



## Effects of treatment with a prostaglandin analogue on developmental dynamics and functionality of induced corpora lutea in goats

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### ARTICLE INFO

#### Article history:

Received 20 April 2009

Received in revised form 19 May 2009

Accepted 26 May 2009

Available online 21 June 2009

#### Keywords:

Corpus luteum

Ovarian follicular dynamics

Prostaglandin analogue treatment

Goats

### ABSTRACT

The aim of this study was to compare morphological and functional features of spontaneous and induced corpora lutea (CLs) in goats. Fourteen adult and cycling Anglo Nubian goats (Argentina) were randomly allocated to two groups: Group N ( $n=7$ ) included goats with natural spontaneous oestrus and Group PG ( $n=7$ ) included does in which oestrus was synchronized by the administration of two i.m. cloprostenol doses, 10 days apart. In both groups, oestrous behaviour was checked twice daily (Day of oestrus = Day 0) and daily transrectal ultrasonographies were performed for evaluating CLs and follicles dynamics through the complete subsequent oestrous cycle; the luteal activity was determined directly, in terms of progesterone (P4) secretion, and indirectly, by assessing effects of CL on follicular dynamics. All goats exhibited oestrous behaviour and ovulation without differences in ovulation rate (N:  $1.67 \pm 0.2$ , PG:  $2.0 \pm 0.1$ ). The total luteal tissue area showed linear growth from Day 4 to Day 15 of oestrous cycle in all goats, but the developmental dynamics differed between groups, treated goats had larger area ( $P < 0.01$ ). Plasma P4 concentrations also increased from Day 0 to Day 15 in all the does; however, from Day 5 to Day 15, treated does had a lower concentrations than the untreated group ( $P < 0.001$ ). There were differences in the development of follicular waves between groups; assessment of size-distribution showed that treated group had a higher number of small and larger follicles ( $P < 0.05$ ). The largest follicles recorded in treated goats had a higher maximum diameter both at the first (PG:  $7.6 \pm 0.8$  mm; N:  $4.9 \pm 0.7$  mm,  $P < 0.05$ ) and second follicular waves (PG:  $6.3 \pm 1.4$  mm; N:  $5.0 \pm 0.4$  mm,  $P < 0.05$ ) and a longer growth phase during the second wave (PG:  $6.5 \pm 1.7$  days; N:  $4.6 \pm 0.7$  days,  $P < 0.05$ ), coincident with the period of maximal luteal secretion. In conclusion, synchronization of oestrus and ovulation by the administration of a prostaglandin analogue causes differences in developmental dynamics and functionality of induced corpora lutea when compared to natural spontaneous ovulation.

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### 1. Introduction

The management of the productive cycles by programing reproduction through synchronization of ovulation is the basis for animal production, in goats as in other species. Synchronization is usually accomplished by using hormonal treatments mimicking the activity of the corpus luteum (progesterone or progestagen-based treatments; Corteel et al., 1988), due to their availability and the possi-

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bility of being used in breeding and non-breeding season. On the other hand, the use of progestagens is limited as a result of considerations related to economy (expensive cost and limited availability in some countries) and, mainly, to animal welfare (by inducing problems like vaginitis and sponge retention) and to food safety (by producing biochemical residues), which are contrary to consumer demands for “clean, green and ethical” products (Martin et al., 2004). The latter has caused the prohibition of progestagen use in farm animals (USA) and a higher restriction in the maximum residue limit allowed in European Union countries.

Thus, the alternative for synchronization of oestrus and ovulation, in cycling does, is the induction of a controlled luteolysis and a subsequent follicular phase by administering two doses of luteolytic agents like prostaglandin  $F_{2\alpha}$  or its analogues, 9–11 days apart (Greyling, 1996).  $PGF_{2\alpha}$  treatments are easily applied by intramuscular injection, avoiding problems of intravaginal devices, rapidly metabolized, and avoiding chemical residues in animal products. However, in goats, fertility at first service after  $PGF_{2\alpha}$  treatments is reduced when compared with progestagen sponges (67% vs. 80%, Kusina et al., 2000). Possible causes for such decreased conception rate remain unclear. A previous study in goats pointed to alterations in the functionality of preovulatory follicles (Fernandez-Moro et al., 2008).

We hypothesize that such defective follicles may give way to defective corpora lutea unable to maintain pregnancy. However, to our knowledge, there are no previous studies comparing the functionality of corpora lutea from oestrus induced with prostaglandin analogues and natural oestrus in goats. Thus, the objective of the current experiment was to compare the characteristics of spontaneous and induced corpora lutea both by a direct evaluation, determining their tisular and endocrine features during a complete oestrous cycle, and by indirect assessment, evaluating the follicular population in the ovaries. Deleterious effect of the presence of an active corpus luteum on dynamics and lifespan of follicles in ruminants is well known (Adams, 1999); thus, evaluation of the follicle population may be likely to be used as an indirect index of the quality of the corpora lutea.

## 2. Materials and methods

### 2.1. Animals and experimental design

Fourteen Anglo Nubian adult goats were used. The does were maintained, under natural day-length and temperature, at the experimental farm of the National University of Río Cuarto, Argentina, latitude  $33^{\circ}07'S$ , which meets the requirements of the Ethic Committee of the University. The study was conducted in May, during the period of breeding season, and all the females were confirmed to be cycling at starting the experimental procedure by ultrasonography and evaluation of plasma progesterone concentrations. All the procedures were approved by the Committee for Scientific Procedures of the University.

Does were randomly allocated to two groups (7 goats per group). The first group included does with natural spontaneous oestrus (Group N); the second included does

treated with a prostaglandin analogue (Group PG), in which oestrus was synchronized by the administration of two i.m. injections of 1 mL of a prostaglandin analogue (263  $\mu\text{g}$  of cloprostenol, Estrumate<sup>®</sup>, Schering-Plough Animal Health Corporation, NJ, USA), 10 days apart.

In both groups, oestrous behaviour was checked with a vasectomized buck, twice a day for 30 min (8:00 a.m. and 6:00 p.m.), throughout the experimental period for detecting oestrus (considered as Day 0 for experimental purposes) and establishing the inter-oestrous intervals.

Each goat was observed through the complete oestrous cycle subsequent to oestrous detection, in both groups. The variables evaluated were corpora lutea and follicular dynamics, by ultrasonography, and luteal functionality, by analysis of plasma progesterone concentration. Ovaries were evaluated again at Day 7 after the second oestrus for determination of ovulation rate.

### 2.2. Ultrasonographic evaluation of follicular and luteal development

The number, position and diameter of all follicles  $\geq 2$  mm in size, and the position and size of corpora lutea (CL) were determined by transrectal daily ultrasonographies, conducted by a single operator, by using an Aloka SSD-500 (Aloka, USA) fitted with a 7.5 MHz linear array transducer, as previously described (Ginther and Kot, 1994). Data were recorded in a diagram of the ovaries to evaluate their development in successive observations. For measuring corpora lutea with cavity, the cavity area was subtracted from corpus luteum area to obtain the total area of luteal tissue.

For every oestrous cycle, ultrasonographic data were summarized to characterize patterns of ovarian follicular development. First, the subordinate, dominant and ovulatory follicles were characterized retrospectively. Follicular waves were characterized by adapting the methodology of Ginther and Kot (1994) and Gonzalez-Bulnes et al. (1999) for goats: (a) wave onset (emergence): day in which follicles were firstly detected at 3 mm, growing to at least 4 mm at the following day; (b) growth phase: time taken by a single follicle to grow from 3 mm to its maximum diameter; (c) regression phase (atresia): time taken by a single follicle to regress from its maximum size until the day that it reach its smallest size; (d) end of the wave: day when the dominant follicle ending its regressing phase and/or ovulate (last wave).

Thereafter, follicles present in the ovaries were classified by their diameter. Four groups were categorized: total follicles  $\geq 3$  mm in size, large ( $\geq 5.5$  mm), medium (3.5–5.4 mm) and small follicles (3–3.4 mm). After this, during the follicular phase, the number of new follicles (not previously detected), growing follicles (those that increased in size with respect to the previous day), and decreasing follicles (those that decreased in diameter with respect to the previous day or disappeared) were also considered.

### 2.3. Hormonal evaluation of luteal activity

The luteal activity was evaluated in terms of progesterone secretion by drawing jugular blood samples coincidentally with ultrasound scannings. Blood sampling

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