JOURNAL OF THE Selva Andina Animal Science
Official Journal of the Selva Andina Research Society.

Research Article

ISSN 2311-3766 (print edition)
JSAAS
ISSN 2311-2581 (online edition)

Determination of hormone concentrations (estradiol and progesterone) during follicular detour in llamas

Determinación de concentraciones hormonales (estradiol y progesterona) durante la desviación folicular en llamas

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Article Data

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Keywords:

ELISA,
estradiol,
follicular deviation,
follicles,
llamas,
progesterone.

J. Selva Andina Anim. Sci.
2025; 12(2):71-78.
Article ID: 148/JSAAS/2025.

Article history

Received May 2025.
Returned August 2025.
Accepted September 2025.
Available online October 2025.

Edited by:
Selva Andina
Research Society

Palabras clave:

Desviación folicular,
ELISA,
estradiol,
folículos,
llamas,
progesterona.

Abstract

The objective was to determine by ultrasonography and concentration of estradiol and progesterone during follicular deviation in llamas, as the first report in South American camelids. 10 females were used in conditions of the Peruvian Altiplano, which were evaluated for follicular deviation from follicular ablation (day 0) to day 6 with daily ultrasonographic evaluations with a SonoStar SS8 equipment with a linear endocavity microconvex transducer at 6.5 MHz in mode 2B, blood samples were to determine estradiol and progesterone by ELISA, all data were analyzed using statistics and descriptive graphs in the Jamovi 2.23 program. Concentrations were evaluated from day 0 to 6, with average estradiol and progesterone concentrations of 0.9105 ng mL^{-1} and 1.11 ng mL^{-1}, respectively, which, although minimal numerical changes are shown, a pattern was observed in both steroid hormones with an increase in their respective concentrations on day 4 after follicular ablation. In conclusion, the llamas follicular deviation began on day 4 after follicular ablation that coincides with the increase in estradiol and progesterone concentrations; however, further studies are needed to determine the process of follicular deviation in llamas.

Resumen

El objetivo fue determinar mediante ultrasonografía y concentración de estradiol y progesterona durante la desviación folicular en llamas, como primer reporte en camélidos sudamericanos. Se utilizaron 10 hembras en condiciones del Altiplano Peruano, las que se evaluaron la desviación folicular desde la ablación folicular (día 0) hasta el día 6 con evaluaciones ultrasonográficas diarias con un equipo SonoStar SS8 con un transductor microconvexo lineal endocavitario a 6.5 MHz en modo 2B, las muestras sanguíneas fueron para determinar el estradiol y progesterona mediante ELISA, todos los datos fueron analizados mediante estadística y gráficos descriptivos en el programa Jamovi 2.23. Las concentraciones fueron evaluadas desde el día 0 a 6 siendo las concentraciones de estradiol y progesterona promedio de 0.9105 ng mL^{-1} y 1.11 ng mL^{-1} respectivamente que, aunque se muestra cambios numéricos mínimos se observó un patrón en ambas hormonas esteroides con un incremento de sus respectivas concentraciones el día 4 post ablación folicular. En conclusión, la desviación folicular en llamas inicio el día 4 post ablación folicular que coincide con el incremento de las concentraciones de estradiol y progesterona, sin embargo, es necesario realizar más estudios para determinar el proceso de desviación folicular en llamas.

Introduction

Reproductive physiology in South American camelids (SAC) has its own characteristics, unlike other domestic species, with follicular dynamics in successive and overlapping waves, as well as induced ovulation as the main particularities[1-3]. The main characteristics of follicular dynamics in CSA are their phases and duration, which classifies them as growth, varying from 3 to 9 days; maturation of 2 to 8 days and regression of 3 to 8 days evaluated in alpacas and llamas[3-5], with average growth rates in alpacas 0.43 mm/day and in llamas 0.5 to 0.9 mm/day[2,5,6], results that allowed to characterize in a general way part of the ovarian activity in CSA, taking into account that there are more phenomena (recruitment, selection, deviation and follicular regression) within follicular dynamics.

In monoovular species such as camelids, only one of many follicles becomes dominant in this process, known as selection, which is characterized by the acquisition of a higher growth rate compared to the other follicles (subordinate follicles). This characteristic is called follicular deviation[7,8], which begins with an average recruitment of 7 to 11 follicles per cohort with diameters of 4 mm (cattle) and 6 mm (mares). reaching follicular deviation diameters of 8.5 to 7.7 mm in cattle and from 22.5 to 19 mm in mares. Current studies in camels report recruited follicle diameters between 4.4 and 3.5 mm and deviated follicles of 7.43 and 7.75 mm in the presence of a single dominant follicle (DF) and codominance, respectively[8,9], important results for the knowledge of female reproductive physiology.

However, there are no reports on follicular deviation in SAC that provide an important contribution to the application of reproductive biotechnologies.

In recent years, the development of reproductive biotechnologies has required greater knowledge of reproductive physiology, with the help of techniques such as hormonal analysis and mainly ultrasonography[6,10]; efficient reproductive management with the use of artificial insemination (AI) and embryo transfer (ET) requires extensive knowledge of follicular dynamics and factors that affect them[4,11], the monitoring of follicular deviation allows to explain some particularities in ET in camelids[12,13]; a response to multiovulation protocols is sometimes reported with the evaluation of the time it takes for follicular deviation and the number of follicles recruited to predict the probable response to the protocols. In this sense, the objective was to characterize the process of follicular deviation in llamas by ultrasonography and concentration of 17β-estradiol, as a first report in SAC.

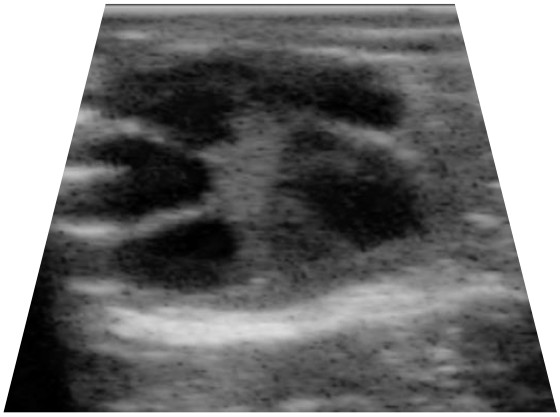
Materials and methods

Animals and food. The study was carried out at the facilities of the Faculty of Veterinary Medicine and Zootechnics, of the National University of the Altiplano Puno, located at an altitude of 3812 meters above sea level (masl), the experiment was carried out between the months of May and August. Ten (n=10) non-pregnant, multiparous females aged between 4 and 6 years were selected, all with a body condition between 2.5 and 3.0 according to the scale recommended by Van Saun[14]. The animals were fed natural pastures, water *ad libitum* and supplemented daily with oat hay.

General characteristics of ovarian ultrasonography. The previous steps were to hold and prepare llamas by sanitizing the perineal area and her vulva, in addition to the transducer since the evaluation was per-

formed transvaginally with a SonoStar SS8 equipment (SonoStar Thecnologies, China) with an endocavity linear microconvex transducer (V6S9 multifrequency: transvaginal examination) at 6.5 MHz in mode 2B to observe one side for each ovary (Figure 1) with the option of *freeze* the images[15-17] were paralyzed for storage and subsequent evaluation, as detailed below:

Figure 1 Ultrasonography of llama ovary with the presence of several follicles (follicular recruitment)



Follicular ablation or follicular wave synchronization. Follicular ablation (day 0) was performed as recommended by various authors[18,19] with some modifications of the vacuum pump pressure of 110 mmHg in our case, then it was evaluated daily to observe the recruitment determined by the presence of a new pool of follicles with diameters between 1.5 and 2.5 mm, the number of follicles and the time elapsed. identifying each of the follicles with the terminology F1, F2, F3 and F4 as the case may be, as recommended by Goodman & Hodgen[20].

Characterization of follicular deviation. Evaluations were performed daily from the day of follicular ablation (day 0) during the mornings to each of the animals, first identifying each of the follicles as F1, F2, F3 and F4 as the case may be, with F1 being for a single DF as the future diverted follicle[9,20]. Identifying the follicles as defined rounded and anechogenic structures, measurements were made inside the follicular walls, taking two diameters using the average of these as the final diameter of the follicles[4,21].

Blood sample and hormonal evaluation (estradiol and progesterone). Samples were obtained by puncture of the jugular vein daily (days 0, 1, 2, 3, 4, 5 and 6 after follicular ablation) in tubes with heparin that were immediately centrifuged, the blood plasma was stored at -20° C until evaluation. The concentration of 17β-estradiol was determined using a commercial ELISA kit (Estradiol ELISA, 17β-estradiol antigen, DiaMetra, Case for quality, Italy) and progesterone (Progesterone ELISA kit Enzo, Farmingdale, NY, USA) following the steps of the product insert for processing with an ELISA kit (Organon Teknica, Microwell System, model Reader 230S).

Statistical analysis. The data were analyzed using descriptive statistics to facilitate their interpretation, for comparison with both a single DF were subjected to normality (Shapiro-Wilk) and homoscedasticity (Levene) tests, resulting in all of them the statistical non-significance with an analysis of variance (one way); estradiol and progesterone concentrations were only described from the day of ablation to follicular deviation. Statistical analyses were performed using the statistical program (R version 4.0.2)[22] with the Rcmdr package and graphs using Microsoft Excel®.

Results

Follicular diameters of a single DF before and after follicular deviation. Table 1 evaluates the follicular diameters before and after follicular deviation, observing before deviation in both cases similar diameters being 2.21±0.89, 2.24±0.89, 2.65±0.93, 2.25±0.67 and 2.37±0.66 for F1, F2, F3, F4 and F5 respectively. The post-follicular deviation diameters show important differences, this phenomenon in llamas of 6.01±0.73 for F1, 2.05±0.52 for F2, 2.25±0.54 for F3, 2.05±0.78 for F4 and 2.07±0.56 for F5 (subordinate follicles).

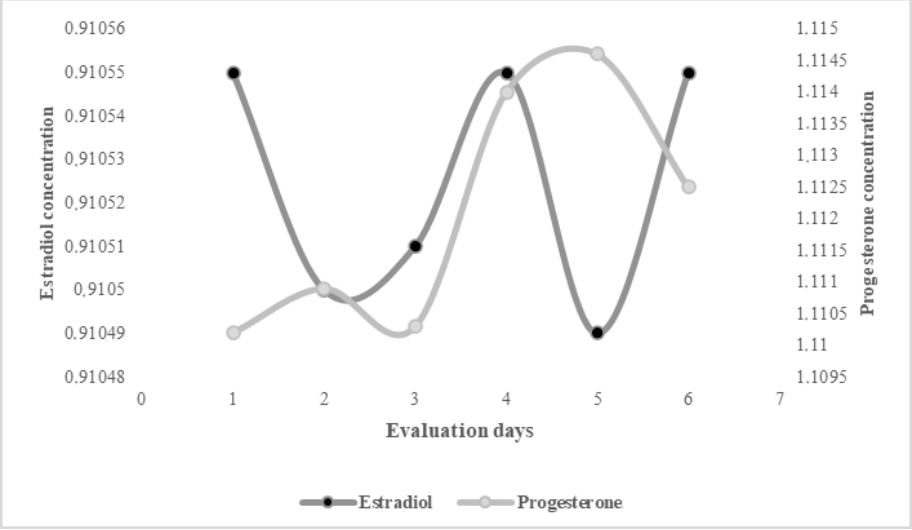
Table 1 Follicular diameters during follicular deviation

	Pre-deviation	Post-deviation (day 5)
F1	2.21 ± .89	6.01 ± .73
F2	2.24 ± .89	2.05 ± .52
F3	2.65 ± .93	2.25 ± .54
F4	2.25 ± .67	2.05 ± .78
F5	2.37 ± .66	2.07 ± .56
Valor de "p"	.9339	**<.0001**

Follicle growth rates pre- and post-follicular deviation. They were in the presence of a single follicle of 0.656 mm/day and 0.58 mm/day in the presence of codominance while the other follicles with a decrease rate that varied from -0.01 mm/day to -0.24 mm/day, which confirm the process of follicular deviation (increase in growth rate) in the llamas evaluated. It is also necessary to indicate that the differentiation of the diameters of the deviated follicles was identified between day 3 and 4 after follicular ablation that coincides with the concentrations of hormones specified below.

Hormone concentration of estradiol and progesterone. Figure 2 shows the relationship between the concentration of estradiol and progesterone at day 4 after follicular ablation, the concentrations of 17β-estradiol have an increase at the beginning (before follicular estradiol ablation) due to the presence of DF of the previous follicular wave, in the same way a more direct relationship is observed by progesterone with the average follicular diameters evaluated for both cases of follicular deviation, day 4 coincides with the beginning of the differentiation of the future DF, increasing their diameter unlike the decrease in diameter of the subordinate follicles (flaming follicular deviation).

Figure 2 Hormonal ratio of estradiol and progesterone in relation to the duration of follicular deviation



Discussion

The presence of simple dominance is a characteristic reported in SAC, camels and cattle[6,9,23], however, co-dominance is reported, which consists of the growth of 2 follicles in the same follicular wave, in both cases we observed a direct relationship with the increase in estradiol and progesterone concentrations from day 3 and 4 after follicular ablation. Follicular deviation is a phenomenon that has been widely studied in cattle. They mention that there is a slight increase in gonadotropins (FSH and LH) before follicular deviation[23,24], the same phenomenon could probably be happening in SAC, however, the behavior of gonadotropins before and after follicular deviation in camelids has not been studied.

Follicular deviation is defined as a change in the growth rate of future DF as opposed to subordinate follicles[8], a similar phenomenon in llamas, we observed differentiated rates of futures for a single follicle in case of codominance, growth rates similar to those reported in llamas and camels[6,9,16] during the follicular wave growth phase and regression rates for subordinate follicles reported in camelids, camels, and cattle[6,8,9]. The onset of follicular deviation in llamas begins on day 4 after follicular ablation both in the presence of a single DF with a diameter of 4.01 mm, and on the day of onset of follicular deviation they were similar to that reported in camels as a species more compatible with llamas[9] as well as in cattle with a similar time of onset of follicular deviation[25-27]. While the future DF differentiated on day 5 after follicular ablation (Table 1) being 6.01 mm, inferring these results as the first findings in llamas.

The levels of estradiol and progesterone during the follicular deviation process have a pattern compatible with the follicular growth observed during follicular recruitment (day 1, 2 and 3) increasing together with the follicular deviation the hormone levels on day 4 after follicular ablation (Figure 2) these results are only descriptive because it is the first report of the flaming follicular deviation, However, this compatible pattern between 17β-estradiol and follicular diameters (growth phases, statics, and regression) is similar to that reported in alpacas, llamas, guanacos, and vicuñas during follicular wave evaluation in these species[6,28-31]. In conclusion, the llamas follicular deviation began on day 4 after follicular ablation with diameters of 4.01 mm in the presence of a FD with a similar pattern of estradiol and progesterone concentrations during follicular deviation, being a first report in South American camelids; however, more articles on the follicular deviation process are needed.

Source of financing

Self-funded with resources from researchers.

Conflicts of interest

The authors have no conflicts of interest.

Acknowledgements

We would like to thank the staff of the Animal Reproduction Laboratory of the Faculty of Veterinary Medicine and Animal Science of the National University of the Altiplano.

Ethical considerations

For the initiation of research activities for this study, mainly for the use of animals and procedures, they were reviewed and approved by the Ethics Committee of the Faculty of Veterinary Medicine and Animal

Science of the National University of the Altiplano Puno.

Authors' contribution to the article

Uri Harold Perez Guerra y Manuel Guido Perez Durand, designed and carried out the experimental part and wrote the manuscript. *Edilberto Mamani Macedo* and *Carlos Washington Bustamante Quispe*, collaborated and carried out the study. *Uri Harold Perez Guerra* and *Edilberto Mamani Macedo*, performed the analysis and interpretation of the statistics and reviewed the manuscript. *Manuel Guido Pérez Durand* and *Eliseo Pelagio Fernández Ruelas*, determined and reviewed the manuscript. *Eliseo Pelagio Fernández Ruelas* and *Manuel Guido Pérez Durand*, collaborated in the study design and reviewed and edited the manuscript.

Limitations in the research

There were no limitations in the research.

Access to data

The data collected and analyzed, which were an important part of the study, and their respective conclusions are available to the authors without any restrictions for any appropriate purposes.

Permissions for publication

The authors, once the research was completed, decided by consensus to publish it.

Use of Artificial Intelligence

The authors declare that they did not use artificial intelligence in writing this document.

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