Ann Med Res 2020;27(10):2534-9

Annals of Medical Research

DOI: 10.5455/annalsmedres.2020.07.683

Original Article

The localization of ERα and ERß in rat testis and epididymis

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Abstract

Aim: It is an indisputable fact that estrogens are essential for the normal functioning of the male reproductive system. Although there are many studies investigating the localization of estrogen receptor α (ERα) and estrogen receptor ß (ERß) in the testis and epididymis, there is no consensus between the results of these studies. Therefore, in this study, it was aimed to investigate the ERα and ERß expression pattern in testis and epididymis using immunohistochemical methods.

Material and Methods: The testis and epididymis tissues removed from 3 adult rats after anesthetized with ether. Then the tissues fixed in Bouin's fixative. The tissues embedded in paraffin were cut 5 µm thickness with microtome and stained with immunohistochemical methods.

Results: In the testis, ERα-positive immunostaining was observed in the nuclei of peritubular myoid cells and in the cytoplasm of Leydig cells. The germ cells other than spermatogonium and spermatid were ERα-negative. ERβ-positive immunoreaction was detected in the nuclei of spermatogonia, peritubular myoid cells and Leydig cells. The cells in which ERα and ERβ positive immunostaining were seen most intensively were spermatogonia. ER (α and ß) positive immunostaining in the epididymis was observed in epithelial cells and interstitial stromal cells. Although most of the epithelial cells (principal, basal, apical) were ERα and ER β positive staining, others were negative.

Conclusion: The results obtained in this study showed that ERα and ERß are localized in somatic and germ cells in the testis and epithelial and stromal cells in the epididymis. In this study, it was observed that ERα and ERß staining intensity in spermatogoniums was higher than other cells. This result can be interpreted that estrogens perform their effects on the testis mainly through spermatogonia. The presence of ER (α and ß) in testis and epididymis, support the view that estrogens play an important role in the development and maintenance of male reproductive functions and fertility.

Keywords: Epididymis; estrogen receptor alpha; estrogen receptor beta; rat; testis

INTRODUCTION

Until recently it has been known that estrogen is femininity hormone and testosterone is masculinity hormone (1,2). Nowadays, it is proven that estrogens have crucial roles in male reproductive system (3,4). Estrogens affect the growth, differentiation and functions of organs such as ovaries, mammary glands, uterus, vagina, testis, epididymis and prostate (5).

In the testis, the estrogens produced by the aromatization of testosterone with cytochrome P 450 aromatase enzyme (6) perform their effects through estrogen receptors (ER). ER has 3 subtypes: ERα, ERβ and G protein - coupled estrogen receptor 1 (GPER1, also known as GPR30). While GPR30 is a membrane receptor, ERα and ERβ are binding-activated receptors which are members of nuclear receptor family (4-8).

As a result of the biochemical and histological studies, it was detected that ER is located in the brain, pituitary and reproductive organs of the females and males (9). ER have been detected in the male reproductive systems of mice, rabbits, rats, goats, monkeys and humans (10). The results of the immunohistochemical studies on the distribution of ERα in the testis are variable. Taylor et al. (11) have declared that ERα is present in Sertoli and Leydig cells, whereas Nie at al. (10) have declared that ERα is localized in peritubular myoid cells and Leydig cells. Pelletier et al. (9) and Zhou et al. (12) stated that ERα is localized only in the nucleus of Leydig cells, while Saunders et al. (13) and Goyal et al. (14) stated that ERα positive immunostaining was not observed in the testis. In immunohistochemical studies to investigate the localization of ERß in the testis, it has been reported that ERβ is expressed in Sertoli (9,11,13,15-17), Leydig (10,11,13,15-17), peritubular

Received: 01.07.2020 **Accepted:** 28.09.2020 **Available online:** 21.10.2020
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myoid cells (10,13,17), germ cells (10,13,15,17) and rete testis epithelium (10,13).

There is no consensus regarding the localization of ERα in the epididymis. In some studies, epithelial cells of epididymis have been reported to be ERα-negative (18,19) and in others ERα-positive (10,12,20). In addition, in some studies reported that some of these cells were negative and some were positive. Also, some studies have reported that stromal cells in epididymis are ERα-negative (19,21), while others have been reported to be ERα-positive (10,12,13). The results of the studies on the localization of ERβ in the epididymis are more consistent. In most of the studies, epididymis epithelium and stromal cells are reported to be ERβ-positive (10,13,19-21) in adults, but there are studies reporting that they are negative (12,22).

The infertility of mutated male mice lacking the functional ERα demonstrated that estrogen is essential for male fertility (23-25). Infertility in this type of mice occurs mainly as a result of development and dysfunction in the ductus deferens (4,6). Also, infertility is formed as a result of disorders in germ cell development in mice without a functional aromatase gene (4). In adult humans, estrogen has been reported to positively affect spermatogenesis by inhibiting post-meiosis germ cells apoptosis. (15).

Saunders et al. (17) reported that estrogens have important roles in the normal functioning of the testis and that estrogen can directly affect the functions and maturation of germ cells by binding to ERβ. Although the role of estrogen in the epididymis is not known for certain, it has been claimed that it regulates the secretory activity of the epididymis epithelium (26,27). In addition to studies reporting the positive effects of estrogen on the male reproductive system, there are studies reporting that exposure to estrogen for environmental reasons harms the development and healthy functioning of the male reproductive system (7,19,28). These results show that estrogen plays an important role in spermatogenesis and male fertility.

Although there are many studies investigating the localization of ERα and ERß in testis and epididymis, the precise localization of estrogen receptors, especially ERα, in these tissues has not been determined exactly. Therefore, in this study, it was aimed to investigate the ERα and ERß expression pattern in the testis and epididymis by using immunohistochemical methods.

MATERIAL and METHODS

In present study, 3 sham group (only laparotomy and sutured) wistar male rats (120 days old) belonging to FÜBAP-2107 project, obtained from Firat University Faculty of Medicine Experimental Research Unit (FÜTDAM), were used. Tissues (testis and epididymis), which were fixed for 36 hours at room temperature in Bouin's fixative, were passed through alcohol and xylol series and embedded in paraffin. Paraffin blocks cut by microtome with a thickness of 5µm were taken into polysine coated slides and stained with immunohistochemical methods.

Immunohistochemistry

Sections were incubated for 1 hour in a 60 °C oven to ensure stronger adhesion of the slides to the tissue, followed by a series of xylol and alcohol. In order to remove endogenous peroxidase, tissue sections were kept in 3% hydrogen peroxide (H_2O_2) (prepared with methanol) for 10 minutes and then washed in distilled water (5 minutes). The antigen retrieval protocol began by transferring tissue sections to the plastic coplin jar containing 0.01M citrate buffer (pH 6.0). The coplin jar was placed at the midpoint of the rotating platform of the microwave oven and heated four times in succession for five minutes at 600W. The amount of buffer in the coplin jar was controlled and the reduced fraction was completed with distilled water in every five minutes. The sections removed from the microwave then allowed to cool to room temperature for 20 minutes. Antigen retrieval was completed by washing the cooled tissue sections in PBS (phosphate buffered saline) for 5 minutes. Sections washed in PBS after antigen retrieval protocol, 10% normal goat or rabbit serum was incubated at room temperature (10 minutes) to prevent nonspecific antibody binding. To determine the localizations of ERα and ERβ, tissue sections were incubated with primer antibodies in a humidified chamber at 4 °C for 16-20 hours and respectively for rabbit anti-ERα (estrogen receptor alpha polyclonal antiserum, bs-2098R, Bios, lot: 9K01V2) and mouse anti-ERβ (estrogen receptor beta monoclonal antiserum, sc-390243; Santa Cruz, CA, lot: B2217). Before this procedure, primary antibodies of ERα and ERβ were diluted 1:200. For negative control, PBS was used instead of primary antibody (Figure 1B,1D,2D). Subsequently, it was incubated with biotinylated secondary antiserum for 1 hour and then with streptavidin horseradish peroxidase for 1 hour in a 37 ºC humid environment. Sections were washed with PBS solution for 10 minutes before each incubation period. Sections were then immersed in AEC (3-amino-9-ethylcarbozole) chromogen substrate, washed with distilled water, stained with hematoxylin (10 minutes) and covered with mounting medium. After, tissue sections were examined by BX53 microscope and photographed.

The intensity of ERα and ERβ immunostaining were scored by semi-quantitative analysis (analysis of 10 microscopic fields at 400X magnification) as strong (+ + +), moderate (+ +), weak (+), negative (-) or variable (discrepancies between the 2 analyses) immunoreactivity.

RESULTS

In this study, the localization of ERα and ERß in adult rat testis and epididymis was examined using immunohistochemical methods in tissue sections embedded in paraffin. The results of this study are summarized in Table 1 and compared with those previously reported in other species.

In testis, ERα positive immunostaining was observed in germinal cells and interstitial tissue. From the germ cells, ERα-positive immunostaining was observed in the

nucleus in spermatogoniums and in the acrosomal region in spermatids. In addition, ERα-positive immunostaining was observed in the nuclei of peritubular myoid cells, while in the cytoplasm of Leydig cells. Some of the peritubular myoid cells were stained positive, while others were negative. The germ cells other than spermatogonium and spermatid were ER alpha negative (Figure 1A).

ERβ positive immunoreaction was detected in the nuclei of spermatogonia, peritubular myoid cells and Leydig cells. Some of the peritubular and Leydig cells were ERβ positive, while others were negative. The cells in which ERα and ERβ positive immunostaining were seen as the strongest were spermatogonia (Figure 1C).

Table 1. A comparison of obtained findings with the results of similar studies

	This study		Mouse (Zhou et al.2002)		Dog (Nie et al.2002)		Cat (Nie et al.2002)	
	ERα	Erß	ERα	Erß	ERα	Erß	ERα	Erß
Testis								
Germ cell	+++/-*	+++/-	-	++/-"	-	++	-	+++
Sertoli cell	-	-	-	++	-	-	-	-
Leydig cell or Interstitium	++/-**	++/-	+++	+++	++	-	+	+
Peritubular myoid cell	+/-	+/-	+++/-	+/-	++	+	-	+++
Epididymis								
Epithelium	++/-^	++/-^	++/+++	+++	-	+++	++	+++
Stroma	++/-	++/-	-/+++	-	++	+	+	++

[a]Staining intensities are scored as follows: +++ = strong, ++ = moderate, +=weak, – = negative, +++/-, ++/–, +/– = variable
*The germ cells other than spermatogonium and spermatid were ER alpha negative. **Stoplasmic staining. ^Nuclear and stoplasmic staining

In the epididymis, ER positive immunostaining was observed in epithelial cells and interstitial stromal cells. Although ERα and ERß positive immunostaining in epithelial cells were seen especially in the nuclei, there was a slight staining in the cytoplasm. Most of the epithelial cells (principal, basal, apical) were ERα and ER β positive, while others were negative. Immunostaining intensity in principal cells was stronger than basal and apical cells. Some of the stromal cells were ERα and ER β positive, while peritubular smooth muscle cells and others were negative (Figure 2A, 2B, 2C).

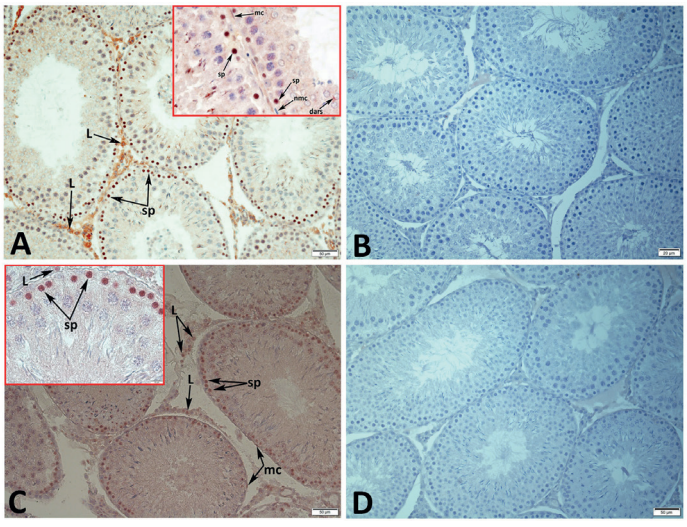

Figure 1. Immunohistochemical localization of ERα and ERβ in testis. ERα (A), ERβ (B) negative controls (C, D). sp: spermatogonia, dars: developing acrosomal region of round spermatid, esp: elongated spermatid, L: Leydig cell, mc: peritubular myoid cell. A, B, C, D: magnification × 200

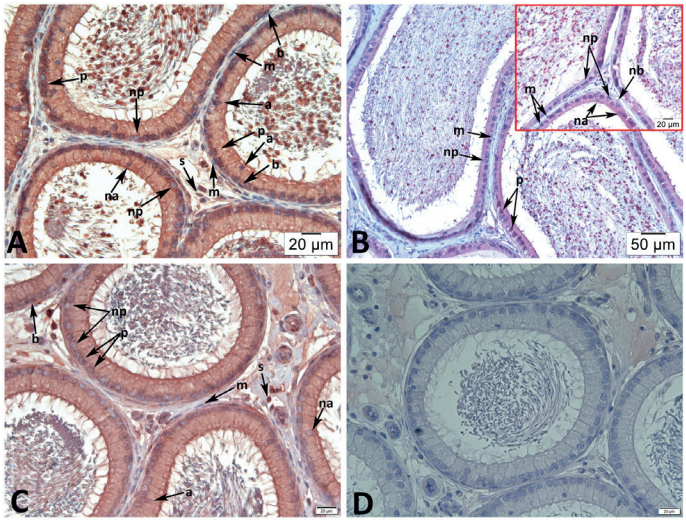

Figure 2. Immunohistochemical localization of ERα and ERβ in epididymis. ERα (A, B), ERβ (C) negative control (D). a: apical cell, na: negative apical cell b: basal cell, nb: negative basal cell, m: negative peritubular smooth muscle cell, p:principal cell, np: negative principal cell, s: stromal cell. A, C, D: magnification × 200, B:magnification × 400

DISCUSSION

In the present study, ERα and ERβ localizations were investigated by using immunohistochemical methods in adult rat testis and epididymis tissues. ER (α and β) positive immunostaining was observed in the nuclei of cells (spermatogonium, peritubular myoid cells), cytoplasm (acrosomal region of spermatids, Leydig cell) or both (epididymal epithelial cells). These results are accordance with previous literature (20,29).

There is no consensus on the localization of ERα in the testis. Saunders et al. (13) stated that human and monkey testes were ERα-negative, while Pelletier et al. (9,16) reported that ERα-positive immunostaining occurred only in Leydig cells in humans, and in cytoplasm of Leydig cell nuclei and spermatocytes and spermatids in rats. Filipiak et al. (30) reported that Leydig and Sertoli cells were Erα-positive and germ cells negative. Nie et al. (10) reported that ERα-positive immunostaining was observed in Leydig cells in cats and in some of the peritubular and Leydig cells in dogs. Also, Zhou et al. (12) and Oliveira et al. (31), reported that ERα-positive immunostaining was observed in peritubular myoid cells and Leydig cells. Cavaco et al. (32) detected that ERα-positive immunostaining was observed in spermatogonium, spermatocyte, round and elongated spermatid, Sertoli and Leydig cells. In the present study, ERα-positive immunoreaction was observed in the nuclei of peritubular myoid cells and spermatogoniums, while in the cytoplasm of Leydig cells, it was observed in the acrosomal region of round spermatids.

The results of the studies for the localization of ERß in the testis tissue are generally agree with each other. In most studies (9-13,16,31), it was reported that ERβ-positive immunostaining was observed only in the cell nucleus, while Saunders et al. (17) also detected immunoreactivity in the secondary spermatocyte and round spermatid cytoplasm. Peach et al. (33) reported that the mouse testis was ERβ-negative. In other studies, ERβ-positive immunoreaction has been reported to be detected in Sertoli (9-13,17,31,34), Leydig (9-13,16,32), peritubular myoid cells (9,10,12,13), spermatogonium (10-13), spermatocyte (10-12,17,32) and spermatids (10,13,17,32). In the current study, ERβ-positive immunoreaction was detected in the nuclei of spermatogonium, peritubular myoid cells and Leydig cells.

The localization of ERα in the epididymis is controversial. In some studies, epithelial and stromal cells have been reported to be ERα-positive (10,12,13,20), while in others have been reported to be negative (18,19,21). In addition, in some studies have suggested that some of the epididymal epithelial cells are ERα-negative and others are positive (12,13). However, the results of studies on the localization of ERβ in the epididymis are generally agree with each other. In most studies, epithelial and stromal cells in the epididymis have been reported to be ERβ-positive (10,13,19-21) in adults. However, there are studies reporting that these are ERβ-negative (12,22). Zaya et al. (20) reported that ER (α and β) expression of epididymis tissue in rats showed similar patterns and that both cell nuclei and cytoplasm of epithelial cells were ER (α and β) positive. The results obtained in the current study regarding the localization of ER (α and β) in the epididymis overlap with the literature (10,12,13,19-21).

Although there is a consensus between the results of studies on ERβ localization in the testis and epididymis, there is not for ERα. It has been suggested that the inconsistent results reported for the localization of ERα in the testis and epididymis may be due to the difference of the animal ages, primary antibodies, and methods (20,32,35). Also, this discrepancy between results may result from differences in dilution rates of primary antibodies used.

Studies have shown that administration of estrogen stimulates gonocyte proliferation (36-38) and causes an increase in the number of type A spermatogonia in rat testis (39). In a study by Shettey et al. (40), It was reported that a decrease in germ cell numbers was observed in male monkeys treated with aromatase inhibitor. In addition, estrogen has been shown to stimulate DNA synthesis in rat spermatogonium (41). In the current study, the cells in which ERα and ERß immunostaining were seen most strongly were spermatogoniums. This result, which shows that ER (α and ß) receptor density in spermatogonium is higher than other cells, when evaluated together with the information given above, it can be interpreted as estrogens perform their effects on the testis mainly through spermatogonia. Although the role of estrogen in the epididymis is precisely not known, it has been reported that its regulates the secretory activity of the epididymis epithelium and luminal reabsorption in the caput epididymis (23,26,27).

CONCLUSION

In the present study, the data obtained on the immunohistochemical localization of ER (α and β) in the testis and epididymis supports the information given above regarding the functions of estrogens in the testis and epididymis.

Competing interests: The authors declare that they have no competing interest.

Financial Disclosure: This article was supported by Firat University BAP 2107 project.

Ethical approval: 2010/11/62 Firat University Ethics Committee.

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