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Gestational period and reproductive cycle in Spix's yellow-toothed cavy (*Galea spixii* Wagler, 1831)

Período gestacional e ciclo reprodutivo em preás (*Galea spixii* Wagler, 1831)

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Abstract
Spix's yellow-toothed cavies are rodents displaying high biological and farming potential. Knowledge of cavy reproductive aspects is paramount for satisfactory breeding. This study aimed to determine the gestation length and characterize the reproductive cycle phases of Spix's yellow-toothed cavies, also investigating potential male effects on these processes. The investigated animals were categorized into three groups: Gestational follow-up (G1), with a 5:1 female-to-male enclosure ratio; estrous cycle (G2), with a 5:1 female-to-male ratio with a male confined to a cage; and G3, consisting of five females and no male. Daily colpocytological examinations were performed, with the presence of spermatozoa on the microscopy slides indicative of copulation. G1 females were separated from the male immediately after copulation, with this being considered day "zero" of the pregnancy. G2 and G3 females were evaluated for two complete estrous cycles and qualitatively assessed through vaginal smears. The gestation length of the Spix's yellow-toothed cavies averaged 59 ± 2.24 days, with a continuous polyestrous cycle lasting 14.8 ± 0.73 days in G2 and 14.6 ± 0.75 days in G3. The proestrus phase was characterized by the dominance of parabasal cells, dyes, bacteria, and leukocytes; the estrus phase, by superficial cells with the predominance of anucleate cells with and without the presence of bacteria; the metestrus phase, by parabasal cells and numerous genuine cells, neutrophils, and bacteria; and the diestrus phase, mainly by basal, parabasal, and mutant cells, as well as high amounts of vaginal mucus, neutrophils, and bacteria. The presence of a male cavy significantly influenced diestrus duration, prolonging this phase, which is potentially attributed to female progesterone production effects.
Keywords: Estrous cycle; estrus; *Galea spixii*; pregnancy; rodent.

Resumo
O preá é um roedor com elevado potencial biológico e zootécnico a ser explorado, sendo o conhecimento sobre os aspectos reprodutivos fundamentais para que sua criação seja satisfatória. Objetivou-se determinar a duração da gestação em preás, e a caracterização das fases do ciclo reprodutivo, verificando, se existe influência da presença do macho neste processo. Os animais foram separados em três grupos: Acompanhamento gestacional (G1), 5:1, proporção de fêmea e macho no box; Ciclo estral, 5:1, com o macho preso em gaiola (G2) e cinco fêmeas em outro box sem o macho (G3). O exame colpocitológico ocorreu diariamente, identificado o espermatozoide na lâmina como cópula. As fêmeas do G1 foram separadas assim que copulavam e contadas como dia “zero” da gestação. As fêmeas do G2 e G3 foram avaliadas ao longo de dois ciclos estrais completos, avaliados qualitativamente pelo o esfregaço vaginal. O período de gestação em preás foi de 59±2,24 dias, com um ciclo poliéstrico contínuo, com duração de 14,8±0,73 dias no G2 e 14,6±0,75 dias no G3. O proestro caracterizou-se pelo predomínio de células parabasais, intermediárias, bactérias e leucócitos; o estro, células superficiais, com predomínio das anucleadas com presença ou não de bactérias; metaestro, células parabasais e grande quantidade de células intermediárias, neutrófilos e bactérias; diestro, predomínio de células basais, parabasais e intermediárias e grande quantidade de muco vaginal, neutrófilos e bactérias. A presença do macho influenciou significativamente a duração do diestro, tornando-se mais longa, fato que pode estar atrelado a influência sobre a produção de progesterona na fêmea.
Palavras-chave: Ciclo estral; estro; *Galea spixii*; gestação; roedor.

1. Introduction

Rodents exhibit a wide variety of shapes and sizes, with particularities identifiable by their teeth, skull, and jaw. This enables these animals to carry out specific feeding, tunnel digging, and seed dispersal activities, validating their importance in many ecosystems[1, 2]. Some particular life habits and reproductive diversities constitute a challenge for scientists who employ rodent species in experiments[3]. However, several genetic,

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ecological, demographic, and physiological studies have applied rodents as experimental models, due to their simple management and breeding[2].

Cavies, encompassing rodents belonging to the family Caviidae, exhibit an elongated body, dark gray dorsal surface, and white belly. This species has achieved commercial interest due to its high farming potential, which may be explored in the near future[4, 5]. Captive cavy breeding has been carried out with the aim of furthering knowledge of this species and its maintenance requirements. Reproductive aspects, especially concerning reproductive periods and estrous cycles, are paramount in this context[6, 7].

Vaginal cytology is a practical and non-invasive method used to obtain reproductive data[8, 9], where smears and staining are performed with Giemsa, Methylene Blue, and Shorr dyes, constituting a relatively quick and efficient method[10]. Morphological and quantitative cell patterns are evaluated post-staining to assess the integrity of the female reproductive system[11, 12]. In this context, this study aimed to determine the duration of pregnancy and characterize the reproductive cycle phases of female cavies. The potential influence of males in this process was also evaluated.

2. Material and methods

Cavies were obtained from the Wild Animal Multiplication Center (CEMAS) at Universidade Federal Rural do Semi-Árido (UFERSA), registered at the Brazilian IBAMA as a scientific breeding site under number 1478912. All experiments were approved by an Institutional Research Committee (approval no. 23091.010264/2015-90).

Five non-pregnant cavy females (Group 1, G1) were allocated in 5.0 m x 4.0 m enclosures and differentiated by painting their fur with hair dye in different anatomical regions. After a 10-day adaptation period, one male was added to the group, and daily vaginal cytology tests were carried out to detect the presence of sperm and the beginning of the gestational period 24 h after encountering the male gamete. Pregnant females were separated from the group and transferred to a male-free enclosure for pregnancy monitoring.

Estrous cycle analyses were performed on ten females, five of which were maintained in an enclosure containing a male trapped in a cage (Group 2, G2), preventing fertilization, whereas the other five were maintained in an enclosure with no males (Group 3, G3). Estrous cycles were determined daily employing vaginal colpocytology smears dyed with Instant-Prov rapid panoptic dye (New Prov®) according to the manufacturer's recommendations.

The first cycle day for females maintained in the presence of a male was considered the estrus, in which females exhibit a swollen vulva and cytological smears present mostly superficial cells. Concerning the other female group, macroscopic vulva aspects and vaginal cytology were analyzed to assess estrogenic phase features. Several microscopic fields were analyzed in each stained cytological slide, and anucleate scales, nucleated superficial cells, and intermediate, parabasal, and basal cells were counted up to a total of 100 cells. Leukocytes, bacteria, and mucus filaments were qualitatively observed through crosses, with one, two, and three crosses being associated with low, moderate, and high amounts, respectively. In total, two complete cycles were monitored for each animal.

Cell typing data were subjected to an Analysis of Variance (ANOVA) applying the least squares method [13] employing general linear models available in Statistical Analysis System (SAS) software[14] to determine male presence and estrous cycle phase effects. Data were expressed as means ± standard errors at a $p<0.05$ significance level and subjected to the Shapiro-Wilk normality test ($p=0.1528$).

The ANOVA was based on the following mathematical model:

$$Y_{ijk} = \mu + M_i + F_j + I_{ij} + \varepsilon_{ijk}$$

Where Y_{ijk} is colpocytological analysis k performed in experimental group i during phase j of the estrous cycle; μ is the overall mean; M_i comprises the fixed effect of treatment i (i=male absent, male present); F_j is the fixed effect of phase j of the estrous cycle (j=proestrus, estrus, metestrus, diestrus); I_{ij} is the nested effect of phase j of the estrous cycle within treatment i; and ε_{ijk} is the residual effect that includes all other variation sources not considered in the model.

Potential male effects concerning the length of each estrous cycle phase were determined by Student's T test using SAS software. Data normality was first verified by the Shapiro-Wilk test, followed by a homoscedasticity of variances test. Non-normally distributed data were transformed into decimal base logarithms.

3. Results

Cavy pregnancies lasted about 59 days, with a standard error of 2.24 for the five analyzed females, which gave birth from one (20%) to two offspring (80%). Females exhibited a continuous polyestrous sexual cycle, averaging 14.8 ± 0.73 days (12-16) (n=5) for females maintained with a male (G2) and 14.6 ± 0.75 days (13-17) (n=5) for the group maintained in the absence of a male (G3). No statistically significant

differences (p>0.05) were detected between groups (Table 1).

On the other hand, when comparing each cycle phase to its respective duration and to the two experimental groups, the findings indicate that the presence of a male cavy significantly influenced the diestrus phase, which was longer in G2 (4.0 ± 0.63 days) compared to G3 (2.4 ± 0.24 days) (Table 1).

Table 1. Male cavy influence on female estrous cycle phase length (means ± standard errors, days).

Estrous cycle phase	Experimental group	
	Male present (G2)	Male absent (G3)
Proestrus	4.0 ± 0.55	3.0 ± 0.89
Estrus	3.2 ± 0.49	4.0 ± 0.55
Metaestrus	3.6 ± 0.68	5.2 ± 0.58
Diestrus*	4.0 ± 0.63	2.4 ± 0.24
Estrous cycle	14.8 ± 0.73	14.6 ± 0.75

*p<0.05

Concerning estrous cycle cellular patterns, vaginal squamous cells were observed in the decreasing maturity order of polygonal anucleate superficial cells (anucleate scales), exhibiting a delimited eosinophilic cytoplasm, with no nucleus, often found as granulations resulting from karyorrhexis (nuclear fragmentation) (Figure 1B, 1C and 1D), polygonal nucleated superficial cells with well-defined cytoplasmic limits, a slightly basophilic or eosinophilic cytoplasm and a small nucleus containing finely granular (Figure 1B) or markedly condensed (nuclear pyknosis) chromatin (Figure 1D), intermediate round to polygonal cells, a basophilic cytoplasm, poorly defined cell boundaries, a small nucleusn (albeit larger than in superficial cells), and finely granular chromatin (Figures 1A, 1E, 1F, 1G, 1H, and 1I), round basophilic parabasal cells with a moderate nucleus/cytoplasm ratio (Figure 1A, 1E, 1F, 1G, 1H, and 1I), and small round markedly basophilic basal cells with a high nucleus/cytoplasm ratio (Figure 1G, 1H, and 1I).

Cavy estrous cycle phases were identified by applying these cellular patterns. The beginning of the cycle is termed proestrus, characterized by mostly parabasal and intermediate cells and the presence of bacteria and leukocytes (Figure 1A). The end of this period was similar to the estrogenic phase. The estrus phase exhibited numerous superficial cells, many times exceeding 80% of the total cell counts. Nucleated superficial cells exhibited a finely granular chromatin (Figure 1B). Nucleated scales were predominant compared to nucleated superficial scales (Figure 1B, 1C, and 1D). No leukocytes were detected in this phase, and bacteria were present in low amounts, in some cases not detected (Figure 1D).

Numerous intermediate cells, neutrophils, and bacteria were observed in the metestrus phase, followed by a standard number of parabasal cells (Figure 1E and 1F). The end of this cycle is characterized by the diestrus phase, composed mostly of basal, parabasal, and intermediate cells. Vaginal smears in the diestrus phase appear “dirty”, due to the intense production of vaginal mucus and high amounts of neutrophils and bacteria (Figure 1G, 1H, and 1I).

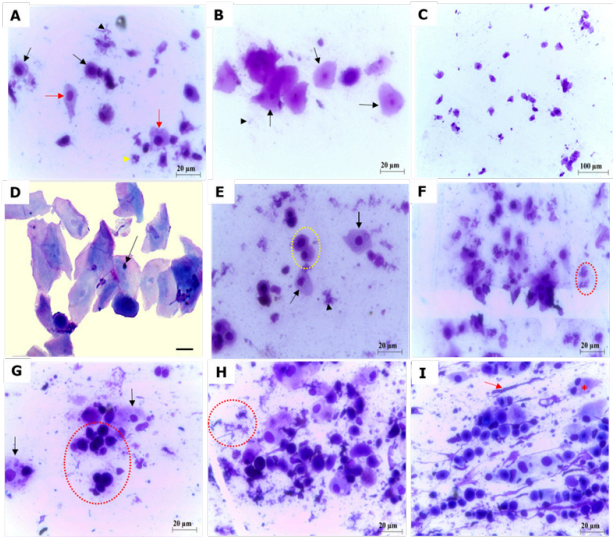

Figure 1. Estrous cycle phases in female cavies. Proestrus - A: Parabasal (black arrows) and intermediate (red arrows) cells, bacteria (black arrowhead), and a neutrophil (yellow arrowhead). Estrus (B, C and D) - B: Nucleated surface cells (arrows) and bacteria (arrowhead); C: Desquamation of superficial cells, presence of bacteria, and absence of leukocytes; D: Anucleate superficial cells and superficial cell containing a pyknotic nucleus (arrow). Metaestrus (E and F) - E: Parabasal cells (dashed ellipse), intermediate cells (arrows), and bacteria (arrowhead); F: Predominance of intermediate cells, parabasal cells, bacteria, and leukocytes (dashed ellipse). Diestrus (G, H and I) - G: Intermediate cells (arrows) and basal cells (dashed ellipse); H: Basal cells (deep) and bacteria (dashed ellipse); I: Mucus filaments (arrow) and binucleate intermediate cell (*).

Enucleated and superficial nucleated scales (59.42 ± 1.48% and 15.84 ± 0.79, respectively) represented over 70% of the total cell counts in the estrus phase, while deep cells (parabasal and basal), averaging 30.65 ± 1.06% and 18.67 ± 0.87%, respectively, constituted almost half of all identified cells in the diestrus phase. Similar cell types, on the other hand, were observed in the proestrus and metestrus phases, with the predominance of superficial nucleated cells in the proestrus phase and intermediate cells in the metestrus phase (Figure 2).

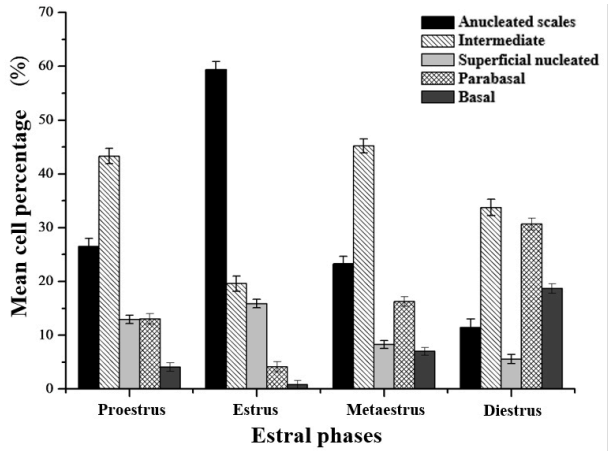

Figure 2. Different cell types determined in each cavy estrous cycle phase by colpocytological analyses.

Enucleated scales were significantly influenced ($p<0.05$) by male cavy treatments and different between estrous cycle phases (Figure 3). A significant difference between treatments was observed in the proestrus phase, with G2 females (35.05 ± 1.97%) displaying higher amounts of enucleated scales compared to G3 females (18.00 ± 2.28%). During the estrus phase, both treatments exhibited the highest averages, 59.19 ± 2.21% for G2 females and 59.65 ± 1.97% for G3 females. G2 females exhibited lower amounts of enucleated scales in the diestrus phase (14.55 ± 1.97%). No significant enucleated scale differences between the proestrus (18.00 ± 2.28%) and diestrus (8.33 ± 2.55%) phases were observed for G3 females.

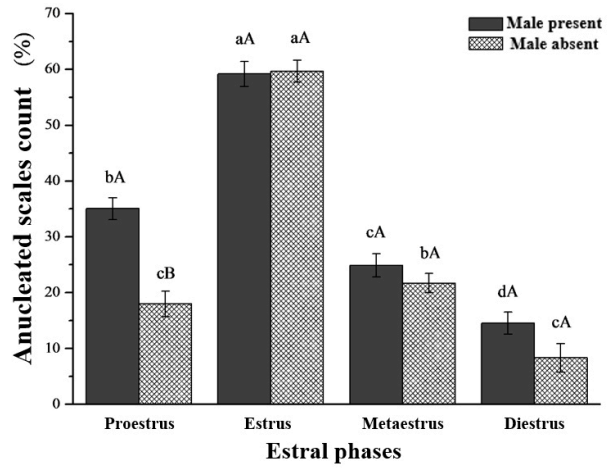

Figure 3. Effects of the presence of a male cavy on enucleated scales determined by colpocytology during different female estrous cycle phases. * The same lowercase letters within each experimental group (male present, male absent) indicate no differences from each other by the Tukey test ($p<0.05$). ** The same upercase letters within each phase of the estrous cycle (proestrus, estrus, metestrus, diestrus) indicate no differences from each other by the Tukey test ($p<0.05$).

Significant ($p<0.05$) interactions between treatments and estrous cycle phase were also observed for intermediate cells (Figure 4). During the proestrus phase, G3 females exhibited significantly higher intermediate cell amounts (48.73 ± 2.17%) compared to G2 females (37.85 ± 1.88%). No significant differences, however, were observed between the proestrus and metestrus phases in G3 females (46.34 ± 1.65%), with the lowest counts observed in the estrus phase (18.45 ± 1.88%). Likewise, no significant differences were observed between the proestrus (37.85 ± 1.88%), metestrus (44.05 ± 1.98%), and diestrus (30.75 ± 1.88%) phases for G2 females, with the lowest counts noted for the estrus phase (20.69 ± 2.10%).

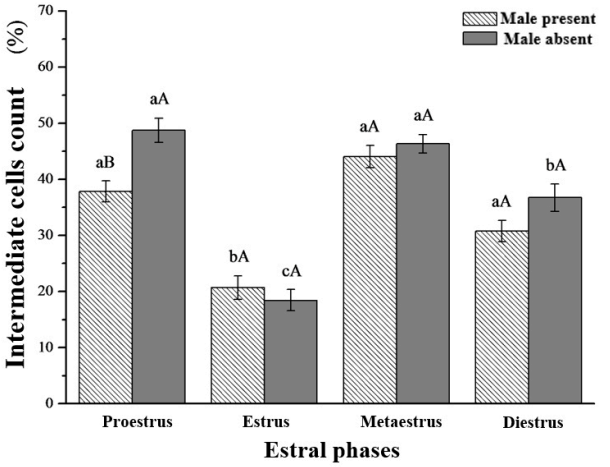

Figure 4. Effects of the presence of a male cavy on intermediate cells as determined by colpocytology during different female estrous cycle phases. * The same lowercase letters within each experimental group (male present, male absent) indicate no differences from each other by the Tukey test ($p<0.05$). ** The same uppercase letters within each phase of the estrous cycle (proestrus, estrus, metestrus, diestrus) indicate no differences from each other by the Tukey test ($p<0.05$).

Significant effects between estrous cycle phases ($p<0.05$) were noted for nucleated superficial, parabasal, and basal cells. Significantly higher nucleated superficial cell counts were observed during the proestrus and estrus phases when compared to the metestrus and diestrus phases (Figure 5). The lowest counts for both parabasal (4.10 ± 0.97%) and basal (0.72 ± 0.79%) cells were noted in the estrus phase. The diestrus phase, on the other hand, was characterized by the highest parabasal (30.65 ± 1.06%) and basal cell (18.66 ± 0.86%) counts. Parabasal cell counts did not differ between the proestrus (13.03 ± 0.99%) and metestrus (16.23 ± 0.89%) phases (Figure 5).

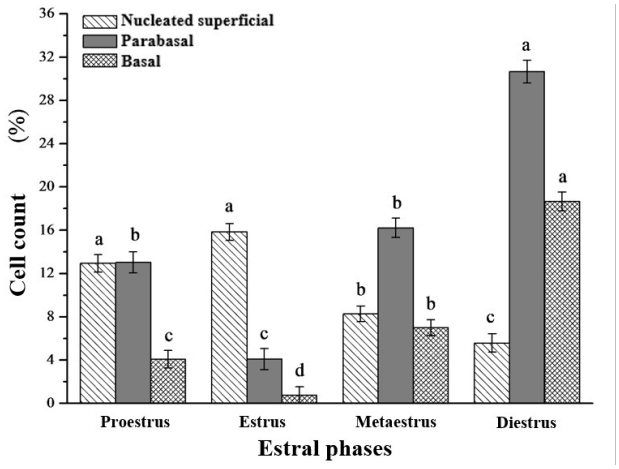

Figure 5. Average nucleated superficial, parabasal, and basal cell counts as determined by colpocytology during different female estrous cycle phases. * The same lowercase letters within each cell type (nucleated surface, parabasal and basal) indicate no differences from each other by the Tukey test ($p<0.05$).

4. Discussion

Murine rodent assessments reveal a short luteal phase, agreeing with the hypothesis that implantation occurs early in the uterine horns. This has motivated gestational period characterization assessments, suggesting an initial blastocyst development in overlapping pregnancies in which females are still breastfeeding the previous litter. Herein, cavy pregnancies were evaluated for less than 10 days, contrasting with the length of non-overlapping pregnancies reported for other rodents, such as *Calomys musculinus* (21 days), *Calomys laucha* (21 days), *Microtus ochorogaster* (21 days), and *Akodon molinae* (25 days), all South American sigmodontine rodents, and *Peromyscus maniculatus bairdii* (25 days), a North American sigmodontine rodent[15].

Additionally, the information converged regarding the gestation duration estimates of the species *Clethrionomys glareolus*, *Mus musculus*, and *Rattus norvegicus*, which were 19, 20, and 22 days, respectively[16, 17], and agoutis around 104 to 120 days[18]. Under natural conditions, cavy gestations last 48 days[6, 19, 20], which has been employed as the reference in *Galea spixii* placentation studies[21]. This contrasts with our results, in which the gestation length was determined as being about 59 days for this species in captivity.

The determined cervicovaginal desquamative type profiles reflect the endocrine dynamics associated with mammalian reproductive cycles. Qualitative and quantitative cell estrous cycle analyses and their associated figurative elements can, therefore, be employed to efficiently predict fertile cavy periods and, consequently, reproductive disorders[22].

In this regard, one study characterized the cavy estrous cycle employing 12 non-pregnant isolated females and another five maintained in the presence of a male. Two complete cycles were evaluated in the first group, with an average cycle length of 15.8 ± 1.4 days, ranging from 14 to 19 days[23]. Females maintained in the presence of the male could not be evaluated, as most became fertilized. This was not an issue in the present study, as the male was maintained in a separate cage and was thus unable to fertilize the females.

In another study, the reproductive cycle of red-rumped agoutis (*Dasyprocta leporina*) raised in captivity in the Brazilian semi-arid region was assessed by vaginal cytology associated with transabdominal ultrasounds for eight complete cycles averaging 28.2 ± 0.7 days, ranging from 24 to 31 days[24]. These results are similar to those reported for black-rumped agoutis (*Dasyprocta prymnlopha*), reported as 30.69 ± 4.65 days, ranging from 19 to 40 days[25], and 29.94 ± 6.77 days, ranging from 18 to 41 days[26]. Another assessment concerning black-rumped agoutis reported an average estrous cycle of 32.05 ± 4.17 days ranging from 25 to 40 days for 20 total cycles, which were non-statistically different in relation to a male effect in the experimental groups during cycles. However, most experimental groups contained adult and young females maintained alongside vasectomized males. Hormonal measurements carried out by the radioimmunoassay method indicated two concentration 17β-estradiol peaks, the first in the metestrus phase and the second in the proestrus phase, suggesting two follicular development phases. Concerning progesterone, a statistically significant difference ($p<0.05$) was noted between phases, which was low in the estrus phase, increasing after 24 h and reaching the highest average in the diestrus phase[27].

In this sense, the presence of a male significantly influenced the diestrus phase in our study, which was longer (4.0 ± 0.63 days) when compared to the group of five females maintained in the absence of a male (2.4 ± 0.24 days) as determined by vaginal cytology. These findings suggest that males can influence progesterone concentrations after ovulation, subsequently acting on corpus luteum activity.

In terms of cytological findings, the criteria applied to determine each cavy reproductive cycle phase and assess cellular morphological aspects included the presence of superficial cells and anucleate scales in the estrus phase, with a minimum requirement of at least 70% representation of these squamous cell types. These cells exhibited eosinophilic cytoplasms, polygonal morphology, low nucleus/cytoplasm ratios, finely granular chromatin or a pyknotic nucleus in the case of superficial and nucleus-free cells. Furthermore, leukocytes and bacteria were detected at low

concentrations or were absent.

In the metestrus phase, the predominant cell type comprised intermediate cells, followed by considerable amounts of bacteria and leukocytes. Intermediate cells varied from oval to polygonal, with a centered nucleus and dispersed and granular chromatin, in addition to a basophilic cytoplasm. In the diestrus phase, the sum of deep cells (parabasal and basal) is expected to exceed 40% and present intermediate cells as the second most prevalent cell type. Furthermore, numerous leukocyte, bacterial, and mucus infiltrates were also evident. Cavy parabasal and basal cells are both round, with a large and central nucleus, granular chromatin, and a high nucleus/cytoplasm ratio. In the present study, however, the latter were smaller and with an intensely basophilic cytoplasm.

The onset of the proestrus phase was marked by the prevalence of intermediate cells and bacteria and mucus. The transition to estrus began when superficial cells once again reached or exceeded 70%. The morphological cellular aspects of each phase were similar to those described in agoutis[24, 25, 27], capybaras[10], pacas[8], and cavies[23]. Figurative elements associated with colpocytological smear cells were not included in the results, and their description is limited herein to abundant bacteria, leukocytes, and mucous filaments between the metestrus and diestrus phases.

Our findings concerning the average distributions of different cell types during the estrous phase in cavies resembled those observed during the metestrus phase. Furthermore, the presence of a greater number of nucleated scales compared to nucleated superficial cells across all cycle phases suggests a maturation stage akin to that observed in agoutis[25] and dissimilar to those of cavies raised in captivity and Rock cavies (*Kerodon rupestris*)[23, 28].

A significant effect ($p<0.05$) concerning enucleated scales during different estrous cycle phases was noted for both experimental groups, with the highest counts observed in the estrus phase and the lowest in the diestrus phases. This contrasts with findings reported for agoutis, where significant differences in enucleated surface cell counts were only noted in the estrus phase, with higher counts when compared to the other phases, while no difference was observed between phases[25]. Concerning cavy male treatments, statistically higher scale counts were observed in the proestrus phase in the mated female group (35.05 ± 1.97%) compared to the group with no male contact (18.00 ± 2.28%).

The practice of introducing a male into a female group to synchronize the estrous cycle is paramount for various purposes, such as embryo cryopreservation, sanitary colony cleaning, and murine transgenesis[29]. In the case of goats and sheep, reproductive performance is directly tied to the profitability of milk and meat production, considering the economic, environmental, and sociological significance of these animals[30]. Therefore, synchronizing estrous cycles offers substantial benefits to livestock producers[31].

Although a limited body of research is available on estrus induction in wild rodents, exploring this area could potentially enhance their reproductive control. For instance, the use of prostaglandin alone or associated with the GnRH analogue for estrus induction displays limited efficiency in both agoutis[32] and guinea pigs[33]. This underscores the need to standardize protocols for better reproductive efficiency in captivity.

Cycle synchronization was not evident for cavies in the present study, although the presence of a male was both directly and indirectly associated with estrogenic stimulation during the proestrus phase. This, in turn, led to an increase in the vaginal epithelium, which tends to stratify from the most differentiated layers, resulting in the desquamation of superficial anucleate cells. In one study in this regard, female rock cavies maintained with a male exhibited statistically different enucleated scale counts in the estrus phase when compared to the other phases according to the Tukey test[28]. This contrasts with our data, as significant enucleated scale differences were observed in all cycle phases in both treatment groups.

Concerning intermediate cells, significant interaction effects between treatments were also observed in the proestrus phase. However, the male-free group exhibited higher intermediate cell percentages (48.73 ± 2.17%) compared to G3 females (37.85 ± 1.88%). Less shedding of superficial anucleate cells was also noted in the G3 group, probably due to lower circulating estrogens. This, in turn, may lead to more intermediate cells due to higher progesterone levels and, consequently, greater intermediate cell detachment. Furthermore, the G3 proestrus and metestrus phases exhibited the highest intermediate cell counts, with no significant differences. However, significant differences were noted when comparing the diestrus and estrus phases, with the latter exhibiting the lowest intermediate cell counts.

In G2 females, the estrus phase also showed the lowest cell counts, differing significantly from the other phases but not between each other. The metestrus phase exhibited the highest intermediate cell counts, similar to that reported for agoutis[25] and pacas[27]. In these animals, intermediate cell counts differed significantly from the other cycle phases, but not from each other. Furthermore, higher nucleated superficial cell counts were expected in the proestrus and estrus phases in cavies, as greater cell differentiation and proliferation stimulation are noted during these phases due to higher estrogen concentrations. As ovulation takes place during the luteinizing hormone peak in the estrus phase, the highest average percentage of this cell type was detected during this phase, although not statistically different from the

preceding phase and differing from the other phases. This corroborates reproductive agouti data except for the proestrus phase findings[25].

A significant parabasal cell production difference was noted between the diestrus phase and the other cycle phases. However, no difference was observed between the metestrus and proestrus phases, with the lowest production occurring in estrus. Similar results were observed for basal cells, which was higher in the diestrus phase and significantly different ($p>0.05$) from the other phases. However, each cycle phase was significantly different, with lower basal cell production in the estrus phase. The observed diestrus phase pattern is consistent with high circulating progesterone concentrations, inducing a thinner vaginal epithelium, which results in a thin germinal layer of deep cells[34].

5. Conclusion

Cavy gestation typically lasted 59 days, resulting in the birth of one to two pups at a time. Female cavies exhibit a continuous polyestrous sexual cycle, lasting about 14.8 ± 0.73 days when exposed to a male and 14.6 ± 0.75 days when kept without a male. The presence of a male did not influence cycle length. The reproductive cycle in cavies is similar to that of other cavids, being categorized as the proestrus, estrus, metestrus, and diestrus phases. The absence of a male significantly influenced the number of intermediate and enucleated superficial cells during the proestrus phase. Conversely, the presence of a male extended the diestrus phase duration, which may be associated with high progesterone levels and reduced circulating estrogen levels.

Conflict of interest declaration

The authors declare no conflict of interest.

Author contributions

Conceptualization: A. M. do Vale and M. F. de Oliveira. *Data curation*: A. M. do Vale. *Formal analysis*: G. B. de Oliveira and F. V. F. Bezerra. *Investigation*: A. M. do Vale, G. B. de Oliveira, H. N. A. Júnior, F. V. F. Bezerra, A. C. F. C. de Sousa, J. A. R. A. Diniz, and I. R. G. Lopes. *Methodology*: A. M. do Vale and M. F. de Oliveira. *Resources*: M. F. de Oliveira. *Supervision:* M. F. de Oliveira. *Validation:* A. M. do Vale and M. F. de Oliveira. *Writing (original draft):* A. M. do Vale. *Writing (proofreading and editing):* A. M. do Vale and M. F. de Oliveira.

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