on morphology and function of the EDs. Benomyl and carbendazim, commonly used fungicides, are known to be reproductive toxicants in male and result in occlusions of the EDs (Hess et al., 1991; Nakai et al., 1992). An exposure to glyphosate, an active component of Roundup (Monsanto Company, St. Louis) herbicide, leads to structural alteration of the EDs, such as epithelial vacuolization and redistribution of PAS-positive granules throughout the cytoplasm of the epithelial cell (Oliveira et al., 2007). In addition, treatment of a synthetic estrogenic compound, diethylstilbestrol (DES), in male results in morphological abnormalities in the EDs, including dilation of lumen and reduction of epithelial height (McKinnell et al., 2001). It is well determined that estrogen-like endocrine disruptors, such as polychlorinated biphenyls, dichlorodiphenyl-trichloroethane, and diozin, influence the male fertility by affecting normal endocrine system of living forms (Sikka and Wang, 2008). However, most of these researches have focused on effects of the endocrine disruptors on the testis of the male reproductive tract (Sikka and Wang, 2008). As discussed in earlier section of this article, the EDs possesses the highest ERα among the male reproductive organs (Hess et al., 1997b). Thus it is no doubt that these estrogenic environmental endocrine disruptors could give an influence on function and morphology of the EDs. Future researches will clarify effects of endocrine disruptors on the EDs, leading to functional alteration of the EDs and so thus male infertility.
REFERENCES


hydroxysteroid dehydrogenases in the male reproductive tract
of the domestic fowl (Gallus domesticus). Histochem. J. 5:57-
65.

tissue structure of efferent ductules in aromatase-deficient

Cellular localization of rat testicular aromatase activity during


Androl. 1:303-311.