

# G protein-coupled oestrogen receptor 1, oestrogen receptors and androgen receptor in the sand rat (*Psammomys obesus*) efferent ducts

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**Background:** The efferent ducts are mainly involved in the reabsorption of the seminiferous tubular fluid. Testosterone and oestrogens regulate efferent ducts functions via their receptors.

**Materials and methods:** This paper presents an experimental investigation on the location of the P450 aromatase, the 17- $\beta$  oestradiol (E2), the androgen receptor (AR), the oestrogen receptor 1 (ESR1), the oestrogen receptor 2 (ESR2) and the G protein-coupled oestrogen 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.

**Results:** 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.

**Conclusions:** Our result shows that the expression of androgen receptor and oestrogen receptors in the efferent ducts vary by season which suggest that they are largely involved in the regulation of the efferent ducts functions. (Folia Morphol 2020; 79, 4: 756–766)

**Key words:** G protein-coupled oestrogen receptor 1, oestrogen receptor, androgen receptor, aromatase, efferent ducts, sand rat

## INTRODUCTION

In order to get into the epididymis spermatozoa cross the efferent ducts, small convoluted tubules [28]. Depending on species, the number of ducts varies

between 1 and 33 [46]. 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

[2, 7, 10, 31, 68]. A conjunctive tissue and one to three layers of smooth muscle cells surround the ducts and help in sperm propulsion to the epididymis [10].

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 [9, 22, 24, 28]. The secretory functions of the efferent ducts are not completely known while spermiophagy function is documented [28]. Androgen regulates efferent ducts functions; its receptor (androgen receptor [AR]) was found in the epithelium [21, 72]. But oestrogen seems being the main regulator of the efferent ducts, considering that oestrogen receptors are widely expressed in the epithelium compared with AR [11, 16, 21, 48, 50, 73, 79]. The efferent ducts have two sources of androgens, either the rete testis, seminal fluid or the circulating blood in the vasculature [50]. Aromatase, the cytochrome P450 (CYP19A1) converts the androgen into the oestrogen, was located in the epithelial cells of efferent ducts as well as in the caput epididymis [33, 55, 59]. Oestrogen exerts its effect through two nuclear receptors family of transcription factors the oestrogen receptor 1 (ESR1) and the oestrogen receptor 2 (ESR2) [23, 67]. ESR1 was largely described in the male reproductive system including the efferent ducts [48, 63, 64], 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 oestrogen receptor recently found, the G protein-coupled oestrogen receptor 1 (GPER1) that belongs to the family of seven-transmembrane G-protein-coupled receptors and triggers rapid non-genomic cellular responses [25, 57, 60]. The GPER1 expression was detected in various sites of the testis [40, 44, 65, 66, 74, 77], as well as in the epididymis of adult rats [25, 42, 54]. Meanwhile, its expression in the efferent ducts is still unknown.

Because of the major role of oestrogen in the male reproductive functions and fertility, the presence of 17- $\beta$  oestradiol (E2) and its receptors need to be explored. Therefore, we performed this study to investigate the location of 17- $\beta$  oestradiol with its receptors (ESR1 and ESR2) alongside with the new player in oestrogen 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 oestrogen 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 145 g 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 euthanised 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  $\mu$ m 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 haematoxylin and eosin [17, 41].

### Immunohistochemistry

Immunohistochemistry was performed to detect the occurrence and cell distribution of aromatase, 17- $\beta$  oestradiol, oestrogen receptors (ESR, ESR2 and GPER1) and 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 phosphate buffered saline (PBS).

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), oestradiol (AB924, Mill-

ipore, CHEMICON), ESR1 (H-184:sc-7207, Santa Cruz Biotechnology, USA), ESR2 (H-150:sc-8974, Santa Cruz Biotechnology, USA), during 1 h for aromatase (ab3504, Abcam plc, Cambridge, UK) and during 2 h 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 oestradiol 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 haematoxylin (haematoxylin 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 oestradiol 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 authorisation 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, characterised 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

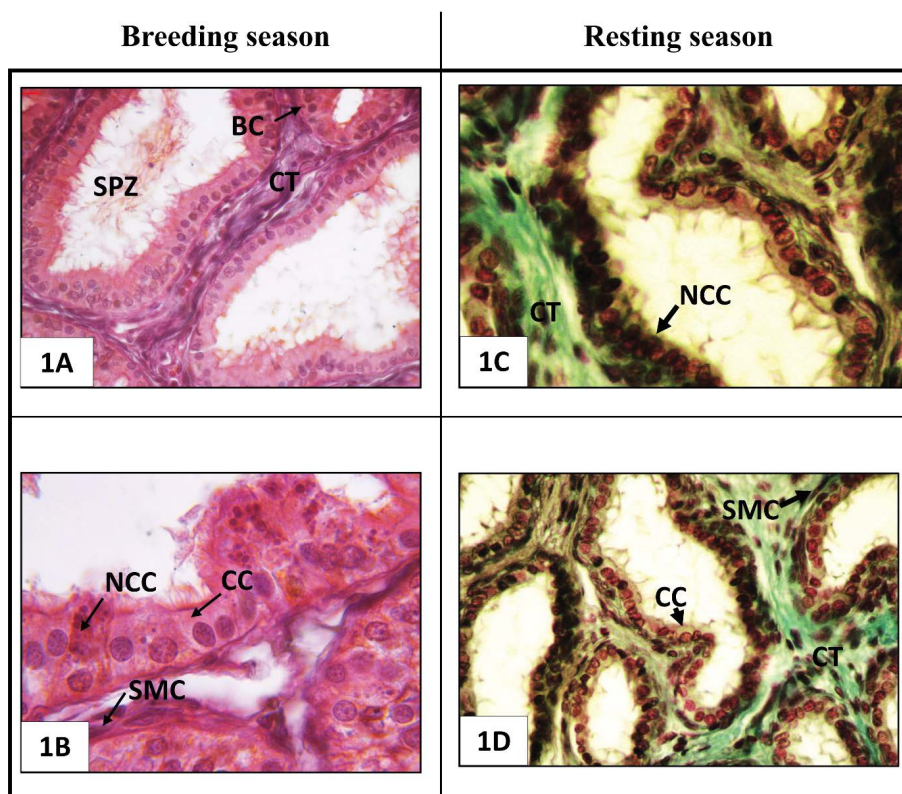
All results of immunohistochemistry for aromatase, E2, AR, ESR1, ESR2 and GPER1 are summarised in Table 1.

#### Aromatase

During the breeding season, a strong immunorepression 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 localised in the cytoplasm of the ciliated cells and nonciliated cells, some nuclei were negative. The smooth muscle cells and the fibroblast remained negative.

#### 17- $\beta$ oestradiol (E2)

During the breeding season, the immunohistological signal of the 17- $\beta$  oestradiol was located in the basal cells, some ciliated cell were moderately marked



**Figure 1.** Histology of the efferent ducts of the sand rat *Psammomys obesus*; **A, B.** Breeding season; **C, D.** Resting season. Panels A and B during the breeding season (haematoxylin and eosin stain). The epithelial sections consist of pseudostratified columnar epithelium with irregular height, formed of three cellular categories: ciliated cells (CC), nonciliated cells (NCC), basal cells (BC). The epithelium of each section is surrounded with two layer of smooth muscle cells (SMC). The lumen contains spermatozoa (SPZ). Panels C and D during the resting season (Masson's trichrome). The connective tissue (CT) 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.

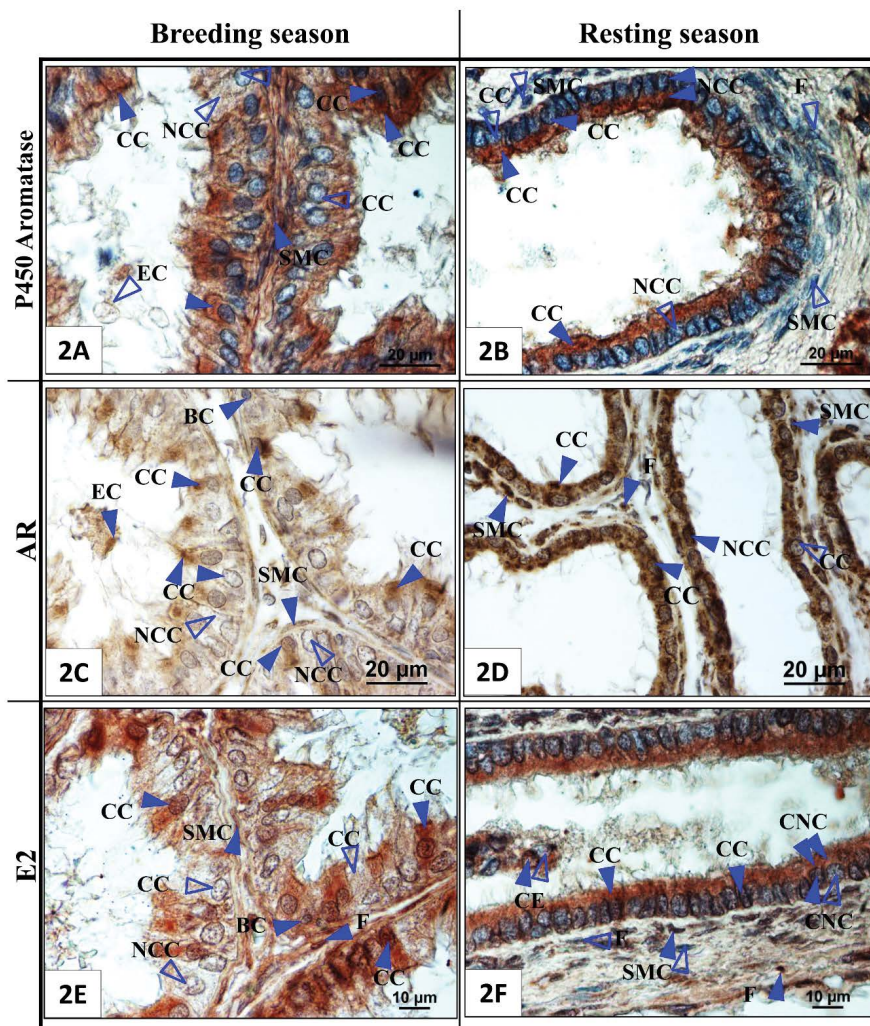
**Table 1.** Immunolocalisation of aromatase (Aro), 17- $\beta$  oestradiol (E2), the androgen receptor (AR), the oestrogen receptor 1 (ESR1), the oestrogen receptor 2 (ESR2) and the G protein-coupled oestrogen receptor 1 (GPER1) in efferent ducts of the sand rat (*Psammomys obesus*) during the breeding 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/-	+++SN/-
Nonciliated cells	N	-	-	-	-	++/-	-	+/-	+++	+/-	+++	++/-	+++/-	+++/-
	C	-	-	-	-	++/-	+SN/-	++	+++	++	++	++	+++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	ND

(-) null, (+) weakly positive, (++) moderately positive; (+++) strongly positive; N — nucleus; C — cytoplasm; ND — not determined; SN — supranuclear

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- $\beta$  oestradiol, but some of them presented either non-marked cytoplasm or non-marked nuclei, while the



**Figure 2.** Immunohistochemistry of aromatase, androgen receptor (AR) and 17- $\beta$  oestradiol (E2) in the efferent ducts of the sand rat *Psammomys obesus*; **A, C, E.** Breeding season; **B, D, F.** Resting season. Panels A and B illustrate immunohistochemistry of aromatase. During the breeding season (A), the immunohistological staining was mainly located in ciliated cells (CC) and smooth muscle cells (SMC). During the resting season (B) some ciliated cells and some nonciliated cells were marked while others were not, smooth muscle cells and fibroblast were negative. Panels C and D illustrate immunohistochemistry of AR. During the breeding season (C), the signal was found in ciliated cells (CC), basal cells (BC), smooth muscle cells and extruded cells (EC), but not in nonciliated cells (NCC). During the resting season (D) ciliated cells, nonciliated cells, fibroblast and smooth muscle cells were stained, whereas some ciliated cells were not. Panels E and F illustrate immunohistochemistry of E2. During the breeding season (E) 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 (F) 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.

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

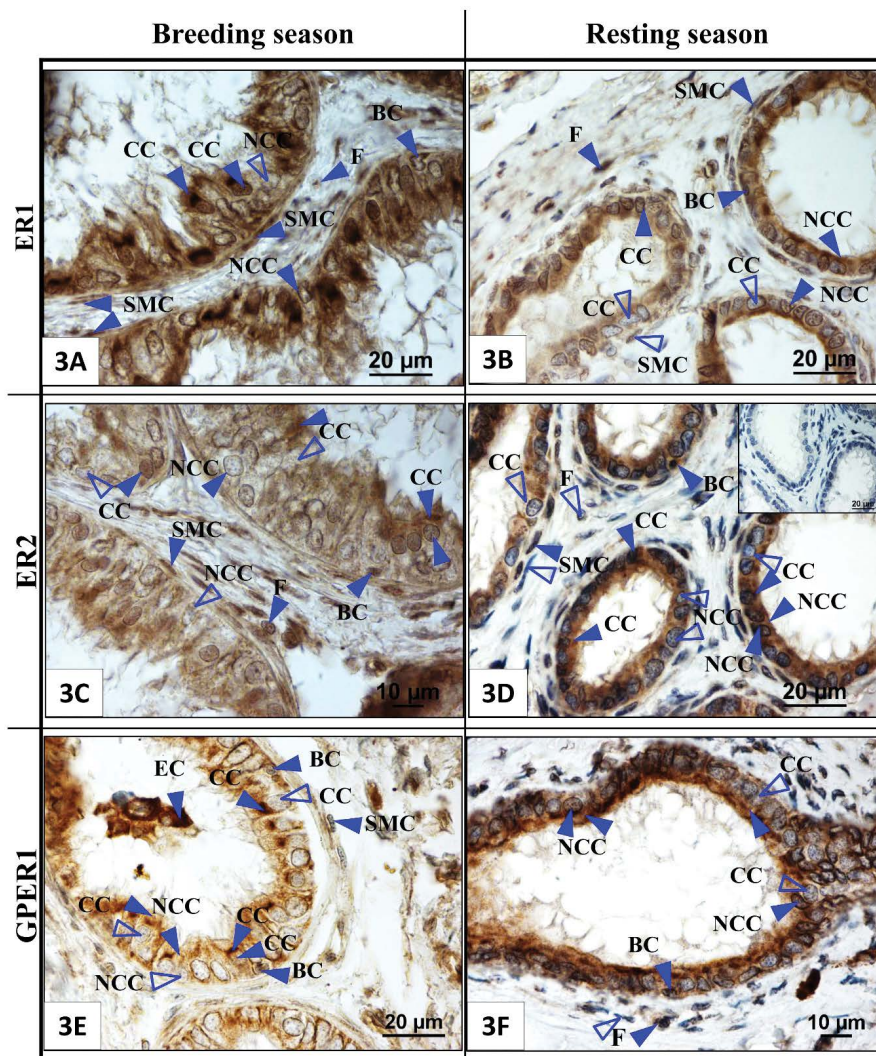
#### Androgen receptor (AR)

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.

#### Oestrogen 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



**Figure 3.** Immunohistochemistry of the oestrogen receptor 1 (ESR1), the oestrogen receptor 2 (ESR2) and the G protein-coupled oestrogen receptor 1 (GPER1) in the efferent ducts of the sand rat *Psammomys obesus*; **A, C, E.** Breeding season; **B, D, F.** Resting season. Panels A and B illustrate immunohistochemistry of ESR1. During the breeding season (A), the immunohistological staining was mainly located in ciliated cells (CC), basal cells (BC), fibroblast and smooth muscle cells (SMC); some nonciliated cells (NCC) were also marked. During the resting season (B) nonciliated cells, basal cells and fibroblast are positive, some ciliated cells and smooth muscle cells are negative. Panels C and D illustrate immunohistochemistry of ESR2. During the breeding season (C), 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 (D), ciliated cells, nonciliated cells, basal cells, fibroblast and smooth muscle cells were immunoreactive, whereas others were not, basal cells were also stained. Panels E and F illustrate immunohistochemistry of GPER1. During the breeding season (E) 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 (F) 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.

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.

**Oestrogen 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 oestrogen receptor 1 (GPER1)**

During the breeding season, a supranuclear immunohistochemical 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 non-ciliated 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

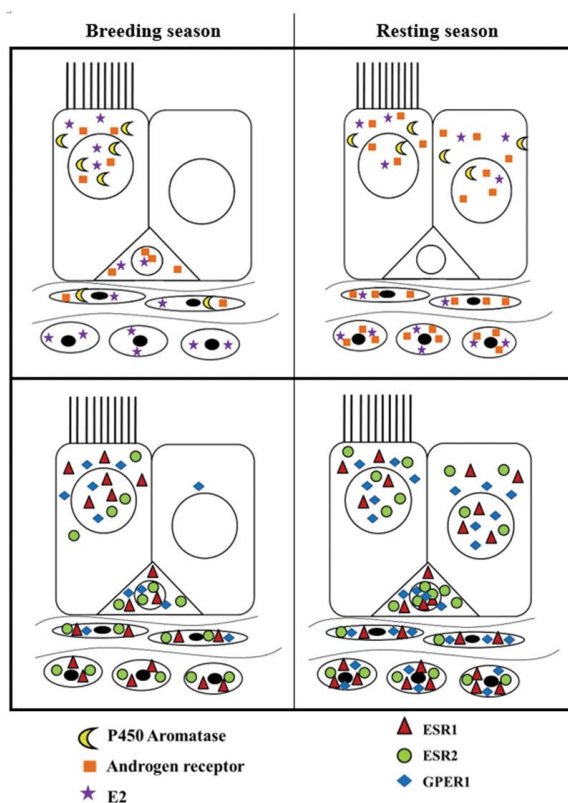
Considering that, *Psammomys obesus* has a seasonal reproduction cycle, several studies performed to describe this cycle in both hormonal [34] and cytophysiological terms [19, 20, 45]. While there are few studies dealing with the effects of seasonal variation on the histology and the cytology of the 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. [52] 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-Spychalowicz [20] and Menad et al. [43] 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. [52] obtained similar results. In the epididymis, such observation was described in the sand rat [43] and the Libyan jirds [5]. The same author reported an accumulation of collagen I and III in seminal vesicles in the jirds [4], 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 matrix metalloproteinases and their inhibitors TIMPS [3].

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 localisation of the P450 aromatase, E2, AR 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).



**Figure 4.** Schematic representation of the expression of P450 aromatase, 17- $\beta$  oestradiol (E2), androgen receptor, the oestrogen receptor 1 (ESR1), the oestrogen receptor 2 (ESR2) and the G protein-coupled oestrogen receptor 1 (GPER1) in the efferent ducts of the *Psammomys obesus* during the breeding season and the resting season.

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. [52] reported similar data in the big fruit-eating bat, which support the idea that oestrogen is locally synthesised in the efferent ducts. In the testis, there was a reduced concentration of aromatase during the regression season [6, 71, 76], the unchanged aromatase localisation 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. [52]. Several studies showed that AR levels in the efferent ducts are lower than in the epididymis [21, 62, 72]. Androgen regulates AR, so during the resting season, even if there is severe changes in testosterone

levels, another AR ligand like dihydrotestosterone or androstenedione may be responsible for controlling levels of AR. This may suggest that efferent ducts functions depends on the androgen during both seasons.

Oestrogen 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 [8, 27] as well as in the epididymis [43]. 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 localisation of this receptor in the ciliated cells, basal cells, fibroblasts and smooth muscle cells. Oliveira et al. [52] and Joseph et al. [33] 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, 29], mice [63], roosters [35], dogs, cats [48], goats [21], monkeys [75] and humans [13]. According to Hess et al. [29] and Hess et al. [25], 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 [29] by controlling ion transporters in the epithelium and altering the proteins involved in fluid reabsorption [32, 36, 37, 70, 78], this regulation is essential for male fertility [12, 26, 49, 51, 78]. In the ESR1 knockout mice, a dramatic dilatation in the epithelium of the rete testis [12], due to the inactivation of ESR1 in the efferent duct [29], a reduce of the epithelium height, the absence of microvilli and the endocytic apparatus, distension of the efferent duct was reported [29]. The recent data provided by Nanjappa et al. [47] indicated both nuclear and membrane localisations 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. [52] 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. [52] 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 [38].

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 localisation in the endoplasmic reticulum or Golgi apparatus [1, 15, 39, 56, 61, 69].

In the sand rat, it seems like the immunoeexpression 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 oestrogenic signal transduction [14, 30, 57]. In fact, the co-expression of ESR1 and ESR2 with GPER in breast cancer cells suggest a possible interaction between these receptors [14, 30, 57] as well as in uterine epithelial cells, where the GPER1 functions as inhibitor of cell growth mediated by E2 through the ESR1 [18].

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 [53]. 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.

#### Funding source

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