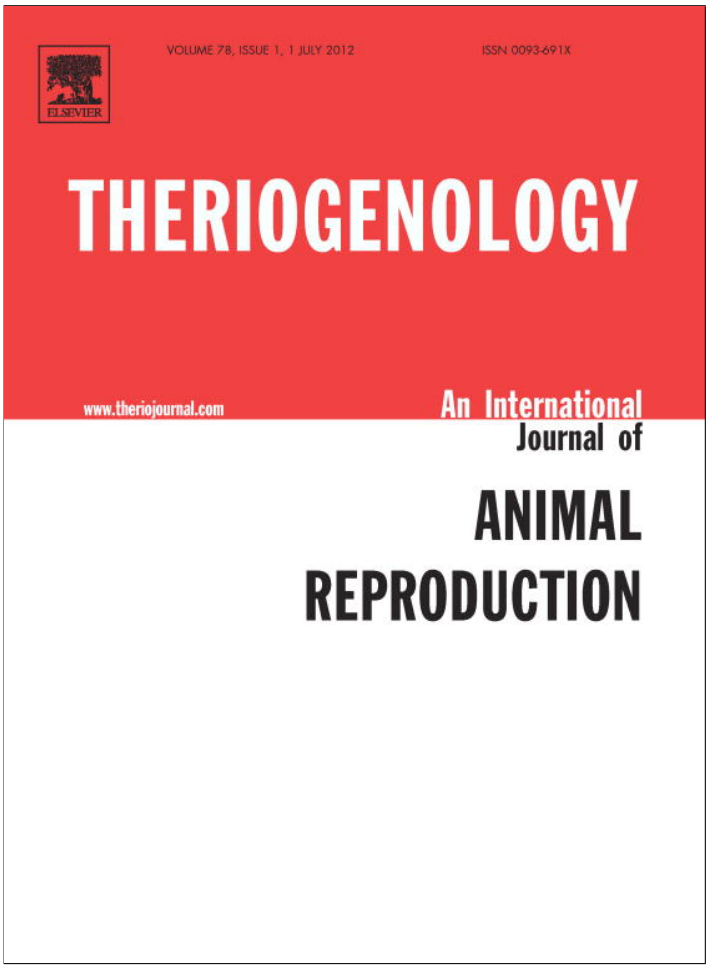

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Review

Reproduction in chinchilla (*Chinchilla lanigera*): Current status of environmental control of gonadal activity and advances in reproductive techniques

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Abstract

A review of the biology of reproduction of chinchilla, focusing on environmental control of the gonadal activity, is presented. Chinchilla is a South American hystricomorph rodent genus currently considered almost extinct in the wild. However, a domestic form is still widespread in breeding farms around the world. Information regarding their reproductive biology has been obtained from studies on captive animals. In the case of *Chinchilla lanigera*, a seasonal reproductive pattern has been frequently reported in breeding facilities, but factors that might trigger gonadal activity have not been identified. The available information on reproductive productivity in farms worldwide shows a range of 1.2 to 2.4 deliveries per female per yr (with up to 2.1 weaned young per female per yr). Indeed, as found in all rodents, chinchillas can multiply at high fecundity and fertility rates (4 to 6 follicles mature during estrous cycles). Some new research avenues are postulated to improve the control of gonadal activity by means of environmental and/or pharmacologic factors. Furthermore, reproductive techniques that have been validated in chinchilla are reviewed (noninvasive hormone monitoring, semen collection, sperm cryopreservation, estrus induction), and several technical steps are proposed to be able to achieve AI. Because domesticated chinchilla still share some genomic characteristics with their counterparts in the wild, validated reproductive techniques in chinchilla males and females might contribute to the success of breeding programs.

Keywords: Chinchilla; Reproduction; Photoperiod; Semen collection; Noninvasive hormone monitoring; Artificial insemination

Contents

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1. Chinchillas (Chinchilla spp.)

The present work compiles all information published regarding the reproductive physiology of *Chinchilla* spp. since the review of Weir in 1970 [1]. In the wild, chinchillas are herbivores and live in colonies, in extensive burrow systems. The environmental conditions of their habitats are typical of tropical desert (15° S/34° S), with climatic variations exhibiting drastic temperature changes (from 30 °C to −22 °C) and low rainfall. Information regarding reproductive activity of this genus in nature is scarce, but it is known that females give birth from September to February in South America [2,3]. However, under captive conditions at similar latitudes, births can occur at any time, with peaks occurring in spring and summer [2,4–6].

Chinchilla spp. belongs to the Chinchillidae family; six species, namely *Lagostomus* spp., *Lagidium* spp., and *Chinchilla lanigera* and *Chinchilla brevicaudata*, are all endemic in South America. However, *Chinchilla* spp. is under threat (IUCN Red List of Threatened Species; http://www.iucnredlist.org) [7], with the existence of remnant colonies in the Argentine Andes being uncertain [8], and some reports regarding the presence of a wild strain in Chile [9–11]. Amori and Gippoliti [7] indicated that a domestic form is widespread in breeding farms around the world, and captive-bred chinchillas still share some genomic characteristics with their wild counterparts (based on cytochrome b sequence analysis). This close relationship of wild and domestic *Chinchilla lanigera* is to be expected, because the captive-bred specimens were mainly derived from a few wild individuals collected in Chile [12,13]. Therefore, we consider that this review provides scientific information that may be helpful for: (1) assessing ecological phenomena (by focusing on environmental endocrinology, i.e., quantifying concentrations of stress hormones in feral individuals of this species); (2) identifying healthy reproductive feral individuals to develop reproductive ex situ programs; and (3) indicating validated reproductive techniques for manipulation of chinchilla gonadal activity to assist diagnosis of reproductive dysfunction in valuable farmed individuals.

Because domestic *C. lanigera* and wild *C. brevicaudata* have low levels of genetic variation [12] and morphologic similarities [3,8], perhaps validated reproductive techniques in *C. lanigera* will also be useful for studying reproductive functions in *C. brevicaudata*.

1.1. Reproductive development and gonadal cycles in Chinchilla lanigera

Information regarding their reproductive biology comes from studies on commercially exploited animals, especially *Chinchilla lanigera*. However, chinchillas have some characteristics that distinguish them from most rodents. Some authors have proposed that the chinchilla's longer reproductive cycles could be a result of adaptations arising during the colonization of ecological niches characterized by harsh environmental conditions, particularly at high altitudes or at high latitudes (highland tropical desert) [14].

Captive-bred chinchilla males have a short and rapid growth cycle (approximately 180 days) [15]. In adults, increased sexual activity in winter was inferred, based on morphometric changes in the male reproductive system [16], with a peak occurring in autumn through winter (under natural photoperiod and ambient temperature conditions) in the seminal glands [17] and urethral bulbs [18]. Moreover, there was a 30% reduction in average testis volume in the southern hemisphere in January and February (under natural photoperiod, 31° S/64° W). In our laboratory, although testis volume had a positive correlation ($r = 0.83$; $P = 0.001$) with body weight under similar experimental conditions, no seasonal changes were detected [19,20].

1.1.1. Testicular activity

Leal and Franca [21] indicated that proliferation of both Sertoli and Leydig cells occur up to 2 mo after birth and then the total number of these cells per testis reaches a plateau. In addition, based on spermatid release from the seminiferous epithelium and the presence of sperm in the epididymis, puberty in chinchilla

occurred at approximately 3 mo of age [21]. However, based on other measurements of testicular variables (e.g., testis weight, tubular diameter, and epithelium height), these authors demonstrated that attainment of sexual maturity takes relatively longer than in other rodent species (17 mo after birth). These data were obtained under a natural photoperiod variation, with statistical differences being minimal after 11 mo of age. Therefore, it is possible that males evaluated at 17 mo (born in March and killed in August), 22 mo (born in December and killed in October), and 30 mo of age (born in February and killed in August) may have been affected by decreasing photoperiod typical of December to March. In fact, Leal and Franca [21] indicated that the gonadosomatic index and the seminiferous volume density per testis were similar from 5 mo of age, which was consistent with the short and rapid growth interval (6 mo) mentioned above [15].

The spermatid/Sertoli cell differentiation index and sperm production per testis gram increased daily from 5 mo of age. In addition, the spermatogenic cycle lasted 10.2 days and the total duration of spermatogenesis was approximately 46 days [22]. Although it is generally accepted that the male remains fertile throughout the year [1], studies related to the sperm concentration in semen of chinchilla have produced variable results. A range of 20 to 200 $\times$ 10^6 sperm per ejaculate was proposed for *C. lanigera* [2], whereas in our laboratory, in conscious animals subjected to electroejaculation twice a month for 1 yr, there were 0.9 to 432 $\times$ 10^6 sperm per ejaculate [19]. The variability in these results may be due to differences in the photoperiod, which was the only environmental factor not regulated during these studies. In a subsequent study, we investigated testicular activity in animals exposed to a natural photoperiod and found seasonal changes in sperm concentration (winter and summer median values of 46 $\times$ 10^6 and 7 $\times$ 10^6 sperm, respectively) and testicular endocrine activity (winter and summer median values of 66.9 $\pm$ 32.1 and 7.5 $\pm$ 1.4 ng of urinary androgen/mg of creatinine, respectively) [20].

1.1.2. Ovarian activity

There are apparently no reports with respect to puberty in female chinchilla. It is well known that the estrous cycle in mammalian females consists of a "cascade" of progressive, synchronized, and repetitive hormonal and behavioral events [23]. However, the estrous cycle has been found to range from 15 to 90 days, using various technical approaches (see below or the following references: [1,2,24–29]). Conaway [24] documented a cycle of 15 to 35 days, whereas Weir [1] reported cycles of 28 to 35 days. Both of these authors indicated that females exhibited spontaneous ovulation, typically with very frequent estrus in winter, consistent with a high birth rate in spring [1,13]. Gestation was 111 days (range, 105 to 118) in *C. lanigera* [2,13]. In a review of the biology of *C. lanigera*, Spotorno et al. [2] recently reported an estrus cycle of 38.1 $\pm$ 0.7 days, with a range of 16 to 69 days (estrus could last 48 h), and Kuroiwa and Imamichi [25] indicated an estrous cycle of 35.7 $\pm$ 7.9 days, with a range of 15 to 62 days.

A preliminary multivariate study (using rectal temperature, vaginal cytology by Quick-Papanicolaou smear and noninvasive monitoring of progestagens and estrogens) reported an estrous cycle of 25 days (with a range of 10 to 56 days) during increasing photoperiods (winter through spring) [27], whereas Brookhyser and Aulerich [28] recorded a mean of 33 days with a range of 11 to 49 days, using the vaginal opening as a marker of the onset of the estrous cycle [1] and progesterone profiles in blood samples. Similarly, an average estrous cycle of 35 days was reported (with seasonal variations of 22 to 27 days in spring, 33 to 36 days in summer, and 70 to 90 days in autumn and winter), based on vaginal cytology (Papanicolaou, 0.1% toluidine blue) and blood progesterone concentrations [29].

Reproductive physiology, reproductive tract morphology and sexual behavior of female chinchilla have been poorly explored. Regarding physiology, no environmental sources of variations (photoperiod, temperature, social cues, and food details) were reported in studies regarding ovarian activity, although, a high coefficient of variation is expected in reproductive variables. We also believe that vaginal opening alone is not indicative of estrus and vaginal cytology is not an accurate tool for detecting ovulation, because changes in these parameters were not sufficient to determine the length of estrous cycles. In fact, vulva color, vaginal opening, and exfoliative cytology are only indirect indicators of female reproductive stage, and should therefore only be considered complementary information. For future studies, we strongly recommend monitoring estrogen and progestagen concentrations. Furthermore, it is noteworthy that estrus is usually characterized by other behavioral and nonbehavioral components [30,31], which to date have not been explored in chinchilla.

1.2. Influence of environmental factors on chinchilla reproduction

In the wild, chinchilla births have been reported to occur in spring and summer in the southern hemisphere

[3,32]. However, in captivity, births occur throughout the year, with two annual activity peaks in spring and summer, usually producing two litters a year [2,4]. Similar results have also been reported on some farms in South America [5,13,33,34].

From an ecological perspective [35], reproduction in mammals is a complicated process, which should occur in harmony within dietary, physical (temperature and photoperiod), and social contexts. In chinchilla, the effects of these environmental factors on gonadal activity have not been individually studied, and consequently, natural reproductive cycles are still poorly understood.

Chinchilla breeding facilities are normally exposed to significant macro- and microclimatic variations. Reproductive performance throughout the year under thermal environmental variations (8 °C to 18 °C in winter and spring, respectively) and variable humidity percentages (colder and more humid winters, with temperatures of 4 °C to 5 °C) were reported [36]. These data were obtained using a polygamic system (from 3 to 8 females per male per family), under a natural light regime (33° S/70° W) and a variable diet, with lactating females receiving greater amounts of feed. We believe that the strong influence of environmental changes on reproductive cycles and reproductive performance (productivity) could lead to the misinterpretation of results in studies regarding chinchilla reproduction.

Under captive conditions, a litter size of up to six has been recorded. Lactation usually lasts 42 to 64 days [2,5]. The highest number of parturitions has been associated with shorter than usual lactating periods [5], and Garcia et al. [5] have suggested that these differences could be due to a greater sexual activity in the reproductive system selected by the breeder (i.e., number of females per male). Breeders' manuals suggest that, under a polygamic system, males are only able to mate starting at 18 mo of age, whereas a female accepts mating at 6 to 8 mo [13]. However, fertile matings can occur at as early as 5 mo [2], and as pointed out above, the gonadosomatic index and seminiferous volume density per testis are similar from 5 mo of age, consistent with a rapid growth phase (6 mo) [13,21].

Regarding fertility (expressed as the percentage of pregnant females), values reported ranged from 75% to 87% (1.2 to 2.4 deliveries per female per yr; up to 2.1 weaned young per female per yr) [5]. The mean age at first delivery was 15 mo and the interval between successive parturitions was 7 mo (based on a gestation of approximately 3.5 mo, which included an interval of approximately 3.5 mo during which conception does not occur), with fluctuations throughout the year under changing environmental conditions [2,5,6,36–38]. Probably the most important factor for any species in the regulation of its lifetime fertility is the interval between successive births. Thus, small mammals like chinchilla have minimized intervals between successive births by conceiving at an estrus that occurs immediately after parturition [14]. Therefore, it may be concluded that chinchilla females do not routinely achieve either maximum fertility or the highest fecundity at breeding facilities.

A central concept in the study of the environmental influence on reproduction in mammals associates reproductive activity with energy balance, and assumes that the animal has the ability to monitor external and internal energy availability [39]. Overall, when food is accessible and the energetic requirements are low, energy is partly expended on vital processes, with the remaining energy allocated to growth and immunologic and reproductive needs [39]. When evaluating chinchillas in terms of reproduction in captivity, the environment where the chinchilla evolved should be considered. In the wild, when animals, such as chinchilla are exposed to situations of high energy expenditure and/or low forage availability, it may lead to the death of the litter and even of the mother. Chinchilla females are folivorous, with the feeding pattern of generalist species. However, this opportunistic feeding behavior may be an adaptation to harsh conditions and high variability in food availability [9]. In the less challenging situation of captivity, delayed puberty, suppressed ovulation, reduced performance during lactation, etc., can occur due to low nutrition and/or high caloric restriction. However, when energy balance is apparently satisfactory in breeding facilities, males and females are able to reproduce at 6 mo of age (puberty begins at 5 mo). Nevertheless, further studies are needed to determine why there are still patterns of reproductive seasonality and delays of 15 mo before first parturition [37].

Chinchilla have apparently evolved in environments with scarce food and water availability, and with drastic short-term temperature variations. To manage this situation, in natural xeric environments of low biomass productivity, physiological (bioenergetic) adaptations resulting in cost-effective use of energy and water have been reported for this species [40,41]. Chinchilla have a very low metabolic rate, consistent with thermal isolation and low water loss through evaporation [9,40,41]. Therefore, where the body temperature of chinchillas has been shown to remain stable up to 25 °C

of ambient temperature, the best thermal performance in this species—at the temperatures evaluated—was between 5 °C and 15 °C ambient temperature (although the recommended values for captive-bred chinchillas are 17 °C to 25°C at 30% to 60% of humidity) [13,42]. Furthermore, it is generally accepted that body heat in mammals can be lost by dissipation into the air (insensitive loss) or by evaporation (sensitive loss), with heat loss by evaporation increasing with temperature. Unlike the tropical arid regions where the wild chinchilla lives, evaporation is negatively affected by the humid environment (with variations of up to 80% to 90%) frequently present on farms, probably producing stress on the bioenergetic balance.

Photoperiodic influences on synchronization of reproductive responses are usually studied using natural photoperiods. However, as mentioned above, although few studies have investigated photoperiod as the source of variation in the experimental design, this factor has a significant influence on testicular volume [19]. Fuentes et al. [43] postulated that photoperiod is one of the most important factors in synchronization of the reproductive cycle in vizcacha, a rodent belonging to the *Chinchillidae* family. Besides, similar effects have been postulated for other rodents [44]. In addition, in recent studies regarding photoperiodic effects on male reproduction, Busso et al. [19,20] postulated that chinchilla might be classified as a photoperiodic species, because the male is sensitive to photoperiod changes. In fact, phenotypic variation has been observed in several experiments, but, to assess whether these animals can be classified as "photoresponsive" or "nonphotoresponsive" a thorough study is required, as previously proposed for other rodents [30,44]. It is also necessary to find animals that can maintain a constant reproductive rhythm and/or are less photosensitive to inhibitory natural photoperiods in breeding facilities. In that regard, optimal artificial light conditions for chinchilla have only been evaluated by Felska and Brzozowski [45]. That study, which employed artificial light within a range of 30 to 270 lux (12 h light/dark cycles with 40 W fluorescent lamps, at 18 °C to 20 °C and 50% to 60% relative humidity), reported similar mean values of reproductive efficiency and survival to those found by Garcia et al. [5] (natural light, 30° S/70° W).

1.3. Reproductive techniques

Reproductive techniques validated for chinchilla are shown (Table 1). Pukazhenthi and Wildt [56] proposed five biological aspects (sex; age and seasonality in gonadal cycles; timing of ovulation and spermatogenesis; types of ovulation; ways to overcome infertility; and protocols for consistently successful assisted breeding) that might contribute to the successful application of assisted reproductive techniques (e.g., AI and/or embryo transfer) in mammals. However, in chinchilla, these aspects are in general still far from being fully understood, with the only exceptions being those of seasonality and spermatogenic activity. Therefore, we

Table 1
Validated reproductive techniques reported for chinchilla.

Technique	Basic characteristics	Reference
Semen collection (electroejaculation)	Voltage applied not stated/success: 67%	Dalziel and Phillips, 1948 [46]
	Voltage applied 9.5 V/success: 92%	Healey and Weir, 1967 [47]
	Voltage applied 22 V/success: 80%	Calderón Fernandez, 1976 [48]
	Voltage applied 12 V/success: 75%	Barnabe et al., 1994 [49]
	Voltage applied 6.5 V/success: 100%	Ponce et al., 1998 [50]
	Voltage applied 6.5–8 V under anesthesia/ success: 60%	Busso et al., 2005 [51]
Oocyte collection (in vitro maturation)	Mature in vitro	Aiudi et al., 2007 [52]
Hormone detection (testosterone RIA)	Blood; total testosterone, Coat-A-Count*	Cepeda et al., 2006 [53]
	Urine/feces, steroidal extraction; total testosterone, Coat-A-Count	Busso et al., 2005 [54]
Hormone detection (progesterone RIA)	Blood, Sephadex extraction, steroid extraction, home-made assay	Brookhyser and Aulerich, 1980 [28]
	Blood, in-house assay	Gromadzka-Osrttowska et al., 1984 [55]
	Urine/feces, steroidal extraction; total estradiol or progesterone, Coat-A-Count	Busso et al., 2007 [26]

For semen collection, information is given as follows: voltage applied for semen collection in conscious or anesthetized animals, success achieved during experiment and authors of reports; for hormone detection: matrix and extraction (if applicable) for steroidal analysis, immunoassay used (in-house assay refers to noncommercially available assays) and authors of reports.

* Diagnostic Products Corporation (DPC), Los Angeles, CA, USA.

consider that genetic improvement in chinchilla cannot yet be successfully achieved by AI.

1.3.1. Noninvasive hormone monitoring

Some of the reproductive aspects mentioned above can only be studied when reproductive techniques are properly validated. At present, it is assumed that steroidal hormone variations in chinchilla are driven by both internal and external factors, such as FSH and LH and by photoperiod, respectively. However, because FSH and LH (protein hormones) are extensively and rapidly degraded during metabolism, noninvasive hormone monitoring is often limited to analysis of steroid hormones [56]. Conversely, blood collection is still an attractive technique for steroid analysis. In that regard, there are reports on this technique providing recommendations for safe, easy, and repeated collections. Nevertheless, the application of repeated sampling (twice a week for 2 mo) affected the overall health of chinchillas [57,58].

Evaluating the steroid metabolite content and/or profiles in either urine or feces represents an alternative technical approach that does not disturb individuals. In that regard, these techniques have been used to achieve a wide range of research goals in captive and free-ranging wildlife, as well as in domestic and laboratory species [59–63]. In our laboratory, chinchilla were subjected to radio-metabolism studies of progesterone, corticosterone, testosterone, and estradiol, for precise monitoring of steroidal metabolites in excreta [26,54,64]. These results were recently reviewed and compared with those obtained in other rodents [65].

Among the few investigations carried out on testicular endocrine activity are those of Busso et al. [20] and Cepeda et al. [53], which analyzed excreta (urine/feces) and plasma, respectively. Both these research groups used the same commercial testosterone immunoassay. However, although methodological strategies were validated, details of the assay performance were only reported for urine samples and fecal steroid extracts ($y = -11.42 + 0.80 \times$ for urine, and $= 15.31 - 1.06 \times$ for feces). From a practical perspective, fecal testosterone analysis yielded more specific results according to the recovery test (observed value $= 1.06 \times$, where $\times$ represents hormone mass), probably due to the presence of native testosterone in feces, whereas immunoreactive androgen metabolites were only present in urine. In addition, feces are easier to collect than urine samples, without the need to determine creatinine to account for day-to-day fluctuations in fluid balance [54]. Although fecal testosterone analysis is more specific, mean hormonal values can be seriously affected by interindividual differences regarding, for example, constipation or different times of steroidal excretion due to changes in diet. Therefore, it is essential to monitor feed administration and defecation rhythms of experimental animals to optimize interpretation of hormonal analyses.

In females, the earliest endocrinological study on ovarian activity used repeated blood sampling [28,29,55,57,66,67]. In lightly anesthetized animals, blood samples were obtained using the orbital sinus bleeding technique, modified by Brookhyser and Aulerich [28]. Progesterone concentrations were then determined by means of a laborious procedure (plasma samples were purified by chromatography and steroids were extracted) and the plasma progesterone concentrations ranged from 0.07 to 6.27 ng/mL. In another study Gromadzka-Ostrowska and Zalewska [29] also employed plasma samples, but these were obtained using another bleeding technique (repeated cuts approximately 5 mm from the tail tip in nonanesthetized animals in various seasons). Using a direct progesterone radioimmunoassay, values ranged from 0.3 to 14.0 ng/mL (annual mean of 5.04 ± 0.49).

In what was the first noninvasive hormone monitoring in chinchilla females, radioactive estradiol was rapidly metabolized and predominantly excreted into urine as polar metabolites, with the radioactive progesterone peak excretion being delayed and steroid metabolites occurring in equal amounts in urine and feces [26,65]. Natural urinary and fecal estrogens and progestagens were measured by commercially validated radioimmunoassays. Although an overestimation of hormone mass (i.e., $1.2 \times$ for urinary estrogens) was detected, this is not a substantial limitation if a longitudinal daily sampling scheme was applied and all excrement collected [26].

Future research may be able to improve accuracy and precision by applying a technique that minimizes interferences in immunoassay [68], or by validating a new immunoassay. Nevertheless, early results on the use of this noninvasive monitoring technique have successfully demonstrated the effect of seasonality (as mentioned for reproductive cycles, especially for progesterone [20] and a significant positive correlation between progestagens and body weight during pregnancy [26]).

1.3.2. Semen collection and sperm cryopreservation

To the best of our knowledge, semen collection has only been attained by applying electroejaculation on animals in varying states of consciousness. In 1998, Ponce et al. [50] were 100% successful for the first time using this method. However, in later experiments on anesthetized animals conducted in the same laboratory, only 60% of animals ejaculated [51]. The volume was also reduced due to the use of anesthesia in males, but

the quantity and quality of sperm obtained was maintained. Chinchillas are unlikely to ejaculate 1 mL of semen (the volume commonly reported in other larger-sized species). Therefore, using the total number of sperm per ejaculate is more appropriate than the concentration (number of sperm per mL). Based on state-of-the-art techniques, we consider that new developments should focus on obtaining semen naturally using trained individuals, as frequent semen collection (for AI) by this means would be less stressful for chinchilla than standard techniques. Furthermore, future research employing this strategy should evaluate if the sperm of chinchilla is deposited in the vagina close to the posterior end of the cervix, as in other rodent species.

As previously reported [51], we have always tried to consider aspects of individual welfare in our work and believe that the methods employing anesthesia are a practical prerequisite for applying reproductive technologies (e.g., AI, sperm cryopreservation, etc.), because this reduces stress induced by electroejaculation. However, electroejaculation using low voltages for a few minutes may be considered a reasonable approach, not only in captive-bred but also in wild chinchillas, to obtain the largest amount of semen possible in a single attempt.

Studies cited in the present review as well as those mentioned by Weir [1] had high variation in the number of ejaculated sperm obtained after electroejaculation. Despite these variations, semen analyses conducted in our laboratory using various animals over 10 yr [50,51,69,70] had similar mean values, with confidence intervals for motility of 92% to 95% and for intact acrosome sperm of 83% to 89%. Therefore, our latest report [19] could be used as a technical reference of ranges and confidence intervals for sperm functional activity obtained in chinchilla (under a natural photoperiod of 10/14 h light/dark cycle; 31° S/64° W; controlled temperature: 20 ± 2 °C; a balanced diet, and the presence of females in the same room).

Technical information on cryopreservation of sperm (such as cooling temperature, cryoprotectors, and freezing time) taken from reports about cryopreservation of ejaculated and epididymal sperm [50,69,71,72] is shown (Table 2). Freezing generally produced deleterious effects on sperm functional activity, which was confirmed by comparing mean values of sperm functional activity with the corresponding frozen-thawed variants in liquid nitrogen (stored for 1 to 6 mo at −196 °C) and also with the corresponding variants after cooling (4 °C) for 24 or 72 h. Interestingly, no differences in sperm functional parameters were detected between 24 and 72 h after thawing, with high rates of activity occurring under in vitro conditions.

Table 2
Chinchilla sperm functional activity after the application of various cooling and cryopreservation methods.

Sperm	Reference	T (°C)	Interval	CPA	Sperm functional activity		
					Variable (%)	In fresh samples	In thawed samples
Epididymal	Ponce et al., 1998 [72]	−196	3 to 6 mo	G, 6%	Motility	95	−41
					Host	78	−61
					IAV	85	−1
Ejaculated	Ponce et al 1998 [50]	−196	3–6 mo	G, 6%	Motility	97	−54
					Host	68	−54
					IAV	83	−52
Ejaculated	Carrascosa et al 2001 [69]	4	24/72 h	EG, G, 2 M	Motility	94	G: −30/−38 EG: −5/−7
					Host	74	G: −20/−47 EG: −6/−0
					IAV	80	G: −36/−35 EG: −0/−0
Ejaculated	Ponzio et al 2008 [71]	−196	1 mo	EG, G, 1 M	Motility	97	G: 41 EG: −44
					Host	68	G: −71 EG: −71
					IAV	83	G: −48 EG: −52

Time indicates period under cryopreservation; fresh, functional activity immediately after ejaculation; thawed, percentage of decreases in comparison with fresh samples; G or EG indicates different CPA in unwashed samples. For G and EG, percentages or final concentration in the cryoprotectant media are indicated.

CPA, cryoprotectant agent; EG, ethylene glycol; G, glycerol; host, hypoosmotic swelling test; IAV, intact acrosome viable sperm; T, temperature.

Although the usefulness of these protocols has not been confirmed (because the fertilizing capability of semen samples has not been evaluated either in vivo or in vitro), successful outcomes in assisted reproduction are to be expected in chinchilla, because the quality of some the seminal biochemical parameters, such as motility and acrosomal integrity, are generally associated with sperm's fertilizing capacity. Indeed, we predict a promising future for in vivo or in vitro fertilization in the use of the cryoprotectant ethylene glycol in sperm populations subjected to cooling (not to freezing) [69]. As acrosomal status was the least affected variable, perhaps these sperm populations are more suitable for use in IVF or in vivo insemination procedures in areas closer to the ovary (e.g., oviducts than in the cervix or vagina). Nevertheless, further studies are still needed to evaluate the true fertilizing capacity of these sperm cell populations, as well as of the semen deposited into female tract, which forms a hostile environment.

Epididymal sperm were cryopreserved using a buffer (according to Ponce et al. [72]). Briefly, this buffer contained sodium citrate (30%), methyl-2-aminoethanesulfonic acid and Tris (24% vol/vol), egg yolk (20%), fructose (2%), and glycerol (6%). In this study, after 24 h of in vitro incubation in Tyrode's medium, the percentages of motile, viable, swollen, and acrosome-intact sperm had decreased significantly with respect to values obtained immediately after extrusion. Again, further studies are necessary to evaluate sperm functional activity after cryopreservation during sperm transit through the female reproductive tract.

Finally, it is generally accepted that male germ plasm cryopreservation facilitates indefinite preservation of the currently available gene diversity represented in both captive and wild populations [73]. In fact, as mentioned for other endangered species, several studies related to chinchilla sperm functional activity at different in vitro experimental conditions have provided useful information for management and research of both captive-bred and wild individuals [74]. Regardless, a better understanding of the highly complex processes between insemination and fertilization in chinchilla is necessary to improve the efficiency of conventional and validated reproductive techniques presented here, as well as to enable development and establishment of new ones [75,76].

1.3.3. Artificial insemination

Species-specific reproductive mechanisms have been shown to inhibit the rapid application of AI in wildlife [56]; in that regard, the chinchilla is no exception. The precise determination of the duration of the estrous cycle and the timing and type of ovulation are particularly important. Furthermore, the location of semen deposition varies among species [75]. We infer that chinchillas deposit ejaculated sperm in the vagina close to the posterior end of the cervix, as in other rodents, but this aspect needs further research. In addition, in domestic mammals, an oviductal sperm reservoir consisting of several thousand sperm must be established for successful fertilization. However, in order to achieve these seemingly moderate numbers using conventional AI techniques requires an insemination dose of several million (perhaps as many as 1 billion) sperm, which greatly limits the efficient use of ejaculated sperm [75]. Therefore, it is essential to evaluate whether 0.9 to 432.6 $\times$ 10^6 sperm per ejaculate and/or a sperm concentration of 2145.9 $\pm$ 365.3 ($\times$ 10^6/mL), reported by Busso et al. [19,20], as well as a range of 20 to 200 $\times$ 10^6 sperm per ejaculate proposed by Spotorno [2], are sufficient for successful AI. Furthermore, it is noteworthy that increasingly good examples of low-dose insemination technology are now available for other species [77].

With respect to ovulation, there is a general consensus that chinchillas undergo spontaneous ovulation. Because the estrous cycle ranged from 15 to 69 days, the application of hormonal treatments to induce estrus at present is proposed as a way of synchronizing reproductive ovarian activity. The technique has already proved valuable for increasing the chances of females becoming pregnant throughout the year [78,79]. Some pharmacological protocols have been applied to chinchilla females, with a combination of exogenous gonadotropins (eCG and human chorionic gonadotrophin) being administered to animals, although births occurred in only 12% of the treated females [80]. Furthermore, gonadotropin analogues have been recently used again to stimulate the reproductive system, resulting in a CL present in the ovaries, in contrast to the controls [80]. Between 62% and 100% of females (N = 8 per group) given various doses of gonadotropin analogues had offspring within 6 mo, whereas the controls did not produce any young. However, as the order of parturitions was not reported by these authors [80], it was not possible to elucidate from this report whether the fertile estrus was produced by the hormone treatment, by ovarian activation and further fertile estrus over the 6-mo interval, or by another cause. Koziorowski et al. [81] gave infertile females eCG and human chorionic gonadotrophin and reported only 18% of pregnant females, including those in the control group. We agree with Weir [79] that the direct action of gonadotropins on the ovary depends on in the stimulation of follicle

growth, leading to follicle rupture and CL formation. The rationale behind the treatment is, therefore, induction of an ovarian cycle. However, the use of different-aged animals, the lack of information on environmental factors, and different experimental designs are among the factors that prevent us from proposing a reliable hormone schedule to induce ovulation.

Finally, with respect to oocyte collection, to best of our knowledge, there is only one study on oocyte recovery using an in vitro maturation protocol (described for bovine oocytes), with only a few oocytes recovered [52]. Despite a paucity of work on egg maturation and collection, we agree with Pukazhenthi and Wildt [56], who argued that IVF or intracytoplasmic sperm injection/embryo transfer technologies along with frozen storage of eggs and embryos could be considered long-term strategies for assisted reproduction and/or genome resource banking for threatened species (e.g., chinchillas).

2. Final remarks

Information regarding chinchilla reproductive biology comes mainly from studies on commercially exploited animals (*C. lanigera*). Some characteristics of this rodent make it very different from most species of the Rodentia order (e.g., long gestation). Consequently, female chinchillas exhibit estrus post delivery as an extraordinary productivity characteristic. In addition, precocious neonates are able to consume solid food at 1 wk of age, and it has been demonstrated that males exhibit short and rapid growth, with daily sperm production per testis gram increasing markedly after 5 mo of age and both sexes being able to mate as early as 5 mo old or after both sexes stop growing (approximately 6 mo). Therefore, we inferred that the reproductive rate may be accelerated and reproductive seasonality reduced by controlling environmental factors.

At present, according to the literature, genetic improvement in chinchilla cannot be successfully put into practice by AI, with little being known regarding reproductive tract morphology and sexual behavior in females. Furthermore, the estrous cycle has not been precisely determined (reported range of 15 to 69 days). Several aspects of the reproductive physiology of females still need to be elucidated by employing noninvasive hormone monitoring, a validated technical approach with great potential for research and management, especially in this species.

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