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Abstract

The efferent ducts are mainly involved in the reabsorption of the seminiferous tubular fluid. Testosterone as well as estrogens regulate efferent ducts functions via their receptors. This paper presents an experimental investigation on the location of the P450 aromatase, the 17-beta-estradiol (E2), the androgen receptor (AR), the estrogen receptor 1 (ESR1), the estrogen receptor 2 (ESR2) and the G protein-coupled estrogen receptor 1 (GPER1) in the efferent ducts using psammomys obesus as an animal model to highlight the effect of the season on the histology and the distribution of these receptors. We observed a proliferation of the connective tissue, decreasing in the height of the epithelium during the resting season compared to the breeding season. Ciliated cells expressed P450 aromatase, AR, E2, ESR1, ESR2 and GPER1 during both seasons. Basal cells showed a positive staining for the ESR1 and the GPER1 during both season, the AR and E2 during the breeding season and ESR2 during the resting season. Our result shows that the expression of androgen receptor as well as estrogen receptors in the efferent ducts vary by season witch suggest that they are largely involved in the regulation of the efferent ducts functions.

Key words: GPER1, ERs, AR, aromatase, efferent ducts, sand rat

INTRODUCTION

In order to get into the epididymis spermatozoa cross the efferent ducts, small convoluted tubules [1]. Depending on species, the number of ducts varies between 1 and 33 [2]. In most species, the epithelium of the efferent ducts is formed of ciliated cells and nonciliated cells, while other species have basal cells and intraepithelial lymphocytes or macrophages [3–6]. A conjunctive tissue and one to three layers of smooth muscle cells surround the ducts and help in sperm propulsion to the epididymis [7].

In addition to transporting sperm, the efferent ducts play a role in the reabsorption of the seminiferous tubular fluid to concentrate sperm by the nonciliated cells. This function involves water transport, the active transport of ions including solute transport, endocytosis and secretion [1,8–10]. The secretory functions of the efferent ducts are not completely known while spermiophagy function is documented [1]. Androgen regulates efferent ducts functions; its receptor (AR) was found in the epithelium[11,12]. But estrogen seems being the main regulator of the efferent ducts, considering that ERs are

widely expressed in the epithelium compared with AR [11,13–18]. The efferent ducts have two sources of androgens, either the rete testis, seminal fluid or the circulating blood in the vasculature[15]. Aromatase, the cytochrome P450(CYP19A1) converts the androgen into the estrogen, was located in the epithelial cells of efferent ducts as well as in the caput epididymis[19–21]. Estrogen exerts its effect through two nuclear receptors family of transcription factors ESR1 and ESR2 [22,23]. ESR1 was largely described in the male reproductive system including the efferent ducts[17,24,25], contrary to the expression of the ESR2, which was ubiquitous, even though it seems more predominant in epithelia than stroma. The expression profile of ESR2 in mouse tract is more like AR than it is to ESR1. Another estrogen receptor recently found, the GPER1 that belongs to the family of seven-transmembrane G-protein-coupled receptors and triggers rapid non-genomic cellular responses[26–28]. The GPER1 expression was detected in various sites of the testis[29–34], as well as in the epididymis of adult rats[27,35,36]. Meanwhile, its expression in the efferent ducts is still unknown.

Because of the major role of estrogen in the male reproductive functions and fertility, the presence of 17-β Estradiol and its receptors need to be explored. Therefore, we performed this study to investigate the location of 17-β Estradiol with its receptors (ESR1 and ESR2) alongside with the new player in estrogen response, the GPER1, as well as AR and aromatase in the efferent ducts of psammomys obesus. All in comparative way between the breeding season and the resting season, we dispatch a query about whether the expression and the location of the estrogen receptors and Androgen receptors as well as the P450 aromatase vary during the season.

MATERIALS AND METHODS

Animals

Adult male fat sand rats *(Psammomys obesus)* of average weight 145g were trapped in the wild region of Béni Abbès (30°07′N 2°10′W) during the breeding season (n = 8) and during resting season (n = 8). They were euthanized by decapitation using a specially prepared laboratory guillotine in the morning, 48 h after capture. The efferent ducts were quickly excised, weighed, fixed in Bouin's solution, dehydrated in increasing concentrations of ethanol (70%, 95%, and 100%), cleaned in toluene, and embedded in paraffin.

Tissue preparation and histology

The samples were sectioned to 5 μm thick sections using a Leitz Wetzlar 1212 microtome. Slices were cut and mounted on histological slides or on "Super Frost" glass slides for immunohistochemistry. After hydration with decreasing concentrations of ethanol, the sections were stained with Masson's Trichrome and Hematoxylin and eosin (H&E) [37,38].

Immunohistochemistry

Immunohistochemistry was performed to detect the occurrence and cell distribution of aromatase, 17-β Estradiol, estrogen receptors (ESR , ESR2 and GPER1) and androgen receptor (AR). Sections were deparaffined with cyclohexane and rehydrated with decreasing concentrations of ethanol. The slides were then washed in tap water for 10 min, then with PBS (Phosphate buffered saline).

For antigen retrieval, the slides were incubated at 95 °C in a 10 mM sodium citrate solution (H-3300, pH 6.0) for 45 min (for ESR1 , ESR2 and GPER1 analysis) or 30 min (for AR). The slides were cooled down during 20 min then washed in distilled water. Endogenous peroxidase was blocked by immersing the slides in 3% peroxydase solution for 20 min followed by two baths in distilled water then were encircled using a DakoPen (Dako, USA). In order to block the nonspecific antibody bindings, sections were incubated with a 10% normal goat serum (S-1000) for 1 h at room temperature. Afterwards, the slides were incubated overnight at 4°C with primary rabbit polyclonal antibodies against human AR(ab74272, Abcam plc, Cambridge, UK), estradiol (AB924, Millipore, CHEMICON), ESR1 (H-184:sc-7207, Santa Cruz Biotechnology, USA), ESR2 (H-150:sc-8974, Santa Cruz Biotechnology, USA), during 1h for aromatase (ab3504, Abcam plc, Cambridge, UK) and during 2h for GPER1 (ab39742, Abcam plc, Cambridge, UK) in wet chamber. All sections were exposed for 1 h to biotinylated secondary antibodies (Anti-Mouse IgG/Rabbit IgG; BA-1400, Vectastain Universal, Vector Laboratories, Burlingame, CA, USA) for 1 h in a wet chamber. After rinsing three times in PBS for 5 min, the slides were incubated with a streptavidin-biotin-peroxidase complex for 1 h. Each tissue section was washed in PBS and stained by the AEC (Amino-Ethyl-Carbazole; Vector Laboratories, SK-4200) for aromatase and estradiol or the DAB chromogen (3, 3 diaminobenzidine, kit for peroxidase; Vector Laboratories) for 1 min. The reaction was

stopped by rinsing in PBS solution. Mayer hematoxylin (Hematoxylin QS, H-3404; Vector Laboratories) for 1 min was used to counterstain the sections, then dehydrated and preserved using VectaMount (AQ Aqueous Mounting Medium, H-5501) for aromatase and estradiol or dehydrated and preserved using the Permount mounting medium (Fisher Scientific, USA) for AR, ESR1, ESR2 and GPER1. The immunostaining was observed under the Nikon Eclipse E 400 light microscope fitted with the Nikon DXM 1200 digital camera.

The intensity of the immunostaining was scored as null (−), weakly positive (+), moderately positive (++) or strongly positive (+++) by two independent observers blinded to the antigen type under analysis.

Ethical note

All experiments complied with the Algerian legislation (Law Number 95-322/1995) inherent to protection of animals designed to experimental and other scientific purposes as well with the guidelines of the Algerian Association of Experimental Animal Sciences (AASEA) and were specifically approved by the latter (AASEA authorization number 45/DGLPAG/DVA/SDA/14).

RESULTS

Histology

During the breeding season, the efferent ducts appear as a set of epithelial sections dispersed in intertubular connective tissue. These tubules relate the rete testis to the caput epididymis.

Each tubular section consists of pseudostratified columnar epithelium with irregular height, formed of three cellular categories: columnar ciliated cells, with clear cytoplasm and basal or apical nucleus, nonciliated cells and basal cells (Fig. 1A). The apical cytoplasm of the ciliated cells contains cilia projecting into the lumen of the efferent ducts. The base of these cilia is marked by the presence of a thick line.

The nonciliated cells, characterized by dark cytoplasm, have thick granules in the supranuclear zone and/or in the apical cytoplasm. These granules are probably secretory granules (Fig. 1B).

The basal cells of a small volume adhere to the basement membrane but do not prolong to the lumen of the duct, interposed between the ciliated cells and the nonciliated cells (Fig. 1A).

The epithelium of each section is surrounded with two layers of smooth muscle cells; these cells are arranged in concentric way around the epithelial tubules. The lumen contains spermatozoa and shows an irregular aspect due to the irregular cellular height.

During the resting season, an important proliferation of the connective tissue and a significant decrease in the height of the epithelium was observed(Fig. 1C). All cellular categories were observed, ciliated cells and nonciliated cells were columnar giving the epithelium the same appearance. We noticed the absence of the granules observed during the breeding season (Fig. 1D).

Immunohistochemistry

All results of immunohistochemistry for aromatase, 17-β Estradiol, AR, ESR1, ESR2 and GPER1 are summarized in Table 1.

Aromatase

During the breeding season, a strong immunoexpression of P450 aromatase was observed in the nuclei of ciliated cells while some other nuclei were not stained, the cytoplasm of the ciliated cells was moderately immunoreactive. However the nonciliated cells and the extruded cells showed no immunohistological staining (Fig. 2). Smooth muscle cells showed a weak immunoreaction. During the resting season, immunohistochemical signal of P450 aromatase was mainly localized in the cytoplasm of the ciliated cells and nonciliated cells, some nuclei were negative. The smooth muscle cells and the fibroblast remained negative.

17-β Estradiol (E2)

During the breeding season, the immunohistological signal of the 17-β Estradiol was located in the basal cells, some ciliated cell were moderately marked some others were not stained. However, the nonciliated cells were unreactive while smooth muscle cells and fibroblasts were positive (Fig. 2). During the resting season, ciliated cells were weakly stained for the 17-β Estradiol, but some of them presented either non-marked cytoplasm or

non-marked nuclei, while the nonciliated cells had a moderately marked cytoplasm and a few weakly stained nuclei. Some fibroblast, smooth muscle cells and extruded cells were positive.

Androgen receptor (AR)

During the breeding season, a moderate immunohistological staining was located in the nuclei and the supranuclear zone of the ciliated cells as well as the basal cells. Nonciliated cells were unreactive, while the smooth muscle cells were weakly marked, and the extruded cells were moderately marked (Fig. 2). During the resting season, the AR was strongly expressed in the nonciliated cells, as well as the cytoplasm of the ciliated cells while some of their nuclei were moderately reactive and others were negatively reacted. However, the smooth muscle cells and the fibroblast presented a strong staining.

Estrogen receptor 1 (ESR1)

During the breeding season, the immunohistological signal was detected in the nuclei and the cytoplasm of the ciliated cells, the basal cells, smooth muscle cells and fibroblasts, signal was absent in the nonciliated cells (Fig. 3). During the resting season, all type of cells were marked while some ciliated cells were not.

Estrogen receptor 2 (ESR2)

During the breeding season, ciliated cells were strongly marked, while basal cells were not, fibroblasts, smooth muscle cells as well some nuclei and cytoplasm of nonciliated cells were moderately marked (Fig. 3). During the resting season, we observed a strong signal in the basal cells, ciliated cells and nonciliated cells were moderately stained, others were not. Some fibroblast and smooth muscle cells were positive.

G protein-coupled estrogen receptor 1 (GPER1)

During the breeding season, a supranuclear immunohistochimical reaction was present in ciliated cells and nonciliated cells, some nuclei of ciliated cells were marked but the nuclei of the nonciliated cells were negative, basal cells were moderately marked, smooth muscle cells and extruded cells were also positive (Fig. 3). During the resting season, some ciliated cells and nonciliated cells presented a strong nuclear and

supranuclear immunohistological reaction while others were not marked, basal cells and smooth muscle cells were moderately stained, some fibroblasts were positive.

DISCUSSION

Considering that, Psammomys obesus has a seasonal reproduction cycle, several studies performed to describe this cycle in both hormonal[39] and cytophysiological terms[40–42]. While there are few studies dealing with the effects of seasonal variation on the histology and the cytology of the efferent ducts. Our results showed a structural reorganisation of the tubular epithelium as well as in the connective tissue, confirming data obtained by Oliveira *et al.* [43] which showed a similar reorganisation in the efferent ducts of the bat.

However, earlier studies carried out on the male excurrent duct system highlighted an important structural reshuffle in the epithelium and the connective tissue. In fact, studies of Gernigon [41] and Menad *et al.* [44] revealed a remarkable epithelial atrophy and a significant proliferation of the connective tissue in the epididymis.

In the sand rat, during the resting season we observed a proliferation of the connective tissue and a blatant decrease in the epithelium height, Oliveira *et al.* [43] obtained similar results. In the epididymis, such observation was described in the sand rat [44] and the Libyan jirds [45]. The same author reported an accumulation of collagen I and III in seminal vesicles in the jirds [46], possibly due to the decrease levels of testosterone. In fact, it has been proven that testosterone regulates the quantity and the quality of the extracellular components via the MMP and their inhibitors TIMPS [47].

Even though, studies focused only on the seasonal variation effects on the androgenic profile, oestrogen from aromatisation of androgen plays a key role in the regulation of the male reproductive functions.

The aim of this study is to reveal the localization of the P450 aromatase, E2, Androgen receptor and oestrogen receptors (ESR1, ESR2, GPER1) in the efferent ducts of the psammomys obesus, during the breeding season and the resting season, in order to define the effects of the seasonal variation on their distribution (Fig. 4).

Aromatase was located in the ciliated cells and the smooth muscle cells, during the breeding season, in the ciliated cells and the nonciliated cells during the resting season in the sand rat, Oliveira *et al*. [43] reported similar data in the big fruit-eating bat, which support the idea that oestrogen is locally synthetized in the efferent ducts. In the testis,

there was a reduced concentration of aromatase during the regression season[48–50], the unchanged aromatase localization in the ciliated cells at resting season possibly because levels of oestrogen need to be maintained in this tissue during this season.

Androgen receptor was present in the ciliated cells, the basal cells and the smooth muscle cells during the breeding season, and present in all cell types during the resting season. This data corroborate with those obtained by Oliveira *et al*. [43]. Several studies showed that AR levels in the efferent ducts are lower than in the epididymis [11,12,51]. Androgen regulates androgen receptor, so during the resting season, even if there is severe changes in testosterone levels, another AR ligand like DHT or androstenedione may are responsible for controlling levels of AR. This may suggest that efferent ducts functions depends on the androgen during both seasons.

Estrogen exert an important role in regulating and maintaining the male reproduction, that's why E2 expression was detected in ciliated cells, fibroblasts and smooth muscle cells during both seasons, while it appeared in nonciliated cells during the resting season. E2 expression was also described in the testis[52,53] as well as in the epididymis [44]. It is possible that oestrogen regulates the functions of the efferent ducts, by maintaining the role of the ciliated cells, fibroblast and smooth muscle cells during both season, the role of the nonciliated cells during the resting season.

ESR1 is largely expressed in the efferent ducts. Our data showed the localization of this receptor in the ciliated cells, basal cells, fibroblasts and smooth muscle cells. Oliveira *et al*. [43] and Joseph *et al.* [20]stated a slightly positive or negative immunoreactivity of the ESR1 during the breeding season and the resting season in the bat, mouse, hamster and monkey. Studies showed the presence of ESR1 in rats[16,54], mice [24], roosters [55]**,** dogs, cats [17], goats [11], monkeys [56] and humans [57]. According to Hess *et al.* [54]and Hess *et al.* [27], ESR1 dominates the efferent ducts. The same author suggests that E2 is not the one indispensable for the efferent duct morphology and function, but its receptor, ESR1.

ESR1 regulates fluid reabsorption in the efferent ducts [54] by controlling ion transporters in the epithelium and altering the proteins involved in fluid reabsorption [58–62], this regulation is essential for male fertility[60,63–66]. In the ESR1 knockout mice, a dramatic dilatation in the epithelium of the rete testis[63], due to the inactivation of ESR1 in the efferent duct [54], a reduce of the epithelium height, the absence of microvilli and

the endocytic apparatus, distension of the efferent duct was reported[54]. The recent data provided by Nanjappa *et al.* [67] indicated both nuclear and membrane localization of ESR1 are necessary for normal morphology of the efferent duct.

The expression of ESR2 in the efferent duct was found in the ciliated cells, nonciliated cells, fibroblast and smooth muscle cells. Oliveira *et al.* [43]reported similar observations in the bat. During the resting season ESR2 appeared in the basal cells possibly because of its expression was inhibited by testosterone during the breeding season. Oliveira *et al.* [43] have shown no seasonal variation of the ESR2 in basal cells. The functions of the basal cells in the efferent ducts are not yet elucidated. However, these cells exert a crucial paracrine role in the epididymal epithelium, indeed basal cells regulate the functions of the principal cells, which are responsible for the sperm maturation [68].

The immunohistological signal of the novel oestrogen receptor GPER1 was strong in the ciliated cells, weak in the nonciliated cells and smooth muscle cells, average in the basal cells. While during the resting season, the staining was ubiquitous in all the epithelium. The supranuclear staining of the GPER1 may reflect its cellular localization in the endoplasmic reticulum or Golgi apparatus [69–74].

In the sand rat, it seems like the immunoexpression of the ESR1, ESR2, GPER1 in the ciliated cells and the smooth muscle cells are constitutive and are not influenced by the season. An interesting observation that these receptors co-existed in ciliated cells and smooth muscle cells, this supports the notion of a crosstalk between the ESR1, ESR2 and GPER1, probably in a common signalosome, for estrogenic signal transduction [75–77]. In fact, the co-expression of ESR1 and ESR2 with GPER in breast cancer cells suggest a possible interaction between these receptors [75–77] as well as in uterine epithelial cells, where the GPER1 functions as inhibitor of cell growth mediated by E2 through the ESR1 [78].

The ERs could have regional distribution in the efferent ducts. Indeed, an increase of immunohistological staining was described in the efferent duct of the rooster [79]. All this data represent strong indicators that oestrogen may have a role in the contraction of the smooth muscle cells as well as in the remodelling of the connective tissue in the efferent ducts.

CONCLUSIONS

Considering that the efferent ducts are an essential part of the male reproductive tract and that their normal function is required for male fertility. It is important to elucidate its different functional mechanism as well as the molecules involved in the regulation of those functions, knowing that oestrogen alongside with androgen interact to control the male reproductive system.

Our study provided for the first time data about the localisation of these main effectors that regulate the male reproductive tissue, establishing the expression profile of the Aromatase, AR, E2, ERs and GPER1. The present data give a preview on the impact of seasonal variation on the expression of these molecules. Further studies are needed to examine the effect of different treatment or inactivation of one of these receptors on the efferent duct functioning.

Conflicts of Interest: There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

1. Hess RA. The efferent ductules: structure and functions. In: The epididymis: from molecules to clinical practice. Springer; 2002. p. 49–80.
2. Mobilio C, Campus A. Osservazioni sull'epididimo dei nostri animali domestici. Arch Ital Anat Embriol. 1912;11:419–79.
3. Arrighi S, Romanello MG, Domeneghini C. Ultrastructure of the epithelium that lines the ductuli efferentes in domestic equidae, with particular reference to spermatophagy. Cells Tissues Organs. 1994;149(3):174–84.
4. BYERS SW, MUSTO NA, DYM M. Culture of ciliated and nonciliated cells from rat ductuli efferentes. J Androl. 1985;6(5):271–8.
5. Ilio KY, Hess RA. Structure and function of the ductuli efferentes: a review. Microsc Res Tech. 1994;29(6):432–67.
6. Stoffel MH, Friess AE. Morphological characteristics of boar efferent ductules and epididymal duct. Microsc Res Tech. 1994;29(6):411–31.
7. Creasy DM, Chapin RE. Chapter 59 – Male Reproductive System [Internet]. Third Edit. Haschek and Rousseaux's Handbook of Toxicologic Pathology. Elsevier; 2013. 2493–2598 p. Available from: http://dx.doi.org/10.1016/B978-0-12-415759-0.00059-5
8. Hermo L, Oka R, Morales CR. Secretion and endocytosis in the male reproductive tract: a role in sperm maturation. In: International review of cytology. Elsevier; 1994. p. 105–89.
9. Clulow J, Jones RC, Hansen LA, Man SY. Fluid and electrolyte reabsorption in the ductuli efferentes testis. J Reprod Fertil. 1998;1–14.
10. Hansen LA, Clulow J, Jones RC. The role of Na+-H+ exchange in fluid and solute transport in the rat efferent ducts. Exp Physiol. 1999;84(3):521–7.
11. Goyal HO, Bartol FF, Wiley AA, Khalil MK, Chiu J, Vig MM. Immunolocalization of androgen receptor and estrogen receptor in the developing testis and excurrent ducts of goats. Anat Rec. 1997;249(1):54–62.
12. Ungefroren H, Ivell R, Ergün S. Region-specific expression of the androgen receptor in the human

epididymis. Mol Hum Reprod. 1997;3(11):933–40.
13. Danzo BJ. Environmental xenobiotics may disrupt normal endocrine function by interfering with the binding of physiological ligands to steroid receptors and binding proteins. Environ Health Perspect. 1997;105(3):294–301.
14. Van Pelt AMM, De Rooij DG, Van Der Burg B, Van Der Saag PT, Gustafsson J-A, Kuiper GGJM. Ontogeny of estrogen receptor-β expression in rat testis. Endocrinology. 1999;140(1):478–83.
15. Oliveira CA, Mahecha GAB, Carnes K, Prins GS, Saunders PTK, França LR, et al. Differential hormonal regulation of estrogen receptors ERα and ERβ and androgen receptor expression in rat efferent ductules. Reproduction. 2004;128(1):73–86.
16. Fisher JS, Millar MR, Majdic G, Saunders PTK, Fraser HM, Sharpe RM. Immunolocalisation of oestrogen receptor-α within the testis and excurrent ducts of the rat and marmoset monkey from perinatal life to adulthood. J Endocrinol. 1997;153(3):485–95.
17. Nie R, Zhou Q, Jassim E, Saunders PTK, Hess RA. Differential Expression of Estrogen Receptors α and β in the Reproductive Tractsof Adult Male Dogs and Cats1. Biol Reprod [Internet]. 2002;66(4):1161–8. Available from: https://academic.oup.com/biolreprod/article-lookup/doi/10.1095/biolreprod66.4.1161
18. Zhou Q, Nie R, Prins GS, Saunders PTK, Katzenellenbogen BS, Hess R a. Localization of Androgen and Estrogen Receptors in Adult Male Mouse Reproductive Tract. J Androl [Internet]. 2002;23(6):870–881. Available from: https://www.researchgate.net/profile/Rex_Hess/publication/11063237_Localization_of_androgen_and_estrogen_receptors_in_adult_male_mouse_reproductive_tract/links/09e4150982cf290762000000.pdf
19. Pereyra-Martinez AC, Roselli CE, Stadelman HL, Resko JA. Cytochrome P450 aromatase in testis and epididymis of male rhesus monkeys. Endocrine. 2001;16(1):15–9.
20. Joseph A, Shur BD, Hess RA. Estrogen, Efferent Ductules, and the Epididymis. Biol Reprod [Internet]. 2011;84(2):207–17. Available from: https://academic.oup.com/biolreprod/article-lookup/doi/10.1095/biolreprod.110.087353
21. Rago V, Maggiolini M, Vivacqua A, Palma A, Carpino A. Differential expression of estrogen receptors (ERα/ERβ) in testis of mature and immature pigs. Anat Rec - Part A Discov Mol Cell Evol Biol. 2004;281(2):1234–9.
22. Heldring N, Pike A, Andersson S, Matthews J, Cheng G, Hartman J, et al. Estrogen receptors: how do they signal and what are their targets. Physiol Rev. 2007;87(3):905–31.
23. Stanišić V, Lonard DM, O'Malley BW. Modulation of steroid hormone receptor activity. In: Progress in brain research. Elsevier; 2010. p. 153–76.
24. Rosenfeld CS, Ganjam VK, Taylor JA, Yuan X, Stiehr JR, Hardy MP, et al. Transcription and translation of estrogen receptor-β in the male reproductive tract of estrogen receptor-α knock-out and wild-type mice. Endocrinology. 1998;139(6):2982–7.
25. Saunders PTK, Sharpe RM, Williams K, Macpherson S, Urquart H, Irvine DS, et al. Differential expression of oestrogen receptor α and β proteins in the testes and male reproductive system of human and non-human primates. Mol Hum Reprod. 2001;7(3):227–36.
26. Rago V, Romeo F, Giordano F, Maggiolini M, Carpino A. Identification of the estrogen receptor GPER in neoplastic and non-neoplastic human testes. Reprod Biol Endocrinol [Internet]. 2011;9(1):135. Available from: http://www.rbej.com/content/9/1/135
27. Hess RA, Fernandes SAF, Gomes GRO, Oliveira CA, Lazari MFM, Porto CS. Estrogen and its receptors in efferent ductules and epididymis. J Androl. 2011;32(6):600–13.
28. Prossnitz ER, Barton M. Estrogen biology: New insights into GPER function and clinical opportunities [Internet]. Vol. 389, Molecular and Cellular Endocrinology. Elsevier Ireland Ltd; 2014. p. 71–83. Available from: http://dx.doi.org/10.1016/j.mce.2014.02.002
29. Sirianni R, Chimento A, Ruggiero C, De Luca A, Lappano R, Andò S, et al. The novel estrogen receptor, G protein-coupled receptor 30, mediates the proliferative effects induced by 17β-estradiol on mouse spermatogonial GC-1 cell line. Endocrinology. 2008;149(10):5043–51.
30. Lucas TFG, Royer C, Siu ER, Lazari MFM, Porto CS. Expression and signaling of G protein-coupled estrogen receptor 1 (GPER) in rat sertoli cells. Biol Reprod. 2010;83(2):307–17.
31. Sheng Z-G, Zhu B-Z. Low concentrations of bisphenol A induce mouse spermatogonial cell proliferation by G protein–coupled receptor 30 and estrogen receptor-α. Environ Health Perspect. 2011;119(12):1775–80.
32. Vaucher L, Funaro MG, Mehta A, Mielnik A, Bolyakov A, Prossnitz ER, et al. Activation of GPER-1 estradiol receptor downregulates production of testosterone in isolated rat Leydig cells and adult

human testis. PLoS One. 2014;9(4):e92425.
33. Zhang K-S, Chen H-Q, Chen Y-S, Qiu K-F, Zheng X-B, Li G-C, et al. Bisphenol A stimulates human lung cancer cell migration via upregulation of matrix metalloproteinases by GPER/EGFR/ERK1/2 signal pathway. Biomed Pharmacother. 2014;68(8):1037–43.
34. Menad R, Smaïa S, Bonnet X, Gernigon-Spychalowicz T, Moudilou E, Khammar F, et al. Seasonal variations of aromatase and estrogen receptors expression in the testis of free-ranging sand rats. Acta Histochem. 119(4):382–91.
35. Pereira MFN, Fernandes SAF, Nascimento AR, Siu ER, Hess RA, Oliveira CA, et al. Effects of the oestrogen receptor antagonist Fulvestrant on expression of genes that affect organization of the epididymal epithelium. Andrology. 2014;2(4):559–71.
36. Menad R, Fernini M, Smaï S, Bonnet X, Gernigon-Spychalowicz T, Moudilou E, et al. GPER1 in sand rat epididymis: Effects of seasonal variations, castration and efferent ducts ligation. Anim Reprod Sci. 183(June):9–20.
37. Martoja R, Martoja-Pierson M. Initiation aux techniques de l'histologie animale. 1967;
38. Gabe M. Histological techniques. Masson; 1976.
39. Khammar F. Variations saisonnières de l'activité endocrine de deuxespèces de rongeurs désertiques, le rat des sables (Psammomysobesus) et la gerbille (Gerbillus gerbillus). USTHB Algiers; 1987.
40. Gernigon T, Malaprade D, Mesbah A, Lécher P. Aspects cytologiques du testicule et de l'épididyme du rat des sables (Psammomys obesus) dans son biotope. Le Rongeur L'esp. 1991;129–42.
41. Gernigon-Spychalowicz T. Etudes cytologiques et biochimiques des variationssaisonnières de l'appareil génital mâle d'un rongeur saharien diurne, le rat des sables (Psammomys obesus). USTHB Algiers; 1992.
42. Menad R. Régionalisation structurale et fonctionnelle de l'épididyme d'un rongeur déserticole diurne, psammomys obesus CRETZSCHMAR, 1828. USTHB Algiers; 2008.
43. Oliveira RL, Nogueira JC, Mahecha GAB, Oliveira CA. Seasonal variation in estrogen receptor ERα, but not ERβ, androgen receptor and aromatase, in the efferent ductules and epididymis of the big fruit-eating bat Artibeus lituratus. Gen Comp Endocrinol [Internet]. 2012;179(1):1–13. Available from: http://dx.doi.org/10.1016/j.ygcen.2012.06.028
44. Menad R, Smaï S, Moudilou E, Khammar F. Immunolocalization of estrogen and androgen receptors in the caput epididymidis of the fat sand rat (Psammomys obesus): Effects of seasonal variations, castration and efferent duct ligation. Acta Histochem [Internet]. 2014;116:559–69. Available from: http://dx.doi.org/10.1016/j.acthis.2013.11.004
45. Belhocine M. Etude histo-cytologique des variations saisonnièresde l'appareil reproducteur male d'un rongeur déserticole nocturne, le mérion du désert (Meriones Crassus) et de son espèce sympatrique, le mérion de Libye (Meriones libycus). USTHB Algiers; 1998.
46. Belhocine M, Gernigon-Spychalowicz T, Robert AM, Schoevaert D, Bennazzoug Y, Exbrayat JM. Ecophysiological responses of the seminal vesicle of Libyan jird (Meriones libycus) to the Saharan conditions: histological, morphometric and immunohistochemical analysis. Histol Histopathol. 2007;22(4–6):603–16.
47. Belhocine M, Gernigon-Spychalowicz T, Jacob MP, Benazzoug Y, Exbrayat JM. Immunoexpression of gelatinase (MMP-2 and MMP-9) in the seminal vesicles and ventral prostate of Libyan jird (Meriones libycus) during the seasonal cycle of reproduction. Histol Histopathol. 2010;25(4):619.
48. Bilińska B, Schmalz-Frączek B, Sadowska J, Carreau S. Localization of cytochrome P450 aromatase and estrogen receptors α and β in testicular cells–an immunohistochemical study of the bank vole. Acta Histochem. 2000;102(2):167–81.
49. Tsubota T, Howell-Skalla L, Nitta H, Osawa Y, Mason JI, Meiers PG, et al. Seasonal changes in spermatogenesis and testicular steroidogenesis in the male black bear Ursus americanus. Reproduction. 1997;109(1):21–7.
50. Zhang H, Sheng X, Hu X, Li X, ZHANG M, LI B, et al. Seasonal changes in spermatogenesis and immunolocalization of cytochrome P450 17α-Hydroxylase/c17-20 lyase and cytochrome P450 aromatase in the wild male ground squirrel (Citellus dauricus Brandt). J Reprod Dev. 2010;1002260252.
51. Roselli CE, West NB, Brenner RM. Androgen receptor and 5α-reductase activity in the ductuli efferentes and epididymis of adult rhesus macaques. Biol Reprod. 1991;44(4):739–45.
52. Hess RA. Estrogen in the adult male reproductive tract : A review. 2003;14:1–14.
53. Carreau S, Lambard S, Delalande C, Denis-Galeraud I, Bilinska B, Bourguiba S. Aromatase expression and role of estrogens in male gonad: a review. Reprod Biol Endocrinol. 2003;1(1):35.
54. HESS REXA, GIST DH, BUNICK D, LUBAHN DB, FARRELL AMY, BAHR J, et al. Estrogen

receptor (α and β) expression in the excurrent ducts of the adult male rat reproductive tract. J Androl. 1997;18(6):602–11.
55. KWON S, HESS REXA, BUNICK D, KIRBY JD, BAHR JM. Estrogen receptors are present in the epididymis of the rooster. J Androl. 1997;18(4):378–84.
56. West NB, Brenner RM. Estrogen receptor in the ductuli efferentes, epididymis, and testis of rhesus and cynomolgus macaques. Biol Reprod. 1990;42(3):533–8.
57. Ergün S, Ungefroren H, Holstein AF, Davidoff MS. Estrogen and progesterone receptors and estrogen receptor- related antigen (ER- D5) in human epididymis. Mol Reprod Dev Inc Gamete Res. 1997;47(4):448–55.
58. Lee K-H, Finnigan-Bunick C, Bahr J, Bunick D. Estrogen regulation of ion transporter messenger RNA levels in mouse efferent ductules are mediated differentially through estrogen receptor (ER) α and ERβ. Biol Reprod. 2001;65(5):1534–41.
59. Lee K-H, Bunick D, Lamprecht G, Choi I, Bahr JM. Differential Expression of Genes Important to Efferent Ductules Ion Homeostasis across Postnatal Development in Estrogen Receptor-慣 Knockout and Wildtype Mice. Asian-Australasian J Anim Sci. 2008;21(4):510–22.
60. Zhou Q, Clarke L, Nie R, Carnes K, Lai L-W, Lien Y-HH, et al. Estrogen action and male fertility: roles of the sodium/hydrogen exchanger-3 and fluid reabsorption in reproductive tract function. Proc Natl Acad Sci. 2001;98(24):14132–7.
61. Toda K, Okada T, Hayashi Y, Saibara T. Preserved tissue structure of efferent ductules in aromatase-deficient mice. J Endocrinol. 2008;199(1):137–46.
62. Joseph A, Hess RA, Schaeffer DJ, Ko C, Hudgin-spivey S, Chambon P, et al. Absence of Estrogen Receptor Alpha Leads to Physiological Alterations in the Mouse Epididymis and Consequent Defects in Sperm Function 1. 2010;957(July 2009):948–57.
63. Eddy EM, Washburn TF, Bunch DO, Goulding EH, Gladen BC, Lubahn DB, et al. Targeted disruption of the estrogen receptor gene in male mice causes alteration of spermatogenesis and infertility. Endocrinology. 1996;137(11):4796–805.
64. Hess RA, Zhou Q, Nie R, Oliveira C, Cho H, Nakai M, et al. Estrogens and epididymal function. Reprod Fertil Dev. 2001;13(4):273–83.
65. Oliveira CA, Carnes K, França LR, Hess RA. Infertility and testicular atrophy in the antiestrogen-treated adult male rat. Biol Reprod. 2001;65(3):913–20.
66. Oliveira CA, Zhou Q, Carnes K, Nie R, Kuehl DE, Jackson GL, et al. ER function in the adult male rat: short-and long-term effects of the antiestrogen ICI 182,780 on the testis and efferent ductules, without changes in testosterone. Endocrinology. 2002;143(6):2399–409.
67. Nanjappa MK, Hess RA, Medrano TI, Locker SH, Levin ER, Cooke PS. Membrane-localized estrogen receptor 1 is required for normal male reproductive development and function in mice. Endocrinology. 2016;157(7):2909–19.
68. Leung GPH, Cheung KH, Leung CT, Tsang MW, Wong PYD. Regulation of epididymal principal cell functions by basal cells: role of transient receptor potential (Trp) proteins and cyclooxygenase-1 (COX-1). Mol Cell Endocrinol. 2004;216(1–2):5–13.
69. Revankar CM, Cimino DF, Sklar LA, Arterburn JB, Prossnitz ER. A transmembrane intracellular estrogen receptor mediates rapid cell signaling. Science (80-). 2005;307(5715):1625–30.
70. Albanito L, Lappano R, Madeo A, Chimento A, Prossnitz ER, Cappello AR, et al. Effects of atrazine on estrogen receptor α–And G protein–coupled receptor 30–mediated signaling and proliferation in cancer cells and cancer-associated fibroblasts. Environ Health Perspect. 2015;123(5):493–9.
71. Prossnitz ER, Arterburn JB, Sklar LA. NIH Public Access. 2008;(505):138–42.
72. Liverman CS, Brown JW, Sandhir R, McCarson KE, Berman NEJ. Role of the oestrogen receptors GPR30 and ERα in peripheral sensitization: relevance to trigeminal pain disorders in women. Cephalalgia. 2009;29(7):729–41.
73. Terasawa E, Noel SD, Keen KL. Rapid action of oestrogen in luteinising hormone- releasing hormone neurones: the role of GPR30. J Neuroendocrinol. 2009;21(4):316–21.
74. Filardo EJ, Thomas P. GPR30 : a seven-transmembrane- spanning estrogen receptor that triggers EGF release. 2005;16(8).
75. Filardo EJ, Graeber CT, Quinn JA, Resnick MB, Giri D, DeLellis RA, et al. Distribution of GPR30, a seven membrane–spanning estrogen receptor, in primary breast cancer and its association with clinicopathologic determinants of tumor progression. Clin cancer Res. 2006;12(21):6359–66.
76. Huang GS, Gunter MJ, Arend RC, Li M, Arias-Pulido H, Prossnitz ER, et al. Co-expression of GPR30 and ERβ and their association with disease progression in uterine carcinosarcoma. Am J Obstet Gynecol. 2010;203(3):242. e1-242. e5.

77. Prossnitz ER, Barton M. The G-protein-coupled estrogen receptor GPER in health and disease. Nat Rev Endocrinol. 2011;7(12):715.
78. Gao F, Ma X, Ostmann AB, Das SK. GPR30 activation opposes estrogen-dependent uterine growth via inhibition of stromal ERK1/2 and estrogen receptor alpha (ERα) phosphorylation signals. Endocrinology. 2011;152(4):1434–47.
79. Oliveira RL, Oliveira CA. Reproductive biology of male bats: anatomy, physiology and endocrinology. Bats Biol Bahavior Conserv Nov Sci Publ New York. 2011;135–75.

Table 1. Immunolocalization of aromatase, 17-β Estradiol, AR, ESR1, ESR2 and GPER1 in efferent ducts of the sand rat (Psammomys obesus) during the breeding season and the resting season.

		Breeding season						Resting season					
		Aro	AR	E2	ESR1	ESR2	GPER1	Aro	AR	E2	ESR1	ESR2	GPER1
Ciliated cells	N	+++/-	++	++/-	+++/-	+++	++/-	+/-	-/++	+/-	++/-	+++/-	-/+++
	C	++	++SN	++/-	+++/-	+++	+++SN/-	++	+++	++/-	++/-	++/-	+++SN/-
Nonciliated cells	N	-	-	-	-	++/-	-	+/-	+++	+/-	+++	++/-	+++/-
	C	-	-	-	-	++/-	+SN /-	++	+++	++	++	++	+++SN/-
Basal cells	N	ND	++	+	++	-	++/+	ND	ND	ND	++	+++	++
	C	ND	++	+	++	-	++/+	ND	ND	ND	++	+++	++
Fibroblasts	N	ND	ND	++	+/++	++	ND	-	+++	++/-	+++	++/-	-/++
Smooth muscle cells	N	+	+	+	++/+	++	+	-	+++	+/-	+/++	+/-	+++
Extruded cells	N	-	++	ND	ND	ND	+++	ND	++	+/-	ND	ND	ND

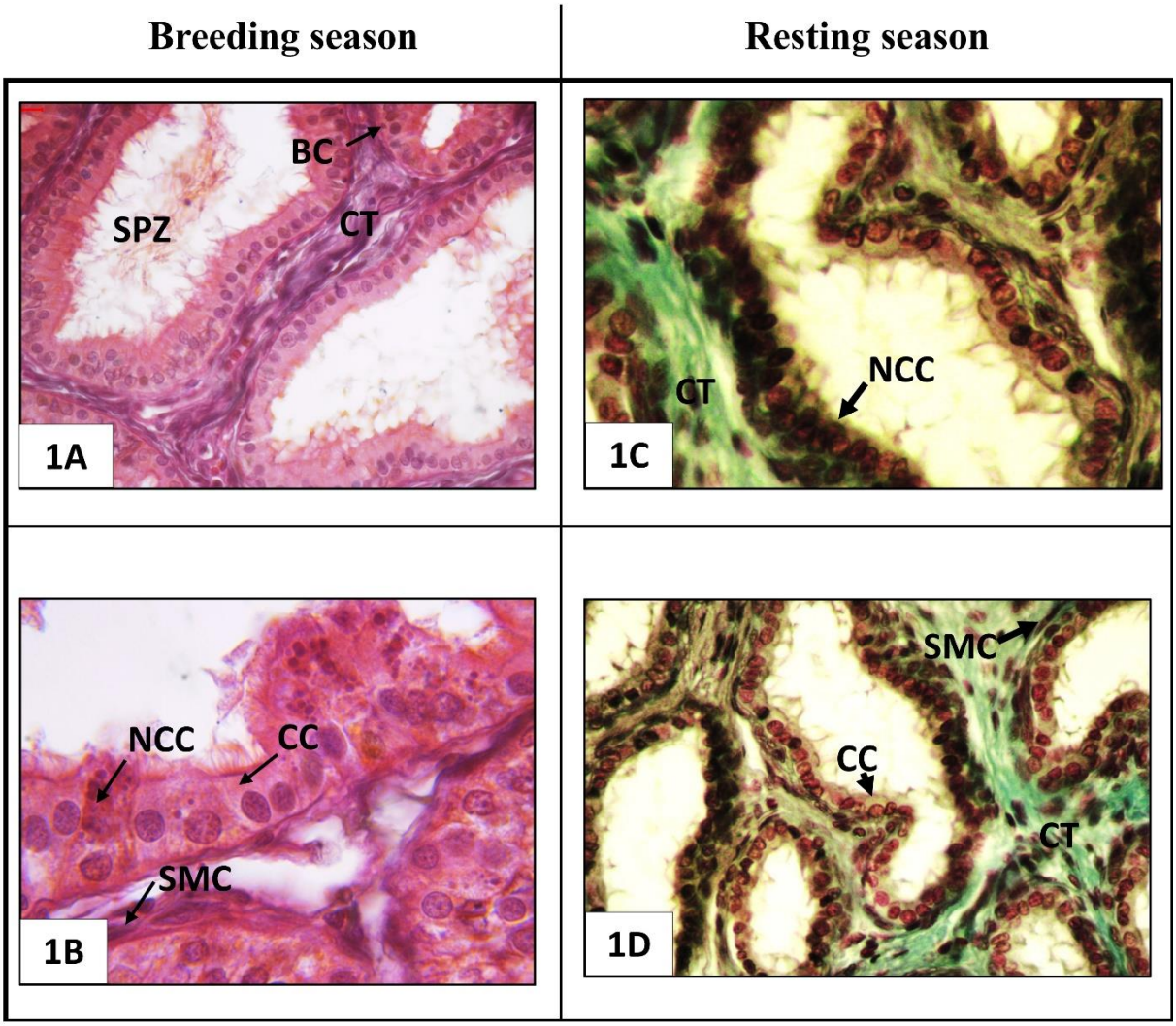

Fig. 1. Histology of the efferent ducts of the sand rat *Psammomys obesus* (breeding season: 1A,1B, resting season :1C,1D)**.** Panels 1A and 1B during the breeding season (H&E stain). The epithelial sections consist of pseudostratified columnar epithelium with irregular height, formed of three cellular categories: ciliated cells, nonciliated cells, basal cells. The epithelium of each section is surrounded with two layer of smooth muscle cells. The lumen contains spermatozoa. Panels 1C and 1D during the resting season (Masson's Trichrome). The connective tissue shows an important proliferation. A significant decrease in the height of the epithelium was observed containing the ciliated cells and the nonciliated cells without the granules observed during the breeding season.

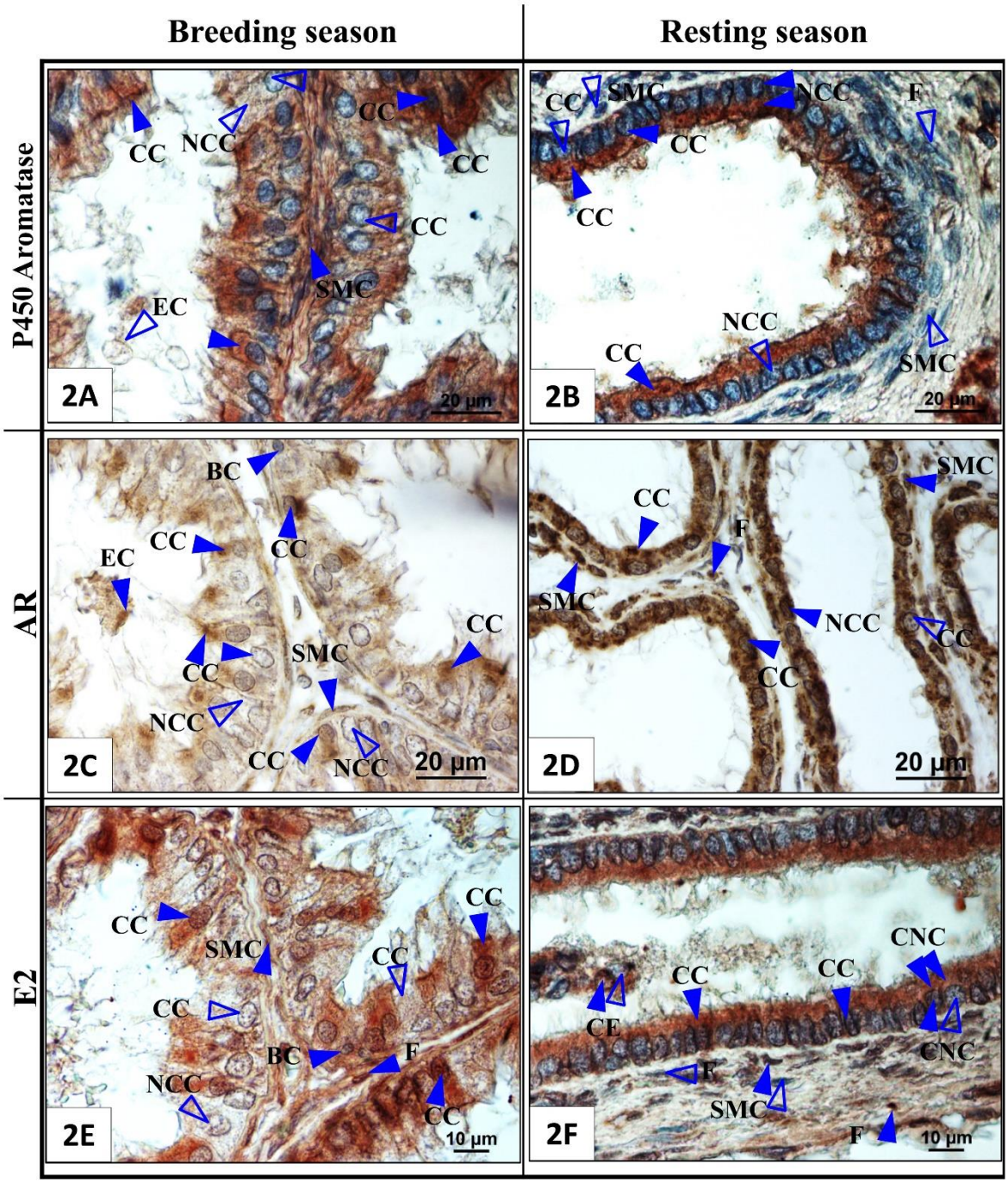

Fig. 2. Immunohistochemistry of Aromatase, AR and E2 in the efferent ducts of the sand rat *Psammomys obesus* (breeding season: 2A, 2C, 2E, resting season 2B, 2D, 2F). Panels 2A and 2B illustrate immunohistochemistry of Aromatase. During the breeding season (2A), the immunohistological staining was mainly located in ciliated cells and smooth muscle cells. During the resting season (2B) some ciliated cells and some nonciliated cells were marked while others were not, smooth muscle cells and fibroblast were negative. Panels 2C and 2D illustrate immunohistochemistry of Androgen receptor.

During the breeding season (2C), the signal was found in ciliated cells, basal cells, smooth muscle cells and extruded cells, but not in nonciliated cells. During the resting season (2D) ciliated cells, nonciliated cells, fibroblast and smooth muscle cells were stained, whereas some ciliated cells were not. Panels 2E and 2F illustrate immunohistochemistry of E2. During the breeding season (2E) the immunohistological reaction was observed in ciliated cells, nonciliated cells, basal cells, fibroblast and smooth muscle cells, some ciliated cells and nonciliated cells were negative. During the resting season (2F) the immunohistological signal was detected in ciliated cells, some extruded cells, fibroblast and smooth muscle cells, the nonciliated cells were positive but some of their nuclei were negative. Hollow arrows signify absence of staining; filled arrows signify presence of staining.

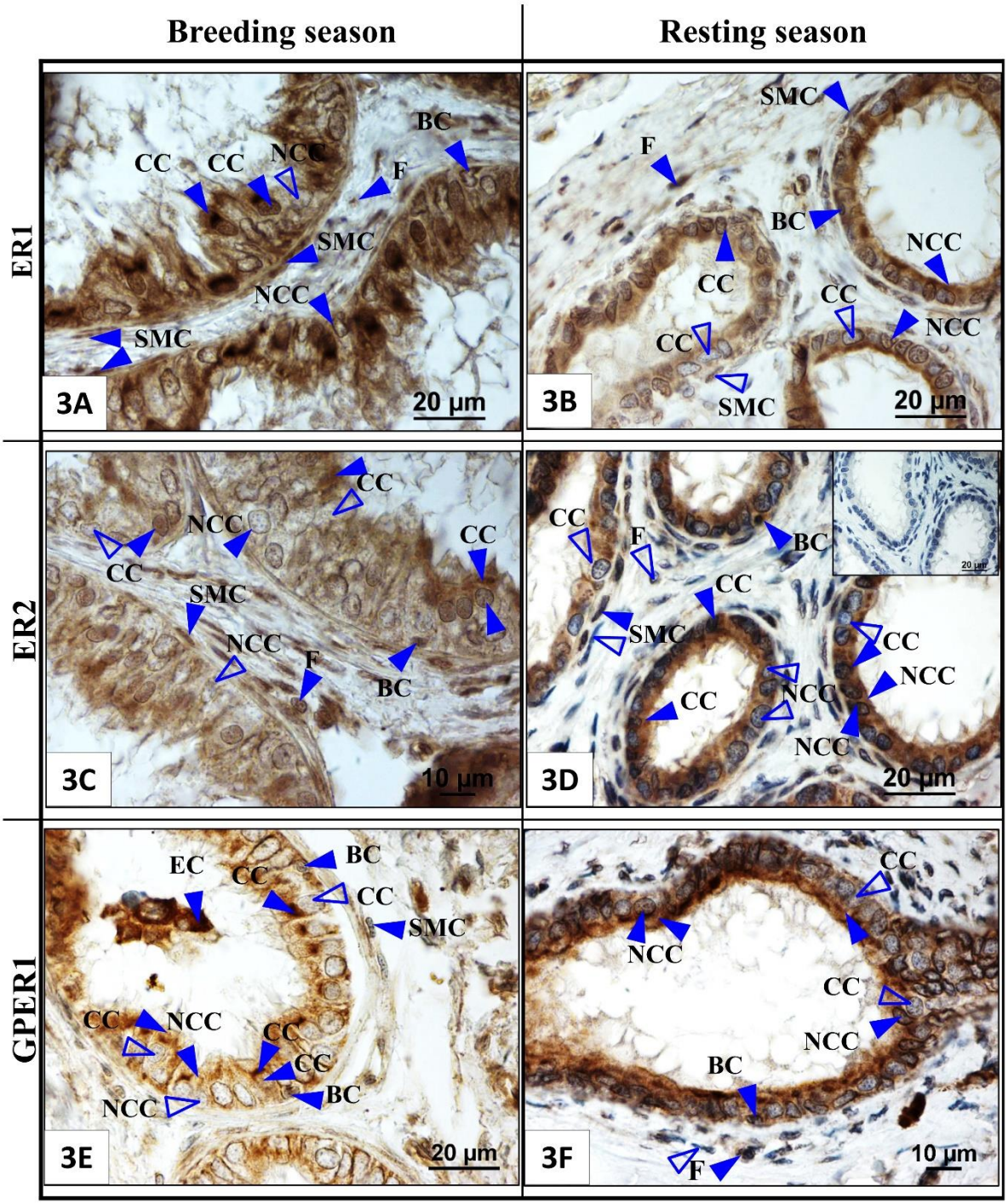

Fig. 3. Immunohistochemistry of ESR1, ESR2 and GPER1 in the efferent ducts of the sand rat *Psammomys obesus* (breeding season: 3A, 3C, 3E, resting season 3B, 3D, 3F). Panels 3A and 3B illustrate immunohistochemistry of ESR1. During the breeding season (3A), the immunohistological staining was mainly located in ciliated cells, basal cells, fibroblast and smooth muscle cells; some nonciliated cells were also marked. During the resting season (3B) nonciliated cells, basal cells and fibroblast are positive, some ciliated

cells and smooth muscle cells are negative. Panels 3C and 3D illustrate immunohistochemistry of ESR2. During the breeding season (3C), the signal was found in ciliated cells, nonciliated cells, basal cells, fibroblast and smooth muscle cells, but some ciliated cells and nonciliated cells were not marked. During the resting season (3D), ciliated cells, nonciliated cells, basal cells, fibroblast and smooth muscle cells were immunoreactive, whereas others were not, basal cells were also stained. Panels 3E and 3F illustrate immunohistochemistry of GPER1. During the breeding season (3E) the immunohistological reaction was observed in ciliated cells, nonciliated cells, basal cells, smooth muscle cells and extruded cells, some ciliated cells and nonciliated cells were negative. During the resting season (3F) the immunohistological signal was detected in nonciliated, basal cells, fibroblast and smooth muscle cells, the ciliated cells were positive but some of their nuclei were negative. Hollow arrows signify absence of staining; filled arrows signify presence of staining.

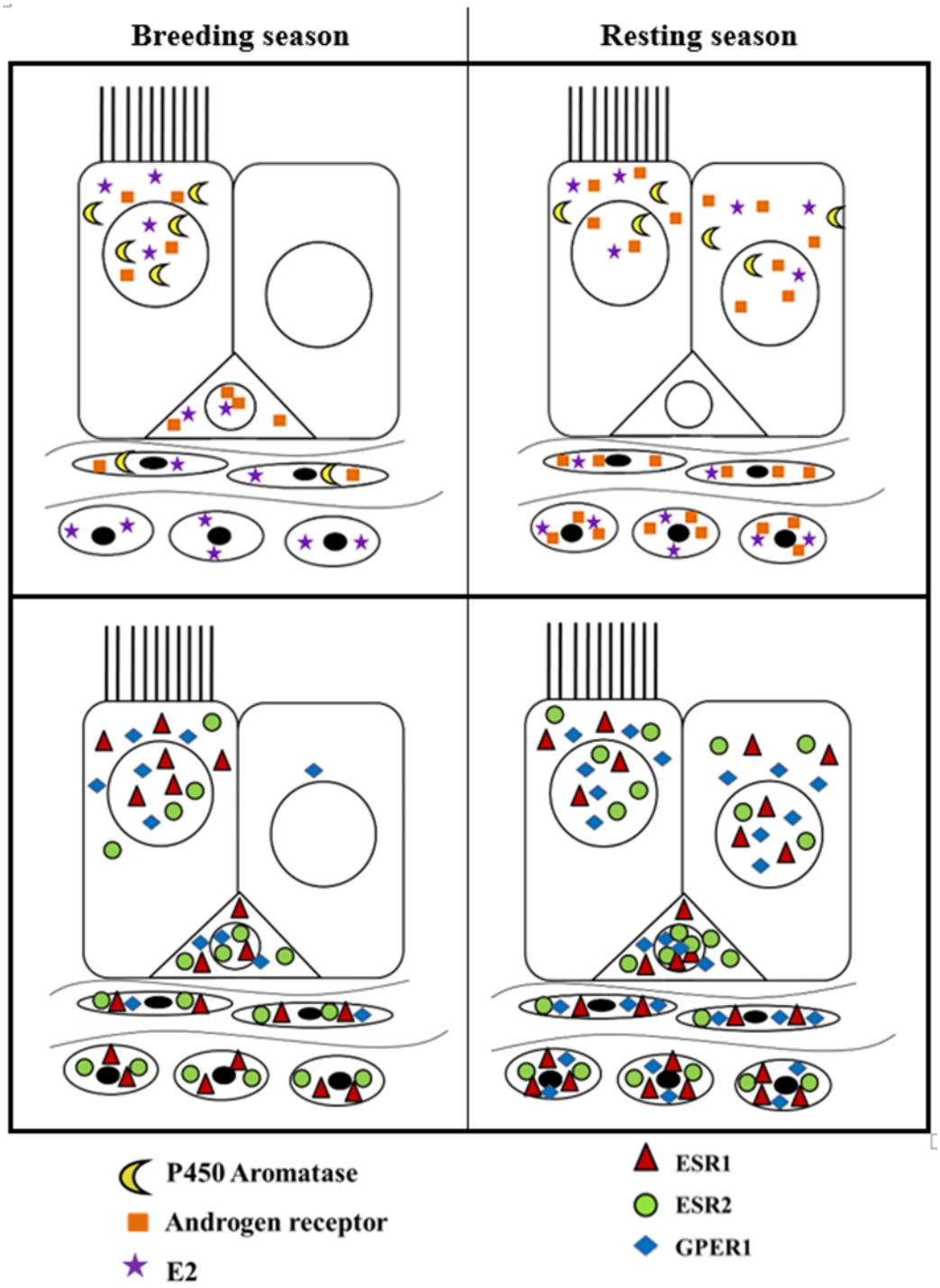

Figure 4. Schematic representation of the expression of P450 aromatase, E2, AR, ESR1, ESR2 and GPER1 in the efferent ducts of the Psammomys obesus during the breeding season and the resting season.