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Original

Effect of Different Oral Doses of Progesterone on Estrus Synchronization and Ovulation in Cavies

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ABSTRACT

Aim. This study looked to assess the effect of different oral doses of progesterone (P4) on estrus synchronization and ovulation of covies. **Methods:** The study was conducted in the high Ecuadoran tropical areas, on a commercial farm located at 2 500 meters above sea level. A total of 35 multiparous Peruvian female covies and five male covies were used in the experiment. The procedure consisted of five treatments: T1= 0.33mg/kg (n=7). T2=0.28mg/kg (n=7). T3=0.22mg/kg (n=7). T4=0.17mg/kg (n=7). T5=0.11mg/kg (n=7). The vaginal membrane opening was evaluated on the last day (15d) of P4 administration every 24 hours (h). A vaginal cytology test was performed to establish the moment of ovulation, which helped determine the moment of estrus and ovulation. A simple ANOVA was performed to analyze the vaginal membrane opening, the vaginal cytology, estrus, and ovulation. A 5% Tukey test was conducted for multiple comparisons. **Results:** The T1, T4, and T5 covies showed 100% vaginal membrane opening, whereas T2 and T3 underwent 85.7% vaginal opening, with a general mean of 94.3%. More than 50% vaginal cells from T1 were superficial at 37.7 ± 4.84 hours following P4 withdrawal; T2 and T3 needed 14.3 additional hours. Ovulation in T1 was observed at 85.7 ± 4.84 hours, with a value similar to T2 (88.0 ± 5.05 hours), and no differences between them ($p>0.05$). The T3 and T4 animals ovulated 20.6 hours after. However, the T5 animals needed more hours for ovulation (113.1 ± 4.42). **Conclusion:** The oral administration of 0.33 mg of progesterone for 15 days in a row helped synchronize estrus at 48 hours following the administration of progesterone.

Keywords: Female covy, synchronization, estrus, ovulation, progesterone (Source: *AGROVOC*)

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INTRODUCTION

Covies constitute an excellent animal model for laboratory studies (Trejo *et al.*, 2019) since they go through an interstitial placental implantation process and the formation of a human-like syncytiotrophoblast (Wang *et al.*, 2019). However, one of the major challenges linked to the utilization of covies for breeding research is the exact day on which mating and ovulation will occur.

The duration of the estrus cycle is approximately 16 days (Araníbar and Echevarría, 2014), with four phases: proestrus (1-1.5 days), estrus (8-24 hours), meta-estrus (1-1.5 days), and diestrus (13-15 days) (Kuhne and Mendoza, 1992). These phases are characterized by progesterone and estrogen interaction, the former has high levels during the estrus, declining at ovulation, whereas the latter prevails during the diestrus period (Wilson *et al.*, 2021). Besides, the existence of two follicular waves during the estrus cycle has been described. One ends on days 10-11, while the other ends at ovulation (Bland, 1980).

The administration of oral progesterone for 15 straight days was assessed, as it is an easy method that increases the number of estrus animals by 90% (Grégoire *et al.*, 2012). However, other species using lower doses of oral progesterone have developed follicular cysts (De Rensis and Kirkwood, 2016).

Consequently, this study aimed to assess the effect of different oral doses of progesterone on estrus synchronization and ovulation in covies.

MATERIALS AND METHODS

Animals and farms

Overall, 35, 4-6 months old, multiparous, and healthy Peruvian covies were selected for the study. The animals weighed 1000-1200 g, with a body condition (BC) of 2.5-3.5 on a scale of 1 (cachectic) to 5 (obese), as described by Ara *et al.* (2012). Moreover, five fertile 1500-2000 g male covies (aged 6-8 months) were included.

The experiment was performed on a commercial farm in the high tropics (2500 meters above sea level), with an average temperature of 11.8 ^{C}. The animals underwent a 15-day adjustment period before the study. The diet consisted of 20% ryegrass (*Lolium multiform*), 70% Alfalfa (*Medicago sativa*), and 10% commercial feed containing 16.27% protein, administered twice a day. The covies were exposed to the light for 12 h, and for the other 12 h they were in the dark.

Experimental design

Five different doses of oral progesterone (Altrenogest, Regumate®, MSD Animal Health, France) were evaluated during the estrus synchronization and ovulation. T1=0.33mg/kg (n=7). T2=0.28mg/kg (n=7). T3=0.22mg/kg (n=7). T4=0.17mg/kg (n=7). T5=0.11mg/kg (n=7). The doses were administered orally using an adjustable (20 µl to 200 µl) automatic pipette (Boeco, Germany) for 15 straight days (Fig. 1). From the last day of the administration on (day 15), estrus and ovulation were evaluated every 24 hours (h).

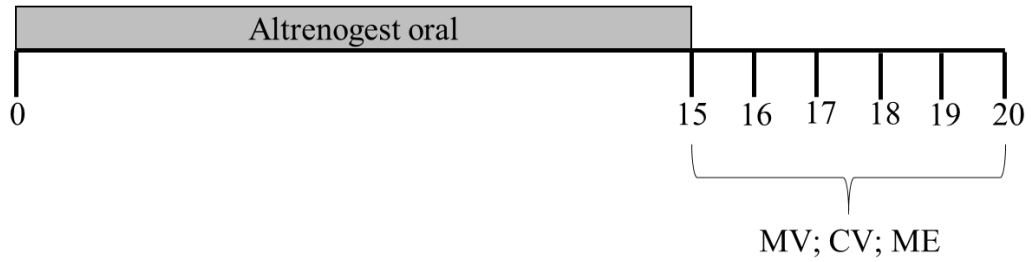

Figure 1. Experimental protocol: day 0=beginning of the protocol, administration of oral progesterone (Altrenogest) up to day 15. Daily assessment of the vaginal membrane aperture. CV=vaginal cytology. ME=determniation of effective mating.

Estrus assessment

The vaginal membrane opening was monitored for five consecutive days (6:00 am), starting on day 15 (Fig. 1), as described by Li *et al.* (2015), with adjustments.

The animal was placed supine decubitus, then the vulva was disinfected and the presence or absence of vaginal membrane was checked. Four categories were established according to the opening percentage: closed (0%), beginning to open (25%), semi-open (50%), and open (100%). The criterion for covy estrus consisted of 50% vaginal membrane aperture.

The samples of the vaginal mucosa (cytology tests) were collected every 24 h from the day the vaginal membrane began to open (25%) to its closure. A 6" sterile disposable swab was dampened and introduced into the mid-third of the vagina, then it was swabbed clockwise. The samples were spread on microscope slides and were stained with Diff-Quick (Quick staining, BIOMED, Ecuador), according to the technical specifications of the manufacturer. The smear was observed at 400x magnification. The results showed that the female covy was in estrus, with 50% or more surface polyhedral-shaped cells and pycnotic nucleous or surface nucleousless cells (Arcila *et al.*, 2019).

The day the female covy showed 50% opening of the vaginal membrane, a male covy was brought in for an hour (8:00 am). Effective mating was evaluated to determine the acceptability of the female, through spermatozoa identification in the vaginal cytology tests made every 24

hours. The mating day and time were determined by subtracting 24h from the day the samples tested positive.

Ovulation assessment

The ovulation time was established by cervical cytology, as described by Grégoire *et al.* (2012), who said that the moment of ovulation is associated with the presence of greater numbers of leukocytes and few intermediate cells.

Statistical analysis

The data were processed through GraphPad Prism, 8.0, (2016, GraphPad Software, USA). The main values of the variables were achieved. Data normality was established through the Shapiro-Wilk test, where $p>0.05$. A simple ANOVA was used for the analysis of the vaginal membrane aperture, the cytology testing, estrus, and ovulation. A 5% Tukey test was conducted for multiple comparisons.

RESULTS AND DISCUSSION

Vaginal membrane (MV)opening

On day 15, the vaginal membrane (MV) turned light pink (Fig. 2; panel 1) and was totally closed (Fig. 3; panel 1). As estrus day approached (day 16) gradual coloring changes were observed in the MV, turning dark pink (Fig. 2; panel 2).

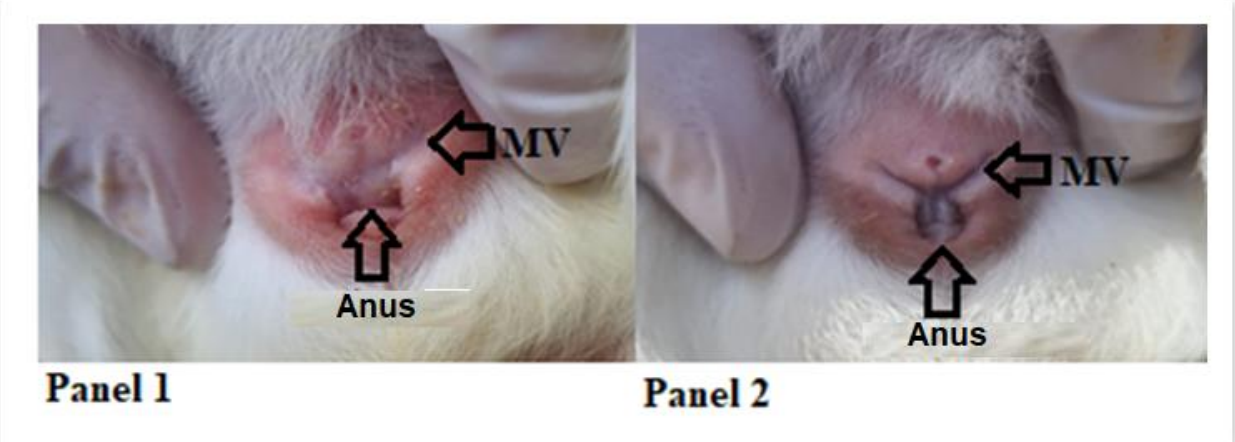

Figure 2. Color changes of the vaginal membrane following the withdrawal of progesterone. Panel 1: MV=vaginal membrane in light pink, below is the anus. Panel 2: vaginal membrane in dark pink, below is the anus.

On day 18, 50% membrane opening was observed (Fig. 3; panel 2), to fully open on days 19 and 20 (Fig. 3; panel 3).

Figure 3. Vaginal membrane opening Panel 1: MV=closed vaginal membrane 0% Panel 2: semi-open membrane (50%). Panel 3: open membrane (100%).

The T1, T4, and T5 covies showed 100% vaginal membrane opening, whereas T2 and T3 underwent 85.7% vaginal opening, with a general mean of 94.3%. These results show that the daily administration of oral progesterone (Altrenogest) in doses of 0.11-0.33 mg/kg body weight, led to estrus synchronization in similar percents to the values described by Grégoire *et al.* (2012), who observed 93% opening when using oral 0,22mg/kg progesterone (Altrenogest). Hence, the currently suggested dose can be cut down to half with the same estrus synchronization percentage in covies.

However, the animals in T5 (78.9 ± 4.43 h) that received low oral progesterone doses (0.11 mg/kg) needed 34.3 more hours to achieve 50% of membrane aperture following the withdrawal of oral progesterone ($p<0.05$) than the the T1 covies (44.6 ± 4.16 h) and T2 (52.0 ± 4.00 h; Fig. 4). The covies in treatments 3 (60.0 ± 5.37 h) and 4 (65.1 ± 4.42 h) showed similar values to the ones in T5 ($p>0.05$; Fig. 4).

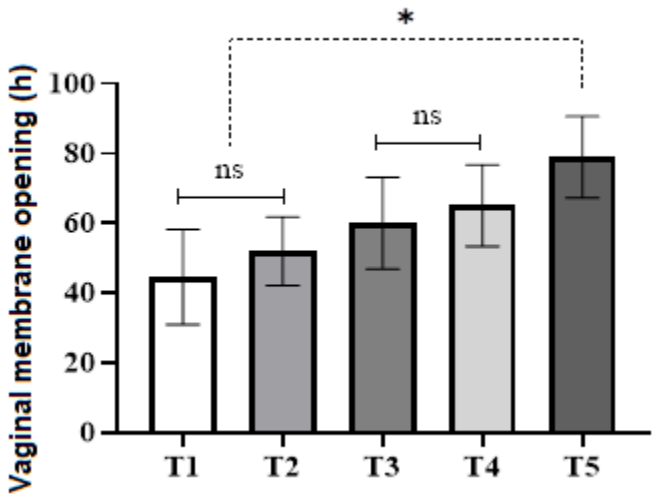

Figure 4. Mean and standard error of hours elapsed following the withdrawal of progesterone (day 15) until the opening of the vaginal membrane in all the treatments. T1= 0.33mg/kg. T2=0.28mg/kg. T3=0.22mg/kg. T4=0.17mg/kg. T5=0.11mg/kg. ns=no significant difference between the treatments*=differences between the treatments Tukey (5%).

Vaginal cytology.

More than 50% of cells were observed (Fig. 5; panel 1) to be superficial at 37.7 ± 4.84 hours in T1 cytology. However, in T2 and T3, it was 52.0 ± 4.00 h, whereas T4 was 54.9 ± 4.43 h. An average of 14.3 was needed to get the surface cell percentage in T1 (Fig. 6).

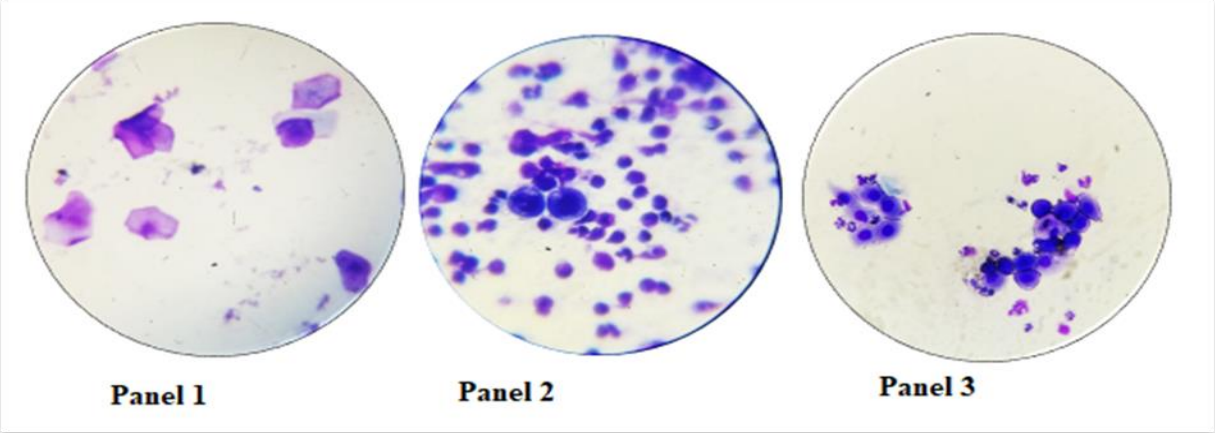

Figure 5. Presence of different types of cells from the day the vaginal membrane began to open (25%) until closure. Panel 1: Presence of surface cells (estrus). Panel 2: the emergence of a large number of neutrophils (ovulation). Panel 3: Parabasal cells (meta-estrus).

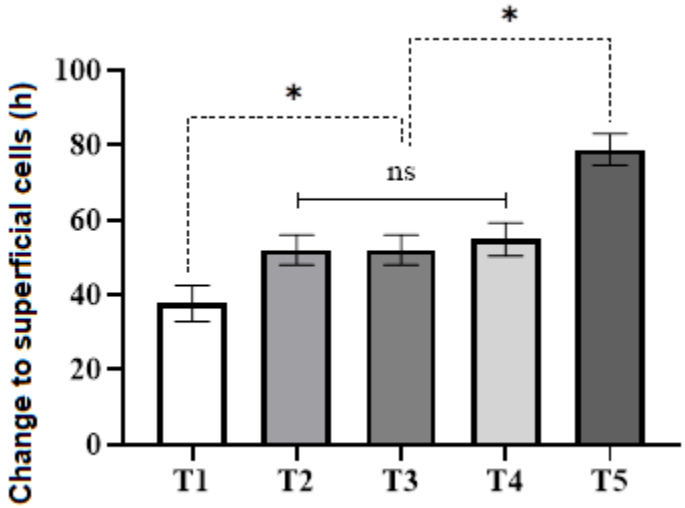

Figure 6. Mean and standard error of hours elapsed following the withdrawal of progesterone (day 15) until the emergence of 50% or more of surface cells in all the treatments. T1= 0.33mg/kg. T2=0.28mg/kg. T3=0.22mg/kg. T4=0.17mg/kg. T5=0.11mg/kg. ns=no significant difference between the treatments*=differences between the treatments Tukey (5%).

The observations of the vaginal membrane aperture and the presence of surface cells combined confirmed that estrus occurred quicker in the female covies under the highest hormone doses.

Mating

Effective mating in T1 was determined at 44.5 ± 6.25 hours and T2 (52.0 ± 4.00 hours), with no significant differences between the two treatments ($p>0.05$. Fig. 7). The T3 covies (68.0 ± 7.37 hours) and T4 (61.7 ± 4.84 hours) needed 24 additional hours on average to accept the male. The T5 animals (78.8 ± 4.40 hours) were mounted 34.3 hours after T1, and 10.8 hours following the T2 and T3 animals, with differences between these two ($p<0.05$). These results confirmed the concepts indicating that most females copulated on the same day their vagina opened, while few of them needed 2 additional hours for effective copulation (Araníbar & Echevarría, 2014).

Therefore, the administration of 0.33-0.28 mg/kg permits the female covy to accept the male approximately 48 hours later. However, as the progesterone doses decreases, the period for the female to accept the male lasts longer (Fig. 7).

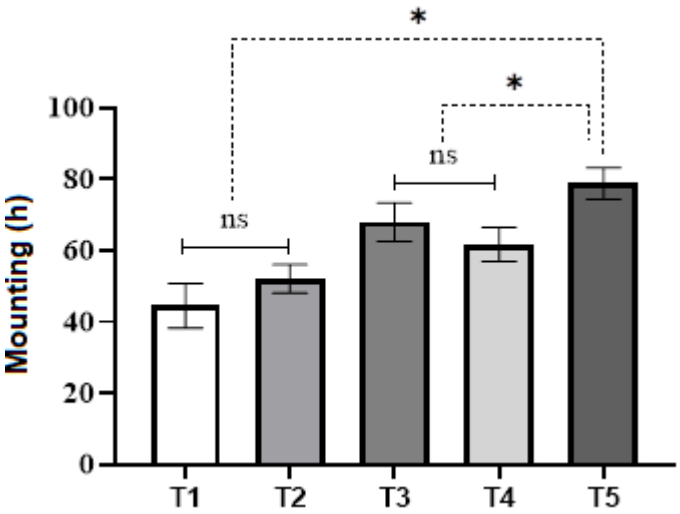

Figure 7. Mean and standard error of hours elapsed following the withdrawal of progesterone (day 15) until the effective mating in all the treatments. T1= 0.33mg/kg. T2=0.28mg/kg. T3=0.22mg/kg. T4=0.17mg/kg. T5=0.11mg/kg. ns=no significant difference between the treatments*=differences between the treatments Tukey (5%).

Ovulation

Ovulation was determined through the presence of over 50% neutrophils in the vaginal smear. 5; panel 2). Upon the withdrawal of progesterone in T1, ovulation was observed at 85.7 ± 4.84 hours, with a similar value to T2 (88.0 ± 5.05 hours); no differences were observed between them ($p>0.05$). The T3 covies (96.0 ± 6.19 hours) and T4 (106.3 ± 7.13 hours) ovulated 20.6 hours following the T1 and T2 animals.

The covies needing more time to ovulate were the ones receiving the lowest progesterone doses (T5=113.1 ± 4.42 h; Fig. 8). The administration of high P4 doses led to a reduction of ovulation in approximately 34 hours (T1 and T2), compared to the results described by Grégoire *et al.*

(2012), whose animals ovulated between days 4-5 (96-120 hours), upon the withdrawal of 0.22 mg/kg Altrenogest administration for 15 days.

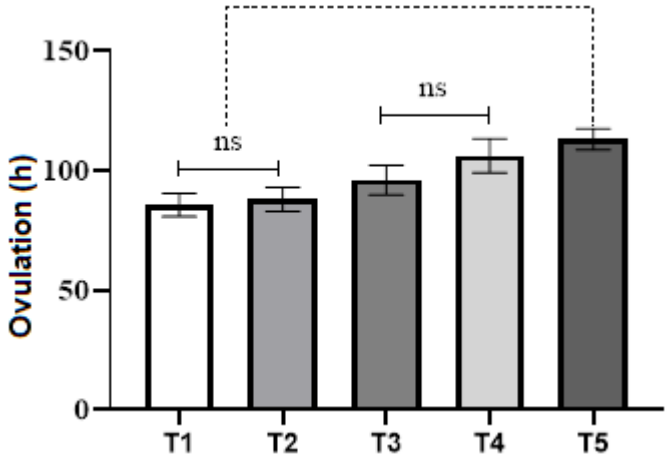

Figure 8. Mean and standard error of hours elapsed following the withdrawal of progesterone (day 15) until the ovulation in all the treatments, confirmed by the emergence of neutrophils in the vaginal smear. T1= 0.33mg/kg. T2=0.28mg/kg. T3=0.22mg/kg. T4=0.17mg/kg. T5=0.11mg/kg. ns=no significant difference between the treatments*=differences between the treatments Tukey (5%).

Consequently, the administration of 0.33 or 0.28 mg progesterone orally influences the earlier presence of ovulation in female covies.

CONCLUSIONS

The oral administration of 0.33 mg of progesterone per female covy weight kg, for 15 days in a row helped synchronize estrus at 48 hours following the withdrawal of progesterone. It caused faster acceptability and ovulation than in the presence of lower doses of this hormone.

Being an orally-administered drug, it can be easily used and applied in the laboratory and the field. It facilitates the existence of animals at different moments of the estrus cycle, which is helpful to conduct breeding biotechniques, such as artificial insemination and superovulation.

REFERENCES

Ara G., M., Jiménez A., R., Huamán C., A., Carcelén C., F., & Díaz C., D. (2012). Desarrollo De Un Índice De Condición Corporal En Cuyes: Relaciones Entre Condición Corporal Y Estimados Cuantitativos De Grasa Corporal. *Revista de Investigaciones Veterinarias Del Perú*, *23*(4), 420–428. https://doi.org/10.15381/rivep.v23i4.948

Araníbar, E., & Echevarría, L. (2014). Número de ovulaciones por ciclo estrual en cuyes (Cavia porcellus) Andina y Perú. *Rev Inv Vet Perú*, *25*(1), 29–36. http://www.scielo.org.pe/scielo.php?script=sci_arttext&pid=S1609117201400010003&

lng=es&nrm=iso

Arcila Q, V. H., César Serrano-Novoa, M. A., Hernández R, M. E., & Luz Meza B Mvz, M. P. (2019). Investigación Estandarización de la citología vaginal exfoliativa correlacionando los niveles séricos de progesterona en perras durante la peri-ovulación. https://revistas.ucc.edu.co/index.php/sp/article/viewFile/565/536

Bland, K. P. (1980). Biphasic follicular growth in the guinea-pig oestrous cycle. *Journal of Reproduction and Fertility*, *60*(1), 73–76. https://doi.org/10.1530/jrf.0.0600073

De Rensis, F., & Kirkwood, R. N. (2016). Control of estrus and ovulation: Fertility to timed insemination of gilts and sows. *Theriogenology*, *86*(6), 1460–1466. https://doi.org/10.1016/j.theriogenology.2016.04.089

Grégoire, A., Allard, A., Huamán, E., León, S., Silva, R. M., Buff, S., Berard, M., & Joly, T. (2012). Control of the estrous cycle in guinea-pig (Cavia porcellus). *Theriogenology*, *78*(4), 842–847. https://doi.org/10.1016/j.theriogenology.2012.03.034

Kuhne, W., & Mendoza, A. S. (1992). Scanning Electron Microscope Investigations on the Vaginal Epithelium of the Guinea Pig during the Estrous Cycle. *Archives of Histology and Cytology*, *55*, 205–210. https://doi.org/10.1679/aohc.55.Suppl_205

Li, J. rong, Wang, W., & Shi, F. xiong. (2015). Induction of follicular luteinization by equine chorionic gonadotropin in cyclic guinea pigs. *Journal of Zhejiang University: Science B*, *16*(12), 980–990. https://doi.org/10.1631/jzus.B1500046

Trejo-Sánchez, F., Mendoza-Martínez, G., Plata-Pérez, F., Martínez-García, J., & Villarreal-Espino-Barros, O. A. (2019). Crecimiento de cuyes (Cavia porcellus) con alimento para conejos y suplementación de vitamina C. *Revista MVZ Cordoba*, *24*(3), 7286–7290. https://doi.org/10.21897/rmvz.1384

Wang, J., Liu, Z., Sun, Q., Xia, S., Cui, J., Yang, L., An, L., Zhang, J., Su, L., Su, Y., & Du, F. (2019). Combined treatment with cysteamine and leukemia inhibitory factor promotes Guinea pig oocyte meiosis in vitro. *American Journal of Translational Research*, *11*(12), 7479–7491.

Wilson, R. L., Lampe, K., Matushewski, B. J., Regnault, T. R. H., & Jones, H. N. (2021). Time mating guinea pigs by monitoring changes to the vaginal membrane throughout the estrus cycle and with ultrasound confirmation. *Methods and Protocols*, *4*(3). HTTPS://DOI.ORG/10.3390/MPS4030058

AUTHOR CONTRIBUTION STATEMENT

Research conception and design: GSGP, PGVA, LEAG; data analysis and interpretation: GSGP, PGVA, LEAG, redaction of the manuscript: GSGP, PGVA, LEAG.

CONFLICT OF INTEREST STATEMENT

The authors state there are no conflicts of interest whatsoever.