BUTTERWORTH HEINEMANN

PLASMA AND FECAL PROGESTAGEN EVALUATIONS DURING AND AFTER THE BREEDING SEASON OF THE FEMALE VICUNA (Vicuna vicuna)

F. Schwarzenberger,[1,a] G. Speckbacher[1] and E. Bamberg[2]

[1]Institut f. Biochemie, [2]Ludwig Boltzmann Institut f. Veterinärmedizinische Endokrinologie
Veterinärmedizinische Universität, Linke Bahngasse 11, A-1030 Vienna, Austria

Received for publication: *July 1, 1994*
Accepted: *December 5, 1994*

ABSTRACT

Plasma and fecal progestagen patterns of female (n=10) vicunas (Vicuna vicuna) were determined about 1 to 2 mo before and until 4 mo after breeding. The vicunas were caught wild and were penned at the Lauca National Park (Chile, 4470 m above sea level) for 7 mo (December to June). Plasma and fecal samples before and during the mating period (January to March) were collected 4 to 5 times weekly, and once or twice weekly thereafter. The samples were analyzed by enzymeimmunoassays (EIA) using antibodies against progesterone and 20α-dihydroprogesterone. High performance liquid chromatography (HPLC) separations confirmed that progesterone and 20α-dihydroprogesterone predominated in the plasma, whereas in the feces several unconjugated, immunoreactive progestagen metabolites containing either a 20-oxo- or a 20α-OH-group occurred. The coefficients of correlation ($n = 409$; $P < 0.01$) between matched plasma and fecal samples were 0.39 and 0.53 for 20-oxo- and 20α-progestagens, respectively. Elevated (5 to 6 d) plasma and corresponding fecal progestagens after mating indicated cyclic corpus luteum activity in 5 of the animals. After the mating period (23.2 ± 3.3 d), corpus luteum function in these 5 animals persisted, as it did in 3 other animals that were not observed to be mating. The persisting corpus luteum function was demonstrated by increased mean plasma and fecal progestagen concentrations (>1 ng/ml and >100 ng/g, respectively). Mean plasma 20α-dihydroprogesterone concentrations exceeded that of progesterone by about 1 ng/ml ($P < 0.01$). The results demonstrated that in addition to plasma progesterone, plasma 20α-dihydroprogesterone and noninvasive fecal progestagen evaluations are useful, valid tools for determining corpus luteum function in vicunas.

Key words: vicuna, luteal function, progestagens, 20α-dihydroprogesterone, induced ovulator

INTRODUCTION

Within the New World camelids, llamas (Lama glama) and alpacas (Lama pacos) represent the 2 domesticated species, while guanacos (Lama guanaco) and vicunas (Vicuna vicuna) are free-ranging animals. The vicuna is the smallest of the South American camelids

Acknowledgments

We thank Dr. B. Urquieta (Santiago, Chile), the Chilean CONAF (Corporation National Forestal Arica) and the staff of Lauca National Park (Region I of Chile) for their help with the animals, and storage of the samples. We also thank A. Aichinger for technical assistance; and Dr. E. Möstl for the preparation of antibodies and enzyme-labels. The field studies of G. Speckbacher were supported by grants from the Eduard Wallnöferstiftung, Tiroler Landesgedächtnisstiftung and Förderungsstipendium der Veterinärmedizinischen Universität, Wien.

[a] correspondence and reprint requests.

Theriogenology 43:625–634, 1995

655 Avenue of the Americas, New York, NY 10010

0093-691X/95/$10.00
SSDI 0093-691X(94)00068-6

(40 to 65 kg), and it inhabits the high central Andean plateau (Altiplano) of Peru, Bolivia, Chile and Argentina (altitudes: 3500 to 4800 m). Vicunas are nonmigratory animals, living in family groups consisting of an adult male leader, 3 to 8 females and their recent offspring (13, 24).

South American camelids are induced ovulators (4, 13, 20). In their natural habitat, breeding and parturition are seasonal, with the breeding season of vicunas lasting from March to May (13,24). However, laparascopic (10) and ultrasonographic investigations (2,3,6) in llamas and alpacas have shown that follicles grow in overlapping waves throughout the year, with an interwave interval of 11 to 18 d in the llama and 8 to 12 d in the alpaca. Follicles can remain at 8 to 12 mm in size for upto 10 to 12 d or until copulation induces ovulation (20). After the induced ovulation, the presence of a functional corpus luteum is indicated by an increase in blood progesterone secretion, which in case of nonfertile mating regresses approximately at Day 11 (4,20). Progesterone concentrations in vicunas mated to a vasectomized male increased from Day 5, reached maximum concentrations on Day 8, and rapidly declined on Days 9 to 10 (25). After fertile mating, plasma progesterone remained elevated (> 1.5 ng/ml) throughout gestation (25).

Investigations of reproductive hormones in New World camelids (other than the vicuna) have been described for llamas and alpacas and include studies on plasma progesterone (1,3,5,6,14,22,23), plasma estrogens (5,6,14,22,23), plasma prostaglandin - $PGF_{2\alpha}$ (22), plasma LH (5,6,8,9), plasma FSH (6), urinary pregnanediol-glucuronide (7,8,15), urinary 20α-dihydroprogesterone (23), and urinary estrogens (6,7,9). Urinary hormone analysis provides the advantage of a noninvasive method, which is especially important in the investigation of free-ranging animals. However, fecal samples are even easier to collect than urine samples, and therefore we used this approach in the present study. Our objectives were 1) to establish a reliable extraction method and to validate assay procedures for measuring fecal progestagens in the vicuna, 2) to compare progestagen concentrations between plasma and feces, and 3) to apply these methods to monitoring corpus luteum function before, during and after the breeding season.

MATERIALS AND METHODS

Animals and Sample Collection

Samples of vicunas were collected at the Las Cuevas Research Station in the Lauca National Park (18° South and 69° West, 4470 m above sea level). Part of the Andean Altiplano ecosystem, the Lauca Park is situated in the north of Chile, close to the borders of Peru and Bolivia. At the beginning of the study, free-ranging male (n=3) and female vicunas (n=10) were captured and penned for 6 mo (December to June, summer-autumn season in the Southern Hemisphere) in 3 corrals (6 x 4 m), in sex and age groupings similar to those of their natural social structure. Three groups of female vicunas (n = 3, 3 and 4/group) were confined with a male. Ages of the female vicunas were estimated from dentition; 1 female was estimated to be about 1 year of age, while the remaining 9 were sexually mature females. One female had a late pregnancy abortion 20 d after capture; this female accidentally died during a breakout in March. All the animals were fed alfalfa hay and were given water ad libitum. Regrettably, the financial funds were not sufficient for a longer period of study, and field studies had to be stopped at the end of June, before winter set in. The animals were released back into the wild. Therefore, pregnancies, which were predicted on the basis of progesterone measurements, and which last approximately 11.5 mo (4,13,20,24), could not be confirmed by observation of the parturitions.

Direct daily observation of estrous behavior and mating activities was carried out for 2 h in the morning and 2 h in the afternoon during the period of the study. In addition,

mounting activities beyond these observation periods were indicated by dye markings on the backs of the females; markings were produced upon mounting by the sire wearing a ram harness. Fecal samples were collected from the rectum. Parallel, heparinized blood samples were taken from the jugular vein and centrifuged immediately after collection. Before and during the mating period, samples were collected 4 to 5 times weekly. After signs of mating ceased (March), fecal samples were collected once or twice weekly; blood samples were taken at weekly intervals. All samples were stored at -20°C and were shipped to and analyzed in Vienna at the end of the collection period.

Extraction of Plasma and Fecal Samples

Before analysis, 0.5 ml plasma was vortexed (30 min) with 5 ml diethyl ether; the ether was then transferred to a new vial, evaporated (40° C), and the residue reconstituted with assay buffer and analyzed. Extraction efficiencies of [^{3}H]-progesterone and [^{3}H]-20α-dihydroprogesterone from a pool of vicuna plasma (n = 8) were 75.3 ± 2.4 and 84.5 ± 2.8%, respectively.

For the extraction of fecal samples a modified method previously described (17) was used; this was followed by a procedure to remove polar substances. Feces (0.5 g), laboratory-grade water (0.5 ml), and methanol (4.0 ml) were mixed and vortexed (30 min). After addition of 3.0 ml petroleum ether, the mixture was vortexed (10 sec) and centrifuged (1500 g x 15 min). To remove polar substances, 0.2 ml of the methanol-extract was transferred into a new vial, diluted with 0.6 ml laboratory-grade water, and vortexed (30 min) with 5 ml of petroleum ether/diethyl ether (v/v 9:1), after which the ether was evaporated (40° C) and the residue reconstituted with assay buffer. Recoveries of [^{3}H]-progesterone and [^{3}H]-20α-dihydroprogesterone from a pool of vicuna feces (n = 12) were 92.0 ± 1.6% and 89.2 ± 1.9%, respectively.

High Performance Liquid Chromatography (HPLC) of Fecal Extracts

To ascertain assay specificity and to obtain indications on the polarity of the immunoreactive progestagens, HPLC separations of selected plasma and fecal samples were done as described previously (16,17). Briefly, the samples were mixed with [^{3}H]-progesterone and [^{3}H]-20α-dihydroprogesterone and were extracted with petroleumether/diethylether (v/v 6:4). The ether was evaporated, the residue reconstituted in n-hexane/chloroform (v/v 7:3), and the extracts separated on a straight phase HPLC system (silica 60 column) using a linear solvent gradient of 0 to 6 % methanol in n-hexane/chloroform (v/v 7:3). Fractions were analyzed with the 2 respective EIA; retention times of immunoreactive substances were compared to those of [^{3}H]-progesterone, [^{3}H]-20α-dihydroprogesterone and different cross-reacting 5α- and 5ß-reduced progestagens containing either a 20-oxo or a 20α-OH group. The [^{3}H]-steroids were determined by liquid scintillation counting; the immunoreactive progestagens were determined by EIA (16,17).

Hormonal Analysis With Enzymeimmunoassays (EIA)

Plasma and fecal extracts were analyzed with 2 previously described EIA using antibodies for progesterone (16) and 20α-dihydroprogesterone (17). The 2 EIA were group-specific progestagen assays, measuring progestagens containing either a 20-oxo- or 20α-OH-group, respectively. The assays were validated for vicuna samples by demonstrating parallelism between standard curves and serial dilutions of plasma and fecal extracts. In the 2 EIA, intra- and inter-assay coefficients of variation for 1 pool of plasma and 2 pools of fecal samples, respectively, varied between 10 and 15%.

Plasma and fecal samples were analyzed without prior chromatographic separation. Results from HPLC separation of plasma samples support the contention that the 2 assays employed were relatively specific in terms of unconjugated progesterone and 20α-dihydroprogesterone. Therefore, the terms used to describe the results of plasma analysis are progesterone and 20α-dihydroprogesterone. In contrast, HPLC separation of fecal extracts and subsequent analysis with the EIA showed the presence of several unconjugated, immunoreactive 5α- and 5ß-reduced progestagens. Accordingly, the results of fecal samples are considered to be measurements of total immunoreactive progestagens containing either a 20-oxo or 20α-OH group and are expressed as 20-oxo-progestagens and 20α-progestagens, respectively.

RESULTS

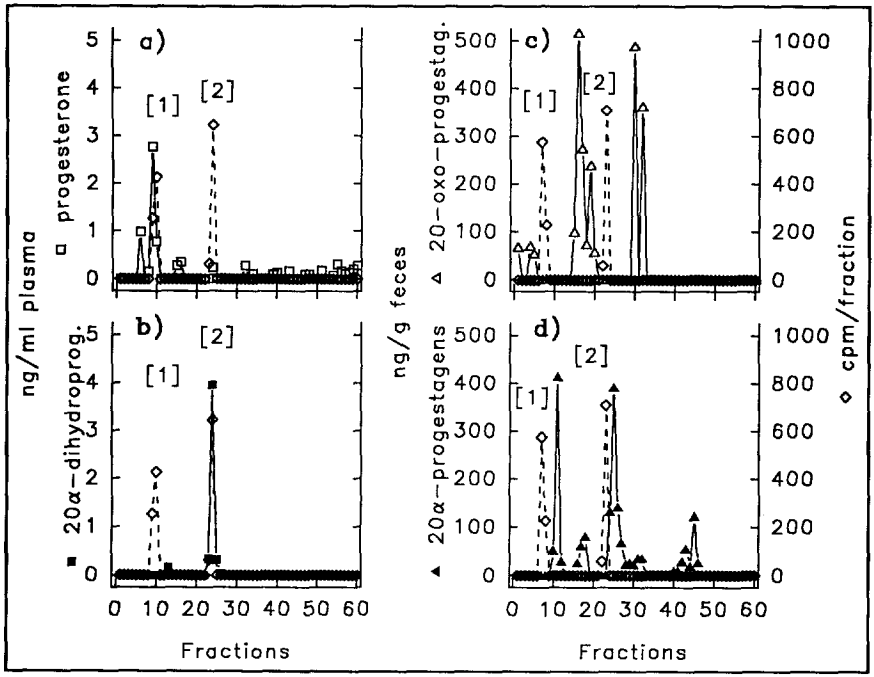

Figure 1. High performance liquid chromatography (HPLC) separations of plasma (left panels) and fecal immunoreactive progestagens (right panels) during the luteal phase of a vicuna. Samples were mixed with [1] [^{3}H]-progesterone and [2] [^{3}H]-20α-dihydroprogesterone, extracted, and chromatographed on a straight-phase HPLC system. Fractions were analyzed with enzymeimmunoassay for progesterone (top panels: a) □ plasma; c) △ feces) and 20α-dihydroprogesterone (bottom panels: b) ■ plasma; d) ▲ feces), and counts per minute (◇ cpm) were measured. Concentrations (ng/fraction) were calculated without correction for methodological losses, for 1 ml of plasma and 1 g of feces, respectively.

The HPLC fractionation of selected plasma and fecal samples, followed by subsequent analysis with the EIA, revealed the presence of several unconjugated immunoreactive peaks (Figure 1). Generally, elution profiles of immunoreactive peaks between plasma and fecal samples were different. Most of the immunoreactive materials present in the plasma eluted in 3 peaks; 2 peaks cochromatographed with [^{3}H]-progesterone and [^{3}H]-20α-dihydro-progesterone, respectively, and 1 peak eluted ahead of and thus with a polarity less than [^{3}H]-progesterone. Compared with that of plasma, several immunoreactive peaks which did not co-elute with the respective [^{3}H]-steroids were present in the fecal samples.

Matched plasma and fecal samples of all animals were used to calculate the coefficients of correlation. The significant ($P < 0.01$; $n = 409$) coefficients were 0.39 ($y = 93.7 + 149.6\ x$) between plasma progesterone and fecal 20-oxo-progestagens; 0.53 ($y = 64.5 + 62.0\ x$) between plasma 20α-dihydroprogesterone and fecal 20α-progestagens; 0.63 ($y = 0.19 + 1.27\ x$) between plasma progesterone and plasma 20α-dihydroprogesterone; and 0.58 ($y = 61.5 + 0.35\ x$) between fecal 20-oxo-progestagens and fecal 20α-progestagens.

Estrous behavior, evidenced by mating marks ($n = 5$) and/or observed matings ($n = 2$), was seen in 5 animals (Figures 2,3); mating marks persisted an average of 23.2 $\pm$ 3.3 d (mean $\pm$ SEM; min: 16; max: 34 d). Temporary periods (5 to 6 d) of elevated plasma and

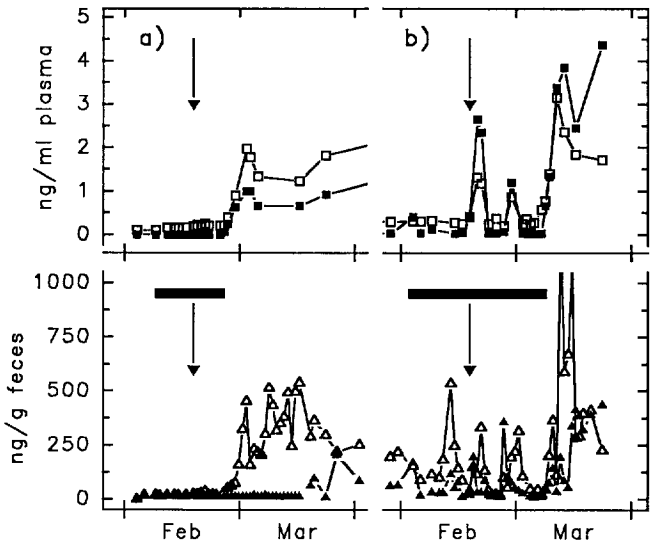

Figure 2. Individual profiles of immunoreactive progestagens in plasma (top panels) and feces (bottom panels) of 2 vicunas (a and b) during the breeding season. Concentrations were determined by enzymeimmunoassay for □ plasma progesterone, ■ plasma 20α-dihydroprogesterone, △ fecal 20-oxo-progestagens and ▲ fecal 20α-progestagens. Observed matings are indicated by arrows; mating marks, produced by a sire marking ram harness, are indicated by closed bars.

corresponding fecal progestagen concentrations indicated cyclic corpus luteum activity in 5 animals; 4 animals (Figure 3) had 1 and another animal (Figure 2b) had 2 transient luteal phases. After the mating marks, and thus signs of estrous behavior, ceased the animals had most probably conceived. Corpora lutea in the vicuna persisted, as was shown by continuously elevated plasma and corresponding fecal progestagen concentrations.

Although mating marks were not observed in 3 of the vicuna kept in the same enclosure (the profile of 1 of these animals is shown in Figure 3d), their plasma and fecal progestagen patterns resembled those of the animals with observed mating marks. In all 3 animals the progestagen concentrations increased sharply by mid February and remained elevated until June. Therefore, plasma and fecal progestagen values of these animals, together with the animals shown in Figures 2 and 3, were standardized to the onset of a persisting corpus luteum function, and mean $\pm$ SEM values were calculated (Figure 4). The periods of estrous behavior (closed bars in Figures 2 and 3) were excluded from calculation. Mean values of plasma progesterone and 20α-dihydroprogesterone before the mating period were < 0.5 ng/ml, while fecal 20-oxo- and 20α-progestagens were < 100 ng/g, respectively. Thereafter the levels sharply increased to > 1 ng/ml and > 100 ng/g, respectively, and mean plasma 20α-dihydroprogesterone concentrations were significantly higher than plasma progesterone values ($P < 0.01$; paired Student's t-test). Fecal 20-oxo-progestagen increased to maximum concentrations about 10 wk after the mating period, whereas fecal 20α-progestagens increased continuously until the end of the investigation period.

Copulations and/or mating marks were not seen in the juvenile animal (Figure 5a). Its plasma and fecal progestagen values throughout the investigation period were < 1 ng/ml and

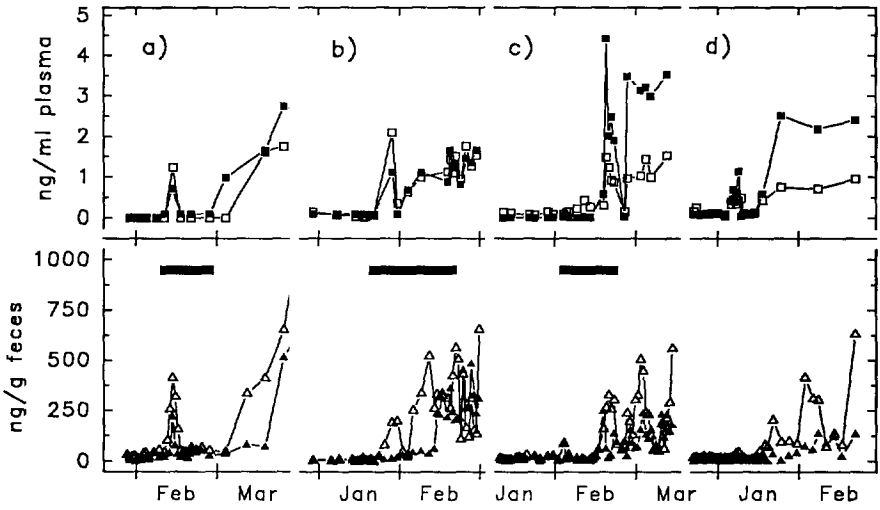

Figure 3. Individual profiles of immunoreactive progestagens in plasma (top panels) and feces (bottom panels) of 4 vicunas (a to d) during the breeding season. Concentrations were determined by enzymeimmunoassay for □ plasma progesterone, ■ plasma 20α-dihydroprogesterone, △ fecal 20-oxo-progestagens and ▲ fecal 20α-progestagens. Mating marks, produced by a sire marking ram harness, are indicated by closed bars.

< 100 ng/g, respectively. The progestagen levels of the animal, which had a late pregnancy abortion 20 d after its capture, are shown in Figure 5b. The progestagen concentrations of the pregnant vicuna decreased to basal values 7 d before abortion and stayed basal during the remaining observation period.

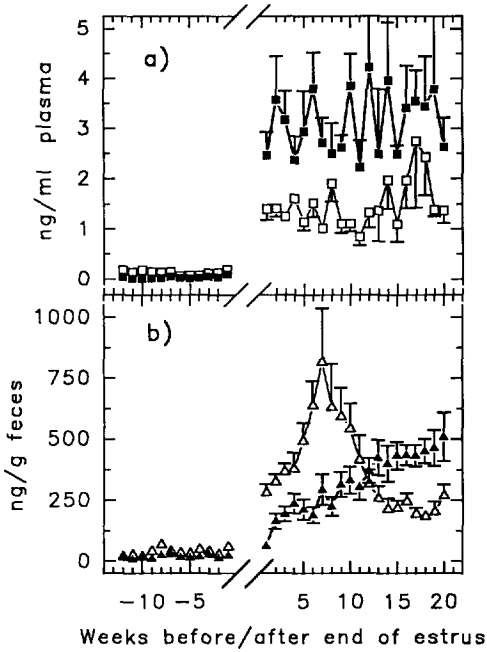

Figure 4. Concentrations (mean ± SEM) of immunoreactive progestagens in a) plasma and b) feces of vicunas (n = 8) before and after the breeding season. Concentrations were determined by enzymeimmunoassay for □ plasma progesterone, ■ plasma 20α-dihydroprogesterone, △ fecal 20-oxo-progestagens and ▲ fecal 20α-progestagens. Individual profiles are standardized to the onset of persisting corpus luteum function; times of estrous behavior (closed bars in Figures 2 and 3) were excluded from calculation.

DISCUSSION

Collection of blood and fecal samples for the present study was conducted during summer and autumn at the Lauca National Park in Chile (4470 m above sea level). Average temperatures were < 6° C, with frosts occuring almost every night. The severe climatic conditions during wintertime, together with limited financial funds, prevented continuation of field studies for an additional 7 to 8 mo. In the vicuna, pregnancy lasts about 11.5 mo and parturition takes place mainly in January and February (4,13,20,24). Therefore, the pregnancies predicted on the basis of progestagen measurements in our study could not be confirmed with observed parturitions.

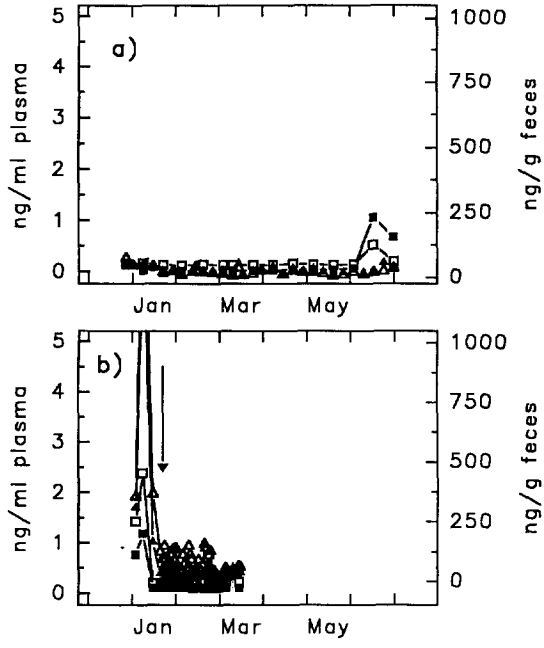

Figure 5. Individual profiles of immunoreactive progestagens in plasma and fecal samples of a) a juvenile vicuna and b) a pregnant vicuna near the time of abortion. Concentrations were determined by enzymeimmunoassay for □ plasma progesterone, ■ plasma 20α-dihydroprogesterone, △ fecal 20-oxo-progestagens and ▲ fecal 20α-progestagens. Abortion is indicated by an arrow.

Despite this draw back, the results of our study demonstrate that fecal progestagen evaluations can be used for noninvasive reproductive monitoring of vicunas. The HPLC separation of plasma samples and subsequent analysis with the EIA support the contention that the 2 assays resulted in yielding relatively specific unconjugated plasma progesterone and 20α-dihydroprogesterone; moreover, the immunoreactive peaks co-eluted with the respective radiolabeled [^{3}H]-steroids. However, retention times of fecal immunoreactive progestagens differed from the respective [^{3}H]-steroids and thus the results are expressed as 20-oxo- and 20α-progestagens. The immunoreactive progestagens showed elution patterns similar to those of 5α- and 5ß-reduced progestagen metabolites. These findings confirm those of our previous investigations of fecal progestagen metabolites in mares (17,18), black rhinoceroses (16) and okapis (19), where we also identified 5α- and 5ß-reduced progestagen metabolites.

Plasma progesterone concentrations in our study (< 0.5 ng/ml before mating and > 1.0 ng/ml after mating) are comparable to those of a recent study in vicunas (25), which described < 0.5 ng/ml before mating and > 1.5 ng/ml after fertile mating, and to plasma

progesterone concentrations in pregnant llamas and alpacas (1,3,5,14). Although 20α-dihydroprogestone has been described in alpacas (11), no investigation of it has been made in vicunas. Urinary 20α-hydroxyprogesterone immunoreactivity has been used to monitor luteal function in llamas (23). Urinary pregnanediol glucuronide, a 20α-hydroxylated steroid, has been used to monitor estrous cycles and early pregnancy in llamas (7,8,15), and it was found that values increased following copulatory-induced ovulation and remained elevated until 60 - 90 d after mating.

Breeding in our study took place between January and March; the range reported in the literature is March to May (24). The onset of the breeding season in our study could have been influenced by the capturing and feeding. Plasma and fecal progestagen values were low before the breeding period, increased sharply thereafter and remained elevated until the end of the observation period. These results further underscore that vicunas are seasonal breeders and have induced ovulations. The transient increases observed in progestagen concentrations during the mating period indicated nonfertile mating, resulting in short luteal phases. Similar to that of our study, Urquieta and Rojas (25) reported that progesterone in vicunas mated to a vasectomized male increased on Day 5, reached maximum concentrations on Day 8, and rapidly declined on Days 9 to 10. The interval from mating to corpus luteum formation in nonpregnant llamas was 3 to 4 d; the corpus luteum reached its maximum diameter 6 d after mating, and was completely regressed by Days 10 to 13 (2,3).

Signs of mating were not observed in 3 females kept with the same male, but their progestagen values increased after the mating period. Therefore, it is not clear whether spontaneous ovulation resulting in pseudopregnancy had occurred, or wether the male did not make mating marks that registered. The incidence of spontaneous ovulation in llamas and alpacas has been reported to range from 3.5 to 10% (1,3,10,12). However, in another study (8), a single mounting with penile intromission caused ovulation in about 90% of female llamas and alpacas.

Since luteal activity in 8 animals persisted after the mating period, we assumed that these animals were pregnant. However, pregnancy diagnosis by means of progesterone analysis gives presumptive not definitive results. It is not clear whether vicunas undergo persistence of the luteal phase without pregnancy, as has been observed in 2 of 13 llamas (1). The corpus luteum, however, is the major source of progesterone throughout pregnancy in alpacas and llamas (21).

In conclusion, results of the present study show that noninvasive fecal progestagen evaluations are a useful, valid approach to investigating reproductive events in the vicuna. Corpus luteum function is readily detected, and distinction between a normal postovulatory luteal phase and the maintenance of the corpus luteum is feasible 15 to 20 d after mating. In addition to plasma progesterone, plasma 20α-dihydroprogesterone concentration provides information about luteal activity of vicunas.

REFERENCES

1. Adam CL, Moir CE, Shiach P. Plasma progesterone concentrations in pregnant and non-pregnant llamas (Lama glama). Vet Rec 1989;125:618-620.
2. Adams GP, Sumar J, Ginther OJ. Effects of lactational and reproductive status on ovarian follicular waves in llamas (Lama glama). J Reprod Fertil 1990;90:535-545.
3. Adams GP, Sumar J, Ginther OJ. Form and function of the corpus luteum in llamas. Anim Reprod Sci 1991;24:127-138.
4. Bravo PW. Basic reproductive physiology in female South American Camelids. In: Fowler ME (ed) Zoo and Wild Animal Medicine. WB Saunders Co, Philadelphia, 1993;532-536.

5. Bravo PW, Fowler ME, Stabenfeldt GH, Lasley B. Endocrine responses in the llama to copulation. Theriogenology 1990;33:891-899.
6. Bravo PW, Fowler ME, Stabenfeldt GH, Lasley B. Ovarian follicular dynamics in the llama. Biol Reprod 1990;43:579-585.
7. Bravo PW, Stabenfeldt GH, Fowler ME, Lasley BL. Urinary steroids in the periparturient and postpartum periods through early pregnancy in llamas. Theriogenology 1991;36:267-278.
8. Bravo PW, Stabenfeldt GH, Fowler ME, Lasley BL. Pituitary response to repeated copulation and/or gonadotropin-releasing hormone administration in llamas and alpacas. Biol Reprod 1992;47:884-888.
9. Bravo PW, Stabenfeldt GH, Fowler ME, Lasley BL. Ovarian and endocrine patterns associated with reproductive abnormalities in llamas and alpacas. JAVMA 1993;202:268-272.
10. Bravo PW, Sumar, J. Laparascopic examination of the ovarian activity in alpacas. Anim Reprod Sci 1989;21:271-281.
11. Fernandez-Baca S, Hansel W, Novoa C. Corpus luteum in the alpaca. Biol Reprod 1970;3:243-251.
12. Fernandez-Baca S, Madden DHL, Novoa C. Effect of different mating stimuli on induction of ovulation in the alpaca. J Reprod Fertil 1970;22:261-267.
13. Fowler ME. Reproduction. In: Fowler ME (ed) Medicine and Surgery of South American Camelids. Iowa State University Press, Ames, IA, 1989;276-312.
14. Leon JB, Smith BB, Timm KI, LeCren G. Endocrine changes during pregnancy, parturition and the early post-partum period in the llama (Lama glama). J Reprod Fertil 1990;88:503-511.
15. Paul-Murphy J, Tell LA, Bravo W, Fowler ME, Lasley BL. Urinary steroid evaluations to monitor ovarian function in exotic ungulates: VIII. Correspondence of urinary and plasma steroids in the llama (Llama glama) during nonconceptive and conceptive cycles. Zoo Biol 1991;10:225-236.
16. Schwarzenberger F, Francke R, Göltenboth R. Concentration of faecal immunoractive progestagen metabolites during the oestrous cycle and pregnancy in the black rhinoceros (Diceros bicornis michaeli). J Reprod Fertil 1993;98:285-291.
17. Schwarzenberger F, Möstl E, Bamberg E, Pammer J, Schmehlik O. Concentration of progestagens and oestrogens in the faeces of pregnant Lipizzan, Trotter and Thoroughbred mares. J Reprod Fertil 1991;44(Suppl):489-499.
18. Schwarzenberger F, Möstl E, Bamberg E, von Hegel G. Monitoring of corpus luteum function by measuring progestagens in faeces of non-pregnant mares (Equus caballus) and Przewalski mares (Equus Przewalskii). Anim Reprod Sci 1992;29:263-273.
19. Schwarzenberger F, Patzl M, Francke R, Ochs A, Buiter R, Schaftenaar W, DeMeurichy W. Fecal progestagen evaluations to monitor the estrous cycle and pregnancy in the okapi (Okapia johnstoni). Zoo Biol 1993;12:549-559.
20. Smith CL, Peter AT, Pugh DG. Reproduction in llamas and alpacas: a review. Theriogenology 1994;41:573-592.
21. Sumar J. Removal of the ovaries or ablation of the corpus luteum and its effect on the maintenance of gestation in the alpaca and llama. Acta Vet Scand 1988;83(Suppl):133-141.
22. Sumar J, Frederikson G, Alarcon V, Kindhal H, Edqvist LE. Levels of 15-keto-13,14-dihydro-$PGF_{2\alpha}$, progesterone and oestradiol-17ß after induced ovulations in Llamas and Alpacas. Acta Vet Scand 1988;29:339-346.
23. Tell L, Paul-Murphy J, Bravo W, Stabenfeldt G, Lasley B, Fowler ME. Urinary and plasma steroid evaluations to monitor non-conceptive and conceptive cycles in the llama (Lama glama). In: Proc of the Joint Conf of the American Association of Zoo Veterinarians and American Association of Wildlife Veterinarians 1988;179 abstr.
24. Urquieta B, Rojas JR. An introduction to South American camelids. In: Livestock Reproduction in Latin America. Proc. of the Final Rresearch Co-ordination Meeting of the FAO/IAEA/ARCAL, Vienna, 1990;389-406.
25. Urquieta B, Rojas JR. Studies on the reproductive physiology of the vicuña (Vicugna vicugna). In: Livestock Reproduction in Latin America. Proc. of the Final Research Co-ordination Meeting of the FAO/IAEA/ARCAL, Vienna, 1990;407-428.