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Seasonal variations of testis anatomy and of G-coupled oestrogen receptor 1 expression in *Gerbillus gerbillus*

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Abstract

The gerbil, *Gerbillus gerbillus*, a nocturnal desert rodent of northern Africa, exhibits a seasonal reproductive cycle with marked anatomical and behavioural shifts between breeding season and resting season. The aim of this study is to investigate key elements involved in these seasonal changes, specifically in males: the histology of the testis as well as the expression of the G-protein-coupled oestrogen receptor 1 (GPER1) in the testis. During the breeding season, the seminiferous tubules were full of spermatozoa, and their epithelium contained germinal cells embedded in Sertoli cells. Amidst tubules, well-developed Leydig cells were observed around blood vessels, with peritubular myoid cells providing structural and dynamic support to the tubules. GPER1 was largely expressed throughout the testis. Notably, Leydig cells, spermatogonia and spermatocytes showed strong immunohistochemical signals. Sertoli cells, spermatozoa and peritubular myoid cells were moderately stained. During the resting season, spermatogenesis was blocked at the spermatocyte stage, spermatids and spermatozoa were absent and the interstitial space was reduced. The weight of the testis decreased significantly. At this stage, GPER1 was found in Leydig cells, spermatocytes and peritubular myoid cells. Sertoli cells and spermatogonia were not marked. Overall, the testis of the gerbil, *Gerbillus gerbillus*, has undergone noticeable histological, cellular and weight changes between seasons. In addition, the seasonal expression pattern of GPER1, with pronounced differences between resting season and breeding season, indicates that this receptor is involved in the regulation of the reproductive cycle.

KEYWORDS
breeding season, free ranging, oestrogen, resting season, testis

1 | INTRODUCTION

The gerbil, *Gerbillus gerbillus*, is a small, primarily nocturnal rodent adapted to extreme environmental conditions that prevail in the arid deserts and semi-deserts of northern Africa. Drastic seasonal climatic fluctuations shape the reproductive cycle of this small mammal. The breeding season starts in early winter (December–January) and peaks in early spring (April) when feeding resources are available. The rapid increase in ambient temperature associated with the decrease in precipitation and foraging opportunities induce the resting season; sexual activity strongly declines in spring (May–June) and reaches its nadir in autumn (September–October) (Amirat et al., 1977). In males, maturity occurs when body mass reaches 17–18 g (Klein et al., 1975), and the testis undergoes radical seasonal changes. Testis mass increases significantly during the breeding season and decreases during the resting season (Amirat et al., 1977). Plasma concentration of testosterone, testicular content of testosterone and androstenedione exhibit parallel seasonal changes with high and low values, respectively, in April and autumn (Amirat et al., 1977).

Besides androgens, other steroids, notably oestrogens, play a key role in male reproductive physiology; they influence the hypothalamic–pituitary-testis axis and spermatogenesis by acting on Sertoli, Leydig and germ cell functions (Hess et al., 1997; O'Donnell et al., 2001). Oestrogens are involved in spermatogonial stem cell division (Miura et al., 1999). They initiate, maintain spermatogenesis (Ebling et al., 2000) and promote germ cells survival (Pentikäinen et al., 2000). In the reproductive tract, oestrogens are mainly secreted by germ cells, spermatozoa and Leydig cells (Carreau & Hess, 2010; Carreau & Silandre, 2007; Payne et al., 1976). The effects of oestrogens, mediated via the nuclear receptors ESR1 and ESR2 (oestrogen receptor 1 and oestrogen receptor 2 genes), are represented by nuclear genomic regulations (Kuiper et al., 1997). These receptors are widely expressed in the testis of mammalian species such as rats, mice, goats, boars, humans and other primates; however, the pattern of cellular localisation differs among lineages and between types of receptor (Chimento et al., 2010; Lucas et al., 2010; Menad et al., 2017; Sheng & Zhu, 2011; Sirianni et al., 2008; Vaucher et al., 2014; Zhang et al., 2014). The seven-transmembrane G protein oestrogen receptor1 (GPER1) is also involved; it prompts rapid non-genomic cellular responses (Hess et al., 2011; Prossnitz & Barton, 2014; Rago et al., 2011) and is mainly found in the endoplasmic reticulum compartment (Prossnitz & Barton, 2014; Sirianni et al., 2008). Thus, oestrogens activate different pathways and trigger different responses when binding to ESRs versus GPER1 receptor. ESRs (oestrogen receptors) control transcription factors and directly regulate gene expression, while GPER1 activates proteins that modulate transcription factor activity (Filardo & Thomas, 2005; Prossnitz et al., 2007; Prossnitz & Maggiolini, 2009; Revankar et al., 2005). For instance, ESR1 can trigger delayed cell proliferation, but GPER1 can prompt rapid anti-apoptotic effects in cultured Sertoli cells. Still, complex crosstalk between these two different regulatory pathways may occur (Lucas et al., 2010). To elucidate the precise functions of oestrogens in male reproductive physiology, notably those mediated by GPER1, a major prerequisite is to locate cellular sites of action, hence the tissular distribution of this receptor, and to track changes in its expression.

Seasonal changes in the localisation and intensity of the expression of oestrogen receptors have been monitored in the testis of one small desert rodent, the sand rat *Psammomys obesus* (Menad et al., 2017). Experimental investigations notably revealed marked seasonal variations in the histology of the reproductive tract, hormone levels and expression of several proteins, oestrogen receptors and other hormone receptors (Menad et al., 2017). These seasonal changes are associated with the fluctuations of trophic and hydric resources; likely, they optimize reproductive success, and thus they are considered adaptive (Menad et al., 2017). However, results obtained in a single species preclude generalisation. Further studies are needed to assess to what extent the patterns observed in the sand rat also exist in other small desert rodents that face similar environmental constraints. Indeed, GPER1 localisation in the reproductive tract has been mainly investigated in laboratory animals but rarely in free-ranging animals. Thus, the implication of oestrogen receptors in the seasonal regulation of the reproduction of free-ranging mammals remains particularly understudied. Species that exhibit drastic seasonal fluctuations in their reproductive activity provide excellent opportunities to fill up this paucity of information.

This study was designed to examine seasonal changes in the histology of the testis during breeding season and resting season of *Gerbillus gerbillus*, a small desert rodent that expresses a strong seasonality in its annual activity cycle (Amirat et al., 1977). We notably aimed to study the expression pattern of GPER1, considering its importance in mediating oestrogen action on the reproductive tract and on spermatogenesis.

2 | MATERIALS AND METHODS

2.1 | Ethical note

All experiments complied with the Algerian legislation (Law Number 95–322/1995) regarding the protection of animals involved in experimental and other scientific purposes, as well as 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).

2.2 | Animals and samples

Sixteen adult males *Gerbillus gerbillus* (mean weight 29.25 ± 2.27 g) were captured in the wild of Béni Abbès area (30°07′N; 2°10′W). Eight animals were caught during the breeding season in January and March (Group 1), and eight were caught during the resting season in June and July (Group 2). They were housed in outdoor enclosures (120 × 60 × 60 cm); natural food (Daly & Daly, 1973), fresh

water and shelter were provided. They were subjected to the natural environment, ambient temperature and photoperiod. They were euthanized using decapitation 48 h after capture, and their testis were quickly removed, weighted and fixed in Bouin's solution.

2.3 | Histology

The testis underwent the classical histology procedure, dehydrated in increasing concentrations of ethanol (70%, 95% and 100%), cleaned in toluene and embedded in paraffin. Samples were then sectioned, using a Leitz microtome, into 5 µm slices and mounted on histological Superfrost®glass slides (Thermo Scientific, Menzel-Gläser, Brausschweig, Germany). Dewaxed sections were rehydrated in decreasing concentrations of ethanol (100%, 95% and 70%) and stained with Masson's trichrome (Gabe, 1976; Martoja & Martoja-Pierson, 1967). The cytoplasm appeared pink; nuclei were black; peritubular myoid cells showed a red colour; and collagen was green.

2.4 | Immunohistochemistry

Immunohistochemistry was used to localize GPER1 in the testis (Menad et al., 2014). After deparaffinisation and rehydration, the slides were washed in tap water for 10 min, then with PBS (phosphate buffered saline), and incubated at 95°C in a 10 mM sodium citrate solution (H-3300, pH 6.0) for 45 min. The slides were left to chill for 20 min, washed in distilled water and immersed in a 3% peroxidase solution for 20 min, followed by two baths of distilled water to block the endogenous peroxidase activity. The testis sections were bordered by drawing circles with DakoPen (Dako, Glostrup, Denmark), incubated with 10% normal goat serum (S-1000) for 1 h at room temperature to block non-specific binding sites, then incubated with the GPER1 (ab39742, Abcam plc, Cambridge, UK, GPER1 antibody was used as 1:50 dilutions in PBS) primary antibody in a wet chamber for 2 h. Other slides were incubated with normal goat serum instead of the primary antibody and thus served as negative controls. All slides were washed in PBS solution before being reintubated again with the secondary biotinylated antibodies (Anti-Mouse IgG/Rabbit IgG; BA-1400, Vectastain Universal, Vector Laboratories, Burlingame, CA, USA) for 1 h in a wet chamber. Lastly, the slides were rinsed three times in PBS for 5 min and incubated with a streptavidin-biotin-peroxidase complex for 1 h. As for staining, a DAB chromogen (3,3-diaminobenzidine, kit for peroxidase; Vector Laboratories) was added for 1 min and washed with PBS before and after staining. Haematoxylin (Hematoxylin QS, H-3404; Vector Laboratories) was used for 1 min to counterstain the slides. Finally, the slides were dehydrated and preserved using the Permount mounting medium (Thermo Fisher Scientific), observed using the Nikon Eclipse E 400 light microscope, and pictured with the Nikon DXM 1200 digital camera. The immunohistochemical signal intensity was rated by two observers unwary of the receptor under study: null (−), weakly positive (+), moderately positive (++) or strongly positive (+++).

2.5 | Statistical analysis

After verifying normality, testicular weights were compared using the Student's *t*-test. All calculations were performed using OriginPro 8.0 software (OriginLab Corp.). A probability below 0.001 was considered significant.

3 | RESULTS

3.1 | Testicular mass

The mean testicular mass was significantly lower during the resting season compared to the breeding season (mean ± SD, standard deviation, respectively: 0.108 ± 0.004 g vs. 0.183 ± 0.005 g; $p < 0.001$; Figure 1).

3.2 | Histology

Masson's trichrome staining revealed differences between breeding season and resting season. During the breeding season, at low magnification, the testis had a lobular architecture composed of well-developed seminiferous tubules with an interstitial compartment (Figure 2, Panel 2a). The testis was encapsulated into a thick, fibrous albuginea (Figure 2, Panel 2b). At higher magnification, the seminiferous tubules revealed a typical functionally high and stratified epithelium containing germinal cells (spermatogonia, spermatocytes, spermatids and spermatozoa) settled between Sertoli cells, while the lumen was full of spermatozoa (Figure 2, Panel 2c). The basement membrane of the tubules was attached to peritubular myoid cells (Figure 2, Panel 2c). In the interstitial compartment, composed of connective tissue, we observed dispersed or aggregated Leydig cells around blood vessels (Figure 2, Panel 2d).

In contrast, during the resting season, at low magnification, the testis was filled up with narrow seminiferous tubules, separated by the interstitial space formed of reduced connective tissue and Leydig cells (Figure 3, Panel 3a). The fibrous capsule surrounding the seminiferous tubules was very thick (Figure 3, Panel 3b). At high magnification, the seminiferous tubules were narrow and empty as spermatogenesis was blocked at the stage of spermatocyte I; therefore, there were neither spermatids nor spermatozoa (Figure 3, Panel 3c). In the interstitial space, we observed Leydig cells, blood vessels and peritubular myoid cells surrounding the seminiferous tubules (Figure 3, Panel 3d).

3.3 | Immunohistochemistry

The results of immunohistochemistry for GPER1 in the testis of *Gerbillus gerbillus* are summarized in Table 1.

During the breeding season, GPER1 was ubiquitously expressed in the testis. Leydig cells, spermatogonia and spermatocytes showed

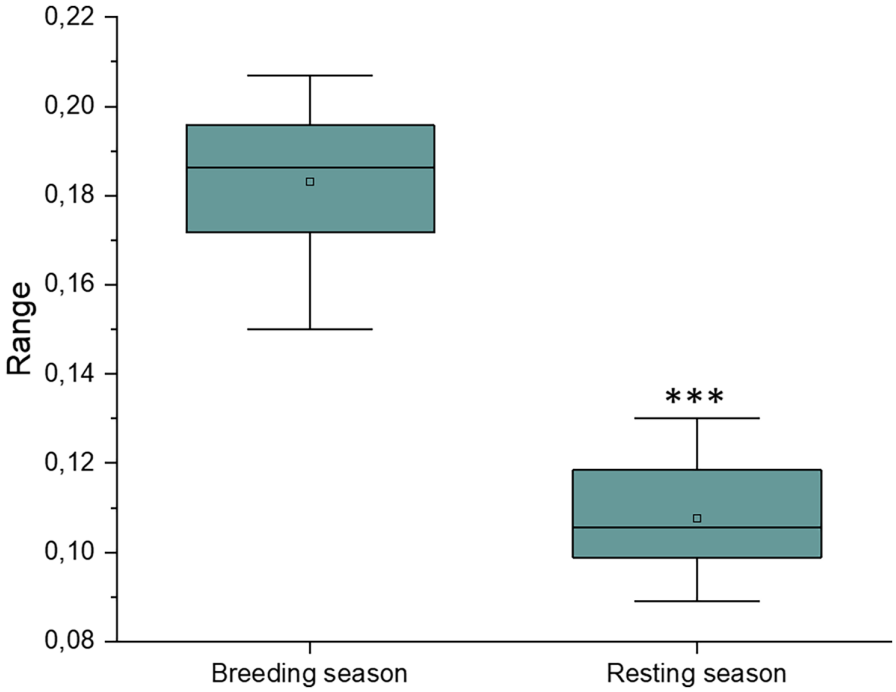

FIGURE 1 Average testicular mass of *Gerbillus gerbillus* during the breeding season and the resting season. *** $p < 0.001$

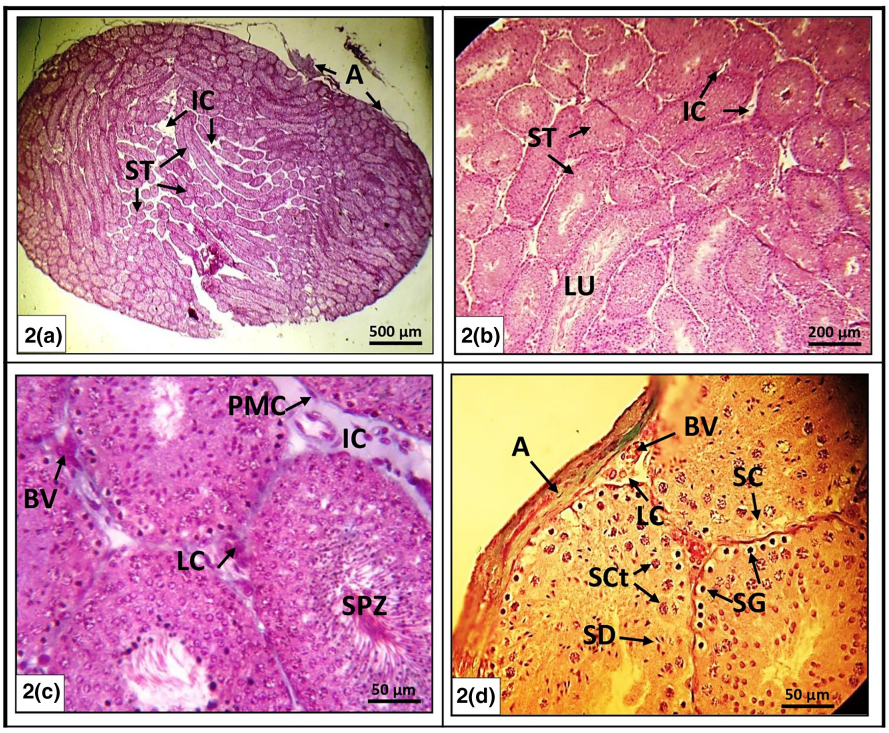

FIGURE 2 Histology of the testis of *Gerbillus gerbillus* during the breeding season. Panels a and b low magnification. The testis was made up of seminiferous tubules (ST) dispersed in interstitial compartment (IC), all surrounded with albuginea (A). Panels c and d High magnification. The seminiferous epithelium was composed of Sertoli cells (SC) that maintain germinal cells: spermatogonia (SG), spermatocytes (SCt), spermatids (SD), the lumen (LU) was full of spermatozoa (SPZ). The interstitial compartment was formed of connective tissue, Leydig cells (LC), blood vessels (BV) and peritubular myoid cells (PMC) around the tubules. Masson's trichrome staining.

strong cytoplasmic immunohistochemical staining; Leydig cells nuclei were weakly marked, and spermatocytes nuclei were not marked (Figure 4, Panel 4a). Sertoli cells, spermatids, spermatozoa and peritubular myoid cells appeared moderately stained (Figure 4, Panel 4b).

During the resting season, Sertoli cells did not express GPER1. However, Leydig cells showed moderate cytoplasmic immunohistochemical staining (Figure 4, Panel 4d). As for germinal cells, GPER1 was not found in spermatogonia but was strongly expressed by spermatocytes. Peritubular myoid cells exhibited a weak immunohistochemical signal (Figure 4, Panel 4e).

4 | DISCUSSION

This study provides new insights regarding histological changes and the distribution pattern of GPER1 in the testis of *Gerbillus gerbillus* during the reproductive cycle (Figure 5). This nocturnal desert rodent

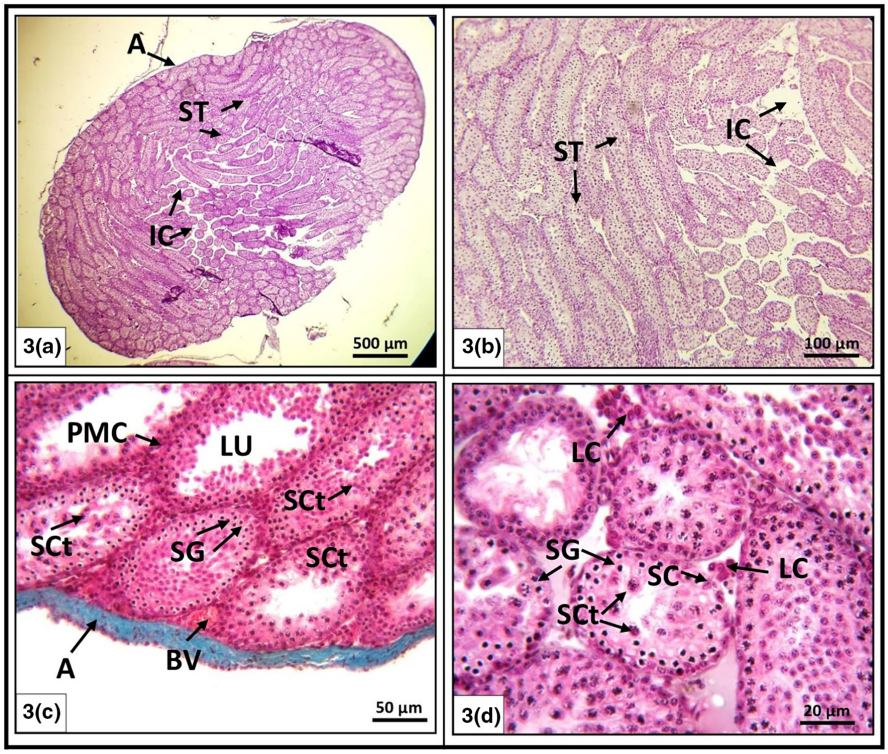

FIGURE 3 Histology of the testis of *Gerbillus gerbillus* during the resting season. Panels a and b Low magnification. The seminiferous tubules (ST) seemed narrow and empty; the interstitial compartment (IC) looked less developed. Panels c and d High magnification. The seminiferous epithelium contained only spermatogonia (SG) and spermatocyte (SCt) maintained by Sertoli cells (SC). Lumen was empty, there was no spermatozoa since spermatogenesis was blocked at the stage of spermatocyte I. In the interstitial compartment, we observed some Leydig cells (LC) in connective tissue around blood vessels (BV). Masson's trichrome staining.

TABLE 1 Immunolocalisation of GPER1 in the testis of *Gerbillus gerbillus*.

Localisation		Breeding season	Resting season
Sertoli cells	N	/	/
	C	+/++	–
Leydig cells	N	+	/
	C	+++	++
Spermatogonia	N	/	/
	C	+++	–
Spermatocyte	N	–	–
	C	++/+++	+++
spermatid	N	/	/
	C	++	/
Spermatozoa	N	/	/
	C	++	/
Peritubular myoid cell	N	/	/
	C	+/++	+

Abbreviations: N, nuclei; C, cytoplasm.

breeds from April to September (Amirat et al., 1977); a similar pattern was also observed in a variety of desert rodents (e.g. *Meriones libycus*, *Massouteria mzabi*, *Jaculus orientalis*, *Mesocricetus auratus* and *Phodopus sungorus*) (Cheniti Lamine, 1974; Hoffman & Reiter, 1965; Hoffmann, 1973; LeBerre & Chevallier, 1990). However, other rodents breed during different periods of the year, including species that live in the same biotope as *Gerbillus gerbillus*. For example, the sand rat (*Psammomys obesus*) breeds from autumn to early spring (Khammar, 1987). The small Gerboise (*Jaculus jaculus*) breeds in Fall–Winter (Ghobrial & Hodieb, 1973), the Dwarf Gerbil (*Gerbillus nanus*) in winter (LeBerre & Chevallier, 1990), the Saharian Goundi (*Ctenodactylus gundi*) in winter–spring (Gouat, 1985) and the white-bellied Gerbil (*Gerbilliscus leucogaster*) in autumn-winter–spring (Muteka et al., 2018). These differences suggest that contrasted regulatory mechanisms allow individuals to integrate the fluctuations of environmental conditions (e.g. ambient temperature, precipitation, humidity and food availability) with photoperiod and possibly with other factors (e.g. predatory pressure) to optimize reproductive output (Aschoff, 1955; Heldmaier et al., 1989). To decipher the diversity of the processes that link complex relationships between environmental variables and reproductive cycles in desert rodents, it is essential to investigate hormonal regulation at different levels.

During the breeding season, in our study, the testis of *Gerbillus gerbillus* included a set of large seminiferous tubules formed by a highly organized epithelium and surrounded by peritubular myoid cells. The seminal epithelium is formed of Sertoli cells that support germinal cells (spermatogonia, spermatocytes, spermatids and spermatozoa). The lumen was full of sperm, and the interstitial space was composed of Leydig cells isolated or grouped around blood vessels. In stark contrast, during the resting season, a marked atrophy of the seminiferous epithelium was associated with a blockage of spermatogenesis, spermatids and spermatozoa were absent, this explains the narrow empty appearance of the lumen. In addition, the interstitial space was reduced. The results of the current work mirror those obtained in sympatric species (*Psammomys obesus*, *Gerbillus tarabuli* and *Gerbilliscus leucogaster*) (Gernigon-Spychalowicz, 1992; Hamidatou Khati & Hammouche, 2021; Khammar, 1987; Menad et al., 2017; Muteka et al., 2018). Moreover, the changes observed in Leydig cells

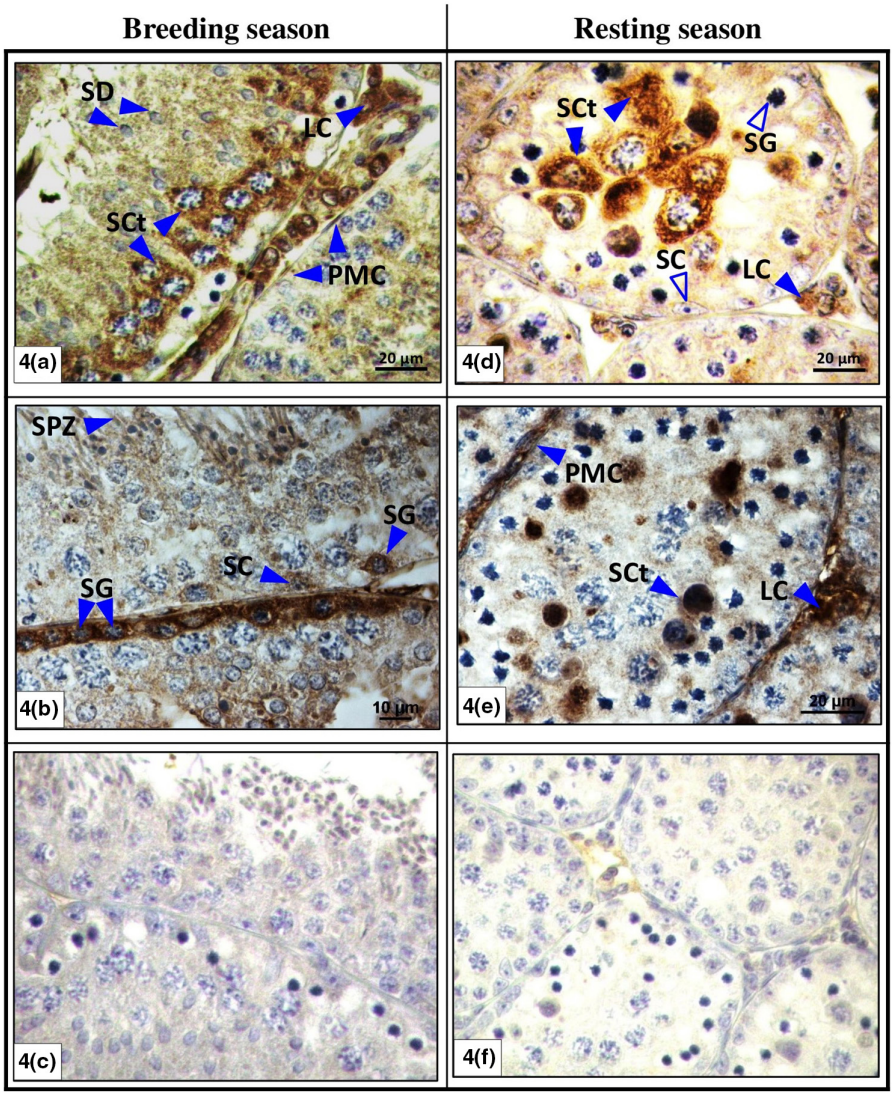

FIGURE 4 Immunolocalisation of GPER1 in the testis of *Gerbillus gerbillus*. Panels a and b during the breeding season, the immunohistochemical signal was found all over the testis. Spermatogonia (SG), spermatocytes (SCt) and Leydig cells (LC) presented a strong staining. While Sertoli cells (SC), spermatids (SD), spermatozoa (SPZ), fibroblasts (F) as well as peritubular myoid cells (PMC) were moderately marked. Panel c negative control. Panels d and e during the resting season, we observed the absence of GPER1in Sertoli cells, spermatogonia (SG). However, spermatocytes (SCt) were strongly marked, Leydig cells (LC) were moderately stained, peritubular myoid cells (PMC) presented a weak signal. Panel f negative control. Empty arrows: absence of immunohistochemical staining. Full arrows: presence of immunohistochemical staining.

in our study, the increase of the nucleo-cytoplasmic ratio and the reduction of cytoplasmic volume noticed during the resting season in *Psammomys obesus* and *Gerbillus tarabuli*, evince the inhibition of the metabolic activity of Leydig cells (Gernigon-Spychalowicz, 1992; Hamidatou Khati & Hammouche, 2021).

Seasonal variations in the testicular activity in seasonal breeders have been documented in desert rodents (*Psammomys obesus*, *Gerbillus gerbillus*, *Gerbillus tarabuli* and *Gerbilliscus leucogaster*); testicular mass, testicular and plasma concentrations of testosterone decrease significantly during the resting season (Amirat et al., 1977; Boufermes et al., 2013; Gernigon-Spychalowicz, 1992; Hamidatou Khati & Hammouche, 2021; Khammar, 1987; Khammar & Brudieux, 1987; Muteka et al., 2018). In these cases, the interplay among histological compartments, hormonal levels and the regulation of receptors provides evidence that breeding is stimulated during a brief period when resources are available. It would be interesting to investigate to what extent this pattern is widespread and modulated in desert rodents, especially in species that exhibit a different annual pattern (e.g. winter breeders).

Actually, there are differences among species. In comparison to the gerbil, in the sand rat, the immunolocalisation pattern of GPER1 was broadly similar but with different signal intensity (Menad et al., 2017). In the absence of comparable studies on other free-ranging animals, we cannot easily interpret these differences. Yet, abundant studies in captive animals and in wild animals that do not face drastic environmental fluctuations during the year provide opportunities to discuss our results. Unfortunately, most captive breeding programs are motivated by conversational objectives, and natural environmental constraints are artificially relaxed (Conde et al., 2011). Captive animals exhibit strong peculiarities regarding their health, genetics, nutrition, behaviour, physiology and reproductive status, making comparisons with free-ranging individuals difficult (Clauss et al., 2008; Edwards et al., 2015; Scheun et al., 2016; Van der Weyde et al., 2016). Furthermore, reproduction is not guaranteed in captivity, likely because the key environmental determinants are lacking (Christie et al., 2012; Farquharson et al., 2021). In breeding depression, inappropriate social development, maternal

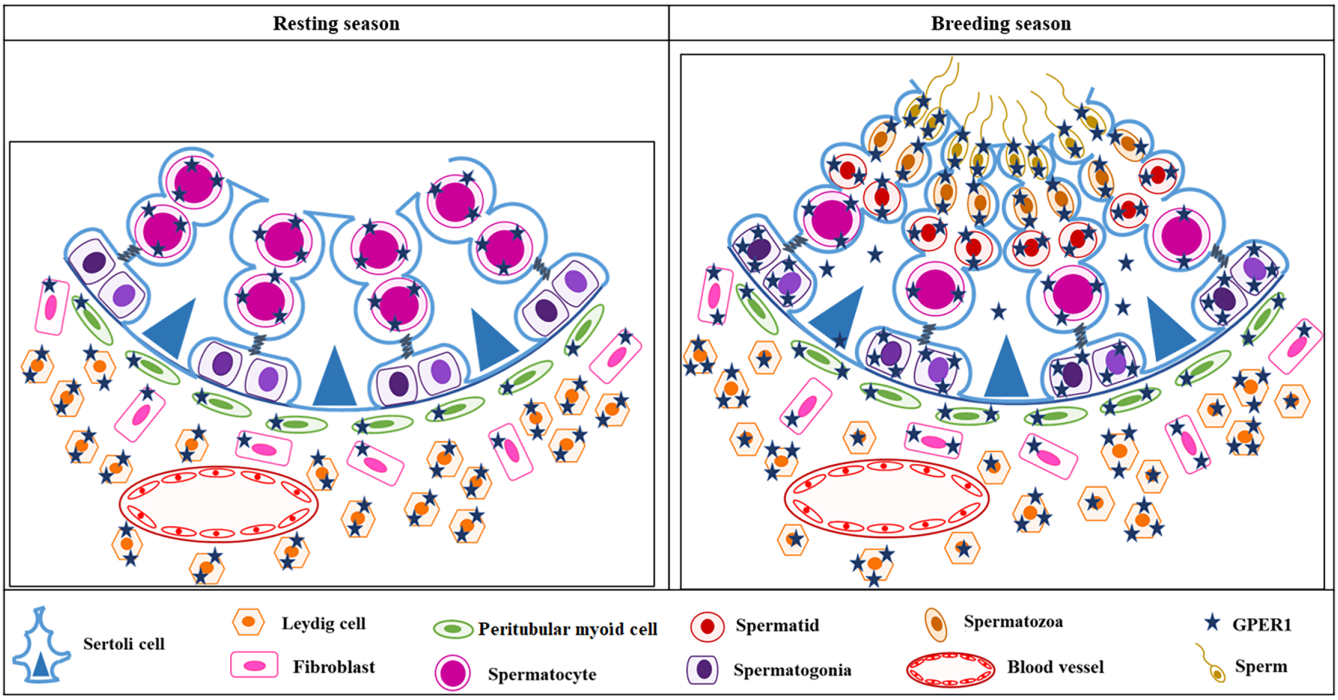

FIGURE 5 Schematic representation of the expression of GPER1 in the testis of *Gerbillus gerbillus* during the breeding season and the resting season.

effects, unbalanced food or stress may all hamper reproduction (Boakes et al., 2006; Kiik et al., 2013; Levallois & De Marigny, 2015; Matos, 2012).

Abundant information suggests that oestrogen plays a key role in male reproductive cycles. In this study, Leydig cells express GPER1 during the whole year, which supports the idea that the activity of Leydig cells is regulated by E2 (Pelletier & El-Alfy, 2000; Saunders et al., 1998). Similar results were found in the sand rat (*Psammomys obesus*), where Leydig cells not only expressed GPER1, but also ESRs, E2 and aromatase (Menad et al., 2017). GPER1 is expressed in the endoplasmic reticulum and Golgi apparatus (Prossnitz et al., 2007), as well as in the plasma membrane (Filardo et al., 2007), so the GPER1 found in the nuclei of Leydig cells during the breeding season may be expressed in the endoplasmic reticulum. Moreover, Leydig cells are the main sources of testosterone (Huhtaniemi & Teerds, 2018), and their expression of the GPER1 may be an indicator of the regulation of their function by E2 via the GPER1. Isolated human and rat Leydig cells expressed GPER1 and released less testosterone when treated with E2 and GPER1 antagonist (G1) (Vaucher et al., 2014), this may explain the decreased levels of testosterone during the resting season in *Gerbillus gerbillus* (Khammar & Brudieux, 1987). Furthermore, studies conducted on mice revealed the contribution of GPER1 with ESRs and aromatase in regulating oestrogen concentration and secretion in Leydig cells. GPER1 blockage decreased GPER1 mRNA levels but increased aromatase, ESR1 and ESR2 mRNA levels. In contrast this treatment had no effect on testis histology but Leydig cells had large mitochondria and numerous lipid droplets (Kotula-Balak et al., 2018). GPER1 subcellular localisation could be found in: plasma membrane, endoplasmic reticulum and Golgi apparatus (Chimento et al., 2014), and it is downregulated in the Golgi apparatus (Cheng et al., 2011) suggesting the importance of the GPER1 in the Golgi complex and mitochondrial functions. Moreover, GPER1 is involved in insulin secretion in β pancreatic cells (Sharma & Prossnitz, 2011) and lipid metabolism (Santolla et al., 2012). Besides, in the testis of *Gerbillus gerbillus*, Leydig cells expressed GPER1 during the whole year, reflecting the importance of GPER1 regulation of Leydig cell functions during the reproductive cycle. Hence, these data may confirm the ability of E2 via the GPER1 to control steroidogenesis in the Leydig cells (Hess, 2003; Vaucher et al., 2014) as found in the fish gonads (Pang & Thomas, 2010; Thomas et al., 2006). In addition, Leydig cells are involved in Sertoli cells proliferation and maturation via the GPER1. Furthermore, it is well known that Sertoli cells play a significant role in maintaining spermatogenesis (Jeégou & Rolland, 2018), and their functions are regulated by E2 (Hess & França, 2005). In our study, Sertoli cells expressed GPER1 only during the breeding season, where spermatogenesis was active, suggesting that E2 regulates Sertoli cells via GPER1 in the testis of *Gerbillus gerbillus*. Moreover, in humans and immature rats, ERs (ESR1, ESR2 and GPER1) are present in the Sertoli cells (Filipiak et al., 2012; Lucas et al., 2008), as observed in the Sertoli cells of the sand rat (Menad et al., 2017). Nevertheless, in Sertoli cells, GPER1 mediates MAPK3/ERK1 (mitogen-activated protein kinase 3/extracellular signal-regulated kinase 1) pathway through the activation of EGFR (epidermal growth factor receptor), hence the activation of the GPER/EGFR/MAPK3/1 signalling pathway increased

Bcl-2 and decreased Bax expression leading to anti-apoptotic effects (Lucas et al., 2011, 2010; Royer et al., 2012). Thus E2 mediates (Lucas et al., 2014), via GPER1, gene regulation and apoptosis, to maintain Sertoli cell functions as well as normal testis development and homeostasis (Lucas et al., 2010). Oestrogens regulate seasonal functions of the testis in free-ranging vertebrates (Beguelini et al., 2014; Caneguim et al., 2013; Oliveira et al., 2009; Schön & Blottner, 2008; Zarzycka et al., 2016). According to Zarzycka et al., 2016, GPER1 expression was higher during the long days (long photoperiods) compared with the short days (short photoperiods), which suggests that GPER1 regulates seasonal testis activity (Zarzycka et al., 2016), while in our study, the breeding season is during the short days of winter and is associated with food availability. In the roe deer bucks, the expression of the GPER1 was similar in the testis compared with the adrenal gland during the pre-rut period (Pawlicki et al., 2023). Leydig cells were weakly marked, and testosterone levels were higher than oestrogen levels. Comparable results found in our study on *Gerbillus gerbillus* were Leydig cells expressed GPER1 during both seasons and testosterone levels decreased during the resting season (Amirat et al., 1977). Research performed by Milon and colleagues reported the presence of a novel cell type, telocytes (TCs), in bank vole testis interstitium. These cells were present regardless of seasonal changes of the photoperiod throughout the year and GPER1 blockage did not affect their number and distribution (Milon et al., 2019). In the peritubular myoid cells, GPER1 was detected during both seasons as documented in the sand rat (Menad et al., 2017). This cell type is responsible for sperm transport along the seminiferous tubules, and it is under the control of E2 (Shughrue et al., 1996). In our study, all germ cells expressed GPER1 during the breeding season, but only spermatocytes were marked during the resting season; thus, E2 may also ensure the blockage of spermatogenesis during the quiescent period. In several species (e.g. roe deer, equine, bank vole and ground squirrel), oestrogens are involved in spermatogenesis (Gancarczyk et al., 2004; Gautier et al., 2016; Li et al., 2015; Schön & Blottner, 2008; Zhang et al., 2010) by interfering in the spermatid's differentiation (Robertson et al., 2002), spermatocyte and sperm maturation (O'Shaughnessy, 2014; Smith et al., 2015). The presence of GPER1 in spermatocytes and spermatids in the testis of *Gerbillus gerbillus* may be an indicator of oestrogen proliferative effect on the germ cells, via the activation of the epidermal growth factor receptor/extracellular signal-regulated kinases (EGFR/ERK) pathway (Chimento et al., 2014), as a result induce spermatogenesis during the breeding season and block spermatogenesis at the stage of spermatocytes during the resting season. In photo-regressed adult males of the Siberian hamster, oestrogens initiate spermatogenesis independently of FSH (Follicle-stimulating hormone) (Pak et al., 2002). E2 inhibits germ cell apoptosis in human (Delbès et al., 2004; Mishra & Shaha, 2005; Pentikäinen et al., 2000), while in immature and adult rats, E2 promotes germ cell apoptosis (Blanco-Rodríguez & Martínez-García, 1997; Walczak-Jedrzejowska et al., 2007). Furthermore, E2 stimulates the mitotic division of spermatogonia (Pierantoni et al., 2009), and has proliferative effects mediated by crosstalk between GPER1 and ESR1 in mouse spermatogonial GC-1 cell line through the activation of EGFR/ERK/fos/cyclin D1 (Sirianni et al., 2008). In Sprague–Dawley rats, E2 and G1 (the selective GPER1 agonist) down-regulate the expression of cyclin A1 and B1 mRNA and upregulate the pro-apoptotic factor Bax (B-cell lymphoma 2-associated X protein) by working through both ESR1 and/or GPER1 in pachytene spermatocytes to control the cell proliferation/apoptosis balance (Chimento et al., 2010; Sirianni et al., 2008). Moreover, in round spermatids, E2 controls apoptosis and differentiation by modulating the transcription of cyclin B1 and Bax through the activation of EGFR/ERK pathway (Chimento et al., 2011). However, this apoptotic effect of E2 was only observed during the breeding season in the toad (Scaia et al., 2015). In addition, germ cells have intense aromatase activity and are an important source of oestrogens (Levallet et al., 1998). Several studies have shown that E2 is important for sperm quality (Arkoun et al., 2014; Gautier et al., 2016; Müller et al., 2012; O'Shaughnessy, 2014) and motility (Aquila & De Amicis, 2014). In this study, spermatozoa expressed GPER1 as observed in sand rat and human (Franco et al., 2011; Menad et al., 2017). Interestingly, ERα (oestrogen receptor α) knockout mice presented immature spermatozoa (Lubahn et al., 1993), and a high pressure was observed in seminiferous tubules caused by fluid excess leading to germ cell alteration (Eddy et al., 1996; Hess, 2000). In contrast, ERβ (oestrogen receptor β) knockout mice displayed normal testis function, because an alternative transcript can replace the neutralized receptor (Antal et al., 2008). Nevertheless, GPER1 can also be involved in several pathogenesis of various cancer types, such as intratubular germ cell tumours, seminomas, embryonal carcinomas and teratomas, where its expression was investigated (Franco et al., 2011; Rago et al., 2011). In testicular germ cell carcinogenesis, GPER1 stimulates cell proliferation through the activation of PKA/MAPK pathways, a non-genomic GPER-dependent mechanism (Bouskine et al., 2009; Chevalier et al., 2012). However, even though GPER1 is expressed in testicular stromal neoplasms such as Leydig and Sertoli cell tumours (Carpino et al., 2007; Rago et al., 2011), its activation induces cell apoptosis. Treatment with G-1 (GPER1 agonist) activated intrinsic apoptotic mechanism ERK1/2 (Chimento et al., 2013).

Overall, although complex pictures emerge from these studies, the importance of oestrogens and their receptors in the regulation of fertility in males is clearly demonstrated. E2 stimulates testicular apoptosis during the reproductive season and influences steroidogenic enzymes in the toad *Rhinella arenarum* (Amphibia, Anura) (Scaia et al., 2015). Furthermore, E2 initiates spermatogenesis in photo-regressed adult males of the Siberian hamster (Pak et al., 2002). These results, in addition to our study about the expression pattern of GPER1 in the testis, may suggest that the regulation of testicular steroidogenesis and spermatogenesis by E2 may be via the membrane receptor GPER1.

This study describes the histological changes and expression pattern of GPER1 in the testis of the seasonal breeder *Gerbillus gerbillus* during the annual reproductive cycle. Our work can provide

more clarifications on the effect of seasonal fluctuations on the reproductive system and GPER1 distribution in free-ranging animals that exert different adaptative behaviours to withstand the environmental constraints. Hence, the histology of the testis, accurately, the seminiferous tubules morphology exhibited a significant atrophy during the resting season, reflecting the state of non-breeding activity during this period of time. Furthermore, our results, along with previous published studies on the expression of ESRs and GPER1, highlight season dependent oestrogen effects on testis physiology in *Gerbillus gerbillus* and *Psammomys obesus*. The influence of oestrogens via GPER1 and the classical ESRs (Menad et al., 2017) might be stimulatory during the breeding season and inhibitory during the resting season, controlling spermatogenesis either by modulating Sertoli cells role in spermatogenesis or by directly regulating germ cells proliferation and maturation. In addition, Leydig cells expressed GPER1 during both seasons, likely because these cells exert differential functions throughout the whole season, for example, to maintain minimal levels of circulating testosterone for the maintenance of territorial behaviours. Further studies are needed to clarify these issues. Nevertheless, studies about the histology of the testis, the morphology of the seminiferous tubules, in addition to exploring the different molecules expressed in the testis, can provide key details about the reproductive physiology in free-ranging animals as well as understanding the correlation between environmental conditions and animal reproduction.

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CONFLICT OF INTEREST STATEMENT

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

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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