

# Endocrine changes during pregnancy, parturition and post-partum in guanacos (*Lama guanicoe*)

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## A B S T R A C T

Plasma concentrations of progesterone (P4), estradiol-17 $\beta$  (E2), estrone (E1) and estrone sulfate (E1S) were measured during gestation in eight guanacos kept in captivity. Gestational length was  $346.1 \pm 9.8$  days. P4 plasma concentrations increased after ovulation and remained elevated until parturition. However, during the last 4 weeks of gestation, a gradual decrease from  $4.17 \times 1.17^{\pm 1}$  nmol/L to  $2.02 \times 1.95^{\pm 1}$  nmol/L on day 5 before parturition was observed, followed by a more abrupt final decline to baseline concentrations which were reached on the day after parturition. Mean E2 plasma concentrations started to increase during the eighth month of gestation, and were significantly elevated up to maximum concentrations of  $484.7 \times 1.21^{\pm 1}$  pmol/L during the last 2 months of pregnancy. Concentrations returned to baseline during the last 2 days of gestation. An increase of E1S concentrations ( $p < 0.01$ ) was observed in the eleventh month of gestation. Mean E1S concentrations remained rather constant during the last 3 weeks of gestation between 4 to 8 nmol/L until parturition, when a steep precipitous decline was observed. E1 concentrations were slightly elevated during the last 4 weeks of gestation, however, maximum concentrations did not exceed 1.5 nmol/L. The results show distinct species specific features of gestational steroid hormone profiles in the guanaco in comparison to domestic South American camelids, such as a more pronounced gradual prepartal

### Keywords:

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Estrone sulfate

## 1. Introduction

The guanaco (*Lama guanicoe*) is a wild South American ungulate belonging to the Camelidae family, which is characterized by a strategy of seasonal reproduction and singleton pregnancy (Franklin, 1982). Conservation plans were implemented for this formerly endangered species during the last three decades, allowing the recovery of wild populations and even their management and reproduction in captivity. The camelids are induced ovulators in which ovulation is normally induced by copulation (Urquieta, 1997) and an ovulation-inducing factor in the seminal plasma for alpacas, llamas (Ratto et al., 2006) and Bactrian camels (Chen et al., 1985). Endocrine changes after ovulation and during early pregnancy (Sumar et al., 1988; Bravo et al., 1990, 1991, 1996a; Aba et al., 1995, 1997) as well as changes related to late gestation, delivery and the early post-partum period (Bravo et al., 1991; Aba et al., 1998; Raggi et al., 1999) have been well defined in domestic South American Camelids (SAC). In llamas and alpacas, the corpus luteum is the main source of progesterone during the complete gestation period (Sumar, 1988). A gestational length of 335–365 days was described in alpacas (Sumar, 1983), 346–359 days in llamas (Leon et al., 1990), 346–356 days in vicuñas (Urquieta and Rojas, 1990), 360–419 days in Bactrian camel (Tibary and Anouassi, 1997) and 315–440 days in dromedary (Ismail, 1987). Maternal gestational progesterone profiles were characterized in llamas and alpacas (Leon et al., 1990; Bravo, 1994; Raggi et al., 1999). Progesterone increases rapidly during the first month of gestation to concentrations  $>8.0$  nmol/L and remains fairly constant thereafter with a tendency to a slight transient decline during months 3–7. It precipitously declines during the last 2–3 days of pregnancy.

Estradiol-17 $\beta$  and combined estrone plus estradiol-17 $\beta$  had a similar secretion pattern in llamas. Serum concentrations were fairly constant from mating until about week 37 of pregnancy, then increased to a maximum at about weeks 48–49 reaching peak values of  $166 \pm 10.0$  pg/mL for estradiol-17 $\beta$  and  $827 \pm 58$  pg for estrone plus estradiol-17 $\beta$ . No significant changes were observed during the 2 weeks prepartum until 24 h before parturition, when a significant decline was observed (Leon et al., 1990). Concerning the early post-partum period conflicting data have been published. Aba et al. (1998) reported about elevated estradiol-17 $\beta$  concentrations on day 7 in alpacas and on day 10 in llamas as an indicator for the reoccurrence of ovarian activity, whereas Leon et al. (1990) have seen no such changes.

Similarly to free estrogens, estrone sulfate was described to increase in the llama and alpaca around 80 days before parturition with peak concentrations of  $15 \pm 3$  nmol/L in llamas and  $18 \pm 5$  nmol/L in alpacas immediately before parturition, and to sharply decrease at parturition (Aba et al., 1998). Due to this observation, the use of estrone sulfate analysis during the last 90 days of pregnancy has been suggested as a tool to monitor the normal progress of pregnancy in alpacas and llamas (Aba et al., 1998).

Much less information on hormonal profiles during gestation, parturition and the post-partum period is available in wild compared to domestic SAC. In guanacos previous studies describe morphological aspects of gestation (Sarasqueta and de Lamo, 1995) and follicular wave dynamics (Riveros et al., 2006). However, no gestational profiles of reproductive hormones have been reported yet in this species.

Although SAC are closely related species (Kadwell et al., 2001), specific features of the guanaco in the hormonal regulation of pregnancy and parturition cannot be excluded. Thus the aim of the present study was to characterize the changes occurring in plasma concentrations of progesterone, estrone sulfate and estradiol-17 $\beta$  during gestation, parturition and the early post-partum period in guanacos.

## 2. Materials and methods

### 2.1. Animals and blood samples

The study was performed using guanacos in captivity in the Mediterranean ecosystem of Chile (33°38'28"S, 70°34'27"W). The observational group was categorized as healthy based on clinical examination. It was formed by one intact 8-year old adult male and eight multiparous 7- or 8-year old females. The animals were fed with alfalfa hay, natural pasture and water *ad libitum*. Blood sampling and ultrasonography were conducted using an infrastructure specially designed for the species, with isolation areas and an immobilization chute.

The day of mating was registered and the development of the dominant follicle was monitored until ovulation by transrectal ultrasonography (Model 100 LC VET, Pie Medical, Maastricht, The Netherlands) in order to exactly calculate gestation length. The observational group was sampled during gestation, parturition and the early post-partum period as follows: blood sampling was performed with animals immobilized in the chute under vision deprivation with a hood in order to minimize stress and to assure the animal's well-being. Samples were obtained every 15 days from first to tenth month of gestation. However, the animals were not available for sampling between days 16 and 78 due to the management of the experimental station herd. Then sampling frequency was increased to three times a week until 1 week after parturition. Blood (10 mL) was collected by direct right jugular venipuncture into EDTA tubes (Vacutainer, Becton Dickinson, NJ, USA) and then samples were centrifuged at  $2000 \times g$  for 15 min for plasma collection which was stored at  $-18^{\circ}\text{C}$  until analyzed.

### 2.2. Hormone assays

Progesterone was determined by a commercial chemiluminescence-based method using ACS 180 Automated System with kit PRGE (Bayer Vital GmbH, Fernwald, Germany). As stated by the supplier, the minimum detectable concentration is 0.35 nmol/L. Intra- and inter-assay coefficients of variation were 6.1% and 7.5%, respectively. The validity of this method in guanacos was confirmed by comparative measurements using a well-established radioimmunoassay after sample extraction with hexane (Hoffmann et al., 1973) as reference method.

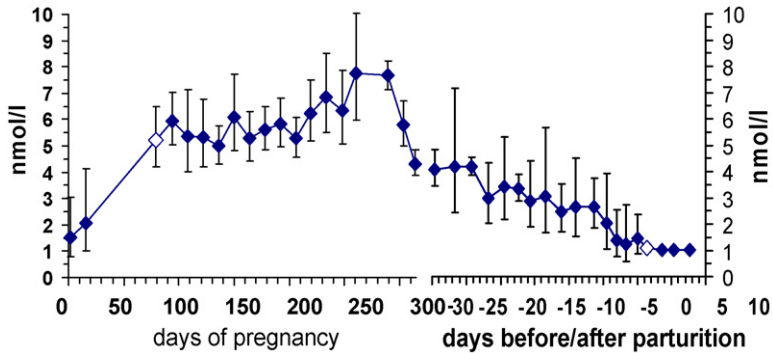
Estradiol-17 $\beta$  was determined using a radioimmunoassay previously described by Hoffmann et al. (1992). Toluene extracts of 0.25 mL plasma were used for the measurements. The radioimmunoassay was set up as a sequential assay, and an antiserum obtained after immunization of a rabbit against estradiol-17 $\beta$ -6-CMO-BSA was applied. The minimum detectable concentration was 7.5 pmol/L. Intra- and inter-assay coefficients of variation were 9.1% and 19%, respectively.

Estrone and estrone sulfate were determined as previously described (Hoffmann et al., 1996, 1997). Briefly, at first samples were extracted with two times 3 mL toluene, yielding the fraction of free estrogens.  $\beta$ -Glucuronidase/arylsulfatase (Roche Diagnostics GmbH, Penzberg, Germany) was added to the remaining aqueous phases, they were then extracted again as mentioned above to yield the fraction of conjugated estrogens. The extracts were evaporated to dryness, redissolved and submitted to radioimmunoassay using an antiserum obtained after immunization of a rabbit against estrone-6-CMO-BSA. The minimum detectable concentration was 0.3 nmol/L. Intra- and inter-assay coefficients of variation were 9.4% and 12.5%, respectively.

### 2.3. Statistical evaluation and presentation of data

Statistical analysis was carried out using the STATA 8.1 software package (Stata Corporation, College Station, TX, USA). Repeated-measures ANOVA test was performed to detect differences in hormone concentrations. A Bonferroni test was used to determine significant differences between means. An error probability of  $p < 0.05$  was considered significant.

To cope with the variability in the length of pregnancy, samples corresponding to the last 30 days of gestation and the early post-partum period were grouped around the day of parturition. To cope with the asymmetrical distribution of hormone concentrations, results were presented as geometric mean  $\times$  dispersion factor $^{\pm 1}$  (Sachs, 1982).



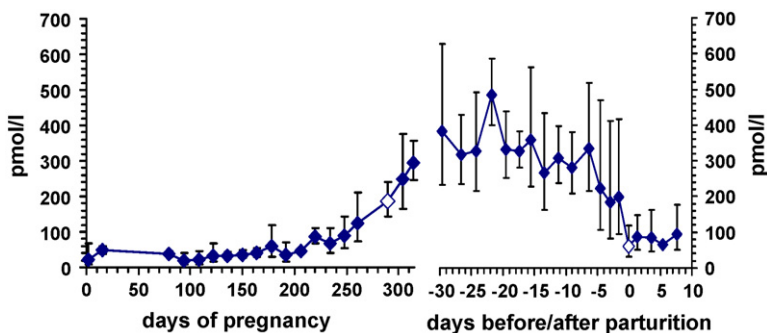
**Fig. 1.** Plasma progesterone concentrations in eight pregnant guanacos. Data are presented as geometric mean  $\times$  dispersion factor $^{\pm 1}$ . Open symbols indicate significant differences with previous samples ( $p < 0.05$ ).

### 3. Results

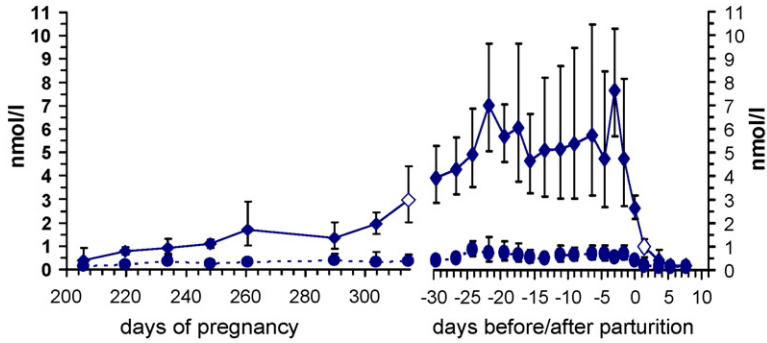
The mean diameter of preovulatory follicles which later developed into a corpus luteum ipsilaterally to the pregnant horn was  $8.0 \pm 2.0$  mm. All gestations observed were singleton and developed in the left uterus horn. The mean gestation length was  $346.1 \pm 9.8$  days with a range of 333–359 days. Seven out of eight females had normal unassisted parturition, one showed a dystocia characterized by a prolonged second phase ending with fetal death.

Plasma progesterone increased from  $1.54 \times 1.97^{\pm 1}$  nmol/L on day 2 after ovulation to  $5.22 \times 1.24^{\pm 1}$  nmol/L on day 79 of pregnancy ( $p < 0.01$ ). Thereafter it showed a mean concentration of  $5.60 \times 1.38^{\pm 1}$  nmol/L until about day 310 (Fig. 1). Maximum values of about  $7.76 \times 1.29^{\pm 1}$  nmol/L were measured between days 260 and 290 of pregnancy. During the last 4 weeks of pregnancy progesterone values declined gradually from  $4.17 \times 1.17^{\pm 1}$  nmol/L to  $2.02 \times 1.95^{\pm 1}$  nmol/L on day 5 before parturition. Then a more abrupt final decline to baseline levels was observed which were reached on the day after parturition. Thereafter, concentrations remained unchanged during the early post-partum period studied.

Plasma estradiol-17 $\beta$  showed a mean concentration of  $21.9 \times 3.07^{\pm 1}$  pmol/L on the second day after ovulation (Fig. 2), started to increase by day 290 of pregnancy to  $186.1 \times 1.29^{\pm 1}$  pmol/L ( $p < 0.01$ ) and reached maximum values of  $484.7 \times 1.21^{\pm 1}$  pmol/L on day 22 before parturition. Thereafter it decreased gradually to  $60.0 \times 1.97^{\pm 1}$  pmol/L ( $p < 0.01$ ) on the day of parturition with the decline from day 2 to parturition being significant. During the first week post-partum a slight increase of estradiol-17 $\beta$  concentrations to  $86.0 \times 1.79^{\pm 1}$  pmol/L was observed, which, however, was not statistically significant.



**Fig. 2.** Plasma estradiol-17 $\beta$  concentrations in eight pregnant guanacos. Data are presented as geometric mean  $\times$  dispersion factor $^{\pm 1}$ . Open symbols indicate significant differences with previous samples ( $p < 0.05$ ).



**Fig. 3.** Plasma concentrations for estrone (●) and estrone sulfate (◆) in eight pregnant guanacos. Data are presented as geometric mean  $\times$  dispersion factor $^{\pm 1}$ . Open symbols indicate significant differences with previous samples ( $p < 0.05$ ).

An increase in plasma estrone sulfate concentrations occurred during the third trimester of gestation (Fig. 3); values increased gradually from  $0.38 \times 2.43^{\pm 1}$  nmol/L on day 206 to  $2.99 \times 1.48^{\pm 1}$  nmol/L on day 315 of pregnancy ( $p < 0.01$ ). Thereafter, concentrations increased to a mean concentration of  $5.39 \times 1.64^{\pm 1}$  nmol/L during the last month of pregnancy, with maximum concentrations of  $7.64 \times 1.34^{\pm 1}$  nmol/L on day 3 before parturition. Plasma estrone sulfate rapidly decreased during the last 2 days of gestation, reaching basal values on the first day post-partum ( $p < 0.01$ ). Concentrations remained basal and without significant changes ( $p > 0.05$ ) during the first week post-partum. Concentrations of free estrone (Fig. 3) basically followed a similar pattern as with estrone sulfate albeit at a substantially lesser concentration. Maximal concentrations of about  $0.64 \times 1.61^{\pm 1}$  nmol/L were measured during the last 4 weeks of gestation. They returned to baseline concentrations during the immediate peripartur period and remained basal thereafter.

#### 4. Discussion

This is the first report describing progesterone, estradiol-17 $\beta$ , estrone sulfate and free estrone profiles during gestation, parturition and the early post-partum period in guanacos. The mean length and range of the gestational period observed in the guanaco are similar to the other SAC (Sumar, 1983; Leon et al., 1990; Urquieta and Rojas, 1990). All pregnancies developed in the left uterus horn as previously reported for the other SAC (Fernandez-Baca et al., 1973; Bravo and Varela, 1993), which could be related to differential luteolysis induction mechanism between left and right uterine horns or may be explained by migration of embryos to the left uterine horn (Fernandez-Baca et al., 1979).

As previously described in the llama (Adams et al., 1989, 1990, 1991), 3–5 days after mating (2–4 days after ovulation) the development of a corpus luteum was observed on the ovary at the site of ovulation and plasma progesterone concentration increased as the diameter of the corpus luteum increased (Adams et al., 1991; Bravo and Varela, 1993). After an initial increase progesterone concentrations showed a slight temporary decrease from months 3 to 7 in alpacas (Raggi et al., 1999) and from months 4 to 7 in llamas (Leon et al., 1990). Rather than a decrease, an increase was observed in the present study in guanacos during this period of time resembling what occurs in dromedaries, where a pronounced peak of progesterone concentrations occurred around mid-gestation (Elias et al., 1984a). While there is a rapid decrease of progesterone concentrations immediately prior to parturition in alpacas (Raggi et al., 1999), in llamas a more gradual decrease has been described to occur during the last 2 weeks of pregnancy from  $12.1 \pm 2.5$  nmol/L to  $4.45 \pm 0.64$  nmol/L on day 1 prepartum (Leon et al., 1990). According to results in the present study, in guanacos this decrease started 1 week earlier than in the llama and was more pronounced, as 11 days before parturition mean progesterone concentrations decreased below 3 nmol/L. Similar to the guanaco, a pronounced gradual decline of progesterone during late gestation was observed in dromedaries (Elias et al., 1984a). In both species, and distinctly prior to parturition, this decline reaches concentrations similar to or less than 3.18 nmol/L, which are considered necessary to maintain pregnancy in Old World Camelids (OWC) and domestic SAC, respec-

tively (Zhao et al., 1998; Bravo et al., 1996a). This indicates species specific features within the group of camelids and within SAC concerning hormonal regulation of pregnancy.

The mechanisms underlying the gradual decrease in progesterone observed in guanacos during the last 3 or 4 weeks of gestation are unclear. For the llama and alpaca corpus luteum function is essential for the maintenance of pregnancy throughout gestation (Sumar, 1988; Bravo et al., 1996b). This, however does not preclude that there is a shift in luteal steroid hormone synthesis at the end of pregnancy from progesterone to another progestagen interacting with the progesterone receptor, such as 5 $\alpha$ -dihydroprogesterone, a placental progestagen replacing luteal progesterone in the late pregnant mare (Fowden et al., 2008). Such a progestagen would not have been detected in the immunoassay used in the present study. Yet, more likely the decrease reflects reduced luteal steroidogenesis with enough progesterone produced to allow for myometrial quiescence. Synergistic factors securing myometrial quiescence might be relaxin, which increases in llamas during late gestation (Bravo et al., 1996a) or the local provision of progesterone by the placenta, as was described for cows (Hoffmann and Schuler, 2002). In camelids, it is still unknown whether or to what extent the placenta is able to produce and secrete progesterone. In SAC, the occurrence of pregnancy-associated estrogens (Aba et al., 1998) clearly points to a significant steroidogenic capacity of the trophoblast. Consistently, the expression of steroidogenic key enzymes was found in the trophoblast giant cells of dromedaries, llamas and alpacas (Wooding et al., 2003).

In the present study in guanacos, a significant increase of estradiol-17 $\beta$  to maximum concentrations of  $484.7 \times 1.21^{\pm 1}$  pmol/L on day 22 before parturition was observed during late gestation. Estradiol-17 $\beta$  concentrations then decreased during the immediate prepartal phase to  $60.0 \pm 43.7$  pmol/L on the day of parturition and tended to increase thereafter, which obviously reflects the resumption of ovarian follicular development during the first week after parturition, at least in some animals. In llamas and OWC, similar gestational profiles were described for estradiol-17 $\beta$ , but with greater maximum values of  $719 \pm 37$  pmol/L in llamas, and of  $2266.1 \pm 119.5$  pmol/L and  $2224.0 \pm 441.4$  pmol/L in the Bactrian camel and dromedary, respectively (Elias et al., 1984b; Zhao et al., 1998).

The estrone sulfate profile in guanacos is different from previous results described in domestic SAC species with respect to its shape and maximal values. After an initial increase estrone sulfate concentrations were fairly constant during the last month of gestation with mean values of  $5.39 \times 1.64^{\pm 1}$  nmol/L. This is clearly different from the profiles established by Aba et al. (1998) in llamas and alpacas, where estrone sulfate concentrations steadily increased during late gestation reaching maximal values of  $13 \pm 5$  nmol/L and  $18 \pm 5$  nmol/L immediately prior to parturition, respectively.

The production of pregnancy-associated estrogens, mainly free and sulfated estrone, is observed in many other ungulate species. In contrast to horses, where maximum concentrations occur around mid-gestation (Hoffmann et al., 1996), in guanacos and other camelids maximum concentrations are observed in late gestation, which is similar to ruminants. However, in contrast to sheep (Tsang, 1978) and cattle (Robertson and King, 1979; Hoffmann et al., 1997), no significant increase of free estrogens was found during the immediate prepartal phase in guanacos. Thus, the regulation and the biological role of pregnancy-associated estrogens are unclear in any species exhibiting this phenomenon (Schuler et al., 2008), and may differ significantly between camelids and other ungulate species.

Results of the present study confirm for the guanaco the basic pattern of steroid hormone profiles during pregnancy previously established in other camelid species. However, some distinct species specific features of gestational steroid hormone profiles were found in the guanaco in comparison to domestic SAC, such as a more pronounced gradual prepartal decrease of P4 concentrations prior to the final decrease to baseline, and clearly lesser E1S concentrations during the last 4 weeks of gestation, which lack a continuous increase during this period as observed in llamas and the alpacas (Aba et al., 1998).

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