Circannual Testis Changes in Seasonally Breeding Mammals

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Abstract

In the non-equatorial zones of the Earth, species concentrate their reproductive effort in the more favorable season. A consequence of seasonal breeding is seasonal testis regression, which implies the depletion of the germinative epithelium, permeation of the blood-testis barrier, and reduced androgenic function. This process has been studied in a number of vertebrates, but the mechanisms controlling it are not yet well understood. Apoptosis was assumed for years to be an important effector of seasonal germ cell depletion in all vertebrates, including mammals, but an alternative mechanism has recently been reported in the Iberian mole as well as in the large hairy armadillo. It is based on the desquamation of meiotic and post-meiotic germ cells as a consequence of altered Sertoli-germ cell adhesion molecule expression and distribution. Desquamated cells are either discarded alive through the epididymis, as in the mole, or subsequently die by apoptosis, as in the armadillo. Also, recent findings on the reproductive cycle of the greater white-toothed shrew at the meridional limits of its distribution area have revealed that the mechanisms controlling seasonal breeding are in fact far more plastic and versatile than initially suspected. Perhaps these higher adaptive capacities place mammals in a better position to face the ongoing climate change.

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Mechanisms Controlling Seasonal Breeding

Seasonal breeding is the lack of continual reproduction throughout the year in particular species or populations inhabiting non-equatorial areas of the Earth, due to circannual fluctuations of the environmental conditions resulting from climatic seasons. This is an adaptive process that ensures that the new individuals are born and grow up during the season that provides the best conditions for survival. In females, ovulation is halted in a particular period of the year, whereas in males testis function is also either reduced or depleted during the non-breeding season. Males undergo a reduction in testis size accompanied by lower testosterone production, spermatogenesis arrest, and inhibited mating behavior. In some species, spermatogenesis is completely arrested, and the animals are unable to breed for several months. This occurs in a variety of species, including hamsters [Bex and Bartke, 1977], deer [Brown et al., 1979; Clarke et al., 1995], brown bears [Tsubota et al., 1997], moles [Dadhich et al., 2010, 2013], and armadillos [Luaces et al., 2013, 2014].

In mammals, reproduction is a complex feature that is induced and controlled by the hypothalamus-pituitary-gonad axis (HPG). The hypothalamic neurons release the decapeptide GnRH (gonadotropin-releasing hormone) in the portal venous system, which in turn causes the release of gonadotrophic hormones LH (luteinizing hormone) and FSH (follicle stimulating hormone) in the anterior pituitary (hypophysis), which activates spermatogenesis and the production of gonadal steroids [Hayes et

al., 2001]. In seasonal breeders, the reproductive status of individuals must change cyclically between the active and the inactive stages, implying that the function of the HPG axis must also be modulated so that reproduction is activated during the breeding season and halted during the resting period. The question arises which factors are responsible for the circannual variations in reproduction. Environmental cues such as temperature, food availability, rainfall, and photoperiod are known to be involved in this process.

Photoperiod provides valuable information about the climatic seasons that remains constant from year to year, but response to photoperiod is species-specific and depends mainly on gestation length. The hamster, for instance, which has a short gestation (3 weeks) and reproduces in the spring and the summer, is a long-day breeder. On the other hand, sheep, goats, and deer, which have longer gestation lengths (5–6 months) mate in autumn during a period of decreasing day length and consequently are short-day breeders. Photoperiod is detected by the photoeuroendocrine system which is composed of the retina, the suprachiasmatic nucleus of the hypothalamus, where the master circadian clock resides, and the pineal gland [Pévet, 1988; Goldman, 2001; Schwartz et al., 2001]. The latter transduces day length into an endocrine signal in the form of rhythmic secretion of the hormone melatonin [Simonneaux and Ribelayga, 2003]. Pineal and plasma melatonin concentrations are low during the daytime and rise massively during the nighttime. Consequently, the profile of the nocturnal melatonin release fluctuates with photoperiod [Bartness et al., 1993; Pitrosky and Pévet, 1997]. Melatonin regulates GnRH secretion via 2 complementary mechanisms [Goldman, 1999]: a change in the steroid negative feedback on GnRH release [Tamarkin et al., 1976; Goodman et al., 1982; Karsch et al., 1993] and a direct steroid independent modulation of GnRH secretion [Bittman and Goldman, 1979; Goodman et al., 1982]. Although the molecular mechanisms controlling these processes are not completely known, one important intermediary component was discovered more recently. The complex formed by kisspeptins, a group of peptides of different lengths encoded by the *KiSS-1* gene, together with their receptor GPR54, represent a powerful activator of the HPG axis [Revel et al., 2006]. In the Syrian hamster, *Mesocricetus auratus*, melatonin regulates the expression of *KiSS-1* in the hypothalamic cells responsible for GnRH production, thereby controlling seasonal reproduction. In this species, exposure to short days results in a dramatic inhibition of reproductive activity manifested by a decrease

in serum LH, FSH and prolactin, accompanied by a complete suppression of spermatogenesis and a massive reduction of gonadal hormone biosynthesis. The visible consequences of these changes are the atrophy of the gonads and the accessory reproductive organs [Pévet, 1988; Bartke and Steger, 1992].

Photoperiod is considered the most common environmental cue controlling seasonal breeding, but a growing number of species are being shown to represent exceptions to this rule. In the Californian mouse (*Peromyscus californicus*), water restriction results in a reduction in the size of the reproductive organs and a halt in reproductive activity independently of the photoperiod and the food availability [Nelson et al., 1995]. Also, it has been reported that in some ruminants such as Merino sheep (*Ovis aries*) the rams do not have fully dependent photoperiodic reproduction. Rather, they behave as opportunistic breeders with a reproductive strategy depending on short-term responses to environmental cues such as food and social factors. In these species, food availability influences adult testis size, a process that appears to be GnRH-independent [Martin et al., 1994]. One of these mechanisms has been discovered more recently in the musk shrew, *Suncus murinus*, a species in which nutritionally challenged females stop breeding as a consequence of complete loss of sexual receptivity, a response controlled by GnRH-II, another form of the GnRH family [Temple et al., 2003].

Circannual Testis Alterations Derived from Seasonal Breeding

The mammalian adult testis contains 2 clearly differentiated compartments: the seminiferous tubules and the interstitial tissue. The seminiferous epithelium, located inside the tubules, is composed of both Sertoli and germ cells. Sertoli cells, which exert sustentacular and nurse functions, are the main targets of the hormones regulating the spermatogenic function, such as FSH and testosterone, and establish regulatory cross-talk with germ cells for the correct timing of spermatogenesis [Jegou, 1993]. They also form the blood-testis barrier (BTB), a specialized junctional complex that defines 2 compartments of the germinative epithelium, i.e. adluminal and basal. This complex provides an immune privilege to the meiotic and post-meiotic germ cells located in the adluminal compartment. The interstitial tissue of the testis is composed mainly of Leydig cells, which represent the steroidogenic component of this organ [Li et al., 2012].

In seasonal breeders, the architecture and function of the testis undergo profound circannual changes that generally imply the elimination of the germinative epithelium during the non-breeding period, a phenomenon in which processes and structures such as the BTB, androgenic production, apoptosis, and cell proliferation are significantly altered. Nevertheless, the mechanisms underlying the transition between the active and inactive periods, the so-called testis regression, vary among species or populations.

The direct consequence of the germinative epithelium depletion during testis regression is the reduction in the diameter of the seminiferous tubules and the subsequent shrinkage in testis size in the males of most seasonally breeding species. The panels in figure 1 depict the aspect of active and inactive testes in 2 mammalian species, the Iberian mole, *Talpa occidentalis*, and the wood mouse, *Apodemus sylvaticus*. In both species, the inactive testis maintains some meiotic activity, as meiosis onset is not completely interrupted and some few primary spermatocytes are still present in the regressed seminiferous tubules [Dadhich et al., 2010, 2013]. This situation appears to be quite common in many species that undergo seasonal testis regression, as observed in the Japanese red-bellied newt, *Cynops pyrrhogaster* [Yazawa et al., 2000], the silver fox, *Vulpes vulpes* [Andersen Berg et al., 2001], the Syrian hamster [Morales et al., 2002, 2007], the Chinese soft-shelled turtle, *Pelodiscus sinensis* [Zhang et al., 2008], and the Japanese jungle crow, *Corvus macrorhynchos* [Islam et al., 2012], as well as in the Mediterranean pine vole, *Microtus duodecimcostatus* [unpubl. data], among others. Contrarily, in other species, the regressed seminiferous tubules contain only Sertoli and spermatogonial cells, showing complete absence of meiosis onset in the inactive period. This is true for the white-footed mouse, *Peromyscus leucopus* [Young et al., 1999], the European starling, *Sturnus vulgaris* [Young et al., 2001], and the large hairy armadillo, *ChaetophRACTUS villosus* [Luaces et al., 2013, 2014].

During spermatogenesis, primary spermatocytes must pass through the BTB to gain access to the adluminal compartment where meiosis is completed. This process implies Sertoli-Sertoli and Sertoli-germ cell interactions at the level of cell junctions, and the BTB acts as a dynamic structure that undergoes cyclical changes of ‘opening’ and ‘closing’ to facilitate germ cell migration. This event is tightly regulated and involves a complex network of signaling cascades and the rapid turnover of junction-associated molecules. The BTB is also of great physiological importance, selectively permitting passage to some mol-

Fig. 1. Haematoxylin and eosin-stained transversal sections of testes from reproductively active and inactive males of the Iberian mole (*T. occidentalis*) (A, B) and the wood mouse (*A. sylvaticus*) (C, D). Seminiferous tubules (ST) in active testes are large and contain a thick, functional seminiferous epithelium, whereas those from inactive testes are reduced in diameter and contain scarce germ cells. Leydig cells observed in the interstitium (arrows) are more abundant in the mole.

ecules that can enter the adluminal compartment. Moreover, it is an immunological barrier that segregates late meiotic and post-meiotic germ cell antigens from the systemic circulation. It creates a unique microenvironment for germ cell development and confers cell polarity. Thus, when the BTB is dysfunctional, germ cell differentiation and development usually fail [Kaur et al., 2014].

The structure and functionality of the BTB has been investigated in 3 seasonally breeding mammals: the mink, *Mustela vison* [Pelletier, 1988], the Djungarian hamster, *Phodopus sungorus* [Tarulli et al., 2008], and the Iberian mole [Dadhich et al., 2013]. Molecular-tracer experiments have shown that in all 3 species the BTB becomes permeable in the non-breeding period, allowing the tracer to reach the adluminal compartment of the seminiferous epithelium (fig. 2). This loss of functionality is clearly associated with reorganization of the cell adhesion proteins involved in the formation of tight junctions, which are the primary components of the BTB. These proteins, mainly Claudin 11, are still present, but abnormally distributed, in the regressed testes of both hamsters and moles. However, Tarulli et al. [2008] reported an increase of mRNA of the Claudin 11 gene (*CLDN11*) in the Djungarian hamster, whereas Dadhich et al. [2013] found a significant reduction in the expression of this gene in the

Fig. 2. Functionality of the BTB in the testes of reproductively active and inactive males of the wood mouse. In the active testis, the biotin tracer does not trespass the BTB, being observed exclusively in the interstitial tissue but not inside the seminiferous tubules. In the regressing testis, the BTB is permeable and the tracer can be seen also inside the tubules.

Iberian mole. In this latter species, the testes of inactive males show severe shrinkage, a fact that makes the number of Sertoli cells per unit of testis volume (numeric density) increase 2.5-fold with respect to those of active males. Accordingly, Dadhich et al. [2013] corrected the expression values of *CLDN11* in the inactive mole testis by dividing them by 2.5, as this gene is expressed exclusively in Sertoli cells. The fact that Tarulli et al. [2008] did not apply a similar correction in the hamster data may account for this discrepancy, although real differences may also exist in this respect between the 2 species.

Inter-species variability also exists regarding the type of Leydig cell remodeling during the testis regression process. Testosterone production by Leydig cells peaks in reproductively active males. Accordingly, the fine structure of Leydig cells reveals that the machinery to synthesize steroid hormones is very active and their cytoplasm contains a conspicuous smooth endoplasmic reticulum (SER), a well-developed Golgi complex, large mitochondria with tubular cristae, and numerous lipid droplets. By contrast, during the quiescent period, SER profiles are sharply reduced, the Golgi apparatus is poorly developed, and the number of lipid inclusions diminishes [Neaves, 1978; Johnson et al., 1987; Hikim et al., 1988; Zayed et al., 1995; Muñoz et al., 1997; Beltrán-Frutos et al., 2014]. In addition, a reduction in the size of Leydig cells has been reported for most seasonal breeders studied to date, including the rock hyrax, the camel, the viscacha, and the hamster during the non-reproductive season [Neaves, 1973; Sinha Hikim et al., 1988; Zayed et al., 1995; Muñoz et al., 1997]. Although this hypotrophy is generally associated with a reduction in the volume occupied by the SER, there are nevertheless some exceptions. In the horse, the

number of Leydig cells declines during the non-breeding period in the stallion, but the average volume of individual Leydig cells and the composition of their cytoplasm does not vary between the breeding and the non-breeding seasons. This lack of seasonal differences in the volume of Leydig cells during the reproductive cycle seems to be related to the fact that stallions produce spermatozoa in the non-breeding season but to a lesser extent. Thus, some testosterone-producing Leydig cells are necessary to maintain the spermatogenic cycle permanently active [Johnson, 1986; Johnson et al., 1987]. Another exception occurs in the European mole *T. europaea* [Suzuki and Racey, 1978; Tähkä et al., 1989]. In this species, no difference was observed between active and inactive males in the size and abundance of Leydig cells and the contents of their cytoplasm, including SER, lipid droplets, and mitochondria. In contrast, the organization of the interstitial tissue and the association with the lymphatic system change throughout the breeding cycle. In the breeding season, clusters of Leydig cells are scattered in abundant connective tissue with extensive peritubular lymphatic sinusoids. However, very abundant, closely packed Leydig cells completely occupying the enlarged intertubular space with little connective tissue and very small interstitial lymphatics are visible in the non-breeding period, as reported also for the Iberian mole (fig. 1) [Dadhich et al., 2010]. Thus, the lowering of testosterone levels in the non-breeding period in moles cannot be attributed to a reduction in the amount of SER, and thus other mechanisms must account for the difference in Leydig cell activity between seasons [Suzuki and Racey, 1978]. Contrarily, other seasonal breeders show reductions in the number of Leydig cells during the non-breeding season, including camels and hamsters [Johnson, 1986; Johnson et al., 1987; Sinha Hikim et al., 1988; Zayed et al., 1995]. The simplest explanation for this circannual variation in the number of Leydig cells would be a wave of cell death during testis regression and a wave of cell proliferation during the recovery period. However, no study has reported either cell death [Strbenc et al., 2003; Hombach-Klonisch et al., 2004; Dadhich et al., 2010] or cell proliferation [Johnson et al., 1987; Hombach-Klonisch et al., 2004; Dadhich et al., 2010] in the interstitial tissue of seasonal breeders throughout the reproductive cycle of several species. In the roe deer, Hombach-Klonisch et al. [2004] proposed a model in which the total number of interstitial cells remains constant, but a number of Leydig cells dedifferentiate during testis regression and then differentiate again during the recrudescence period. Further studies in other seasonal breeders will confirm whether this is a general

mechanism or whether species-specific mechanisms affect Leydig cell dynamics in seasonal breeders. Hence, more attention should be focused on this cell type as little information is being reported lately on their fate throughout the circannual reproductive cycle of mammalian species, compared with the cells located inside the seminiferous tubules, i.e. Sertoli and germ cells, which are being thoroughly studied.

Cellular Mechanisms of Testis Regression in Vertebrates: The Role of Germ Cell Apoptosis

The causes of seasonal testis regression have been investigated in a number of vertebrate species, including reptiles, amphibians, birds, and mammals. The generally accepted hypothesis derived from these studies states that apoptosis (programmed cell death) is the main cellular process mediating seasonal testis involution [reviewed by Young and Nelson, 2001; Pastor et al., 2011]. Apoptosis permits the elimination of damaged, diseased, or superfluous cells from many parts of the body during tissue remodeling and differentiation [Raff, 1998; Cohen, 1999; Lockshin and Zakeri, 2004], playing essential roles in reducing cell numbers during embryogenesis, tissue homeostasis, and the elimination of expendable or potentially harmful cells. Studies that have attempted to inhibit apoptosis (pharmacological or genetic knockouts) during crucial stages of development lead to severe defects, organ dysfunction, and/or mortality [Wyllie et al., 1980; Thompson, 1994; Vaux and Strasser, 1996; LeGrand, 1997]. Spermatogenesis is characterized by high proliferation rates, and the coincidence of spermatogonial proliferation and spontaneous degeneration of spermatogenic cells seems to be normal in the mammalian testis [Allan et al., 1992; Kerr et al., 1992]. Thus, up to 75% of potential spermatozoa have been estimated to die in the testis of some adult mammals. Although spermatogonia and spermatocytes have been described in many species as the main cell types undergoing apoptosis, this may affect all types of male germ cells, including spermatogonia, primary and secondary spermatocytes, spermatids, and sperm [Blanco-Rodriguez and Martinez-Garcia, 1996a, 1998; Erickson et al., 2015]. In the rat testis, spontaneous apoptosis of A2, A3, and A4 spermatogonia occurs regularly [Allan et al., 1988], while primary and secondary spermatocytes as well as spermatids occasionally undergo apoptosis [Kerr et al., 1992; Brinkworth et al., 1995; Blanco-Rodriguez and Martinez García, 1996b]. However, A1, intermediate, and B sper-

Fig. 3. TUNEL assay marking apoptotic cells (arrows) in reproductively inactive testes of the Iberian mole (*T. occidentalis*) (A) and the starling (*Sturnus vulgaris*) (B). Arrowheads indicate interstitial, non-specific, pigment. Panels A and B were adapted from Dadhich et al. [2013] and Young et al. [2001], respectively and reprinted with permission from the Society for the Study of Reproduction.

matogonia rarely degenerate [Bronson, 1988]. The comprehensive analysis of available data indicates that spermatogonial apoptosis is important in the regulation of spermatogonial population density, as well as in the maintenance of the required homeostasis among the various germ cell types that can be supported and nursed by Sertoli cells. In addition, germ cell apoptosis seems to safeguard the genetic integrity of the male gamete and the synchronization between the spermatogonial and the spermatocyte cycles, eliminating harmful, irreparably damaged cells that are not able to pass checkpoint-monitored transitions due to improper synapsis between homologous chromosomes [reviewed in Hamer et al., 2008; Tripathi et al., 2009; Pastor et al., 2011; McClusky, 2012; Shukla et al., 2012].

Variation in the number of apoptotic germ cells has been reported in the testis of some seasonal breeders, including reptiles, amphibians, birds, and mammals (fig. 3). Several authors reported that apoptosis contributes to testicular regression in the European brown hare [Strbenc et al., 2003; Blottner et al., 1995] and hamsters [Furuta et al., 1994; Morales et al., 2002, 2007]. However, conclusive data in the Syrian hamster was only recently provided by Seco-Rovira et al. [2014] showing that apoptotic incidence peaks during the period of testis regression and not later when the process has been completed, and the increase is high enough to explain a massive depletion of the germinative epithelium. The same authors also showed that apoptotic germ cells are phagocytized by Sertoli cells [Seco-Rovira et al., 2015]. An inverse rela-

Fig. 4. Germ cell desquamation during testis regression. Meiotic and postmeiotic germ cells are seen in the lumen of the seminiferous tubules of the large hairy armadillo (*C. villosus*) (A) and in the epididymis of the Iberian mole (*T. occidentalis*) (B). A Adapted from Luaces et al. [2014] and reprinted with permission from the Society for the Study of Reproduction. S = Spermatocyte; SP = sperm; ES = elongated spermatid; RS = round spermatid.

relationship between germ cell proliferation and apoptosis has been found during seasonal testicular changes in roe deer [Blottner et al., 1995, 1996; Schön et al., 2004]. Nevertheless, in this species it was later found that apoptosis does not cause massive germ cell depletion taking place during testis regression [Blottner et al., 2007]. In the Iberian mole, apoptosis varies in a season-dependent manner, affecting mainly late zygotene and pachytene spermatocytes, but not Sertoli cells, during the non-breeding period. However, apoptosis is not responsible for the massive germ cell depletion during mole testis regression as the highest apoptosis rate was not found during the involution process but later, in the inactive testis, once regression had been completed. A wave of spermatogonial cell proliferation probably restores the number of spermatogonial cells lost during the period of testis inactivity [Dadhich et al., 2010]. Similarly, Luaces et al. [2014] found that apoptosis is not the primary cause of germ cell depletion in the regressing testis of the large hairy armadillo.

The discovery of species in which apoptosis is clearly not the cause of testis regression challenges the apoptosis paradigm. Available data indicate that apoptosis could plausibly mediate testis regression in birds [Young et al., 2001; Jenkins et al., 2007; Islam et al., 2012], turtles [Zhang et al., 2008], most amphibians [Yazawa et al., 1999, 2000], and some mammals [Seco-Rovira et al., 2014, 2015]. Contrarily, in one frog species [Sasso-Cerri et al., 2006] and several mammals [Young et al., 1999; Young and Nelson, 2001; Hingst and Blottner, 1995; Nonclercq et al., 1996] apoptosis was associated with but not conclusively shown to trigger testis regression

[Dadhich et al., 2013], and in others apoptosis is not the effector of testis regression [Blottner et al., 2007; Dadhich et al., 2010; Luaces et al., 2014]. In conclusion, apoptosis is not a general mechanism of seasonal testis regression mainly in mammals, which is not a homogeneous group in this respect.

Germ Cell Desquamation-Based Mechanisms of Testis Regression in Mammals

The process of testis regression has been comprehensively studied, including morphological (with both light and electron microscopy), hormonal, ultrastructural, molecular, and functional analyses in 2 mammalian species, the Iberian mole [Dadhich et al., 2010, 2013] and the large hairy armadillo [Luaces et al., 2013, 2014]. In the mole, a new mechanism of testis regression was reported based on massive desquamation (sloughing, exfoliation) of live, non-apoptotic meiotic and post-meiotic germ cells, which are eliminated through the epididymis and the urethra of males undergoing testis regression (fig. 4). This indicates that in this species testis regression is regulated by modulating the expression and/or distribution of the cell adhesion molecules connecting Sertoli and germ cells in the seminiferous epithelium. This process is mediated by low intra-testicular testosterone levels and implies that Sertoli cells lose their nursing and supporting function and that the BTB becomes disorganized and permeable. In fact, the expression patterns of several cell adhesion molecules appear to be clearly altered in the inactive mole testis, including Connexin 43, β -catenin, N-cadherin, and E-cadherin, which are components of the multi-protein complexes forming ectoplasmic specialization and other Sertoli-germ cell adhesion complexes, indicating that the control of the expression of these molecules may play an essential role in the mechanisms governing seasonal breeding. Dadhich et al. [2013] conjectured that germ cell desquamation and BTB permeation must occur sequentially and be regulated separately so that the latter occurs once most of the adluminal germ cells have disappeared. To explain this phenomenon, the authors proposed that low levels of intra-testicular testosterone alter the expression of cell adhesion molecules. In this situation, Sertoli-Sertoli and Sertoli-germ cell adhesion relaxes, resulting in the desquamation of meiotic and post-meiotic germ cells of the adluminal compartment. During this process the BTB maintains its functionality and only later, when the adluminal compartment is al-

most empty and spermatogenesis does not proceed beyond the primary spermatocyte stage does the disassembly of the BTB take place. Under this situation, mature germ cells are never exposed to the basal environment, thus preventing auto-immunization that would lead to permanent infertility. In addition, the apoptosis peak observed in the inactive mole testis probably participates in this process by removing all germ cells that continue to enter meiosis when they reach the zygotene-pachytene stages.

The armadillo represents a model quite similar to that described for the mole in some fundamental features. During testis regression, the germinative epithelium undergoes massive germ cell depletion and, consequently, seminiferous tubules finally contain only Sertoli and spermatogonial cells [Luaces et al., 2013]. In 2014, these authors reported that the extension of vimentin filaments associated with desmosome-like junctions are reduced at the interface between Sertoli and germ cells, and that the immunosignals of nectin-3, Cadm1, N-cadherin, and β -catenin appeared diffused, suggesting the loss of Sertoli-germ cell adhesion and subsequent germ cell desquamation at the beginning of the germinative epithelium regression [Luaces et al., 2014], as described in the mole. In contrast to the scenario in this species, the loss of Sertoli-germ cell adhesion appears to induce apoptosis in the already sloughed germ cells of the armadillo, showing that apoptosis is not the primary effector of testis regression in this species either. Moreover, the observation of cytoplasmic engulfment of cell debris and lipid droplets within Sertoli cells suggest a phagocytic activity contributing to the elimination of the residual germ cells still present in the latest regression phases. Hence, Sertoli cell phagocytosis in the armadillo could have the same role as apoptosis in the mole.

The question arises as to whether this mechanism of testis regression based on massive germ cell desquamation is frequent in mammals. Clearly, thorough studies similar to those performed in the mole or in the armadillo need to be undertaken in other mammalian species to answer this question.

Conserved Features in the Regressed Testes of Seasonally Breeding Species

All seasonally breeding species undergoing complete testis regression during the non-breeding period exhibit reduced levels of circulating testosterone, which appears to be a condition necessary for this process to occur. We

have seen that testicular cell junction dynamics is regulated by hormones and that impairment of Sertoli-germ cell junctions is the main testis regression effector in moles as well as in armadillos [Dadhich et al., 2013; Luaces et al., 2014]. It is known that both testosterone and gonadotropins regulate the expression of testicular cell adhesion molecules [Gye, 2003; Florin et al., 2005; Xia et al., 2005; Kaitu'u-Lino et al., 2007; Tarulli et al., 2008; McCabe et al., 2010; Meng et al., 2011]. In the seasonally breeding species analyzed to date, the androgenic function of males is diminished during the non-breeding season, suggesting that the depressed production of androgens is probably the hormonal signal inducing seasonal germ cell depletion.

Another constant feature in the regressed testes of inactive males is the permeation of the BTB, a process also subject to hormonal control, although it is controversial whether the control is exerted by FSH, testosterone, or both [Xia et al., 2005; Meng et al., 2005, 2011; Kaitu'u-Lino et al., 2007; Tarulli et al., 2008]. In the Iberian mole, Dadhich et al. [2013] have suggested that a reduction of the intra-testicular testosterone levels beyond a given threshold would lead to the disassembly of Sertoli cell apical ectoplasmic specialization and thus to a massive germ cell desquamation, whereas a further reduction to an even lower threshold would be necessary to induce BTB permeation.

Profound remodeling of testicular somatic cells is also a well-conserved feature during testis regression in seasonally breeding males. The loss of most of the germinative epithelium implies a drastic reduction in the seminiferous tubule diameter, forcing Sertoli cells to reduce their cytoplasmic volume and becoming tightly packed in the tubular periphery. In the mole, it has been shown that Sertoli cells in the regressed testis retain most of their cytoplasmic membrane by forming deep, superposed infoldings. With regard to Leydig cells, the 2 mole species mentioned above, *T. europaea* and *T. occidentalis*, are exceptional in showing a particular remodeling pattern in the inactive testis, leading to a continuous, dense matrix of interstitial cells in the inactive testis where the seminiferous tubules are embedded, losing contact to each other (fig. 1B) [Suzuki and Racey, 1978; Dadhich et al., 2013]. This unique feature has not been described in any other mammalian species. The most frequent situation in other species, including rodents, hamsters, deer, and rabbits, is the presence of clusters of Leydig cells occupying the interstitial spaces between adjacent, spermatogenically inactive, seminiferous tubules (fig. 1D).

Adaptive Features of Shrews Evidence High Plasticity in the Control of Seasonal Breeding

The greater white-toothed shrew, *Crocidura russula*, is a seasonal breeder, as evidenced in 2 European populations from the Ebro delta of Spain [López-Fuster et al., 1985] and from Switzerland [Jeanmaire-Besancon, 1988]. Massoud et al. [2014] recently studied males from some populations from near the city of Granada, southern Spain, in order to determine the mechanism of testis regression in this species. The authors expected to find a shorter non-breeding period in these southern populations as the length of the reproductive period of a particular species is known to increase as latitude decreases, as shown in talpid moles [Jiménez et al., 1990]. Surprisingly, they found no testis regression during any season in southern populations of this species, even though data from females showed that most of them stop breeding in the summer, thus evidencing that there is in fact a non-breeding season which is determined exclusively by females. This finding is consistent with the data reported by Brambell [1935], who showed that the beginning, and probably also the end, of the breeding season in another shrew, *Sorex araneus*, is determined by the female and not by the male. Thus, the seasonal breeding cycle in the southern populations of *C. russula* is inverted with respect to that of northern ones, where reproduction is halted in winter, as occurs in populations of other mammalian species living in the same region, including the Iberian mole [Jiménez et al., 1990; Dadhich et al., 2010, 2011, 2013] and 2 rodents [unpubl. data]. Massoud et al. [2014] suggested that the extreme summer drought in south-eastern Spain probably causes this reversed cycle, small mammals facing better life conditions in the moderate winters of this region.

To explain the female-driven non-breeding period of *C. russula* in southern populations, Massoud et al. [2014] suggested that this species, and probably many others, tend to reproduce continuously throughout the year, but females may undergo a transient receptivity drop if they face survival difficulties such as food restriction, as evidenced in the musk shrew *S. murinus* [Temple, 2004]. This phenomenon is controlled by the GnRH-II hormone, a different and an evolutionarily well-conserved form of the GnRH family [Kauffman, 2004], which has been shown to modulate the mating behavior in this species [Temple et al., 2003].

In male shrews, the question is why no testis regression occurs in southern populations during the summer period when females are not receptive. Massoud et al. [2014] considered that this is a new adaptive mechanism not described before. The authors hypothesized that in southern

populations the non-breeding period is short enough to make testis regression inefficient in terms of energy savings for 2 reasons. First, the testes of *C. russula* are very small as a consequence of their monogamy, which leads to low investment in spermatogenesis [Hosken et al., 2001; Parapanov et al., 2009]. Therefore, maintaining those small testes for a short period is not energetically costly. Second, the spermatogenic cycle of this species is relatively slow and long compared with that of other shrew species [Parapanov et al., 2007, 2008], so that once depleted, the time required for complete recovery of the germinative epithelium could be longer than the female resting period.

When species are living in locations where the environmental conditions are stable (e.g. central Europe), their circannual reproductive pattern is constant and repeated every year, leading to the deduction that the mechanisms controlling seasonal reproduction are strict and thus highly susceptible to any environmental change. However, when we study populations located at the limits of the distribution areas of particular species, as in the intriguing case of *C. russula* where the environmental conditions are quite more unstable and stressful, then a higher adaptive capacity is manifested, evidencing that those mechanisms are in fact far more plastic and versatile than initially suspected. The study of more species living in these areas is needed to test this hypothesis.

Species are frequently classified as winter or summer breeders, although the shrew also shows that the circannual reproductive pattern is not a constant feature for each particular species. The less favorable conditions for the reproduction of *C. russula* are found during winter in the north and during summer in the south, and therefore the non-breeding period is inverted in northern with respect to southern populations. The inflexion latitude for this species must be located somewhere between the northern and southern Iberian Peninsula, as shrews from the Ebro delta stop breeding in winter [López-Fuster et al., 1985], whereas those from Granada (only 400 km towards the south) do so in summer. Hence, a particular pattern of seasonal reproductive cycle should be attributed to particular populations of a given species but not the species itself.

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