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Superovulatory response to gonadotrophin FSH/LH treatment and effect of progestin supplement to recipients on survival of transferred vitrified embryos in goats

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ABSTRACT

Two experiments were carried out in goats to evaluate the effects of the FSH/LH ratio during treatment on ovarian response and embryo production (experiment 1) and the efficiency of progestin supplementation on pregnancy and the survival of vitrified embryos (experiment 2). In experiment 1, 30 goats were synchronized and allocated to 2 groups (n = 15) corresponding to the following superovulatory treatments with p-FSH (250 IU, over 3 days) having different doses of purified FSH and LH: (group A) control, FSH/LH ratio of 1, kept constant during treatment; (group B) FSH/LH ratio of 2 and daily FSH/LH ratio of 5.0:1.0:0.3 for the first, second, and third days of treatment, respectively. Ovarian response and embryo production were assessed 7.5 days after estrus. In experiment 2, 46 vitrified blastocysts from p-FSH–superovulated donors were transferred to 26 recipients (2 blastocysts per goat) 7.5 days after estrus. The recipients were synchronized with donors and allocated to 2 experimental groups (n = 13). Group C received progestin supplement as fluorgestone acetate (FGA) inserted into the vagina at the time of embryo transfer, replaced with a new one 16 days later, and maintained until the 45th day of pregnancy; group D, no treatment (control). Pregnancy was diagnosed by transrectal ultrasound scanning on Days 30 and 45 after estrus and followed to term. The results indicated that the increase in FSH/LH ratio from 1 to 2 with decreasing daily FSH/LH (treatment B) did not improve the superovulatory response. Superovulatory treatment A (control) advanced ($P < 0.05$) the onset of estrus and showed a higher ovulation rate compared to group B (14.9 vs. 10.9; $P < 0.05$). Fertilization rate, embryo yield, and mean number of transferable embryos in group A (7.5) were higher ($P < 0.05$) than those in group B (3.2). Recipient goats receiving progestin supplementation (group C) showed a higher ($P < 0.05$) pregnancy rate and embryo survival (kids born per embryos transferred; 69.3% and 73.1%) than the controls (group D; 23.3% and 19.2%). In conclusion, regimen A with FSH/LH ratio of 1 kept constant during the treatment gave the best ovarian response and embryo production. The progestin supplementation as FGA-pessary administered at embryo transfer time to the 45th day of pregnancy improved the pregnancy rate, kidding rate, and embryo survival of transferred vitrified embryos. Intravaginal progestin supplement has the potential to reduce the incidence of pregnancy losses during early pregnancy.

1. Introduction

Multiple ovulation and embryo transfer (MOET) is widely used to increase genetically superior offspring produced from selected females. However, the variation in the superovulatory response of the donor makes the MOET technique rather unreliable [1], limiting its application in

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field conditions. The use of gonadotrophic pituitary extracts, commonly of porcine origin (p-FSH), results in a greater superovulatory efficiency than obtained using eCG [1,2], likely due to its half-life (10–15 hours) [3]. FSH is usually administered twice daily for 3 to 4 days because of its short half-life (5 hours in cows) [4].

The high variability in the biological activity of FSH and LH in the pituitary extracts [5], which are considered to contribute to the variability in responses to superovulatory treatment, has led to the use of more purified preparations with a defined biological activity for both FSH and LH fractions [6]. The daily FSH/LH ratio during treatment also appears to be important in the superovulatory response [1,6,7], taking into account the physiological hormonal pattern from the luteal regression and preovulatory peak in LH [8]. The results on the amount of LH needed to produce a higher ovulatory response and embryo production are rather contradictory.

In MOET biotechnology, embryo cryopreservation is useful for storing embryos in cases in which there is an insufficient number of recipient animals, and it enables them to be easily transported. Among the various procedures, vitrification has been found to be a reliable method for the cryopreservation of embryos [9,10].

In sheep and goats, it has been estimated that during the first 3 weeks of pregnancy, 30% to 40% of fertilized eggs are lost [11]. It is believed that most embryonic deaths occur within the peri-implantation period and may be attributable to inadequate circulating progesterone (P4) concentrations and the subsequent downstream consequences on uterine function or failure of the conceptus to develop appropriately, on signal pregnancy recognition and/or implantation and placentation [12,13].

Luteal insufficiency is an important cause of the reduction in pregnancy rate after embryo transfer (ET) [14] and is especially useful when frozen embryos are being transferred because of their lower survival rate compared to fresh embryos [15]. Systems to enhance embryo survival during early pregnancy involve direct supplemental progesterone or induction of a supplementary CL. Progesterone supplementation before the maternal recognition of pregnancy, either with a progesterone-releasing device or by the induction of accessory CL through the administration of hCG and GnRH, has been used in cows and sheep [12,16]; however, the results are controversial. In goats, little information is available on the effects of progesterone or progestin supplementation to recipients on pregnancy rate and embryo survival of transferred embryos [17].

The aim of the present study was to evaluate in goats (1) the effects of the FSH/LH ratio on superovulatory response and *in vivo* embryo production and (2) the efficiency of progestin supplementation, *via* fluorgestone acetate (FGA)–vaginal sponges, administered to recipients at ET time to the 45th day of pregnancy, on pregnancy rate and survival of vitrified transferred embryos.

2. Materials and methods

The experiments were conducted according to protocols approved by the Italian Ministry for Scientific Research in accordance with European Commission regulations.

2.1. Animals and general information

The study was carried out in southern Italy (41°N latitude) in October during the breeding season [18]. A total of 56 adult nonlactating goats (3- to 4-year old; body weight, 43.9 ± 1.6 kg) and 10 bucks (3- to 4-year old) of Ionica breed were used. The goats were allocated to the experimental groups described in the following sections, through random stratification according to body weight and age. Bucks of proven fertility were used in turn for hand mating (n = 6) of the superovulated does (experiment 1) or as aproned teaser (n = 4) to check for estrus recipient does (experiment 2).

2.2. Experiment 1: effect of FSH/LH ratio regimen on embryo production

The experiment investigated the effects on ovarian response and embryo production of superovulatory treatment with FSH/LH ratio of 2, decreasing daily during the treatment, compared to the regimen of FSH/LH ratio of 1:1 kept constant during the treatment. This design was established on the basis of previous studies in which the superovulatory regimen with FSH/LH of 1 kept constant during the treatment provided a higher transferable embryo yield than the decreasing daily FSH/LH ratio [19], also compared to the regimen of FSH/LH ratio of 2:1 kept constant during the treatment [10].

A total of 30 goats were used. Estrus was synchronized by a progestin (17α-acetoxy-9-fluoro-11βhydroxyl pregn-4-ene-3,20-dione; FGA 45 mg, in vaginal sponges; Intervet, Milan, Italy) [20], for 9 days, and PGF2α (50 μg intramuscularly [im]; ICI cloprostenol, Estrumate; Schering-Plough, Milan, Italy) on the Day 7.

For the treatment, all goats received a total superovulatory dose of 250 IU purified p-FSH (Laboratorios Calier, Madrid, Spain) supplemented with different purified p-LH (contamination 0.1%; Laboratorios Calier) in relation to the experimental FSH/LH ratio. The treatment was performed after a regimen of gonadotrophic administration over 3 days (2 injections im per day, 12 hours apart), starting on the Day 7 of FGA treatment. The goats were allocated to the following 2 treatment groups (n = 15). In group A (control), the FSH/LH ratio of gonadotrophic preparation was 1.0 (250 IU p-FSH:250 IU p-LH) kept constant during the 3 days of treatment; the preparation was administered in 6 decreasing doses as follows: 71.5-71.5, 35.7-35.7, and 17.8-17.8 IU. In group B, the FSH/LH ratio was 2.0 (250 IU p-FSH:125 IU p-LH), decreasing during the treatment as follows: 5.0 (Day 1), 1.0 (Day 2), and 0.3 (Day 3). In the 3 days of treatment, the preparation was administered in 6 decreasing doses as follows: 100.0-20.0, 20.0-20.0, and 6.0-20.0 IU, respectively. The does were checked for estrus with the aid of teaser bucks, every 6 hours beginning 12 hours after sponge removal, and hand mated every 10-12 hours until the end of estrus.

Ovarian response (CL and preovulatory follicles >4 mm) and embryo production were assessed 7.5 days after estrus using a semilaparoscopy procedure under general anesthesia [21]. Briefly, 2 stab incisions were made 3 to 5 cm on either side of the midline about 10 to 12 cm anterior to the