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Levels of 15-keto-13,14-dihydro-PF$G_{2\alpha}$, Progesterone and Oestradiol-17β after Induced Ovulations in Llamas and Alpacas

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Sumar J., G. Fredriksson, V. Alarcón, H. Kindahl and L.-E. Edqvist: Levels of 15-keto-13,14-dihydro-PF$G_{2\alpha}$, progesterone and oestradiol-17β after induced ovulations in llamas and alpacas. Acta vet. scand. 1988, 29, 339–346. – Six llamas and 6 alpacas were mated to vasectomized males; ovulation and corpus luteum formation followed. Progesterone in blood was elevated from day 5 and reached maximum concentrations of 10–20 nmol/l on day 7–8. A rapid decline in progesterone levels occurred on day 9–10 in connection with repeated surge releases of prostaglandin $F_{2\alpha}$. Oestradiol-17β levels were > 100–200 pmol/l during oestrus when the animals were mated. These high levels might have been caused by coital stimulation. A temporary increase was detected in connection with the rise in progesterone levels in the early luteal phase. With this exception levels of oestradiol stayed low, 20–40 pmol/l during the luteal phase but rose in most animals after luteolysis to 40–60 pmol/l.

prostaglandin $F_{2\alpha}$; sterile mating.

Introduction

The llama (*Lama glama*) and alpaca (*Lama pacos*) are two domesticated Camelidae species in the New World. They are economically very important for providing transport, meat, fibre and pelt to a large part of the Andean highland population.

Some of the reproductive physiology and pathology in the llama and alpaca has been described earlier (e.g. *Fernández-Baca* 1970a, *Sumar* 1983). Both species have induced ovulation. During the breeding season from December to April they can show continuous heat between 2 and 36 days in duration and interrupted by short periods of male rejection that can last up to 48 h (*San Martín et al.* 1968). Mating will induce ovulation after approximately 26 h and formation of a progesterone-secreting corpus luteum (*Leyva & Sumar* 1982, *Vivanco et al.* 1985). A fertile mating results in the formation of a corpus luteum of pregnancy which secretes progesterone throughout the gestation period. In the case of a non-fertile mating a short luteal phase seems to be induced. The progesterone levels during the short luteal phase have been reported previously, but the reported data are not in total agreement (*Fernández-Baca et al.* 1970c, *García et al.* 1986, *Sumar & García* 1987).

Also in the llama and alpaca the uterus is considered to have a specific role in luteolysis. It has been found that the luteolytic effect of the right horn is only local, whereas, differently from other species, the effect of the left horn is both local and systemic (*Fernández-Baca et al.* 1979). The role of the left uterine horn is special also in another way, as 98.5 % of the pregnancies are located there (*Fernández-Baca et al.* 1973). It is not known if repression of the corpus luteum is dependent on endogenous prostaglandin $F_{2\alpha}$ synthesis and release as in other domestic animals. It is however known that exogenously administered prostaglandin $F_{2\alpha}$ will induce parturition (*Sumar et al.* 1979). This study was conducted in order to document the secretory profiles of prostaglandin $F_{2\alpha}$, progesterone and oestradiol-17β in the llama and alpaca following sterile mating.

Materials and methods

Animals

Six alpacas and 6 llamas were used. They had earlier been checked for normal reproductive functions. The animals belonged to the herds kept at La Raya research station in the Andes in Peru. They were kept on natural pasture and looked after by a shepherd. They had been kept isolated from male animals during 1 year prior to this study.

Oestrous control

All the animals were in oestrus the day the study started (day 1). They were mated to a vasectomized male during an average time of 20 min in order to induce ovulation and subsequent corpus luteum formation. All the animals were checked for oestrus on days 9, 12 and 18 after sterile mating in the presence of a vasectomized male.

Blood sampling

Permanent catheters were placed in the jugular vein in all animals 2 days before the beginning of the study. In a few cases the catheters were obstructed and the blood sampling was continued by venopuncture.
Blood sampling was performed at 8 h and 16 h during the first 7 days starting immediately after service on day 1. From day 8 to day 12 blood samples were taken 6 times daily (4, 8, 12, 16, 20 and 24 h) and from day 13 to day 18 blood sampling was again done at 8 h and 16 h.
The blood samples were drawn into heparinized Vacutainer tubes (Becton-Dickinson, Rutherford, N. J., USA) for immediate centrifugation. Plasma was removed and stored at –20°C until hormone analyses. during the transport to Uppsala, Sweden, where the hormonal analyses were done, they were stored in dry ice and arrived unthawed to their destination.

Laparoscopy

Laparoscopy was performed on day 3, 9, 12 and 18 after sterile mating to document the ovarian status. The animals were anaesthetized using xylasin (0.2 mg/kg), and a laparoscopy instrument was introduced into the abdominal cavity through a 1 cm incision in the abdominal wall (*Sumar et al.* 1985).

Hormone analysis

Plasma levels of progesterone were determined by RIA using an antiserum to 11α-hydroxyprogesterone-hemi-succinate-bovine serum albumin (*Bosu et al.* 1976). The plasma was extracted by petroleum ether with an extraction recovery of 80 %. Dextran-coated charcoal was used for the separation of the free and antibody-bound hormone. The antiserum cross-reacted < 1 % with progestagens, oestrogens, androgens and corticoids except for deoxycorticosterone (3.8 %) and 5β-pregnane-3,20-dione (11 %). The practical detection limit was 0.5 nmol/l

for analysis of 250 µl plasmas. The intra-assay coefficient of variation varied between 8.5 and 11.5 % for different ranges of the standard curve.

The main plasma metabolite of $PGF_{2\alpha}$, 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$, was analysed by RIA according to *Kindahl et al.* (1976). The relative cross-reactions of the antibody were 16 % with 15-keto-$PGF_{2\alpha}$, 0.4 % with $PGF_{2\alpha}$, 4 % with 13,14-dihydro-$PGF_{2\alpha}$ and 1.7 % with 15-keto-13,14-dihydro-PGE_2. Other prostaglandins tested cross-reacted < 0.1 %. The practical detection limit of the assay is 30 pmol/l. The intra-assay coefficient of variation ranged between 6.6 and 11.7 % for different ranges of the standard curve and the inter-assay coefficient of variation was 14 % (mean value of 320 pmol/l).

Oestradiol-17β was determined by RIA using an antiserum against 6-keto-oestradiol-17β which cross-reacted 11 % with oestrone (*Boilert et al.* 1973, *Lindberg et al.* 1974). Plasma was extracted by diethyl ether with a recovery of about 90 %. Dextran-coated charcoal was used for the separation of the free and antibody-bound hormone. The practical detection limit of the assay was 20 pmol/l. The intra-assay coefficient of variation varied between 3.2 and 12.0 % for different ranges of the standard curve.

All hormone concentrations reported are the mean of duplicate determinations corrected for procedure losses.

Results

All of the sterile mated females formed a corpus luteum as evidenced by elevated progesterone concentrations and visualized by laparoscopy. The luteal phase was characterized by an increase in plasma progesterone concentrations starting around day 5 after sterile mating and reaching maximum concentrations of 10–20 nmol/l 2–3 days later (Figs. 1–3). Luteolysis as indicated by a de-

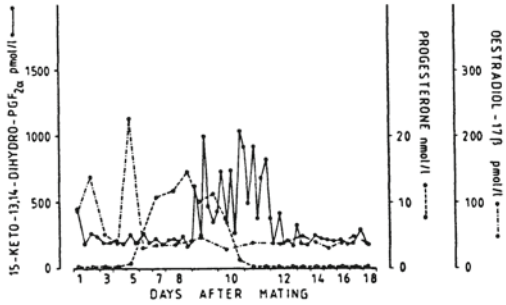

Figure 1. Levels of 15-keto-13,14-dihydro-$PGF_{2\alpha}$ ●—●, oestradiol-17β ○–.–.–○ and progesterone ○---○ in one llama after mating with a vasectomized male on day 1.

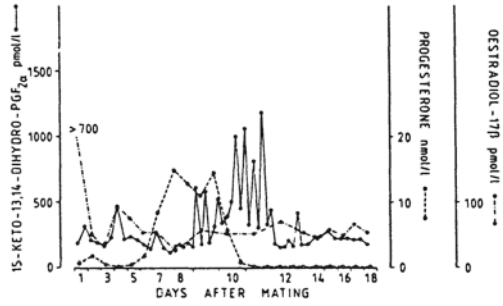

Figure 2. Levels of 15-keto-13,14-dihydro-$PGF_{2\alpha}$ ●—●, oestradiol-17β ○–.–.–○ and progesterone ○---○ in one llama after mating with a vasectomized male on day 1.

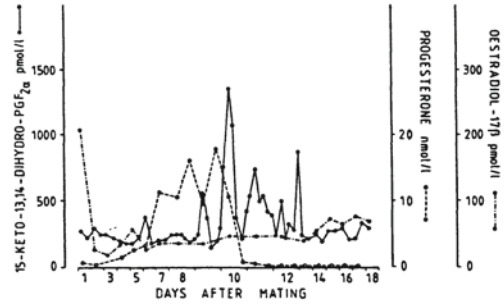

Figure 3. Levels of 15-keto-13,14-dihydro-$PGF_{2\alpha}$ ●—●, oestradiol-17β ○–.–.–○ and progesterone ○---○ in one llama after mating with a vasectomized male on day 1.

cline in plasma progesterone concentrations to around 1 nmol/l occurred during days 9–10 after sterile mating. The time span of the luteal phase arbitrarily defined as the time period with progesterone concentrations above 1 nmol/l was approximately 6 days. However, in 2 alpacas progesterone concentrations rose already on day 3–4 after sterile mating (Fig. 4). The maximum concentrations determined in these cases were 4–5 nmol/l and the length of the luteal phase was around 4 days.

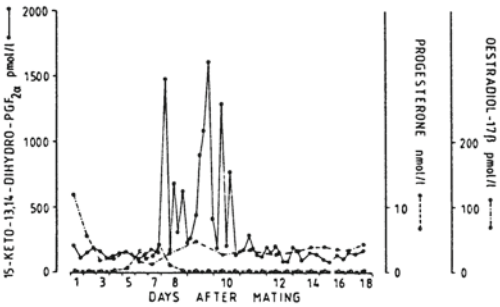

Figure 4. Levels of 15-keto-13,14-dihydro-$PGF_{2\alpha}$ ●—●, oestradiol-17β ○–.–.–○ and progesterone ○---○ in one llama after mating with a vasectomized male on day 1. Note the short luteal phase.

Baseline values of the prostaglandin metabolite of around 200 pmol/l were recorded from the start of the experiment through the early luteal phase. In the interval from day 9 to 12–13 repeated surge releases of prostaglandin $F_{2\alpha}$ were detected. The peaks continued to occur in all animals beyond completion of luteolysis (progesterone < 1 nmol/l). After day 12 baseline values were again recorded until day 18.

In the two alpacas having low progesterone concentrations and somewhat shorter luteal phases the prostaglandin release pattern was not different from the other animals.

Oestradiol-17β levels varied from 100–200 pmol/l up to more than 700 pmol/l on the first day when the animal were mated. These high values were generally only monitored in the morning sample which was taken on the first day of the study in connection with the mating. The general tendency in most animals was that the oestradiol-17β concentration rose gradually from 20–40 pmol/l during day 2–10 to 40–60 pmol/l during day 10–18. In connection with or just before the rise in progesterone levels on day 5 a temporary increase in oestradiol-17β concentrations up to 70–250 pmol/l was monitored. In one alpaca one high value of > 250 pmol/l of oestradiol-17β was monitored on day 9 during the luteal phase in connection with the onset of the release of prostaglandins. The duration of the peak was around 6 h.

The laparoscopy examination performed on day 3 revealed developing corpus luteum and one or several follicles in each animal. On day 9 a large functional corpus luteum was found in all animals. On day 12 a corpus luteum in regression was seen as well as a few growing follicles. On day 18 several follicles, sometimes together with a corpus albicans, were found. In one of the two animals with short luteal phases a functional corpus luteum was detected on day 3. On day 9 the corpus luteum was already in regression in these animals.

On day 9 two of the alpacas postured for mating when exposed to the male. One of them was identical with 1 of the 2 alpacas described separately above and had progesterone values < 1 nmol/l. The other, however, was in the middle of the luteal phase but had a temporary drop of progesterone on that particular day (Fig. 3).

On day 12 all animals accepted the male, although some variation occurred in reaction time (= time from introduction of the male until female postured for copulation).

On day 18 ten of the animals readily ac-

cepted the male whereas 2 had a somewhat prolonged reaction time. Their hormonal picture however did not differ from the others but they had both had a prolonged reaction time also on day 12.

Discussion

The results of the present study did not reveal any specific differences which could be attributed to the two species investigated. In the following the results are thus discussed without differing between the llama and the alpaca.

All animals had high oestradiol-17β levels at the day of mating. We have no obvious explanation for these high values but have paid particular attention to them from an analytical point of view in that the samples have been reassayed several times with the same result. This was an unexpected finding and the experimental design was not made to closely monitor folliculogenesis by oestradiol analysis. These high levels may be a consequence of a mating stimulus, since at day 18, when the animals again were accepting the male but not allowed to mate, no such high oestradiol-17β concentrations were seen. It is known that ovulation occurs around 26 h following mating and the data of the present study thus indicate that a considerable oestrogen secretion occurs during this time. More detailed studies with timed mating and blood sampling at short intervals are required to further eludidate this physiology.

The oestradiol pattern in the dromedary seems to be different from that observed here. In this species no obvious surge releases in oestradiol is found following mating, although oestradiol concentrations in dromedaries in oestrus are high (around 250–350 pmol/l; *Elias et al.* 1984a).

The induction of ovulation and subsequent corpus luteum formation depressed the follicular activity as evidenced by the laparoscopy findings and the relatively low values of oestradiol-17β during the luteal phase. Following lysis of the corpus luteum follicles again started to develop, concentrations of oestradiol-17β increased somewhat and the animals again accepted the male. The observed changes in gross ovarian morphology and the measured oestradiol-17β concentrations thus correlated well with the sexual behaviour of the animals. However, acceptance of the male animal is more correlated to low progesterone values than to high oestradiol-17β levels. The temporary increase in oestradiol-17β concentration when progesterone concentration starts to rise is described also in other species (*Cole & Cupps* 1977). No further explanation can at present be given to this phenomenon.

All animals had a luteal phase following sterile mating and this luteal phase was terminated in conjunction with release of $PGF_{2\alpha}$ as evidenced by the pulsatile release of its main metabolite. The data in this study suggest a shorter lifespan of the corpus luteum than what was reported earlier (for ref. see introduction). Also compared to other large domestic species its lifespan is short. Luteolysis occurs already around day 9–10 and progesterone values fall quickly. The release pattern of $PGF_{2\alpha}$ seen here shows great similarity with those described in other species in which prostaglandin release from the uterus acts luteolytically, e.g. cow (*Kindahl et al.* 1984), goat (*Fredriksson et al.* 1984), sheep (*Zarco et al.* 1983) and water buffalo (*Danell* 1987). Also in these species substantial amounts of prostaglandins are released following lysis of the corpus luteum as indicated by basal progesterone concentrations in blood.

In dromedary the existence of a luteal phase following non-fertile mating has been questioned. Thus *Elias et al.* (1984b) found ca-

mels which did not conceive following mating with intact males. These camels had a continuation of the follicular phase with no ovulation. It is likely also in the New World cameloids that a non-fertile mating can result either in an ovulatory failure without corpus luteum formation or in ovulation and formation of a corpus luteum resulting in a relatively short luteal phase as documented here. The former possibility probably also exists in the dromedary but remains to be proven. The low progesterone concentrations in the two alpacas are probably due to an insufficient formation of luteal tissue after ovulation or due to lack of ovulation followed by only partial luteinization of the follicle. It is also possible that different males provide different sexual stimulation during coitus which could influence the formation of the corpus luteum and perhaps its lifespan (*Fernández-Baca et al.* 1970b).

Taken together the following conclusions can be drawn from the results of the present study:

- sterile mating in the llama and alpaca results in elevations of blood plasma levels of oestradiol likely as a result of follicular development; ovulation and corpus luteum formation follows,
- the duration of the luteal phase is around 6 days,
- corpus luteum function is terminated through release of prostaglandin $F_{2\alpha}$.

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Sammanfattning

Blodnivåene av 15-keto-13,14-dihydro-$PGF_{2\alpha}$, progesteron och östradiol-17β efter inducerade ovulationer på lamor och alpackor.

Sex lamor och 6 alpackor betäcktes med vasektomerade handjur med ovulation och bildning av gulkropp som följd. Progesteron steg på dag 5 och nådde maximal koncentration på 10–20 nmol/l på dag 7–8. Ett snabbt fall i progesteronnivån inträffade på dag 9–10 samtidigt med en upprepad frisättning av prostaglandin $F_{2\alpha}$. Östradiol-17β ni-

vån var > 100–200 nmol/l under brunsten när djuren betäcktes. Dessa höga nivåer kan ha orsakats av den sexuella stimulansen från betäckningsakten. En kortvarig ökning iakttogs också i samband med progesteronstegringen i lutealfasens början. Med undantag av detta var östradiolnivåerna låga under lutealfasen, 20–40 pmol/l, men steg hos de flesta djuren till 40–60 pmol/l efter luteolysen.

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