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Ovarian follicular dynamics and hormonal secretory profiles in guanacos (*Lama guanicoe*)

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ABSTRACT

The objective of the present study was to describe ovarian activity in 11 adult non-mated guanacos, evaluated every second day for 40 days by transrectal ultrasonography and by plasma estradiol-17β and progesterone concentrations. An inverse relationship ($r = -0.29$, $P < 0.001$) was observed between the diameter of the largest ovarian follicle and the total number of follicles indicating that follicular growth in guanacos occurs in waves. The mean duration of follicular wave was 15.1 ± 4.2 days with a range from 9 to 26. The follicular growth phase was 7.0 ± 2.4 days, the static phase 3.0 ± 1.2 days, the regression phase 5.2 ± 2.1 days and the inter-wave interval was 12.6 ± 5.6 days. The maximum follicular diameter in each follicular wave was 10.2 ± 2.1 mm with a range from 7.2 to 16.1 mm. Inter-wave intervals of longer duration were associated with a larger maximum follicle diameter ($P < 0.001$). Follicular activity alternated between ovaries in 93% of the waves with 48% of dominant follicles occurring in the left and 52% in the right ovary without differences ($P > 0.05$). Plasma estradiol-17β concentrations showed a wave-like pattern, varying from 20.0 to 92.1 pmol/L. Plasma progesterone concentrations remained below 1 nmol/L without any ultrasonic evidence of ovulation during the study. These results in guanacos suggest a follicular wave pattern more similar to the llama (*Lama glama*) than previously described in other South American and Old World camelid species.

1. Introduction

The guanaco is one of the wild South American Camelids. Its natural habitat is the extreme ecosystems of South America from Chaco to Patagonia. Like other camelid species it requires copulation to induce the ovulatory process (Riveros et al., 2006). Previous works described that sexual receptivity in guanacos occurs during the rainy season from December to March in Patagonia where the basic reproductive social group is formed by one adult male and a variable number of 6–15 of adult females with their prepubertal offspring (Raedeke, 1978; Franklin, 1982).

Previous studies in other camelids showed that 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). If no ovulatory stimulus occurs, the dominant follicle also undergoes

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atresia. The time required to complete a wave was reported in South American Camelids (SAC) to be 20–25 days (Adams et al., 1990), describing an inverse relationship between the diameter of the largest follicle and the number of follicles (Adams et al., 1990; Chaves et al., 2002). Like SAC (Adams et al., 1990; Miragaya et al., 2004; Vaughan et al., 2004), Old World Camelids (OWC) exhibited overlapping follicular wave development that alternates between ovaries (Skidmore et al., 1996).

Generally a positive correlation between follicular size and estradiol-17β plasma concentrations was found during camelid follicular waves (Bravo et al., 1990; Chaves et al., 2002; Miragaya et al., 2004) and concomitantly long periods of estrous behavior were observed (Bravo and Sumar, 1989; Adams et al., 1990; Skidmore et al., 1996). In the absence of an ovulatory stimulus progesterone concentrations remain basal (Adams et al., 1990).

In contrast to the other wild and domestic camelid species, in guanacos little is knows about the reproductive physiology and the main features of ovarian activity have not been described. However, a thorough understanding of reproductive biology of guanacos is important for conservation and sustainable use of the species. This wild species was seriously endangered until strict protection policies were established, after which populations recovered.

Thus the aim of the present study was to characterize ovarian follicular activity in non-mated guanacos in relation to gonadal and hormonal changes as evaluated by ultrasonic techniques and measurements of progesterone and estradiol-17β, respectively.

2. Materials and methods

2.1. Animals and sampling

The study was performed in a guanaco herd kept in captivity in the Mediterranean ecosystem of Chile (33°38′28″S, 70°34′27″W). The experimental group was formed by 11 adult, non-pregnant, non-lactating, healthy 7 to 8-year-old female guanacos. The animals were fed alfalfa hay, natural pasture and water *ad libitum*. Sampling was conducted with infrastructure specially designed for the species, with isolation areas and an immobilization chute.

Sampling was performed with animals immobilized in the chute under vision deprivation with a hood to minimize stress and to assure the animal's well-being. Ovarian follicular dynamics was monitored every second day for 40 consecutive days by transrectal ultrasonography and by plasma measurement of estradiol-17β and progesterone concentrations. Ultrasonic evaluations were performed using a real-time, B-mode scanner (Biomedical® model Scanner 6500C LC) 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®) centrifuged (2000 × g for 15 min) for plasma collection and stored at −18 °C until analysis.

2.2. Hormone assays

Estradiol-17β was determined using an inhouse RIA as previously described in detail (Hoffmann et al., 1992). Briefly, prior to the radioimmunologic measurement, plasma (0.25 mL) was extracted with toluene. The antiserum was obtained after immunization of rabbits against estradiol-17β-6-CMO-BSA. The radioimmunoassay was set up as a sequential assay. The minimum detectable concentration was at 7 pmol/L. Intra- and inter-assay coefficients of variation were 9.1 and 19.0%, respectively.

Progesterone was determined using a commercial chemiluminescense-based method (ACS: 180 Automated System with kit PRGE, Bayer Vital GmbH, Fernwald, Germany). The validity of this method in guanacos was confirmed by previous comparative measurements using a well established RIA method after sample extraction with hexane (Hoffmann et al., 1973). The minimum detectable concentration was at 0.3 nmol/L. Intra- and inter-assay coefficients of variation were 8.7 and 10.6%, respectively.

2.3. Analysis of data

Based on ultrasonographic observations, the inter-wave interval was defined as the time between two consecutive dominant follicles exceeding a diameter of 7 mm. Moreover, each follicle profile was divided into growing, static and regressing phases. The growing phase was defined as the period from the last time the diameter of the largest follicle was ≤3 mm until the dominant follicle reached its maximum diameter. The period of maintenance of maximum diameter with minimal changes in follicle size for at least 2 days, was defined as the static phase. The regressing phase, started with declining measurements until the follicle diameter decreased to less than 3 mm. The characteristics of follicular waves were expressed as mean ± SEM.

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

To cope with the asymmetrical distribution of plasma hormone concentrations, results were normalized by log transformation to the base 10 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.

All statistical analyses were carried out using the STATA 8.1 software package (Stata Corporation, College Station, TX, USA). An error probability of $P \leq 0.05$ was considered significant.

3. Results

3.1. Follicular wave dynamics

Twenty-five complete follicular waves from 3 mm to regression (range: 2 or 3 waves per animal) were analyzed. Follicular activity was observed to occur in waves characterized by the continuous emergence and regression of follicles. Although the follicular wave pattern was variable among animals, phases of growth, static and regression were distinguished in all cases.

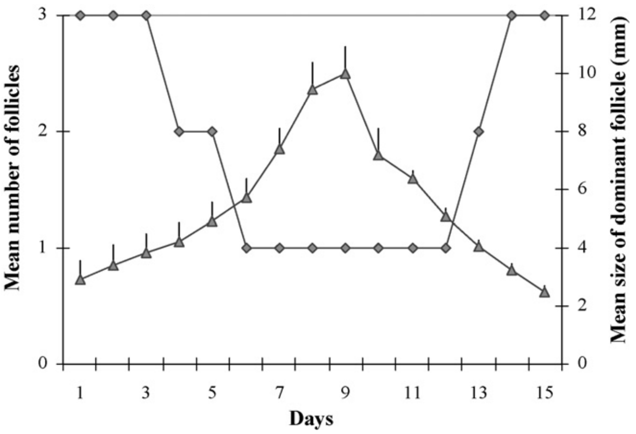

Fig. 1. Inverse relationship between the size of the dominant ovarian follicle (mm, mean ± s.e.m.; triangle) and number of follicles observed in both ovaries of eleven guanacos (diamond; $r = -0.60$, $P < 0.05$). Follicular waves ($n = 25$) were normalized, considering day 0 as the first day of a dominant follicle measured at 3 mm in diameter.

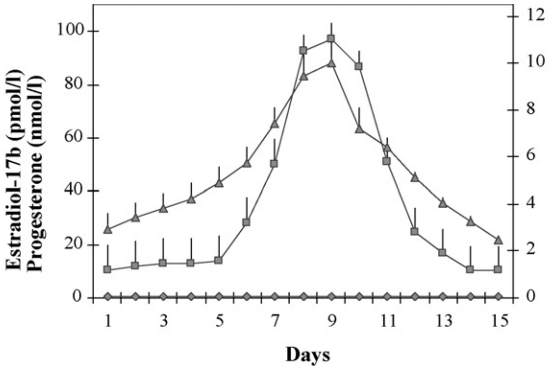

Fig. 2. Mean follicle size (square), plasma estradiol-17β concentrations (triangles) and plasma progesterone concentrations (diamond) during 25 follicular waves in guanacos ($n = 11$). Follicular waves were normalized considering day 0 as the first day a dominant follicle was measured at 3 mm in diameter. Values are expressed as mean ± s.e.m.

The mean duration of follicular waves was 15.1 ± 4.2, with a range of 9–26 days. The follicular growth phase averaged 7.0 ± 2.4 days, the static phase 3.0 ± 1.2 days and the regressing phase 5.2 ± 2.1 days. An inter-wave interval of 12.6 ± 5.6 days was observed. The mean growth rate during the growth phase was 1.0 ± 0.3 mm/day. The mean maximum diameter of dominant follicle was 10.2 ± 2.1 mm (range: 7.2–16.1 mm). There was an inverse relationship between the size of the largest follicle and the total number of follicles ($r = -0.60$, $P < 0.05$; Fig. 1).

Follicular activity alternated between ovaries in 93% of the waves. The emergence of the next successive dominant follicle in the same ovary occurred only in two occasions. A dominant follicle appeared in the left ovary on 12 occasions (48%) and on the right ovary on 13 occasions (52%). Double dominant follicles in both ovaries of an individual were observed in 3 of 25 (12%) waves. Table 1 includes data for follicular wave phases and dominant follicle characteristics. The growing phase of a follicle overlapped with the regression phase of the previous dominant follicle in 60% of the waves.

3.2. Hormone secretory patterns

The estradiol-17β plasma concentration profile exhibited a wave-like pattern. There was a close relationship between follicle size and estradiol-17β concentration ($P \leq 0.05$; Fig. 2). The mean estradiol-17β plasma concentrations steadily increased from 20.0 ± 9.3 pmol/L at the beginning of the follicular growth phase ($r = 0.62$) to maximum plasma concentrations of 92.1 ± 6.2 pmol/L in association with the largest diameter of the dominant follicle ($r = 0.80$). This peak in plasma concentration of estradiol-17β was attained approximately 8.5 ± 3.0 days after the beginning of the growing phase. Finally, concentrations declined to 22.7 ± 8.8 pmol/L by the end of the regressing phase. Plasma progesterone concentrations remained below 1 nmol/L and there was no ultrasonographic evidence of spontaneous ovulation during the study.

4. Discussion

To our knowledge this is the first study of follicular activity in guanacos. As previously reported in other SAC (Agüero et al., 2001; Miragaya et al., 2004; Vaughan et al., 2004; Riveros et al., 2006) and OWC (Skidmore et al., 1996), guanacos also showed a continuous emergence of new follicles in the absence of an ovulatory stimulus and an inverse relationship between dominant follicular size and total number of follicles.

Follicular wave patterns exhibited some differences in guanacos compared to other camelids based on the same definitions of phases as used in the present study (see Table 1). The mean total duration of follicular waves was shorter than in bactrian camel (19.1 ± 4.3 days) (Chen and

Table 1
Comparison of follicular wave phases and dominant follicle characteristics established in the present study for guanacos with data from other SAC species (references indicated in parentheses). Values are expressed as mean ± s.e.m.

Follicular phases	Species			
	Alpaca (Vaughan et al., 2004)	Llama (Bravo et al., 1990)	Vicuña (Miragaya et al., 2004)	Guanaco (Present study)
Growth phase	7.0 ± 1.0 days	4.8 ± 1.5 days	3.0 ± 0.2 days	7.0 ± 2.4 days
Static phase	4.5 ± 0.5 days	5.0 ± 1.6 days	1.4 ± 0.1 days	3.0 ± 1.2 days
Regressing phase	3.0 ± 1.0 days	4.0 ± 1.1 days	2.9 ± 0.3 days	5.2 ± 2.1 days
Dominant follicle characteristics				
Growth rate	1.4 mm/day	0.7 mm/day	1.8 mm/day	1.0 mm/day
Maximum follicular size	8.8 ± 0.3 mm	10.0 ± 2.0 mm	8.4 ± 0.3 mm	10.2 ± 2.1 mm

Yuen, 1979) and dromedary (30.0 ± 2.1 days) (Skidmore et al., 1996), but similar to those of other SAC species. Corresponding differences in different phases of follicular waves were reported among camelid species. Results of the present study indicated that the duration of the growing phase is shorter than in dromedaries (10.5 ± 0.5 days) (Skidmore et al., 1996), and the duration of the static phase in guanacos is similar to the other SAC species and less variable compared to OWC, where this phase showed mean values from 4.6 ± 0.5 to 7.6 ± 0.8 days (Skidmore et al., 1996). The regression phase in guanacos was shorter than that reported in dromedaries (11.9 ± 0.8 days) (Skidmore et al., 1995). The inter-wave interval defined as the period between the emergence of successive dominant follicles found in guanacos in our study was shorter than in alpacas (Vaughan et al., 2004) and dromedaries (18.2 ± 1.0 days) (Skidmore et al., 1996), but similar to llamas (Bravo et al., 1990) and longer than in vicuñas (Agüero et al., 2001; Miragaya et al., 2004).

Follicle activity in guanacos alternated between ovaries in 93% of waves. This rate is greater than that reported in llamas (65% or 81% of the waves) (Bravo et al., 1990; Chaves et al., 2002) and in vicuñas (77% of the waves) (Miragaya et al., 2004). However, other studies in SAC reported a different pattern without significant alternation between ovaries (San-Martin et al., 1968; Fernandez-Baca et al., 1970; Adams et al., 1990; Vaughan et al., 2004). Guanacos showed development of the dominant follicle in the left ovary in 48% of cases similar to the situation in llamas (53%) (Chaves et al., 2002) and alpacas (52%) (Vaughan et al., 2004).

Additionally in the guanaco dominant follicles developed ipsilaterally in 8% of cases and 92% in the contralateral ovary, indicating a different pattern than previously reported in other SAC with about 60% of dominant follicles in the ipsilateral ovary and 40% in the contralateral one (San-Martin et al., 1968; Fernandez-Baca et al., 1970; Adams et al., 1990; Vaughan et al., 2004). Despite considerable similarities in follicular development features between the two wild SAC species, guanacos had a lesser frequency of double dominant follicles (12%) compared to vicuñas (25%) (Miragaya et al., 2004).

The estradiol-17β secretory profiles observed in guanacos clearly reflect follicular waves. Plasma concentration of estradiol-17β increased gradually concomitant with follicular diameter development, and positive correlations were observed between the two variables during growing and static follicular wave phases. There are similar relationships in alpacas (Aba et al., 1995), llamas (Bravo et al., 1990; Aba et al., 1995; Chaves et al., 2002) and vicuñas (Urquieta et al., 1995; Miragaya et al., 2004). This secretory profile exhibits differences between camelid species with mean values from 17.6 to 46.9 pmol/L in llamas (Chaves et al., 2002) and from 28.7 to 43.1 pmol/L (Miragaya et al., 2004) in vicuñas, with the greatest values from 52.5 to 143.2 pmol/L in dromedaries (Skidmore et al., 1996). Thus results from the present study are within a range previously described in other SAC species and are less than those reported in OWC.

Plasma progesterone concentrations remained basal during follicular wave development confirming the absence of ovulation during the study. This pattern was reported previously in other SAC (Bravo et al., 1990, 1995; Aba et al., 1995; Chaves et al., 2002; Miragaya et al., 2004) where in the absence of mating or any other ovulatory stimulus plasma progesterone remained below <3.18 nmol/L.

The detailed characterization of follicular activity in guanacos by the present research suggests that the wave pattern observed in guanacos is more similar to llama than to other SAC species and reveals particular differences in maximum follicular diameter and growth rates compared to OWC patterns (Skidmore et al., 1996). In conclusion, the present study provides the first characterization of follicular activity in non-ovulating guanacos using ultrasound monitoring and hormonal measurements. These results are relevant for the understanding of reproductive physiology that could facilitate the introduction of biotechnology techniques to improve the status of this endangered wild species, promote its management for sustainable use and contribute to the understanding of camelid reproductive biology.

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