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Differences in preovulatory follicle dynamics and timing of preovulatory LH surge affect fertility of maiden sheep reared in semi-arid extensive conditions

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ABSTRACT

In the current study follicular dynamics, pituitary function, ovulatory response and luteal activity of 30 maiden Barbarine sheep were analyzed according to oestrus occurrence and lambing outcome after oestrus synchronisation with cloprostenol. Animals were retrospectively classified in three groups named as O− ($n = 7$, ewes not displaying oestrus), O+L− ($n = 7$, ewes showing oestrus but failing to lamb) and O+L+ ($n = 16$; ewes showing oestrus and lambing thereafter). All the sheep ovulated and daily transrectal ultrasonographies revealed that preovulatory follicles were present at cloprostenol injection in all the animals. In sheep O+L+ and O+L−, 50% and 57% of the ovulatory follicles were the largest follicles at cloprostenol treatment (mean size of 4.1 ± 0.26 mm and 4.3 ± 0.74 mm, respectively). In O− ewes, the same percentage was higher (86%, $P < 0.05$ when compared to group O+L+; mean size of 4.0 ± 0.46 mm). The number of large follicles and the final diameter of the ovulatory follicles at oestrous tended thereafter to be higher in group O+L+ (1.4 ± 0.1 and 6.4 ± 0.2) than in groups O+L− (1 ± 0.2 and 5.7 ± 0.36) and O− (0.9 ± 0.2 and 5.9 ± 0.5, respectively). Conversely, the number of medium follicles at oestrus detection was higher in the group O+L− (2.1 ± 0.3, $P < 0.05$) than in the other two groups (1 ± 0.2 and 1 ± 0.3 for O+L+ and O− respectively). Timing of preovulatory LH surge was earlier for ewes O− (24.0 ± 4.75, $P < 0.05$) than for sheep O+L+ and O+L− (37.9 ± 2.45 h and 38.0 ± 4.75 h, respectively) and 94% of O+L+ ewes had a LH surge between 16 h and 64 h after cloprostenol injection compared to 57% in O+L− and O− groups ($P < 0.05$). Thus, maiden Barbarine sheep failing to display oestrus or conceive showed alterations in their follicular dynamics and, thereafter, pituitary function and ovulatory response.

1. Introduction

Reproduction of young female sheep is characterized by a low efficiency. Even in highly selected breeds reared in temperate regions of Europe and New Zealand (Hare and Bryant, 1985; Davies-Morel and Beck, 2003), reproductive performance of yearlings is much lower than adults and up to 20–40% of maiden ewes presented to rams fail to produce a lamb. These deficiencies are higher in breeds reared under harsher climatic and nutritional conditions e.g. in Tunisia where in commercial flocks of the Barbarine breed, the conception rate of 4263 maiden ewes aged 18 months when first mated, representing 35 flocks and 5 consecutive years, averaged 71%; varying between 38% and 86% according to several management and environmental factors (Ben Salem et al., 2005). Furthermore, Ben Salem et al. (2008)

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reported that up to 14% of Barbarine maiden ewes display oestrus but fail to lamb. Such data are similar to what is reported in commercial Merino flocks in South Australia (Kleemann and Walker, 2005).

Fertility is determined by the regularity of oestrus, the number and quality of ovulations and by the incidence of embryo losses. Studies in breeds from temperate regions have shown similar incidence, between maiden and adult females, of irregular oestrus manifestation (Drymundsson, 1983) and anovulatory cycles (McMillan and McDonald, 1985). Hence, reproductive features in young female sheep are mainly influenced both by a reduced ovulatory success (Drymundsson, 1983) and by embryo losses in pre- (Davies, 1988) and peri-implantation periods (Beck and Davies, 1996). In ruminants, both causes may be related to deficiencies in follicular development; leading to follicles being unable to ovulate (Gonzalez-Bulnes et al., 2005) or, if so, releasing oocytes with disturbed prematuration and maturation (Revah and Butler, 1996; Mihm et al., 1999) and, thus, alterations in fertilization and early embryo development (Greve et al., 1995).

Therefore, the objective of the current study was to characterize and compare follicular dynamics, pituitary function, ovulatory response and luteal activity during the follicular phase and the subsequent luteal phase of maiden Barbarine sheep treated with a PGF analogue and which succeeded or not in displaying oestrus and, thereafter, lambing. These data would give way to the understanding of factors limiting reproductive features of maiden sheep reared in harsh conditions.

2. Materials and methods

2.1. Animals and experimental design

The experiment was carried out at the farm of the Office de l'Elevage et des Pâturages, in Jebibina (Central Tunisia, latitude is 35°N). The area is semi-arid with a very erratic pattern of rainfall and an annual rainfall average of 390 mm. It experiences a Mediterranean-type climate with cool winters and hot dry summers.

Thirty maiden ewes of the fat-tailed Barbarine breed, all aged 18 months and weighing 39 ± 0.6 kg, were used. These females were randomly chosen to be cycling, as assessed by ultrasonographic determination of the presence of a corpus luteum (CL) in the ovaries, within a flock of approximately 180 females. Just immediately after ultrasonography, the ewes were treated with a single im dose of 125 μg of cloprostenol (0.5 ml, Estrumate®, Schering-Plough Animal Health, Friesoythe, Germany) for inducing CL regression and a fertile oestrus, which was detected by 4 harnessed trained Barbarine males. When in oestrus, each female was allowed to be naturally mated twice, 12 h apart, with 2 different males of the same breed for avoiding male effects on fertility.

Determination of follicular dynamics was performed daily by transrectal ultrasonographic assessment of the number and size of all follicles ≥2 mm, from the day of cloprostenol injection to the day following onset of oestrus (day of oestrous being considered day 0) or for 5 days after treatment in those sheep that failed to exhibit oestrus.

Pituitary function was determined by assessing plasma LH levels and, thus, preovulatory LH surges in jugular blood samples drawn every 4 h, from 16 h to 64 h following injection of cloprostenol. Occurrence of preovulatory LH surge was established in agreement with definition of Pearce et al. (1987), but adapted to Barbarine sheep (Lassoued, 1998); hence, a preovulatory LH surge was defined as a sustained increase in LH above 10 ng/ml over two successive samples.

The presence and number of CL were assessed 9 days after cloprostenol injection, by transrectal ultrasonography. Luteal function was determined by assessing plasma progesterone levels in jugular blood samples taken every 72 h between 3 and 19 days after cloprostenol injection.

2.2. Assessment of preovulatory follicular development and presence and number of corpora lutea

Ultrasonographic observations of the ovaries were performed as previously validated in sheep by Gonzalez-Bulnes et al. (1994). All the observations were performed by the same experienced operator using a 7.5 MHz transducer for transrectal ultrasonography (Aloka SSD-500, Ecotron, Madris, S). In brief, observations were conducted with the sheep placed in dorsal position as during laparoscopy. After introducing a hydrosoluble contact gel into the rectum to enhance the ultrasound transmission, the probe was placed in the rectum with the transducer orientated perpendicularly to the abdomen wall. When the urinary bladder was surpassed and the uterine horns were located, the probe was rotated laterally 90° clockwise and 180° counterclockwise to observe both ovaries and their structures. With regard to follicles, ultrasonographic data were summarized to characterize patterns of follicular development. All follicles recorded by ultrasonography were classified as total (≥2 mm), large (≥5.5 mm), medium (3.5–5.4 mm) or small follicles (2–3.4 mm). CLs were identified through their echogenic pattern and their presence and number during the early luteal phase were undertaken according to the method described by Gonzalez de Bulnes et al. (2000).

2.3. Assessment of LH and progesterone secretion

Jugular blood samples were collected by using vacuum blood evacuation tubes with heparin (Vacutainer Systems Europe, Becton Dickinson, Meylan Cedex, France). Thereafter, samples were centrifuged at 1500 × *g* for 15 min and plasma was stored at −20 °C until assayed for progesterone and LH.

Plasma progesterone concentrations were determined in duplicate using a Coat-A-Count® radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, USA), according to the manufacturer's instructions. The limit of detection was 0.02 ng/ml. inter- and intra-assay variation coefficients were 5.6% and 3.8%, respectively.

Plasma LH was measured in duplicate using a solid phase two-site enzyme-immunoassay kit specifically developed for sheep (LH Detect®, INRA Tours, Nouzilly, France). The assay sensitivity was 0.01 ng/ml and the intra- and inter-assay variation coefficients were 6.8% and 7.6%, respectively.