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Prolific strains of Barbarine sheep are characterized by increased ovulation rate due to extended period of ovulatory follicle recruitment and co-dominance effects

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ABSTRACT

Preovulatory follicle dynamics and plasma oestradiol around the time of onset of oestrus as well as ovulation rate were assessed, during non-breeding season, in prolific (W; $n = 20$) and non prolific (AB; $n = 20$) strains of the Barbarine sheep. Oestrus cycles were synchronized with progestagen sponges for 14 days followed by introduction of rams. Transrectal ultrasonographic monitoring of the ovaries was carried out from the day of pessaries removal up to oestrous exhibition. Blood samples were collected every 4 h and were analyzed for plasma concentrations of oestradiol by radioimmunoassay. Laparoscopy was performed 11 days after introduction of rams to assess number of corpora lutea (CL). Results indicate that along the first 5 days after introduction of rams, 20 and 14 ewes of respectively the AB and W ewes exhibited oestrus ($P < 0.01$). Ewes of the W strain had more medium (3.5–5.4 mm) follicles than AB ones during the follicular phase; the difference being significant ($P < 0.01$) 24 h before oestrus. The number of medium follicles prior to oestrus changed little in AB ewes but declined rapidly from 5.8 ± 0.7 to 4.0 ± 0.6 in W females ($P < 0.05$). Differences in medium follicles between strains were not reflected in the number of large (≥ 5.5 mm) or the growing large follicles during the follicular phase. Around oestrus, plasma oestradiol concentrations were higher for W in comparison to AB ewes (22.3 ± 12.6 *vs.* 11.8 ± 6.2 pg/ml; $P < 0.01$) and mean ovulation rates were 1.8 ± 0.8 and 1.1 ± 0.3 for respectively W and AB ewes ($P < 0.01$). In conclusion, the increased ovulation rate in the W strain is related to an extended period of follicle recruitment with appearance of co-dominance effects.

1. Introduction

The fat-tailed Barbarine sheep is the predominant breed in arid areas of North Africa, mainly Tunisia, Libya and Eastern Algeria. In the home breeding area of the Barbarine breed, productive outputs are limited, within other causes, by a low reproductive efficiency when compared to Western breeds; mainly, a delayed onset of puberty, large anoestrus periods and low fertility and prolificacy. In a study carried out in central Tunisia on 25 Barbarine flocks during 15 years, Khaldi (1989) calculated an average fertility rate of 89% while prolificacy averaged 1.2 varying between 1.0 and 1.4 and this means that numerous sheep in a herd are delivering less than 1 lamb/year. The constraints of the arid environments largely contribute to this low productivity but scarcity of genetic selection for improvement of reproductive and productive yields should also be noted (Gonzalez-Bulnes et al., 2010). Recently, all local sheep breeds of Tunisia were mapped in order to study the genetic structure at BMPR 1B, BMP15 and

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GDF9 prolificacy genes and the study revealed absence of $FecB^B$ (BMPR 1B), $FecX^R$, $FecX^H$, $FecX^I$, $FecX^L$, $FecX^G$, $FecX^B$ (BMP15) and $FecG^H$ (GDF9) mutations (Vacca et al., 2010).

The Barbarine sheep is a seasonal breeder. The breeding season extends from mid-July to late February with a 242 day-mean duration. Anoestrous is of moderate depth, since a variable proportion of ewes remains sexually active during spring time (25–40% of adult ewes). Ovulation rate, with an annual mean of 1.3, also shows seasonal variation being lowest (1.10) between March and May (Khaldi, 1984).

Work in the Ouesslatia station (National Agricultural Research Institute, Tunisia (INRAT)) lead to the selection in 1979 of a flock of prolific ewes of the Barbarine. The flock started with 66 prolific females selected in other non-prolific flocks. The ewes were then mated to rams born to prolific ewes and the size of the flock then gradually increased to reach more than 170 ewes during the nineties. Mating is managed within 7 families. Over a period of 20 years (1979/1998) calculated mean prolificacy of the flock is 1.6 reaching 1.4 and 1.9 as minimum and maximum values respectively (Khaldi, 2007). Heritability and repeatability estimates of prolificacy for this prolific strain are 0.2 ± 0.2 and 0.4 respectively (Abdennebi, 1990). So far, there are no reports on the existence of specific genes associated to prolificacy in this strain.

Previous unpublished studies found that ovulation rate for the prolific strain varies between 1.7 and 2.4 corpora lutea (Lassoued, unpublished data of INRAT), which would explain the increase in litter size of the prolific strain. However, there is no information on intrinsic mechanism determining higher ovulation rate in prolific Barbarine sheep. In small ruminants, prolificacy is essentially determined by ovulation rate which, in turn, is determined by preovulatory ovarian follicular development (Bartlewski et al., 1999). Specifically in sheep, increases in ovulation rate have been mainly attributed to an extended period of ovulatory recruitment (the period of time in which recruitment of ovulatory follicles takes place; Driancourt, 2001). Thus, we hypothesized that differences in ovulation rate between the AB and W strains of the Barbarine breed may be related to differences in the extent of the period of ovulatory recruitment and/or differences in preovulatory follicle development. Hence, the objective of this study was to determine whether the background of selection has induced differences in the preovulatory ovarian follicular dynamics of the breed that may explain differences in ovulation rate. Thus, we compared preovulatory follicle dynamics and ovarian function around the time of onset of oestrus of the prolific vs. the non prolific strains of Barbarine ewes reared in a common habitat.

2. Materials and methods

2.1. Experimental location and animal management

The trial was carried out during non-breeding season, April–May, since this is the usual breeding period for producers in the area. The work was performed at the experimental station of Bou Rebiaà (INRAT; latitude 36° 38′ N; longitude 10° 07′ E). Average annual rainfall (mean of last 30 years) is 350 mm. A total of 40 non-pregnant adult ewes of the Barbarine breed were used. All of them were drafted from the selection flocks established at INRAT. Half the animals were of the non prolific AB strain and the other half belonged to the prolific W strain, which was described in the introduction section. The animals were 4–5 years-old, had a mean live weight of 38 ± 2.2 and 41 ± 3.9 kg and a body condition of 2.5 ± 0.2 and 2.3 ± 0.2 (on a scale of 1–5) at the start of the experiment for respectively AB and W ewes (no significant differences).

Throughout the experiment, the animals of the two strains were kept together, grazed natural rangeland and were daily supplemented with 0.3 kg/ewe of barley. When not grazing, animals were kept in fully open sheds exposed to natural photoperiod. Sheep had free access to clean water during the entire trial.

2.2. Experimental design and procedures

On April the 26th, oestrous cycle was synchronized, in all the ewes, by using intravaginal progesterone pessaries (20 mg cronolone; Chronogest CR®, Intervet International, Schering Plough, UK) for 14 days. At sponge withdrawal, 8 adult harnessed Barbarine rams were introduced and permanently remained with ewes until the end of the experiment, when the number of corpora lutea (CL) was assessed. Appearance of oestrus behaviour was checked continuously day and night for the first 5 days following introduction of rams.

Determination of follicular dynamics was performed daily by transrectal ultrasonographic assessment of the number and size of all follicles $\geq$2 mm, from the day of pessaries removal to the day of oestrus onset (considered day 0 for experimental purposes) or for 3 days after sponge removal in those sheep that failed to exhibit oestrus. Starting 18 h after removal of the pessaries and until 28 h after exhibition of first signs of oestrus for each ewe, jugular blood samples were collected every 4 h, by using vacuum blood evacuation tubes with heparin (Vacutainer Systems Europe, Becton Dickinson, Meylan Cedex, France). Thereafter, samples were centrifuged at $1500 \times g$ for 15 min and plasma was stored at −20 °C until assayed for oestradiol.

The presence, number and age of CL were assessed 11 days after removal of the pessaries by subjecting ewes to a mid-ventral laparoscopy. Animals were placed in dorsal recumbence in a metallic cradle with their heads tilted downwards at a 30–45° angle. Laparoscopies were performed with a 5 mm endoscope (Karl Storz, Tuttlingen, Germany), under local anaesthesia, using 1 ml of 2% procaine (Lidoject; Labesfal, Campo de Besteiros, Portugal) injected subcutaneously on the mid-ventral area, 3–4 cm anterior to the udder.

2.3. Evaluation of ovarian activity and oestradiol levels

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, Madrid, Spain). 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° counter-clockwise 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 ($\geq$2 mm), large ($\geq$5.5 mm), medium (3.5–5.4 mm) or small follicles (2–3.4 mm).

Plasma oestradiol concentrations were determined in duplicate using a ultrasensitive double antibody radioimmunoassay with reagents and techniques provided by Coat-A-Count® (IMMUNOTECH, Prague, Czech Republic), according to the manufacturer's instructions. Analyses were made in duplicate, and the sensitivity of the assay was 5.2 pM (1.4 pg/ml). The calculated intra- and inter-assay coefficients of variation were 3.5 and 4.9%, respectively.

2.4. Statistical analyses

Effects of strain and time on the number and size of follicles and plasma oestradiol concentrations were assessed by analysis of variance (ANOVA) for repeated measures, followed by Kruskal–Wallis test, when Levene's test showed non-homogeneous variances.

The period of recruitment was determined by the difference between the day in which the preovulatory follicles were first detected and the day