

Localisation of oestrogen receptors (ER α and ER β) in the human and rat epididymides

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The aim of the study was to compare the localisation of oestrogen receptors α and β (ER α and ER β) in the human and rat epididymides. In the human epididymis the immunoexpression for ER α was detected in the nuclei of the caput epithelial cells, while positive reaction for ER β was observed in the nuclei of the cauda epithelial cells. In the rat epididymis, immunoexpression for ER α showed nuclei of the caput and cauda epithelial cells. However, the reaction was stronger in cells of the caput epididymis. A positive reaction for ER β was observed in the nuclei of smooth muscle cells of the epididymal duct and in the nuclei of interstitial tissue cells of the rat caput and cauda epididymis. We have demonstrated that localisation of ER α and ER β is cell-, region- and species-dependent.

key words: oestrogen receptor α and β , immunohistochemistry, human and rat epididymis

INTRODUCTION

Immature spermatozoa leave the testis together with testicular fluid and are transported to the epididymis via the rete testis. During the traverse through the epididymis the spermatozoa remain in a special microenvironment, necessary for sperm maturation and storage. The microenvironment is created by epididymal epithelial cells. The epithelial cells of the proximal caput of the epididymis (the efferent ductules and the first part of the ductus epididymis) [3], reabsorb nearly 90% of the fluid leaving the testis [9], thereby increasing the concentration of sperm in the epididymis. The function of the cells is regulated mainly by androgens — testosterone (T) and dihydrotestosterone (DHT), acting through androgen receptors (ARs) [8, 10]. However, the epithelium of the human and animal epididymides also contains both α and β oestrogen receptors (ER α and ER β) [7]. There is documentary evidence that the function of the epididymis is regulated additionally by oestrogens [9].

MATERIAL AND METHODS

Epididymides were obtained from mature male Wistar rats (3–4 months old). The animals were anaesthetised with 90 mg/kg pentobarbital sodium by intraperitoneal injection. The epididymides were taken to prepare cryostat sections. The human epididymides, which were obtained from patients who were undergoing orchidectomy, were fixed in Bouin's solution and embedded in paraffin.

The paraffin sections of the human and the cryostat sections of the rat epididymides were incubated with primary antibodies as follows: monoclonal, mouse antibody ER α -1D5 (1:100) (DAKO A/S, Glostrup, Denmark); polyclonal rabbit antibody ER β (1:100) (Affinity BioReagents, Golden CO, USA) overnight at 4°C in humidified chamber. Next, the specimens were incubated with secondary antibody (Vector Lab., Burlingame CA). The reactions were visualised with ABC/HRP (DAKO/AS Denmark) and DAB method. To avoid a non-specific reactivity,

Table 1. Localisation of oestrogen receptors α and β (ER α and β) in the human and rat epididymides

	Caput epididymis		Cauda epididymis		Smooth muscle cells		Interstitial tissue cells	
	Human	Rat	Human	Rat	Human	Rat	Human	Rat
ER α	+	+	-	+	-	-	-	-
ER β	-	-	+	-	-	+	-	+

control sections were incubated without primary antibody.

The experiment received the approval of Local Ethics Committee.

RESULTS AND DISCUSSION

The positive reaction for ERs was localised in the nuclei of the cells. There were differences in the localisation of ER α and ER β between the human and the rat epididymides. The results are presented in Table 1.

Oestrogen receptors α were detected in the nuclei of epithelial cells of the caput of the human (Fig. 1A) and rat (Fig. 2A) epididymides, and in the nuclei of the cauda epithelial cells of the rat epididymis (Fig. 3A). ER β was localised only in the nuclei of the human cauda epididymis (Fig. 1B). Our results for the localisation of ERs in the epididymal epithelium are comparable to the observations of other authors [6, 7]. The same localisation of ER α in the human and rat caput epididymides is indicative of its similar function in both species. As has been mentioned above, the proximal caput of the epididymis takes part in the reabsorption of testicular fluid [9]. From the studies carried out in α ERKO male mice it is well documented that this process is controlled by oestrogens through ER α [9]. The presence of ER α is necessary for the maintenance of the morphological components of the endocytotic apparatus in the epithelial cells of this part of the epididymis [5]. Moreover, α ERKO male mice are infertile [9]. The function of ER β is not fully understood. It is suggested, that one role of ER β is to modulate ER α transcriptional activity [2].

We have shown the immunoexpression of ER β in the nuclei of smooth muscle cells of the duct wall and in the interstitial tissue cells of the rat epididymis (Fig. 2B, 3B). However, studies of other authors have demonstrated the localisation of both ER α and β in the stroma of the mammalian epididymis [1, 6, 7]. The presence of the only ER β in rat stroma cells in our experiment may be attributable to the different histochemical procedures employed. The function of target stroma cells for ERs has not been precisely evaluated. It can be supposed that, as in the accessory sex glands [4], the cells may modulate the function of the epididymal epithelial cells.

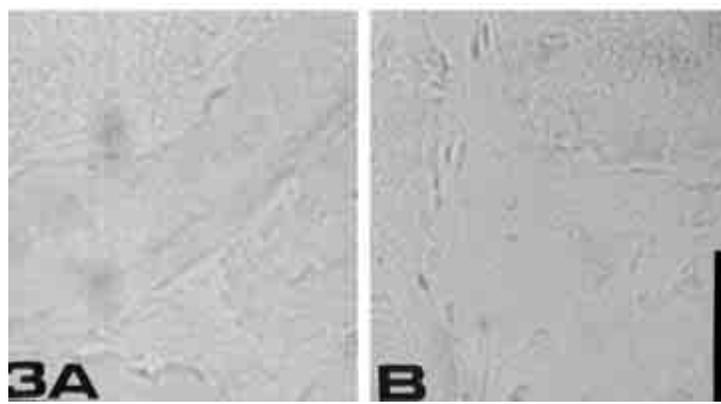
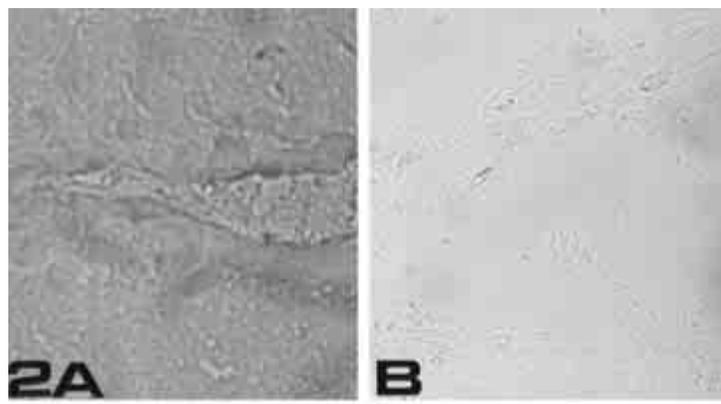
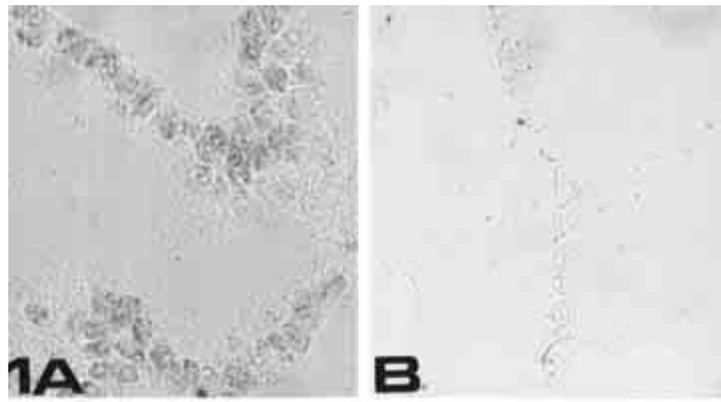
In summary, we concluded that the localisation of ER α and ER β is cell-, region- and species-dependent. The differences in distribution of ERs in the human and rat epididymides indicate that oestrogens could fulfill different functions in each region of the epididymis.

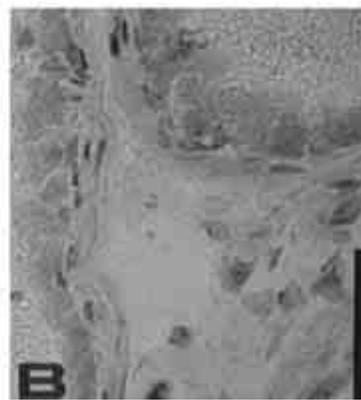
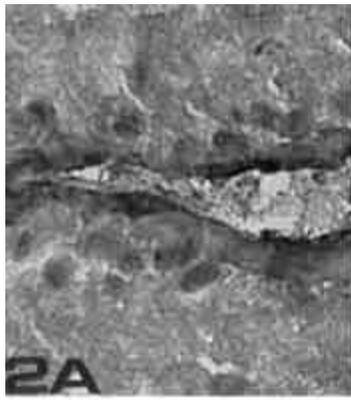
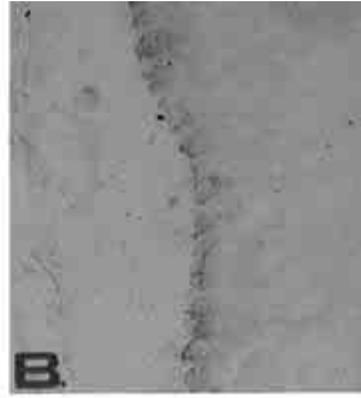
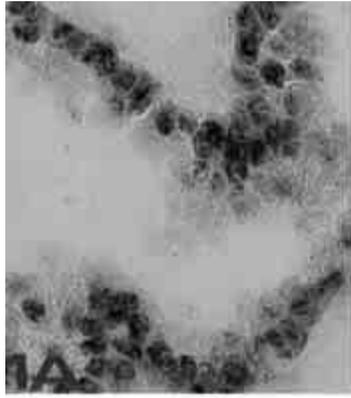
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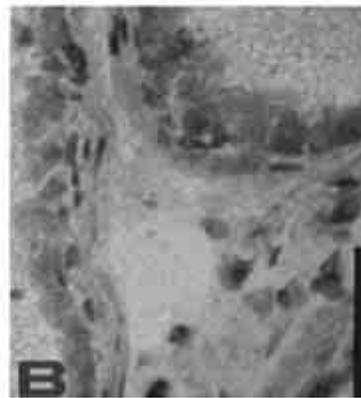
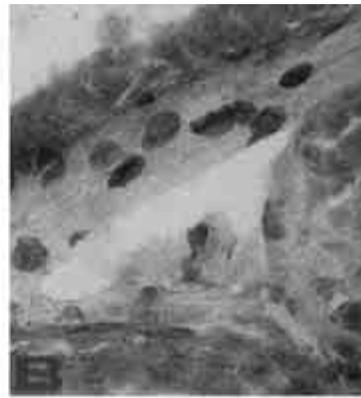
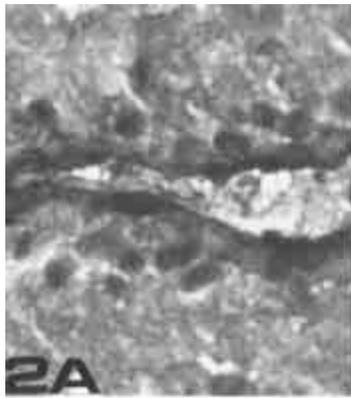
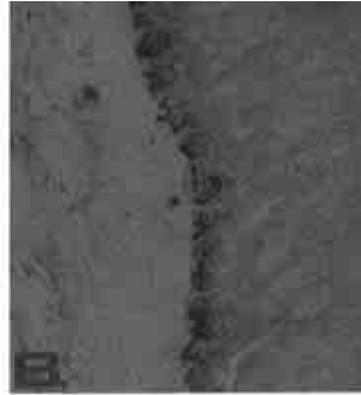
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REFERENCES

- Choi I, Ko C, Park-Sarge OK, Nie R, Hess RA, Graves C, Katzenellenbogen BS (2001) Human estrogen receptor beta-specific monoclonal antibodies: characterization and use in studies of estrogen receptor beta protein expression in reproductive tissues. *Mol Cell Endocrinol*, 181: 139–150.
- Hall JM, McDoonnell DP (1999) The estrogen receptor β -isoform (ER β) of the human estrogen receptor modulates ER α transcriptional activity and is a key regulator of the cellular response to estrogens and antiestrogens. *Endocrinology*, 140: 5566–5578.
- Ilio KY, Hess RA (1994) Structure and function of the ductuli efferentes: a review. *Microsc Res Tech*, 29: 432–467.
- Mäkelä S, Strauss L, Kuipier G, Valve E, Salmi S, Santti R, Gustafsson J-Å (2000) Differential expression of estrogen receptors α and β in adult rat accessory sex glands and lower urinary tract. *Mol Cell Endocrinol*, 164: 109–116.
- Nakai M, Bouma J, Nie R, Zhou Q, Carnes K, Lubahn DB, Hess RA (2001) Morphological Analysis of endocytosis in efferent ductules of estrogen receptor- α Knockout male mouse. *Anat Rec*, 263: 10–18.
- Sar M, Welsch F (2000) Oestrogen receptor α and β in rat prostate and epididymis. *Andrologia*, 32: 295–301.
- Saunders PTK, Sharpe RM, Williams K, Macpherson HU, Urquart H, Irvine DS, Millar MR (2001) Differential expression of oestrogen receptor α and β proteins in the testes and male reproductive system of human and non-human primates. *Molec Hum Reprod*, 7: 227–236.
- Zhu L-J, Hardt MP, Inigo IV, Huhtaniemi I, Bardin CW, Moo-Young AJ (2000) Effects of androgen on androgen receptor expression in rat testicular and epididymal cells: a quantitative immunohistochemical study. *Biol Reprod*, 63: 368–376.
- Zhou Q, Clarke L, Nie R, Carnes K, Lai L-W, Lien Y-HH, Verkman A, Lubahn D, Fisher JS, Katzenellenbogen BS,







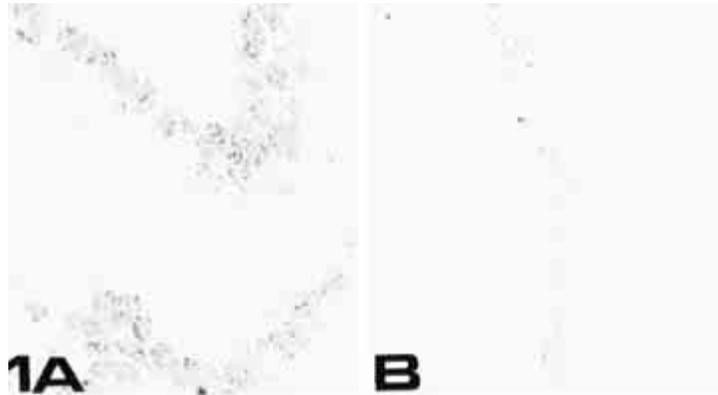


Figure 1. Oestrogen receptors in the human caput and cauda epididymis. The strong positive immunohistochemical reaction for ER α in the nuclei of epithelial cells of the caput epididymis (A) and the weak reaction for ER β in the nuclei of epithelial cells of the cauda epididymis (B). Scale bar, 50 μ m.

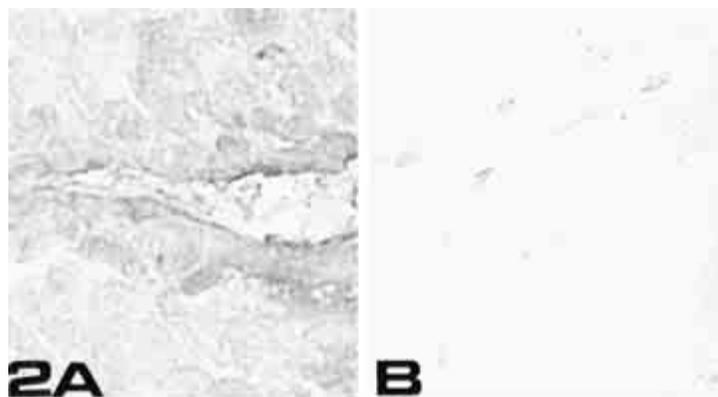


Figure 2. Oestrogen receptors in the rat caput epididymis. The immunoreactivity for ER α is visible in the nuclei of epididymal epithelial cells (A) and the expression of ER β in the nuclei of smooth muscle cells and in interstitial tissue cells (B). Scale bar, 50 μ m.



Figure 3. Oestrogen receptors in the rat cauda epididymis. The weak immunoreactivity of ER α in the nuclei of epididymal epithelial cells (A) and the expression of ER β in the nuclei of smooth muscle cells of the epididymal duct and interstitial tissue cells (B). Scale bar, 50 μ m.

Hess RA (2001) Estrogen action and male fertility: Roles of the sodium/hydrogen exchanger-3 and fluid reabsorption in reproductive tract function. *Proc Natl Acad Sci*, 98: 14132–14137.

10. Zhou Q, Nie R, Prins GS, Saunders PT, Katzenellenbogen BS, Hess RA (2002) Localization of androgen and estrogen receptors in adult male mouse reproductive tract. *J Androl*, 23: 870–881.