Niger. J. Physiol. Sci. 32(December 2017) 191-195
www.njps.com.ng

Niger. J. Physiol. Sci.

Immunolocalization And Distribution Pattern of Estrogen (ERα and ERβ) and Progesterone (PR) Receptors along the Excurrent Duct of Male Greater Cane Rat (*Thryonomys swinderianus*)

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Summary: The excurrent duct, which plays vital roles in the reproductive biology of all male mammals, shows some structural variations among different species. Some hormones such as testosterone, estrogen and progesterone, through their different receptors, have been known to be involved in the normal functioning of the excurrent duct. Here we evaluated the presence, localization and patterns of distribution of three hormone receptors, estrogen alpha (ERα), estrogen beta (ERβ) receptors and progesterone receptors (PR) along the excurrent duct of sexually matured male greater cane rats. Immunohistochemistry revealed presence of ERα in epididymal stroma but not epithelium, selective ERβ staining in narrow & apical cells as well as unique presence of PR in caudal epididymis, which to the best of our knowledge, is the first report on the cellular localization of progesterone receptor in the cauda epididymis. The result suggests the possible involvement of not only estrogen but also progesterone in the modulation of epididymal function in greater cane rat.

Keywords: Excurrent duct, Receptors, Immunohistochemistry, Greater cane rat

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Manuscript Accepted: November, 2017

INTRODUCTION

The excurrent duct of the male reproductive system in mammalian species which comprises of the efferent duct, epididymis and vas deferens, is a channel responsible for sperm maturation, concentration, transportation and storage thereby conferring fertilizing ability on the spermatozoa (Hess, 2003; Robaire *et al*, 2006; Joseph *et al*, 2009). In most studied mammals, these ducts are divided into functional regions and zones that portray distinct zonal intra-luminal environments necessary for the ultimate development of the spermatozoa (Gatti *et al*, 2004; Adebayo & Olurode, 2010; Adebayo *et al*, 2016). These functional zones vary from one species to another and are under hormonal control (Arroteia *et al*, 2012).

Besides androgen, it is now clear that the functioning of these zones is also regulated by estrogen mediated by its two receptors (ERα & ERβ). Recent studies have also established the possible involvement of progesterone in sperm hyper-motility necessary for fertilization (Baidi et al 2011). According to Joseph *et al*., (2009) and Arroteia *et al.*, (2012) the cellular localization, pattern of distribution, and expression of these hormonal receptors are fundamental to the understanding of their roles in the reproduction as well as in the reproductive pathologies of different mammalian species (Walker *et al*, 2012) particularly in those wild rodents that are currently undergoing domestication.

The greater cane rat (*Thryonomys swinderianus*) is a hystricomorphic wild rodent of African origin. This rodent is currently undergoing domestication and captive rearing in some parts of Africa as it can serve as animal model for biomedical research in this continent. In furtherance to some of the research work done on the male reproductive system of the cane rat (Adebayo and Olurode, 2010; Adebayo *et al*, 2015; Adebayo *et al*, 2016), this work elucidates the cellular localization and distribution patterns of ERα and ERβ and progesterone receptors (PR) in the excurrent duct of this rodent.

MATERIALS AND METHODS

Animals

Ten (10) sexually matured, captive-reared, male greater cane rats of known reproductive and medical

records were used in experiment. All the animals had brownish perineal staining which is usually used as index of sexual maturity in male cane rat (Adu and Yeboah, 2003). The animals were maintained on commercial cane rat feed, elephant grasses and water was give *adlibitum.* The experimental protocol followed the ethical principles in animal research adopted by the Animal Ethics Committee, Faculty of Veterinary Medicine, University of Ibadan, ethical code no: ethics 03/14/04

Sample collection and processing

Each animal was weighed, anaesthesized and dissected open after a transcardial perfused-fixation using 4% paraformaldehyde at pH 7.4. After opening the abdominal and pelvic cavity, the entire excurrent duct was dissected out and samples were obtained and routinely prepared for histology.

Immunohistochemistry

Paraffin-embedded samples of each segment of the excurrent duct, fixed in 4% paraformaldehyde were deparaffinised and rehydrated in decreasing ethanol concentrations. Antigens were retrieved in citrate buffer-Tween 20 solution; endogenous peroxidase inhibited by 3% H_2O_2. The sections for estrogen receptors were incubated overnight at 4^0C with 1:100 dilution of 1µl rabbit polyclonal antibody for Estrogen receptor alpha (ERα – Ab37438, abcam®, UK) and Estrogen receptor beta (ERβ – Ab3577, abcam®, UK) on separately labelled slides. These sections were then rinsed in Tris-buffered solution (TBS) and treated for 30 min at room temperature with 1:300 dilution of biotinylated goat anti-rabbit secondary antibody (Vector Laboratories Inc., Burlingame, CA, USA) made in TBS. Sections for progesterone receptors were incubated at 4^0C with 1:60 dilution of mouse monoclonal antibody (PR-AT 4.14) to progesterone receptor and then stir-washed in TBS before being incubated for 30 min at room temperature in 1:300 dilution of biotinylated polyclonal goat anti-mouse secondary antibody (Dako, Glostrup, Denmark) in TBS. Peroxidase was developed with 1.74% (w/v) 3'3-diaminobenzidine (DAB) and sections counterstained with haematoxylin. Negative controls for immunostaining for each receptor were obtained by incubating additional sections with the TBS in the stead of the primary antibodies.

RESULTS

In the greater cane rat, the immunohistochemistry of the efferent duct showed positive staining for both ERα and ERβ with the intensity of staining increasing from proximal to distal efferent duct (Fig. 1). However, no labelling for progesterone receptor (PR) was observed. While there were slight nuclear stainings for ERα in both ciliated and the non-ciliated cells, the cytoplasm of the ciliated cells showed intense staining for this receptor (Fig. 1). Interestingly the ERα was also intensely expressed in the stroma of the efferent duct in the cane rat (Fig. 1A).

Immunohistochemistry revealed no nuclear labeling for ERα throughout the epididymal epithelium. However, whereas low-intensity staining was observed in the cytoplasm of initial segment, caput and corpus epididymis (Fig. 2 & 3), appreciable cytoplasmic labeling was seen at the cauda epididymis (Fig. 4). Immunostaining for ERα was also present in the stroma of the initial segment, caput and cauda epididymis but very weak in that of corpus epididymis (Fig 2, 3 & 4).

Table 1: Summary of localization of the 3 receptors along the excurrent duct in the greater cane rat

	PR	ERα	ERβ
Efferent. duct	-	+	+
Epididymis			
-Initial segment	-	+	+
- Caput	-	+	+
- Corpus	-	+	+
- Cauda	+	+	+
Vas deferens	-	+	+

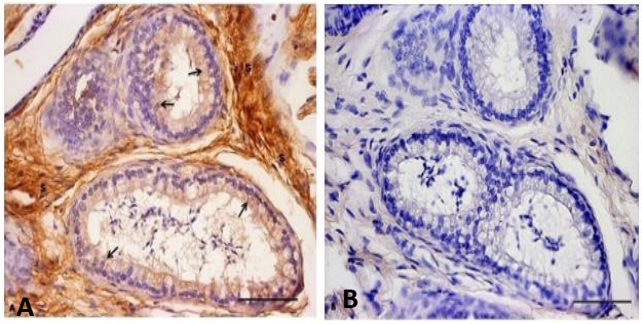

Fig. 1: Photomicrographs of the ERα immunohistochemistry in the efferent duct of the greater cane rat. Figure A shows intense ERα-positive staining of the ciliated cells (arrows) and the stroma (S) while Figure B is the negative control. **Scale bar: 50µm**

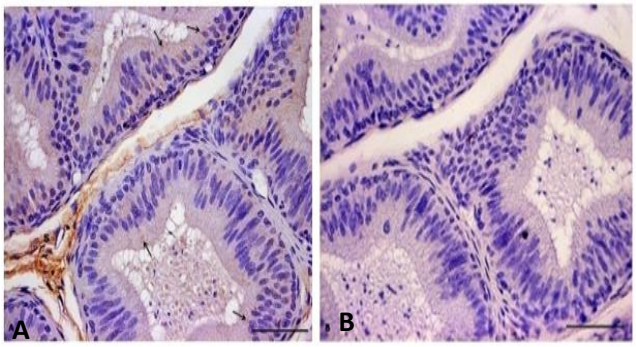

Fig. 2: Photomicrographs of the ERα immunohistochemistry in the initial segment of the epididymis of the greater cane rat. Figure A shows the stroma ERα-positive staining (S) with faint labeling of the epithelial cytoplasm (arrows). Figure B is the negative control. **Scale bar: 50µm**

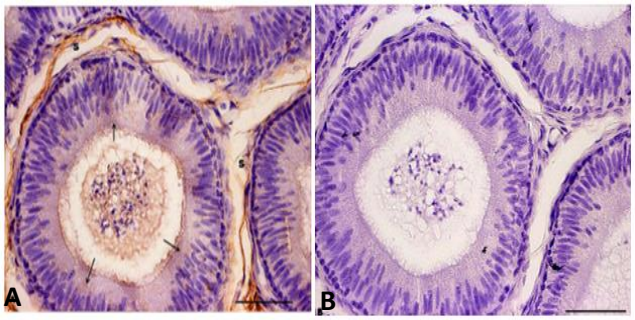

Fig. 3: Photomicrographs of the ERα immunohistochemistry in the caput epididymis of the greater cane rat. Figure A shows the stroma ERα-positive staining (S) with faint labeling of the epithelial cytoplasm (arrows). Figure B is the negative control. **Scale bar: 50µm**

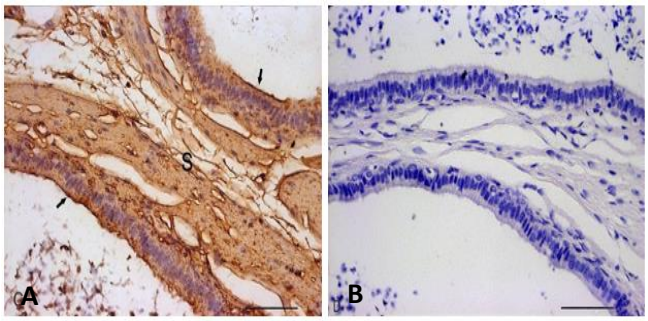

Fig. 4: Photomicrographs of the ERα immunohistochemistry in the cauda epididymis in the greater cane rat. Figure A shows the intense cytoplasmic staining of the epithelium (arrows) as well as the strong stromal labeling (S). Figure B is the negative control. **Scale bar: 50µm**

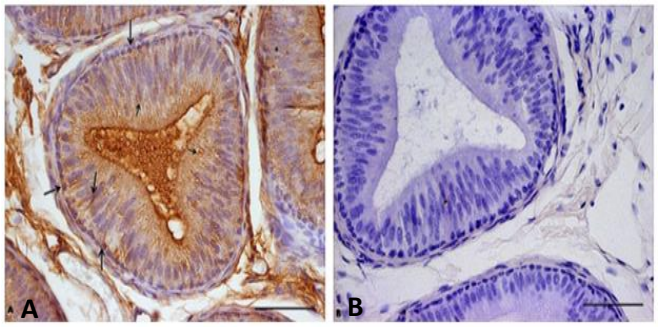

Fig. 5: Photomicrographs of the ERβ immunohistochemistry in the initial segment of the epididymis of the greater cane rat. Figure A shows the selective ERβ-positive nuclear staining of the narrow and basal cells (long arrows) and the labeling of the epithelial cytoplasm (short arrows). Figure B is the negative control. **Scale bar: 50µm**

The ERβ, though immunolocalized throughout the epithelium and stroma of the entire epididymal regions, showed selective nuclear staining for some specific cells. In the initial segment, nuclear staining was observed in the narrow and basal cells while cytoplasmic and membrane staining was in the principal cells (Fig. 5). At the caput and corpus epididymis, there were nuclear staining of the apical, clear and basal cells with labeling of the cytoplasm and membrane in the principal cells (Fig. 6). In the cauda

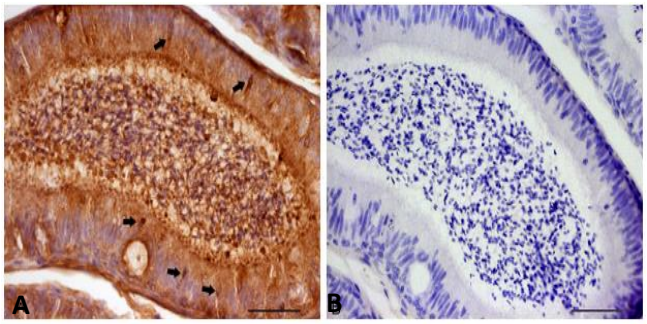

Fig. 6: Photomicrographs of the ERβ immunohistochemistry in the caput epididymis of the greater cane rat. Figure A shows the selective ERβ-positive nuclear staining of the apical cells (arrows) and the labeling of the epithelial cytoplasm. Figure B is the negative control. **Scale bar: 50µm**

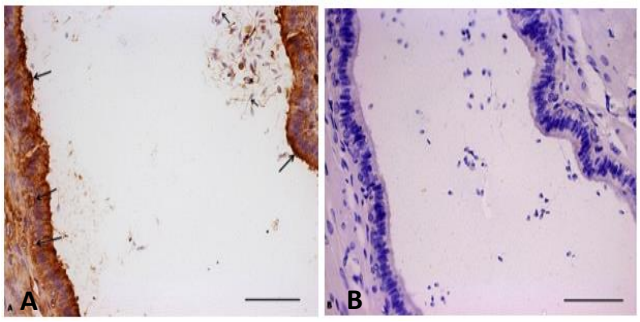

Fig. 7: Photomicrographs of the ERβ immunohistochemistry in the cauda epididymis of the greater cane rat. Figure A shows the ERβ-positive nuclear staining of the basal cells (thin long arrow), cytoplasmic and membrane labeling (thick long arrows) as well as the labeling of the midpiece of the sperm cells (short arrows). Figure B is the negative control. **Scale bar: 50µm**

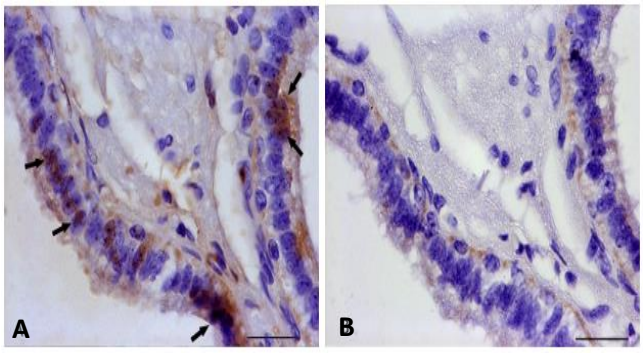

Fig. 8: Photomicrographs of the cauda epididymis in the cane rat showing in **(A)** PR-positive nuclear staining of the principal cells (arrows) while **(B)** is the negative control**. Scale bar: 50µm**

epididymis, nuclear staining was seen in the basal cells with intense membrane and cytoplasmic staining of the principal cells. Also, immunostaining was observed at the middle pieces of the sperm cells present in the cauda epididymis (Fig. 7). A significant finding in this study was the localization of nuclear progesterone receptor (PR) on the epithelium of the cauda epididymis (Fig. 8). While ERα and ERβ were localized in the epithelium as well as the stroma of the vas deferens of the cane rat, PR labeling was not detected in the vas deferens of this rodent.

DISCUSSION

The observed immunolocalization of the estrogen receptors (ERα & ERβ) in the efferent duct of the greater cane rat is consistent with what has been observed across all mammalian species (Hess, 2003; Joseph *et al*., 2011), thereby identifying this duct as an estrogen responsive organ in the cane rat. Not only is the efferent duct a conduit pipe for sperm transport from the testis to the epididymis, it is also involved in the re-absorption of testicular fluids and proteins thereby influencing sperm concentration and seminal fluid composition (Hess and Carnes, 2004; Lazari *et al*., 2009). It has been established that estrogen, through its receptors, ERα and ERβ, plays a crucial role in the maintenance of the structure and function of the efferent duct (Lazari *et al*., 2009; Joseph *et al*., 2011). The presence of these receptors in the cane rat efferent duct suggests that estrogen also regulates the structural and functional status of this duct in the greater cane rat.

In the rat and mouse (Oliviera *et al*., 2004), ERα and ERβ are expressed in both the ciliated and non ciliated cells of the efferent duct epithelium. Whereas ERβ is sometimes found in the peritubular and stromal cells in the rat (Oliviera *et al*., 2003), there are cases in humans where the epithelial cells were stained negative for ERα but the stromal cells were stained positive (Hess, 2003). The observed immunolocalization pattern in the cane rat seems to follow that of the rat as well as the occasional human pattern. The significance of this pattern as well as the presence of positive staining in the ciliated cell cytoplasm of the cane rat efferent duct is yet to be elucidated.

The immunoexpression of the three receptors, estrogen alpha and beta (ERα and ERβ) and progesterone (PR) receptors present unique pattern of distribution in various regions of the epididymis in the greater cane rat. The expression of ERα in the epididymis is highly variable among mammalian species (Joseph *et al*., 2011). While nuclear labeling of ERα is completely lacking in the epididymal epithelium of several species, it is present in mouse, cat and monkey (Nie *et al*., 2002; Hess, 2003; Lazari *et al*., 2009; Joseph *et al*., 2011). Where present, ERα is said to participate in the regulation of narrow cells in the initial segment and clear cells in the remaining epididymal segments (Hess *et al*., 2001; Lazari *et al*., 2009). In some other species like Marmoset, ERα labeling has been shown in the cytoplasm (Lucas *et al.*, 2008; Schon *et al*., 2009), but its significance at this location is yet unknown. From this work, it can be said that the greater cane rat belongs to the class of mammals without nuclear labeling for ERα. Although the import of the cytoplasmic and stromal labeling for ERα particularly at the cauda epididymis of the cane rat is yet to be elucidated, such cytosol immunostaining for this receptor at the cauda epididymis has been reported in Marmoset monkey (Shayu *et al*., 2005). According to Joseph *et al*, (2011), these variations can sometimes be as a result of differences in antibodies and tissue processing techniques.

The expression of estrogen beta (ERβ) throughout the epididymis in the cane rat is similar to that reported for most studied species (Nie *et al*., 2002; Zhou *et al*., 2002). However, the pattern of labeling of ERβ in the cane rat is unique because of its selective nuclear staining for narrow and apical cells in the epididymal epithelium. Selective localization of the ERβ has been observed in boar where only the principal and basal cells of all three epididymal regions are labeled (Pearl *et al*., 2007). Although selective intense staining of the epididymal narrow, apical and clear cells for ERα has been associated with the involvement of this receptor in the regulation of these cells in mouse (Lazari *et al*., 2009), the implication of selective labeling of these cells for ERβ in the cane rat is yet unknown. However, the ERβ localization in the stroma is consistent with that observed in the rat (Atanassova *et al*., 2001)

The observed immunolocalization of the ERβ in the middle pieces of spermatozoa and the nuclear labeling for progesterone receptor (PR) in the cauda epididymis of the greater cane rat are unusual. According to Carreau *et al*, (2011) estrogen receptors have only been localized in the spermatozoa of primates and man but not rodent spermatozoa. As reported by Solakidi *et al*, (2005) and Carreau *et al*, (2011), the localization of aromatase and estrogen receptors in the midpiece of the human spermatozoa could be related to a potential role for estrogen in mitochondria and energy production for motility, as well as for participation in capacitation and/or the acrosomal reaction of sperm. In the same vein, the presence of ERβ especially in the middle pieces of cane rat spermatozoa opens new considerations about the possible role of estrogen in mobility and fertilizing ability of spermatozoa not only in humans but also in this rodent. Similarly, with the localization of PR in the cauda epididymis of the cane rat, which has never been reported in any mammalian species, it is possible that progesterone might be involved in the modulation of epididymal function as it is with sperm hypermotility and fertilization (Baldi *et al*., 2011).

In conclusion, while further studies are on-going on the gene expressions by these receptors, the distribution pattern of the ERα and ERβ as well as the presence of PR in the cauda epididymis presented in the work, suggest the possible involvement of not only estrogen but also progesterone in the modulation of excurrent duct functions in greater cane rat.

Acknowledgements

This work was funded by University of Ibadan Senate Research Grant (SRG/FVM/2010/1[b]) to research team headed by VO Taiwo and partly by the Switzerland-South Africa Joint

Research Programme (SSAJRP) held by AO Ihunwo. We acknowledge Mrs. Hasiena Alli of the University of the Witwatersrand, Johannesburg and Mr E. O Anise of the Federal University of Agriculture, Abeokuta, Ogun State for their technical assistance.

REFERENCES

Adebayo, A. O. and Olurode S. A. (2010) The morphology and morphometry of the Epidydimis in the greater cane rat (*Thryonomys swinderianus* Temminck). *Folia morphol*.; 69 (4): 246-252

Adebayo, A. O., Akinloye, A. K, Ihunwo, A. O and Oke, B. O. (2015). Anatomical and Immunohistochemical characteristics of the Prostate gland in the greater cane rat (*Thryonomys swinderianus*). *Anat. Histol. Embryol.*; 44 (1): 138-145

Adebayo, A. O., Akinloye, A. K, Ihunwo, A. O and Oke, B. O. (2016). Zonal Changes in the Ultrastructure of the Epididymal Principal cell of the greater cane rat (*Thryonomys swinderianus*). *Alex. J. Vet. Sci.*; 48 (1): 99-106

Adu, E. K and Yeboah, S**.** (2003). On the use of perineal stain as an index of sexual maturity and breeding condition in the male greater cane rat (*Thryonomys swinderianus*, Temminck). *Tropical Animal Health and Production*.; 35(5): 433-439

Arrotéia K. F., Garcia P. V., Barbieri M. F., Justino M. L and Pereira L. A. V. (2012). The Epididymis: Embryology, Structure, Function and Its Role in Fertilization and Infertility, Embryology - Updates and Highlights on Classic Topics, Prof. Luis Violin Pereira (Ed.), ISBN: 978- 953-51-0465-0, InTech, Available from: http://www.intechopen.com/books/embryology-updates-and-highlightson- classic-topics/the-epididymis-embryology-structure-function-and-its-role-in-fertilization-and-infertility.

Atanassova N, McKinnell C, Williams K, Turner K. J, Fisher J.S, Saunders P.T. (2001). Age-cell-and region-specific immunoexpression of estrogen receptor alpha (but not estrogen receptorbeta) during postnatal development of the epididymis and vas deferens of the rat and disruption of this pattern by neonatal treatment with diethylstilbestrol. *Endocrinology*.; 142 (2):874-86

Baldi E., Luconi M., Krausz C and Forti G. (2011). Progesterone and Spermatozoa: a long-lasting liaison comes to definition. *Human Reproduction*, 2011; doi:10.1093/humrep/der289

Carreau, S., Bois, C., Zanatta L., Silva F. R. M. B., Bouraima-Lelong H and Delalande, C. Estrogen signaling in testicular cells. *Life sciences.*; 89: 584-587

Gatti, J.L.; Castella, S.; Dacheux, F.; Ecroyd, H.; Métayer, S.; Thimon, V. and Dacheux, J. L. (2004). Post-testicular sperm environment and fertility. *Animal Reproduction Science*.; 82-83: 321-339.

Hess, R. A. & Carnes, K. (2004). The role of estrogen in testis and the male reproductive tract: a review and species comparison. *Anim. Reprod*.; 1, 5–30.

Hess, R. A. (2003). Estrogen in the adult male reproductive tract: A review. *Reproductive Biology and Endocrinology*.; 1: 52

Joseph, A., Shur, B. D. and Hess, R. A. (2011). Estrogen, Efferent Ductules, and the Epididymis. *Biol. Reprod.*; 84: 207–217

Joseph, A., Yao, H., and Hinton, B. T. (2009). Development and morphogenesis of the Wolffian/Epididymal duct, more twists and turns. *Developmental Biology*.; 325**:** 6- 14.

Lazari, M.F.M., Lucas, T.F.G., Yasuhara, F., Gomes, G.R.O., Siu, E.R., Royer, C., Sheilla Alessandra Ferreira Fernandes, S.A. F. and Porto., C.S. (2009). Estrogen receptors and function in the male reproductive system. *Arq Bras Endocrinol Metab*.; 53(8): 923-933

Lucas, T. F., Siu, E. R., Esteves, C. A., Monteiro, H. P., Oliveira, C. A., Porto, C. S. and Lazari, M. F. (2008). 17beta-estradiol induces the translocation of the estrogen receptors ESR1 and ESR2 to the cell membrane, MAPK3/1 phosphorylation and proliferation of cultured immature rat Sertoli cells. *Biol. Reprod*.; 78: 101–114.

Nie, R., Zhou, Q., Jassim, E., Saunders, P. T. & Hess, R. A. (2002). Differential expression of estrogen receptors alpha and beta in the reproductive tracts of adult male dogs and cats. *Biol. Reprod.*; 66, 1161–1168.

Oliveira, C. A., Nie, R., Carnes, K., Franca, L. R., Prins, G. S., Saunders, P. T. and Hess, R. A. (2003). The antiestrogen ICI 182,780 decreases the expression of estrogen receptor-alpha but has no effect on estrogen receptorbeta and androgen receptor in rat efferent ductules. *Reprod. Biol. Endocrinol.*; 1:75.

Pearl C. A., At-Taras E, Berger T and Roser J. F. (2007). Reduced endogenous estrogen delays epididymal development but has no effect on efferent duct morphology in boars. *Reproduction.*; 134(4): 593-604

Robaire B., Hinton B. T and Orgebin-Crist, M. C. (2006). The Epididymis. In: *The physiology of reproduction*, Knobil and J. Neil, (Ed.), pp. 1071-1148, Elsevier, ISBN 978-0-12-515- 400, New York.

Schon J, Neumann S, Wildt D E, Pukazhenthi B S, Jewgenow K. (2009). Localization of oestrogen receptors in the epididymis during sexual maturation of the domestic cat. *Reprod Domest Anim.*; 44 (suppl 2): 294–301.

Shayu D, Kesava C. C, Soundarajan R and Rao A. J. (2005). Effects of ICI 182780 on estrogen receptor expression, fluid absorption and sperm motility in the epididymis of the bonnet monkey. *Reprod Biol Endocrinol*.; 3:10.

Solakidi, S., Psarra, A. M., Nikolaropoulos, S. & Sekeris, C. E. (2005). Estrogen receptors alpha and beta (ERalpha and ERbeta) and androgen receptor (AR) in human sperm: localization of ERbeta and AR in mitochondria of the midpiece. *Hum. Reprod.*; 20, 3481–3487. (doi:10. 1093/humrep/dei267)

Zhou Q, Nie R, Prins G. S, Saunders P. T, Katzenellenbogen B. S and Hess R. A. (2002). Localization of androgen and estrogen receptors in adult male mouse reproductive tract. *J Androl.*; 23: 870–881