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CONSERVATION OF GERMPLASM DERIVED FROM SPIX'S YELLOW-TOOTHED CAVIES (Galea spixii WAGLER, 1831)

Conservación del germoplasma en conejillo de los dientes amarillos o cui (Galea spixii WAGLER, 1831)

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RESUMEN

La importancia ecológica y económica de la *Galea spixii*, un roedor histricomorfos, ha despertado el interés de los investigadores en el desarrollo de estudios centrados en la conservación de su germoplasma. En este sentido, esta revisión presenta información publicada sobre métodos para obtener y caracterizar gametos masculinos y femeninos. Además, se muestran datos interesantes sobre la congelación de los espermatozoides y la vitrificación del tejido testicular y ovárico. Estos estudios prometedores evidencian las posibilidades de mejorar otras técnicas de reproducción asistida centradas en la multiplicación y conservación de *G. spixii*.

Palabras clave: Rodentia, cavia, vida Silvestre, espermatozoide, ovocito.

ABSTRACT

The ecological and economical importance of the Galea spixii, a hystricognath cavid rodent, has aroused the interest of the researchers at developing studies focused on the conservation of its germplasm. In this sense, this review presents published information regarding methods for obtaining and characterizing both male and female gametes. Moreover, interesting data regarding sperm freezing and vitrification of testicular and ovarian tissue are shown. Such promising studies evidence the possibilities for improving other assisted reproductive techniques focused on G. spixii multiplication and conservation.

Keywords: Rodentia, cavy, wildlife, sperm, oocyte.

INTRODUCTION

The Spix's yellow-toothed cavy (*Galea spixii*) is a small wild rodent that is an endemic species in the Brazilian northeastern region. It is a member of the Caviidae family (Reis *et al*., 2006), the same as the cuy (*Cavia porcellus*), whose meat consists of a delicacy widely appreciated by Peruvian cuisine. Despite being culturally poached for use as animal protein source by local communities (Santos *et al*., 2009), Spix's cavies present a stable population according to international criteria (IUCN, 2017). Because of their small features, easy to adapt to captivity, low maintenance costs and a short gestation period (Oliveira *et al.,* 2008; Bjorkman *et al.,* 1989), their captive breeding has been stimulated as an alternative for the conservation and improvement of local income (Santos *et al.,* 2009). Moreover, Spix's cavies are highlighted as adequate experimental models for the development of conservative strategies for endangered cavids as the Santa Catarina's Guinea Pig (*Cavia intermedia*) (Roach, 2016a), the Patagonian cavy (*Dolichotis patagonum*) (Roach, 2016b), and the Shipton's Mountain cavy (*Microcavia shiptoni*) (Jayat & Ojeda, R. 2008).

The ecological and economical importance of this rodent has aroused the interest of the researchers. In this context, some studies focused on the conservation of *G. spixii* germplasm have been recently published. Such studies aims both the formation of germplasm banks and the future improvement of cavies' reproductive performance. Therefore, the aim of this review is to present such innovative information regarding characterization and cryopreservation of germplasm derived from the Spixii's yellow-toothed cavies.

Reproductive aspects

The Spix's yellow-toothed cavies (Figure 1) are characterized as small rodents, with variation of the size and body weight between males and females (Oliveira *et al.,* 2008). They present a mating system classified as polygamous (Taraborelli, 2009), and are able to reproduce the whole year in captivity, with no report regarding the influence of the seasonality on their reproduction (Santos *et al.,* 2012).

Figure 1. Spix's yellow-toothed cavies (*Galea spixii*)

The male presents ovoid testicles located in the inguinal canal, abdominal cavity or in the inguinal position, with a well-defined scrotum. According to Santos *et al*. (2012), their testicular length and weight are 1.53 ± 0.21 cm and 1.17 ± 0.40 g on average, respectively. Regarding their penis, it is caudally folded, like a U-shaped flexion lying down, with the presence of spicules. Male individuals also present prostate, seminal vesicles and bulbourethral glands as accessory organs (Rodrigues *et al.,* 2013).

The male reproductive maturation occurs at 1.5 months on average (Santos *et al.,* 2012). At this moment, sperm can be visualized in the ejaculates, characterizing the complete spermatogenesis, which involves eight stages of cycles of the seminiferous epithelium (Santos *et al.,* 2014a). *G. spixii's* sperm (Figure 2) presents a rounded head, with a base narrower than the apex, with a very prominent acrosome in the apical surface of the cell head (Silva *et al.,* 2017a). Regarding sperm morphometry, the *G. spixii* sperm presents a head that occupies 9.4% on average of the total sperm length, 48, 87 ± 0.1 µm (Silva *et al.,* 2017a).

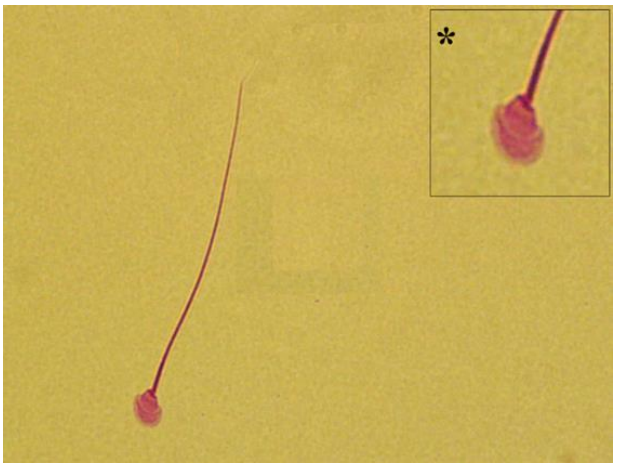

Figure 2. Morphology of Spix's yellow-toothed cavies (*Galea spixii)* epididymal sperm stained with Bengal rose. Normal head and acrosome in zoon (*).

Regarding female, ovaries are dorso-ventrally flattened ovoid structures, with length of 2.7 cm on average (Praxedes *et al.,* 2017), located in the abdominal cavity, in the sublumbar region, surrounded by adipose tissue (Almeida *et al.,* 2003). The ovarian follicles in several stages of development are located in the cortical region, while medullary region is comprised of vessels and fine tissues (Santos *et al.,* 2014b). In addition, female presents uterine tubes, uterus and two uterine horns with glands, which open into a single cervix, connecting with the vagina through the fornix, and a vulva with a clitoris pierced by the urethra. As a peculiarity, females do not present a vaginal vestibule, but a vaginal occlusion membrane is present (Santos *et al.,* 2015).
The female reaches sexual maturity at 55-90 days on average (Larcher, 1981), presenting a continuous poliestric cycle with a mean duration of 15.8 ± 1.4 days (Santos *et al.,* 2015). The estrus can lasts from 6 to 11 days, and the predominance of cornified superficial epithelial cells can be highlighted through the vaginal cytological examination (Santos *et al.,* 2015), associated to the presence of preovulatory follicles in the ovaries (Santos et al., 2017). Their gestation period lasts 68 days on average, culminating in the birth two to four pups per litter (Larcher, 1981).

Male germplasm

In those animals, as the small rodents, in which techniques for obtaining the ejaculate were not developed (Ferraz *et al.,* 2011), the recovery of epididymal sperm from accidentally dead valuable individuals appears as an option for the germplasm conservation. In this context, Silva *et al.* (2017a) recently demonstrate either flotation or retrograde flushing methods are suitable for the recovery of sperm from cauda epididymis of Spix's yellow-toothed cavies. Both methods were able to provide similar values for all the sperm parameters, aiming the recovery of more than 300 million sperm, presenting more than 50% motile sperm, with normal morphology and functional membrane. In spite of the similar results, authors suggest the use of the retrograde flushing method for assisted techniques in order to avoid sperm contamination with blood cells.

As an initial step for the development of sperm conservation protocols, Silva *et al.,* (2017b) evaluate the performance of solutions based in TES or Tris for *G. spixiis*' epididymal sperm recovery and thermal resistance. Authors prove the efficiency of Tris extender for the maintenance of sperm parameters quality during 60 min with 70% motile sperm.

In order to freeze the *G. spixii's* sperm, Silva (2016) adapted a method previously described for the agouti Dasyprocta leporina, also a hystricognath rodent (Silva et al. 2011). Such method consisted on an initial dilution in the extender containing 20% egg yolk at 27 °C, followed of an equilibrium for 40 min at 15 °C and additional 30 min at 4 °C in an incubator. At this occasion, cryoprotectants agents are added and samples are packed into 0.25-mL plastic straws, subjected to nitrogen vapors. Finally, the straws are stored in biological containers containing nitrogen.

Using the above referred method, Silva (2016) freeze the G. spixii's sperm using Tris extender added of 20% egg yolk and various concentrations (3, 6 and 9%) of glycerol or dimethyl sulfoxide (DMSO). Author proved that glycerol at 6% provides adequate conservation of sperm motility (60%), viability (68%), normal morphology (82%), and binding capability (227 sperm per perivitelline membrane fragment) (Silva *et al.,* 2015). Additionally, Moreira (2016) verified the effect of egg yolk or Aloe vera supplementation at various concentrations to Tris or coconut water-based extenders on the cryopreservation of G. spixii's sperm. Author confirmed the superiority of Tris extender for such purpose (Moreira *et al.,* 2016). In addition, he demonstrated that egg yolk remains as a more adequate external cryoprotectant for this cavy's specie than Aloe vera.

In last decade, the cryopreservation of male gonads has been highlighted as an interesting alternative for the conservation of genetic variability, safeguarding the spermatogonia, which allows an unlimited production of sperm (Ning *et al.,* 2012). In this context, Lago *et al.* (2016) first described the cryopreservation of G. spixii's testicular tissue (Figure 3) at using a solid surface vitrification method comparing ethylene glycol, DMSO and dimethyl formamide as cryoprotectants. They suggested that DMSO at 3M concentration is the more adequate cryoprotectant to be used for this purpose once it is able to preserve the structure of the germinative cell nucleus and epithelium as evaluated by classical histology. Of course, these are yet initial data, but they are promising results.

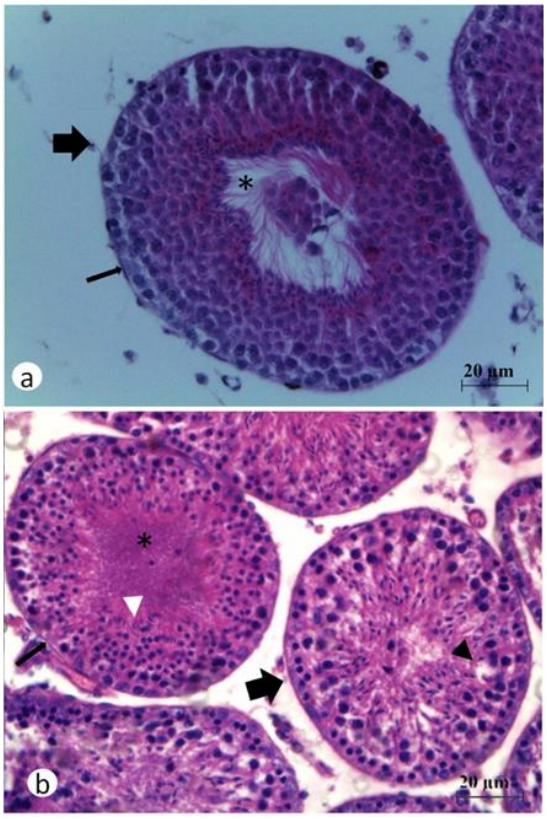

Figure 3: Vitrification of Galea spixii testicular tissue. (a) Fresh control tissue showing Intact basal membrane (thick arrow), Sertoli cells with intact nucleus (fine arrow) and repletion of sperm (*) into seminiferous tubule lumen; (b) Tissue vitrified with 3M dimethylsulfoxide showing intact basal membrane (thick arrow), Sertoli cell with preserved nucleus (fine arrow), but absence of a well-defined seminiferous tubule lumen (*). The presence of spermatozoa (white arrow head) and vacuolization (black arrow head) re noted.

Female germplasm

For G. spixii female, application of assisted reproductive technologies remains very limited. It is known that the ovarian follicles are the morphological and functional unities of the mammalian ovaries. In this context, the development of protocols for ovarian tissue preservation would be a good alternative for the preservation of genetic material, aiming the formation of cryobanks (Santos *et al.,* 2010).

As an initial step for female gamete manipulation, the knowledge of the ovarian follicle population is fundamental for any species. In this context, Praxedes *et al.* (2017) recently demonstrated that the mean population per ovarian pair of G. spixii female is estimated to be around 416.0 preantral follicles. Differently from other mammals, authors emphasize that the primary follicles, characterized by an oocyte surrounded by a single layer of cuboidal granulosa cells, were the predominant ovarian follicular category (64%) found in *G. spixii* ovaries.

In the same manuscript, Praxedes *et al.* (2017) established a vitrification process for the preservation of ovarian tissue of this species. They demonstrated that a solid surface vitrification method using sucrose and dimethyl sulfoxide as

cryoprotectants is able to provide 69.5% normal follicles after freezing-thawing procedures. Moreover, ultrastructural analysis confirmed the conservation of oocytes and granulosa cell membranes and the morphological aspect of follicles. These promising results highlights the possibility of conserving the G. spixii's genetic material in germplasm banks for future use in other assisted reproductive techniques.

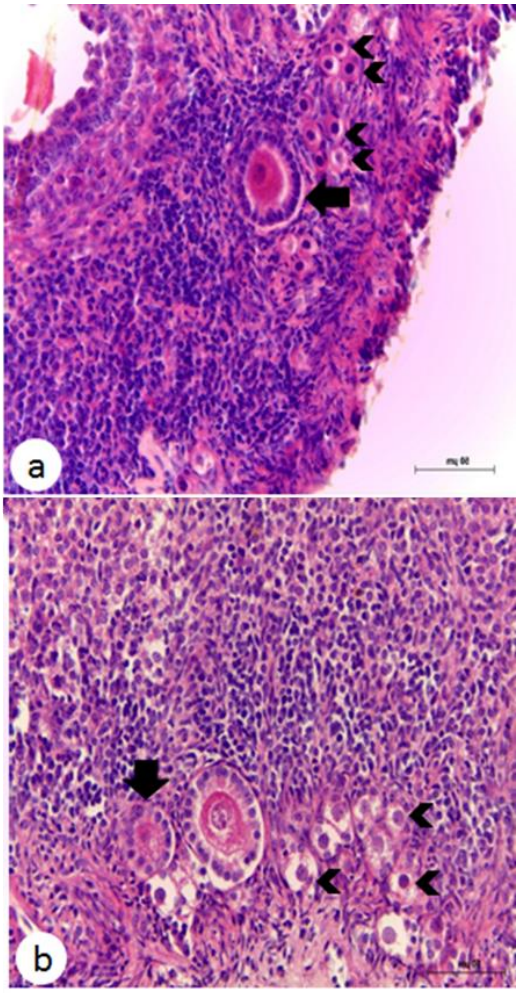

Figure 4: Photomicrographs of Galea spixii ovarian sections. (A) Aggregates of primordial follicles displaying an oocyte surrounded by one layer of flattened cells (white arrows) and primary follicle, displaying an oocyte with homogenous cytoplasm surrounded by one complete layer of cuboidal granulosa cells (arrow). (B) Degenerated follicles displaying oocyte cytoplasm retraction and disorganization of granulosa cells (arrows head) and primary follicle morphologically normal (arrows).

Final considerations

Even if the cryotechnology had allowed significant advances for the germplasm conservation applied to Spixii's yellow-toothed cavy, there are still a large way to be followed. This is mainly because there are limited efforts for the development of other additional reproductive technologies for these and other wild rodents as artificial insemination or in vitro embryo production. In this context, the development of strategies for improving the knowledge on their reproductive characteristics is necessary to be conducted in parallel to the development of strategies for their multiplication and conservation. Besides, preservation of their habitat remains as the main and concrete alternative for providing conditions for the natural reproduction of these wild rodents.

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