

Ovarian Follicular Activity During Late Gestation and Postpartum in Guanaco (*Lama guanicoe*)

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Contents

This study evaluated ovarian activity in late gestation and post-partum in guanacos in captivity. Follicular dynamics was monitored every second day from 40 days before and other 40 after delivery by transrectal sonography and by plasma steroids concentrations. Seven out of eight (87.5%) of gestating females presented ovarian follicular activity under progesterone levels >3 nmol/l with maximum follicular size of 8.42 ± 0.83 mm from days 23 to 1 before delivery. After delivery, all females have follicular wave development from day 0 to 38, with larger follicular size and longer follicular wave phases and interwave interval when compared with pre-partum data. During post-partum period, there was a close relationship between follicle size and estradiol-17 β concentration, with $r = 0.69$ at the beginning of growth phase and $r = 0.86$ in association with the largest dominant follicle. Plasma estradiol-17 β concentration varied from 11.92 to 198.55 pmol/l. Plasma estrone sulfate, free estrone and progesterone returned to baseline concentrations during periparturition period and remained basal thereafter. The results described follicular activity during late gestation and early post-partum period. These findings provide relevant information to understand physiological changes occurring during this reproductive key period in seasonal breeders with long gestation duration as New and Old World camelids.

Introduction

The guanaco is a wild South American camelid (*Lama guanicoe*), widespread from Paraguay and Peru to Southern Chile and Argentina. It adapts to different ecosystems from desert and arid to forest and cold. As environment constraints affect ungulate species everywhere, guanacos developed a strategy of seasonal reproduction and singleton gestation (Franklin 1982).

The South American camelids are the alpaca (Vaughan et al. 2004), llama (Adams et al. 1990), vicuña (Agüero et al. 2001) and guanaco (Riveros et al. 2010) being all induced ovulators. In the absence of an ovulatory stimulus, follicular activity occurs in consecutive waves, with the synchronous emergence of

several follicles, one of which becomes dominant while subordinate follicles regress (Adams et al. 1990). As the ovarian follicles grow and differentiate, increasing amounts of oestrogen are produced, which upregulate the synthesis and release of the pituitary gonadotrophins, thereby promoting the final stages of ovarian follicular growth (Rosenfeld et al. 2001). The mean time required to complete a wave in non-gestating guanaco was reported to be 15.1 ± 4.2 days, showing an inverse relationship between the diameter of the largest follicle and the number of follicles and plasma estradiol-17 β concentration wave-like pattern (Riveros et al. 2010).

As has been shown for many domestic ungulate species, ovarian activity is reduced during pregnancy when progesterone concentrations are more or less constantly high (Peluso 2006). In pregnant llamas, distinct changes folliculogenesis were observed under high progesterone levels, such as lower number of follicles, smaller dominant follicle diameter, shorter interwave periods (Adams et al. 1990) and lower estradiol-17 β levels. The end of pregnancy in guanaco is characterized by a gradual decrease of progesterone during the last 4 weeks of gestation, followed by a final decline to baseline concentrations which were reached on the day after parturition (Riveros et al. 2009). Resumption of postpartum ovarian activity shows distinct species-specific features with postpartum acyclicity observed in many domestic animal species, depending on various factors such as suckling and nutritional status, calving season and age (Yavas and Walton 2000). For domestic South American camelids, it has been reported that resumption of follicular dynamics may occur as early as day 5 to 7 postpartum (Sumar et al. 1972; Bravo et al. 1991; Aba et al. 1998) with the earliest sexual activity and ovulation occurring approximately 10 days after delivery (Bravo et al. 1991).

In general information on the resumption of ovarian activity, postpartum in camelids must be considered scarce (Manjunatha et al. 2012). However, a profound understanding of follicular dynamics during this period is especially important in a seasonal breeder with long gestation period (333–359 days), as the guanaco (Riveros et al. 2010).

The study was performed in a guanaco herd kept in captivity in the Mediterranean ecosystem of Chile ($33^{\circ}38'28''S$, $70^{\circ}34'27''W$).

In guanacos, the resumption of follicular dynamics may occur early during the postpartum period, with significant follicular growth and maturation a few days after partition, suggesting that in some cases, the underlying follicular waves may have emerged already prepartum. Thus the aim of this study was to compare ovarian activity during late gestation with early postpartum period by means of sonography and steroid profiles.

Materials and methods

Animals and blood samples

The study was performed during spring and summer in a guanaco herd kept in captivity in the Mediterranean ecosystem of Chile (33°38'28"S, 70°34'27"W). The observational group was formed by eight healthy, pregnant-lactating 7- to 8-year-old, guanacos exhibiting a body condition score between 3 and 4. Guanacos were fed with access to alfalfa hay, natural pasture and water *ad libitum*. Sonography and blood sampling were conducted using an infrastructure specially designed for the species.

The development of the dominant follicle was monitored three times a week by transrectal sonography, and each accomplished mating and duration of copulation were recorded. Ovulation was confirmed by sonography and registered to estimate delivery date. Thereafter, females were kept isolated from males during gestation and sampling period. Each female was sampled from 40 days before the estimated delivery date to 40 days postpartum (Riveros et al. 2009).

Sampling was performed with females immobilized in the chute under vision deprivation with a hood to minimize stress and easier handling. Ovarian follicular dynamics were monitored three times a week by sonography and by plasma measurement of steroids concentrations. Sonographic evaluations were performed using a real-time, B-mode scanner (Biomedical® model Scanner 6500C LC, Pie Medical Scanner 100 UltraSound, Esaote Europe B.V., Maastricht, the Netherlands) equipped with a 6 MHz linear array electronic transducer. Blood samples of 10 ml were collected by right jugular venipuncture into vacuum tubes with EDTA (Vacutainer®, Franklin Lakes, NJ, USA) centrifuged ($2000 \times g$ for 15 min) for plasma collection and stored at -18°C until analysis.

Hormone assays

Assay of estradiol-17 β , estrone and estrone sulphate was determined by inhouse radioimmunoassays as previously described (Riveros et al. 2009). Estradiol-17 β minimum detectable concentration was 7.5 pmol/l. Intra- and interassay coefficients of variation were 9.4% and 17.5%, respectively. The minimum detectable concentration of estrone and estrone sulfate was 0.3 nmol/l. Intra- and interassay coefficients of variation were 9.3% and 11.5%, respectively.

Progesterone was determined by a commercial chemiluminescence-based method using ACS 180 Automated System with kit PRGE (Bayer Vital GmbH, Fernwald, Germany) (Riveros et al. 2009). The suitability of this method for measurements in guanacos was confirmed by comparative determinations applying a well-established radioimmunoassay after extraction of the samples with hexane as a reference (Hoffmann et al. 1973). The minimum detectable concentration is 0.35 nmol/l. Intra- and interassay coefficients of variation were 6.3% and 7.4%, respectively.

Analysis of data

Based on sonographic observations, the interwave interval was defined as the time between two consecutive dominant follicles exceeding a diameter of 7 mm. Moreover, for each follicular wave, the total follicular number was registered, and each follicle profile was divided into growing, static and regressing phases as previously described by Riveros et al. (2010). The data on characteristics of follicular waves were expressed as mean \pm SEM.

Spearman's correlation analysis was carried out to test the relationship between size of the largest follicle, the total number of follicles and hormonal data. Chi-square analysis was used to compare the proportion of dominant follicles present between gestation and postpartum period, and in the left compared to right ovary.

To cope with the asymmetrical distribution of hormone plasma concentrations, results were normalized by log to the base 10 transformation prior to statistical evaluation. Repeated-measures ANOVA test was performed to detect differences in hormone concentrations. A Bonferroni test was used to determine significant differences between means.

Statistical analysis was carried out using the STATA 8.1 software package (Stata Corporation, College Station, TX, USA). An error probability of $p < 0.05$ was considered significant.

Results

Follicular wave dynamics

Gestation period

All pregnancies were observed in left uterine horn with an ipsilateral corpus luteum. Accessibility of the left ovary and uterine horn was hampered by uterine enlargement during late gestation and postpartum period demanding additional time to perform sonographic evaluations.

Nine complete follicular waves starting from a follicular diameter of 2 mm until regression were analysed during late gestation in seven of the eight pregnant females (87.5%). In the remaining animal, a partial wave without any dominant follicle occurred. Follicular growth was observed with mean plasma progesterone of 4.18 ± 0.5 nmol/l during the late gestation period with mean maximum follicular diameter 8.42 ± 0.83 mm

Table 1. Follicular wave characteristics during gestation and postpartum period in guanaco

Variable/Period	Gestation	Postpartum
Follicular wave (days)	15.44* ± 2.65	17.08^ ± 3.69
Growth phase (days)	8.11* ± 1.27	9.21^ ± 2.80
Static phase (days)	1.89* ± 1.36	2.25^ ± 1.11
Regressing phase (days)	5.44* ± 1.24	5.63^ ± 1.69
Dominant follicle (mm)	8.42* ± 0.83	9.22^ ± 2.07
Growing rate (mm)	0.41 ± 0.24	0.30 ± 0.12

Values are expressed as mean ± SEM. Significant differences are indicated as * and ^ (p < 0.05).

(range: 7.6–10.20 mm) from day 20 to 1 before delivery in the complete follicular waves. Only one of the pregnant females showed a follicular wave on the (ipsilateral) ovary carrying the corpus luteum (11.1%); in this animal, follicular size was smaller when compared to the follicles on the contralateral ovaries (p < 0.01). Seven of the eight pregnant females developed follicles ≥7 mm by day 7.38 ± 7.42, with a range from day -23 to -1 before delivery.

There was an inverse relationship between the maximum follicular diameter and the total number of follicles (r = -0.29, p < 0.05). Follicular waves during gestation period showed a shorter duration and smaller dominant follicle diameter than during postpartum period (p < 0.01) (Table 1).

Postpartum period

Twenty-six complete follicular waves from 2-mm follicles to regression were analysed in eight of the eight females between parturition and day 40. Emergence of the first dominant follicle after delivery was highly variable from day 0 to 38.

There was an inverse relationship between the size of the largest follicle and the total number of follicles (r = -0.25, p < 0.05). Mean duration of follicular waves was 17.08 ± 3.69 days. The mean maximum diameter of the dominant follicle was 9.22 ± 2.07 mm (range: 6.80–13.60 mm).

Follicular activity was different (p < 0.01) between ovaries, with 58.7% of the waves observed on the left and 41.67% on right ovary. Ovarian activity alternated between ovaries in 62.57% of the evaluated waves. The growing phase of a follicle overlapped with the regression phase of the previous dominant follicle in 25% of the waves. A dominant follicle appeared in the ipsilateral ovary on 14 of 26 waves (53.85%). Unilateral double dominant follicles were observed in 1 of 26 (3.85%) waves. Bilateral double dominant follicles occurred in 3 of 26 (11.5%) waves. Table 1 includes data for follicular wave phases and dominant follicle characteristics.

Hormone secretory patterns

Gestation period

Hormonal data during pregnancy have been published earlier (Riveros et al. 2009), with a mean oestradiol-17β concentration of 21.9 pmol/l at the beginning of gestation that increased gradually to maximum concentrations of 186.1 pmol/l on day 290 of pregnancy, followed by a decrease to 60.0 pmol/l on the day of parturition (p < 0.01). Estrone sulphate increased to 3.0 nmol/l by day 315 of gestation (p < 0.01), remained elevated >4 nmol/l during last month of gestation, decreased significantly around parturition day and returned to basal level by day 1 after parturition (p < 0.01). Progesterone concentrations are presented in Fig. 1 to provide information on its availability during the pregnancy/puerperium transition.

Postpartum period

During the postpartum period, there was a close relationship between follicle size and estradiol-17β concentration. Plasma estradiol-17β concentration varied from 11.9 to 198.6 pmol/l. The mean estradiol-17β plasma concentration increased steadily from 11.9 ± 19.4 pmol/l at the beginning of the follicular growth phase (r = 0.69) to 29.3 ± 38.9 pmol/l during growth

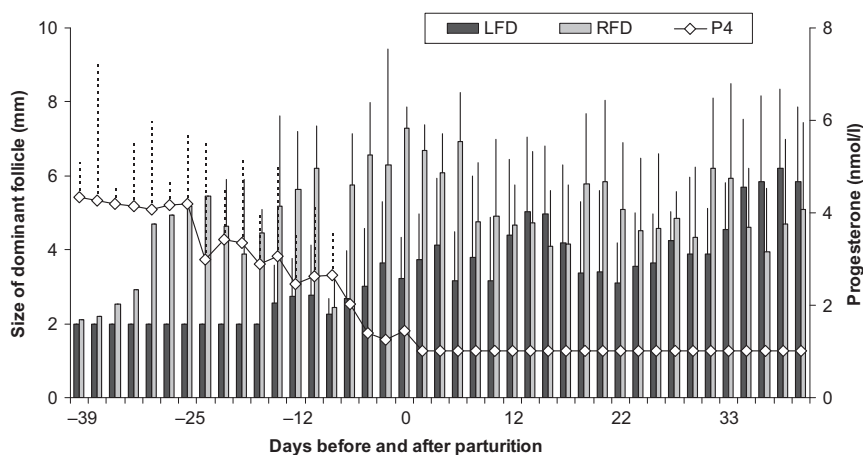


Fig. 1. Mean follicle size (bars) and plasma progesterone concentration (diamond) 40 days before and after parturition in guanacos (n = 8). Follicular waves were calculated considering as day 0 the first day a dominant follicle measured 2 mm. Values are expressed as mean ± SEM. LFD: left follicles diameter; RFD: right follicles diameter; P4: progesterone

phase and then to maximum concentration of 89.1 ± 33.9 pmol/l in association with the largest diameter of the dominant follicle ($r = 0.86$).

Concentrations of free estrone and estrone sulphate returned to baseline levels during the immediate peripartum period and remained basal thereafter.

Plasma progesterone concentration remained below 1 nmol/l after parturition, and there was no sonographic evidence of spontaneous ovulation during this period (Fig. 1).

Discussion

To our knowledge, these results present the first detailed description of the transition phase of follicular dynamics from gestation to postpartum period in guanaco, showing an early follicular development after a long gestation length according to the seasonal breeding strategy suggested for wild camelid species. As in dromedary camels (Manjunatha et al. 2012), in guanaco, the follicular wave during late pregnancy and early postpartum periods was characterized by a common pattern of emergence of a cohort of small follicles of 3–4 mm in diameter with an initial common growth phase, yielding one dominant follicle that continued to grow. This pattern showed a negative correlation between the diameter of the largest dominant follicle and number of follicles during both periods.

The estradiol 17β secretory profile observed in guanaco during the postpartum period was consistent with a wave-like follicular activity. As the dominant follicle size increases the estradiol 17β concentration also increases and reached a peak concentration when the dominant follicle reached to the largest diameter.

As has been reported by Tibary and Anouassi (1996), after establishment of pregnancy, follicular activity continues in pregnant Old World camelids regardless of the presence of an ipsilateral or contralateral corpus luteum. However, our results clearly indicate that in pregnant guanacos, the presence of corpus luteum has an effect on follicular development, resulting in the reduction of maximum follicular size and a shorter follicular wave length. Similar progesterone effects have been described for follicular wave dynamics in llamas, alpacas and vicuñas (Adams et al. 1990; Vaughan 2001; Chaves et al. 2002; Aba et al. 2005). However, no information is available regarding the general patterns of secretion of FSH and LH. Thus, it is open whether these effects are due to a lack of gonadotropins or a direct influence of progesterone on follicular development.

During gestation, in South American camelids, progesterone has been stated to inhibit the development of follicular waves (Vaughan 2011). Post-mortem studies in pregnant alpacas have only shown the presence of anovulatory dominant follicles between 5 and 7 mm in diameter up to 6 months of gestation. Then, maximum follicular size decreased to 3 to 4 mm

in diameter at 7 months of gestation indicating that follicular development would be substantially decreased in advanced stages of gestation (Bravo and Varela 1993).

A decrease in follicular activity has also been described after the first 3 months of pregnancy in cattle (Ginther et al. 1996). In mares, the mechanisms responsible for the dramatic reduction in follicular growth during mid- and late pregnancy were associated to a temporally decrease in circulating FSH (Donadeu and Pedersen 2008). However, in seasonal breeders as horses, further complexity into the regulation of follicle growth during pregnancy is provided by the observation that the effects of season on hormones and follicles continue to occur during pregnancy (Ginther 1992).

In guanaco, follicles growing to a diameter ≥ 7 mm were observed in the presence of progesterone concentration > 3 nmol/l in 87.5% of females during last 3 weeks of gestation. However, our results suggest that on the average follicular wave phases recorded during gestation exhibited smaller maximum follicular size and shorter static follicular phase length than those observed by Riveros et al. (2010) in non-pregnant females.

The natural pressure to produce a foal each year in a species with long gestation period is reflected, for example by an early postpartum ovulation in the mare typically within 2 weeks of parturition (Donadeu and Pedersen 2008). Previous studies in camelids on ovarian follicular development during postpartum yielded highly variable results. In llamas, follicular waves were described to be resumed 3- to 4-d postpartum, with 3 to 4 follicles < 4 mm in each ovary and with a dominant follicle ≥ 7 mm at 4- to 14-d postpartum (Bravo et al. 1995). A similar pattern has been reported in alpacas with follicles of 8 mm by 6-d postpartum (Sumar et al. 1972). In dromedary camel, a variable number of mature follicles have been reported between 39–42 days after birth (Elias 1990), and in bactrian camel, follicular development at the same period has been observed only in 33% of females with follicles from 10 to 14 mm (Chen and Yuen 1979). More recent studies in dromedary camel (Vyas and Sahani 2000) described follicles ≥ 5 mm between 30- and 60-d postpartum and the absence of follicular development during the first 90 days of postpartum in 29.4% of females. In cattle, Perry et al. (2004) described that the first postpartum follicular phase is shortened due to the absence of appropriate LH pulses preventing the final maturation of the dominant follicle (Wulff et al. 2002; Zimmermann et al. 2003; Hunter et al. 2004). In mares, the postpartum increase in follicular growth is induced by an increase in gonadotropin secretion at the time of parturition (Hines et al. 1987; Ginther et al. 1994). In guanacos, follicular wave development recorded during the early postpartum period basically showed a pattern basically similar to the one of non-pregnant females (Riveros et al. 2010). However, the mean follicular wave length, as the

interwave period was longer in non-pregnant females than those observed during gestation, was associated with an increased maximum dominant follicular size. Unlike the diameter of follicles recorded during gestation, the maximum dominant follicle diameter reached in the first wave postpartum was independent of the previous presence of a contra or ipsilateral corpus luteum, indicating that the potential local inhibitory effect exerted by the corpus luteum ended after luteolysis.

Our results suggest that ovarian follicular dynamic in guanaco is characterized by the persistence of the periodic development of follicular waves even during late pregnancy until parturition and by the rapid development of dominant follicles competent to ovulate during the early postpartum period. However, follicular dynamics during this transition period may be modulated by several factors such as energy balance and photoperiod so that different types of follicular waves, minor or major and different levels of activity within waves may develop under different physiological conditions.

Further research for a better understanding of the transition phase will need the characterization of changes in gonadotropin patterns and/or in the

sensitivity of follicles to circulating gonadotropins, which would help to further clarify the physiological mechanisms controlling this key reproductive period in camelids.

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Conflict of interest

None of the authors have any conflict of interest to declare.

Author contributions

Riveros designed the study, analysed the data and drafted the manuscript. Schuler and Urquieta analysed the data and drafted the manuscript. Hoffmann and Bonacic drafted the manuscript.

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