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Short communication

Ovarian response and pregnancy rate following different doses of eCG treatment in Chall ewes

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ABSTRACT

The purpose of the study was to investigate the effects of different doses of equine chorionic gonadotropin (eCG) treatment on follicular development, ovulation and pregnancy rate during the breeding season in fat-tailed Chall ewes. Seventy-two cycling (62.5 ± 2.5 kg), multiparous Iranian Chall ewes were used in the trial. The ewes were randomly allocated to 6 groups (n = 12/group). Estrus was synchronized with the aid of controlled intravaginal drug release (CIDR) devices, inserted for 14 days. At the time of CIDR removal (day 14), the ewes received i.m. either 0 (control group, G0), 450 (G450), 550 (G550), 650 (G650), 750 (G750) or 850 (G850) IU eCG. Vasectomized rams were used to detect estrus in the ewes from 24 h after CIDR removal. Ovarian follicular activity was monitored with the aid of transrectal ultrasonography on the day of CIDR insertion (day 0) and daily from the day of eCG treatment (day 14), until estrus (day 16). During these days, blood samples were collected for the determination of plasma progesterone and estradiol concentrations. Laparoscopic intrauterine inseminations were conducted 54–60 h after CIDR removal. The number of CL's and pregnancy diagnosis was recorded using ultrasonography 7 and 54 days following AI, respectively. Half of ewes in control group and most of the ewes treated with eCG showed signs of estrus within 36 h of CIDR removal. The ewes in groups G750 and G850 recorded the highest number of large follicles at estrus and CL's 7 days later. The pregnancy rate in groups G550 (75.0%) and G650 (75.0%) was higher (P < 0.05) than that in the other groups. The ovarian response and estradiol concentration, as well as pregnancy rate showed that 550 or 650 IU eCG treatment is the most effective doses in improving the pregnancy rate in Iranian Chall ewes.

1. Introduction

Laparoscopy has been introduced as an essential technique for the use of frozen semen AI in sheep genetic improvement programs (Luther et al., 2007). However, using AI reduces the pregnancy rate (40–60%), compared to natural mating in ewes (Byrne et al., 2000; Fair et al., 2005). Therefore, the estrous synchronization protocol should be performed efficiently and accurately, to achieve a high pregnancy rate. Equine chorionic gonadotropin (eCG) is known to be the most commonly used hormone to improve fertility in controlled breeding (Cline et al., 2001; Barrett et al., 2004). The use of this hormone has however also been associated with negative effects on the pregnancy rates obtained (Menchaca and Rubianes, 2004; Zeleke et al., 2005). Despite conflicting results, the majority of estrous synchronization protocols in sheep have continued to use a single dose of eCG near the end of progesterone treatment (Hill et al., 1998; Roy et al., 1999). It is more convenient

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and cheaper, but may have variable responses, compared to other gonadotropins (Samartzi et al., 1995).

Numerous causes have been stated for the variation in ovarian responses recorded following eCG treatment in controlled breeding programs, e.g. the different doses used, different sheep breeds, seasonal variation and nutritional levels, ovarian status on the day of gonadotropin treatment and time of eCG administration during estrous synchronization (Gordon, 1997; Cognié et al., 2003; Gonzalez-Bulnes et al., 2003; Mossa et al., 2007; Ali, 2007; Shipley et al., 2007).

Low doses of eCG (300–400 IU), included in estrous synchronization protocols generally reduce the interval to the onset of estrus (Botha et al., 1975; Barrett et al., 2004). Evidence has also shown that low doses of eCG usually increase the number and size of large follicles, but it possibly has little effect on the ovulation rate (Bartlewski et al., 1998, 1999a,b). In contrast, increasing the dose of eCG (500–700 IU) could enhance the ovulation rate (Samartzi et al., 1995), and also pregnancy rate and litter size (Botha et al., 1975; Timurkan and Yildiz, 2005). Furthermore, superovulatory doses of eCG (1000–2000 IU) may cause the induction of a high number of large follicles (8–10 follicles) and ovulations (6–8 CL's) (Gonzalez-Bulnes et al., 1999). Due to the long half-life of eCG, the estradiol levels increase and remain high even during the luteal phase. Consequently, high early luteal phase concentrations of estradiol have been reported to have deleterious effects on the oviducts and ultimately decrease fertility (Driancourt and Fry, 1992).

The Iranian Chall ewe (fat-tailed) is one of the most important sheep breeds in Iran. Notable characteristics of the Chall breed are its high growth rate and tolerance to unfavorable environmental conditions. Other characteristics include a mean adult body weight of 64.5 kg, reasonable wool production annually, and a 105 l milk yield during a 5-month milking period. Currently there exists no research regarding eCG treatment in Iranian Chall ewes. The aim of this study was thus to investigate the effects of various doses of eCG on follicular development, ovulation rate and pregnancy following laparoscopic AI in Iranian fat-tailed Chall ewes.

2. Materials and methods

The experiment was conducted from February 5 to March 15 (during breeding season), at the institute's farm located in Saveh city, at 50.19 ± 21°E longitude, latitude 35.01 ± 13°N and at an altitude of 995 m above mean sea level – in a semi-arid area of the Iran. The annual rainfall in this region ranges from 220 to 288 mm, with an erratic distribution throughout the year.

2.1. Animals and eCG treatment

Seventy-two cycling, multiparous fat-tailed Iranian Chall ewes, weighing 62.5 ± 2.5 kg, were used in the trial. The ewes were randomly allocated in equal numbers ($n = 12$) to 6 treatment groups. None of the animals had not previously been used for any eCG treatment, superovulation or as embryo recipients in a MOET (multiple ovulation and embryo transfer) programme. Animals were provided with water and fed alfalfa hay, supplemented with grain pellets, ad libitum (CNCPS, 2003). The estrous cycles were synchronized using CIDR's (EAZI-BREED™, CIDR®, New Zealand), inserted for a period of 14 days. The day of CIDR insertion was considered as the onset of the experiment (day 0). At the time of controlled intra-vaginal drug release (CIDR) device removal (day 14), the ewes were treated with 0 (control group, G0), 450 (G450), 550 (G550), 650 (G650), 750 (G750) or 850 (G850) IU eCG (Folligon, Intervet, The Netherlands) as a single i.m. injection. Vasectomized rams were used to detect estrus in all ewes from 24 h following CIDR removal.

2.2. Ultrasonography and laparoscopic AI

Ovarian follicular activity was monitored by transrectal ultrasonography (Piemedical, Falco100; Holland, 8 MHz), on the day of CIDR insertion (day 0) and then daily from the day of eCG treatment (day14), until the occurrence of estrus (approximately day 16). The ultrasonographic scanning of both ovaries being recorded using a MP4 player (Marshal X720, China). In brief, during the ultrasonographic evaluations, ewes were kept in a darkened room and restrained in a fostering crate, in a standing position. After introducing a hydrosoluble contact gel into the rectum (to enhance the ultrasound transmission), the probe was placed into the rectum with the transducer oriented perpendicularly to the abdominal wall. When the urinary bladder was surpassed and the uterine horns located, the probe was rotated laterally clockwise for 90°, and then counterclockwise for 180° to evaluate both ovaries and their structures. One experienced operator performed all the recordings. The ovaries were scanned in several planes to identify all visible follicles (>1 mm in diameter). All follicles larger than 1 mm were recorded and classified according to their diameter, in one of the following classes: small (<2 mm), medium (2 to <4 mm) and large (≥4 mm) follicles.

The ovarian response in terms of number of corpora luteal (CL's) and pregnancy diagnosis was assessed by ultrasonography, 7 and 54 days after the onset of the estrus, respectively. The presence of at least 1 CL on the ovary at day 7 after estrus was indicative of ovulation in the ewe.

Laparoscopic artificial insemination (LAI) was performed 54–60 h following CIDR removal. The ewes were being fasted for 24 h and having restricted access to water for 16 h before laparoscopy. Laparoscopy was performed under general anaesthesia using xylazine (Xylazine 2%, Alfasan, Holland; 3.5 mg i.m.).

2.3. Blood sampling and hormone analyses

Blood samples were collected once daily from the jugular vein into heparinized tubes on days 14, 15 and 16 of the trial. Plasma was harvested within 1 h of collection and stored at −20 °C until assayed for estradiol (E2) or progesterone (P4) concentration. Concentrations of plasma E2 and P4 were determined using a RIA kit (BioSource Europe, S.A. Nivelles, Belgium).

2.4. Statistical analyses

The profiles of the number of ovarian follicles (i.e. small, medium and large follicles) and the mean plasma concentrations of estradiol and progesterone were compared by least square means analysis of variance – using the MIXED procedure of SAS software (SAS Institute, Cary, NC, Version 9.1). The analysis included sources of variation due to treatment groups, days (repeated measures), and their interactions. The ovarian response included the large follicles and CL's on both ovaries being analyzed by least square means analysis of variance – using the general linear model (GLM) procedure of SAS. The mathematical model included a fixed effect, due to the treatment groups and residual error. The data expressed as a percentage, were analyzed using the chi-square test. Significant differences between treatments were determined at the $P < 0.05$ level of confidence. Data were expressed as mean ± SEM, unless otherwise stated.

3. Results

The responses following eCG treatment in the ewes are set out in Table 1. Half of ewes in the control group and nearly all of the ewes treated with eCG exhibited overt signs of estrus within a 36 h period after CIDR removal. The lowest number of ewes that ovulated was observed in the control and the G450 groups. A higher number of large follicles in the G550 and G750 groups and the higher number of CL's only in the G850 group ($P < 0.05$) were recorded on the right versus the left ovary (Table 1). Ewes in the G550