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Oocyte pick-up in juvenile lambs affects neither onset of puberty nor their future fertility

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Abstract

A study was designed to evaluate whether the time of onset of puberty and fertility of young ewe lambs would be affected by oocyte pick-up conducted in single or repeated sessions during the first months of lambs' live. Five groups of lambs from the Karagouniko breed were used (A–E each $n = 12$). In group A no treatments were applied (control group) while, laparoscopical follicular aspiration (OPU) was performed early in the third, fourth and fifth month of lambs age (groups C–E, respectively). From the second to fifth month of their age, group B lambs were aspirated four times in monthly intervals. All lambs were weighed at birth, weaning, at second month and monthly thereafter until the eighth month of age. Progesterone priming and ovarian stimulation by serial FSH administrations proceeded each OPU session. To determine onset of puberty blood progesterone concentration was assayed in samples collected initially every week and after the seventh month of age twice weekly. From the seventh month a fertile ram was introduced in each group and oestrous behavior/mating was daily monitored and recorded. Pregnancy diagnosis was carried out by transabdominal ultrasound scanning 55 days after rams' removal. At the fourth and fifth month of age group B lambs were lighter ($p < 0.05$) than controls, but this difference was later equalized. The time of onset of puberty did not differ between groups ($p = 0.069$) and ranged between 224 and 270 days. Some animals ($n = 15$) entered puberty with a full-length luteal phase having progesterone concentration greater than 1 ng/ml, while others ($n = 32$) exhibited one or two short luteal phases before luteal length restoration. During the first breeding season 41 animals were fertilized and maintained pregnancy to term, without noticeable differences between groups ($p = 0.555$). During the second breeding season, all ewes were naturally served and lambed at the expected time.

It is concluded that OPU in young dairy lambs does not affect the time of onset of puberty, the endocrine profile of the lambs and it does not compromise their future fertility even if it is applied at four successive months.

Keywords: Oocyte pick-up; Puberty; Progesterone; Sheep; Fertility

1. Introduction

In the recent years the demand for accelerated genetic gain of farm animals has been triggering the use of juvenile animals as a source of oocytes for in vitro embryo production. In vivo oocyte retrieval is, in

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general, preceded by ovarian stimulation, which is accomplished by a variety of hormonal regimes, resulting in increased follicular number and size. Based on the number and quality of collected oocytes, good oocyte-donor lambs, could be stimulated and aspirated repeatedly [1,2]. We have recently shown that the number and developmental competence of oocytes collected at particular ages from Karagouniko lambs can be improved by a follicle ablation conducted 1 month earlier [2]. Moreover, the latter study indicated that the adjustment of FSH dose to body weight of juvenile lambs results in an increased number of oocytes suitable for fertilization.

Follicular aspiration in ewe lambs is carried out either by laparotomy or by laparoscopic techniques because. The former approach is believed to minimize the formation of post-operative adhesions [3,4]. Nonetheless, it has been found that repeated transvaginal ovum pick-up in prepubertal calves caused histological lesions in their ovaries. These lesions, if in great extent, could alter the normal ovarian function and could possibly affect their future fertility [5].

The onset of puberty is a complicated process which is regulated by genetics (breed), growth rate, metabolic cues, nutrition, stress, social cues, photoperiod and gonadal steroids [6–8].

Experimental data have shown that premature exposure to short days can lead either to early or late onset of puberty depending on the age at which the lambs were exposed to the artificial photoperiod [7,9,10]. Delayed onset of puberty also occurs in undernourished lambs [7,10]. Hormonal treatments may also affect the timing of onset of puberty; early puberty can be induced in prepubertal heifers treated with progestagens [11] and in bull calves after systemic FSH administration [12], while estradiol treated lambs showed delayed onset of puberty [13].

Karagouniko is an indigenous lowland Greek dairy sheep, with mean litter size of 1.3 lambs/ewe/year. The breed is raised mainly on the plains of Thessaly, Central Greece [14]. Karagouniko lambs attain puberty approximately at 238 days old [15]. The mean duration of breeding season of Karagouniko adult ewe, in the place of its origin (39°26′N), lasts approximately 190 days (from early August to mid-February). In this period ewes usually Exhibit 11–12 oestrous cycles [16].

Although the importance of juvenile oocyte donors is undisputable, their potential to produce and naturally reproduce during their mature life is equally or even more important both from the production and welfare point of view. Thus, the objectives of this study were to evaluate the effect of single or repeated follicular aspiration after ovarian stimulation in Karagouniko ewe lambs on their (a) onset of puberty, (b) body weight and (c) future reproductive performance.

2. Materials and methods

2.1. Animals and treatments

All experiments described in this study were approved and carried out under a special license issued by the Hellenic Ministry of Rural Development and Food (license no. 1520/14-4-2003).

Sixty Karagouniko breed ewe lambs born during winter of 2003–2004 were kept in an open paddock (latitude 39°26′N). After weaning the lambs were fed alfalfa hay (up to 1 kg/head/day) and a commercial concentrate mixture (up to 0.5 kg/head/day). At the age of 7–8 weeks, ewe lambs were divided into five equal groups (A–E; $n = 12$). In lambs of group A no treatment was applied (control group). Laparoscopic follicular aspiration (OPU) was performed one time in lambs aged 12–13 weeks (group C), 16–17 weeks (group D) or 20–21 weeks (group E). In group B lambs, starting from the eighth to ninth week of age four OPU replicates were performed in monthly intervals. Before each OPU, ovarian stimulation was carried out with the same hormonal regime. Before each OPU session lambs received two injections of 25 mg of progesterone 3 days apart [Day 0 (first P4 injection) and Day 3 (second P4 injection), Proluton Depot, Schering AG, Germany]. Beginning on D3, ovarian stimulation was carried out by 3.52 mg ovine FSH (Ovagen, Bodinco B.V., Holland), given in six occasions at 12 h intervals [2]. Prior to each scheduled OPU, lambs were deprived of food and water for 24 and 12 h, respectively. On D6, OPU was performed with the aid of laparoscopic observation (Scholly 110057, 30°, Denzlingen, Germany) under general anesthesia [combination of xylazine (0.1 mg/kg, Rompun, Veterin, Greece) and thiopentone (10 mg/kg, 2.5%, Thiopental, Nycomed, Germany)]. The aspiration of the follicular contents was carried out by a 20G needle connected to a vacuum pump (Medela-Vario model 8, Switzerland) adjusted to aspirate 13–15 ml water/min.

2.2. Assessment of the onset of puberty and fertility

To determine the age at the onset of puberty P4 concentration was assayed in repeatedly collected blood samples. Starting at the second month of age, blood samples (5 ml) were collected weekly up to the seventh month of age and then biweekly until the 10th (groups A, C, D and E) or until the 13th month (group B).