Animal Reproduction Science 138 (2013) 180–187

Contents lists available at SciVerse ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Endocrinological profile and follicular development in cyclic ewes subjected to repeated ovum pick-up

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ARTICLE INFO

Article history:
Received 24 October 2012
Received in revised form 22 February 2013
Accepted 28 February 2013
Available online 14 March 2013

Keywords:
Ewes
Follicular aspiration
FSH
Oestradiol
Progesterone
Oestrous cycle

ABSTRACT

Blood concentrations of progesterone, FSH and oestradiol in Karagouniko ewes subjected to laparoscopic ovum pick-up (OPU) at specific stages of induced oestrous cycle, were measured. Twenty-four cyclic ewes were randomly allocated into four equal groups (A, B, C and D). Oestrus was synchronized with progestagen intravaginal sponges and detected by teaser rams (oestrus: day 0). In group A, during the induced oestrous cycle, OPU was performed on days 4, 9 and 14 (sessions A1, A2 and A3, respectively). In group B and group D, OPU was performed once, on day 9 and 14, respectively. In group C (controls), endoscopic observation of follicular population was performed three times, as in group A. Starting at sponge removal, progesterone was measured in blood samples collected on 22 daily occasions and oestradiol in samples collected on 27 occasions collected at various time-points starting 2 h before to 24 h after OPU. Follicular populations did not differ among A1, A2, A3 or between C1, C2, C3 and A1, A2, A3 or A1, B, D, respectively. Oocytes of better quality (category '1' or '2') were collected at A3 session compared with A1 ($P < 0.05$). Progesterone concentration and oestrous cycle length did not differ among groups. Decreased oestradiol concentrations followed by FSH increase were recorded 3–5 h after OPU. The results confirm the regulatory role of oestradiol on FSH secretion. The quality of collected oocytes was improved in subsequent pick-up sessions in the oestrous cycle. Moreover, OPU at specific stages of the luteal phase of the cycle, even when applied repeatedly, do not affect the normal oestrous cycle length of ewes.

1. Introduction

Embryo production by *in vitro* techniques has increased steadily during recent decades. Oocyte recovery from live sheep may be performed by laparotomy or by laparoscopic follicular aspiration (Cognié et al., 2003; Valasi et al., 2005, 2006, 2007, 2009). Laparoscopic OPU can be applied repeatedly in ewes and ewe-lambs, in order to increase oocyte yields (Stangl et al., 1999; Morton et al., 2005; Valasi et al., 2007) and to provide a high number of good quality cumulus oocyte complexes for *in vitro* embryo production over a long period. The technique also guarantees maintenance of fertility of donor animals, even after repeated OPU (Stangl et al., 1999; Morton et al., 2005; Valasi et al., 2006). By using the method more offspring from genetically valuable animals can be produced than by following traditional multiple ovulation and embryo transfer procedures, with no need for hormonal stimulation (Cognié et al., 2003). By eliminating hormonal treatment, the application of the procedure becomes simpler and less costly.

Apart from assisted reproduction, OPU may also be used in the study of follicular dynamics and hormonal

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http://dx.doi.org/10.1016/j.anireprosci.2013.02.026

interactions during the oestrous cycle, as reported by Amiridis et al. (1999). In ewes, follicular growth occurs in a wave-like pattern (3–6 waves) during the breeding season (Noël et al., 1993; Ginther et al., 1995; Souza et al., 1998; Bister et al., 1999; Evans et al., 2000) and the seasonal anoestrous period (Noël et al., 1993; Bartlewski et al., 1998, 2000; Huchkowsky et al., 2002), as well as during the transition period from the seasonal anoestrous period into the reproductive season (Bartlewski et al., 1999), with a periodicity of approximately 3–6 days. Emergence of a wave is triggered by a transient increase in FSH secretion during (Ginther et al., 1995; Souza et al., 1998; Evans et al., 2002) or outside (Bister et al., 1999; Bartlewski et al., 1998, 2000) the breeding season.

Repeated OPUs have been performed with various frequencies, *e.g.*, 5 times at weekly intervals (Kuhholzer et al., 1997), 4 times at weekly intervals (Tervit et al., 1992), up to 20 times once, twice weekly or every fortnight (Stangl et al., 1999), thrice every fortnight (Morton et al., 2005) in untreated or eCG-treated ewes and up to 4 times at monthly intervals in FSH-treated ewe-lambs (Valasi et al., 2007, 2009). So far, it has been shown that follicular fluid aspiration during the first follicular wave of the oestrous cycle affects the mode of FSH, LH, oestradiol and inhibin A secretion, as well as follicular development (Evans et al., 2002). However it is important to study, if repeated follicular aspiration within an oestrous cycle could lead to varying hormonal and follicular aberrations affecting the animals' cyclicity. Therefore, the aim of the present study was to examine the effects of single or repeated OPU conducted at early, mid or late stage of the luteal phase of an oestrous cycle on (i) follicular development, (ii) oocyte quality, (iii) oestrous cycle length and (iv) circulating progesterone, FSH and oestradiol concentrations in ewes.

2. Materials and methods

2.1. Animals and treatments

All experiments described in this study were carried out under license, issued by the relevant Greek authority (the Hellenic Ministry of Rural Development and Food), within the specifications of EU legislation for animal experimentations.

In total, 24 3–4 years old dairy ewes of Karagouniko breed (55–65 kg bodyweight) were included in the study, which was performed during the breeding season, after the lactation period. Animals were housed (latitude: 39°26′ N) and fed with alfalfa hay and a concentrate compound feed. Oestrous cycles were synchronized with the use of intravaginal sponges containing 60 mg medroxyprogestrone acetate (Veramix; Pfizer Animal Health, New York, NY, USA), which remained *in situ* for 14 days. After oestrus (D0) was detected by teaser rams, ewes were randomly allocated into four equal groups (A, B, C, D; $n = 6$).

In group A ewes, OPU was performed on D4 (early luteal phase; OPU session A1), on D9 (mid luteal phase; OPU session A2) and on D14 (late luteal phase; OPU session A3) of the oestrous cycle. In group B and D ewes, OPU was performed once, on D9 (mid luteal phase) or on D14 (late luteal phase) of the oestrous cycle, respectively. In group C ewes (controls), only endoscopic observation of follicular population (*i.e.*, no OPU) was performed thrice during the luteal phase of the induced oestrous cycle, on D4 (early luteal phase, observation session C1), D9 (mid luteal phase, observation session C2) and D14 (late luteal phase, observation session C3).

2.2. Follicular aspiration-oocyte evaluation

Before each scheduled OPU, feed and water were withdrawn from the ewes for 24 and 12 h, respectively. Following pre-medication with xylazine (0.1 mg kg^{-1} bw iv) (Rompun; Bayer, Leverkusen, Germany), anesthesia was induced in experimental animals with 2.5% thiopentone (10 mg kg^{-1} bw iv) (Thiopental; Takeda Pharmaceuticals, Glattpark-Opfikon, Switzerland). All OPU sessions were carried out by the same operator, who was blinded to the treatment used.

The animal was restrained in a cradle in dorsal recumbence and inclined in an angle of 30° in the head-down position. Initially, observation of the ovaries was performed laparoscopically with a rigid endoscope (model: 250.2 H; Schölly Fiberoptic, Denzlingen, Germany). During observation, total number and diameter of all visible follicles were recorded and classified according to their size as small (diameter < 2 mm) or large (diameter ≥ 2 mm) by using improvised endoscopic calipers. Subsequently, follicular aspiration was performed with a 20 G/75 mm aspiration needle (Spinocan; Braun Medical, Melsungen, Germany), connected by an elastic tube (internal diameter 1.5 mm) to a portable aspirator vacuum pump (Medela-Vario 6002601; Medela, McHenry, IL, USA), which was adjusted to aspirate 13–15 mL water min^{-1}.

The oocytes were collected into a 10 mL sterilized glass tube containing 1 mL phosphate buffer saline pH 7.2 at 37 °C. Immediately after OPU, cumulus oocyte complexes (COCs) were collected and observed in a stereoscope for classification, by using the criteria of de Loos et al. (1989), into categories '1', '2', '3' or '4'. Oocytes classified into '1' or '2' were considered of high quality, while those classified into '3' or '4' were considered of inferior quality.

2.3. Blood sampling

For measurement of progesterone concentration, blood samples were collected from all ewes, daily for 22 days, starting on the day of removal of the intravaginal sponges while for measurement of oestradiol and FSH concentrations, 27 blood samples were collected from each ewe as follows: every 20 min starting 2 h prior to OPU until 2 h after it and continuing every 60 min for the next 8 h and every 120 min thereafter for the next 14 h (*i.e.*, each sampling cycle covering 26 h). The above schedule was repeated three times for group A ewes (once for each OPU session) and once for group B and D ewes. All samples were left for 3 h at 4 °C to clot; serum was then separated and stored at −20 °C until assaying.