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Research Article

Investigation of the Effect of a Progesterone Free Superovulation Protocol on Embryo Yield and Quality in Goats

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ABSTRACT

The main objective of the present study is to determine the effects of progesterone and progesterone-free superovulation protocols on embryo count and quality in goats. The animal material of the study is consisted of 1-3-year-old 22 goats and 3 bucks. During the breeding season, the goats were divided into two groups of 11, and superovulation was applied. In the control group, goats were synchronized with intravaginal sponges containing 20 mg of fluorogestone acetate for 12 days. Four days after oestrus in the experimental group and on the 9th day after sponge application in control group decreasing doses of FSH (Follicle stimulating hormone) (50; 30; 20 mg) were injected intramuscularly at 12 hour intervals. The sponge was removed on the 12th day in the control group. In both groups, oestrus was observed 24 hours after the fifth dose of FSH and the uterus flushing was performed on the 7th day after mating. In the study, superovulation response was obtained 100% in control group and 25% in experimental group. The recovery rate and transferable embryos were determined 61.5; 82.81 and 23.3%; 71.43% respectively and there was no difference between the groups (P>0.05). In the non-progesterone-applied group of animals, it was observed that the cycle cannot be fully dominated, sufficient progesterone level could not be reached and gonadotropin application was, therefore, insufficient. Although progesterone administration is thought to have a negative effect on embryo quality, applying a superovulation by using progesterone has been shown to be more successful on superovulation and embryo collection rates.

Key words: Embryo, Goats, Progesterone, Superovulation

INTRODUCTION

The progesterone that is a hormone released from CL has critical roles in the regulation of reproductive cycle in mammals. The primary function of the progesterone is to maintain the pregnancy with uterine milk released after the embryo reaches the uterus and is implanted (Delmen and Eurell 1988, Uslu *et al*., 2016). Progesterone also plays a key role in embryo development because it is effective in a number of molecular, biochemical, and morphological events for became (Conceiving) and maintaining the pregnancy. However, the increase in progesterone concentration in the early luteal phase has been reported not to affect the rate of blastocyst progression of embryos (Parr *et al*., 2017). In order to organize the sexual cycle; negative and positive feedback effects of progesterone are used in synchronization protocols during the breeding season and stimulation of ovaries during an anestrous period (Kaya 1996, Robert *et al*., 2004). Progesterone containing intravaginal sponges are the most common application in an oestrus and breeding season synchronization in small ruminants. When progestogen is administered fora time when as long as CL is active in an oestrous cycle (12-14 days), it suppresses oestrus and ovulation. As a result of the application, oestrus, ovulation and the peak of LH are synchronized (Jainudeen *et al*., 2000; Alaçam 2010).

Although progesterone is beneficial by ensuring (providing)synchronization, it is reported that it has negative effects on the number of ovulation and embryo quality after superovulation protocols (Mayorga *et al*., 2011).

Superovulation is a hormonal treatment method that increases the number of the ovum by developing numerous follicles and provides this follicles are ovulated in the ovaries. Superovulation increases the number of

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available oocytes released from the follicles, as well as ovulation and oocyte maturation (Rahman *et al*., 2008, Tekeli 2010, Sousa *et al*., 2011). The objective of the superovulation is to arrest atresia in an ultra-high number of follicles, thus facilitating them to be ready for ovulation (Callajas *et al*., 2008).

Gonadotropic hormones are used for superovulation (Emsen, 2004, Lehloenia and Greyling, 2009). Follicle stimulating hormone (FSH) and pregnant mare gonadotropin (eCG or PMSG) are the most commonhormones that is used (Rahman *et al*., 2008, Emsen, 2004, Emsen *et al*., 2009). The success of superovulation programs is influenced by the variability of ovarian response and embryo yield. The ovarium response to be obtained after the superovulation protocol is related to the follicular phases of the ovary at the onset of FSH administration in cattle, sheep, and goats (Menchaca *et al*., 2007). Hence, the presence of a dominant follicle at the commencement of superovulation treatment may have negative effect on response (Callejas *et al*., 2008)

Conventional superovulation protocols applied on the goats are performed by administering intravaginal medroxyprogesterone acetate (MAP) or fluorogestoneacetate (FGA) sponges for 9-14 days and and by removing the sponges 2 days after the first application of gonadotropins to synchronize the oestruses. After 24-48 hours the end of gonadotropin injections artificial insemination or natural mating is applied to animals (Lehloenya *et al*., 2008, Rahman *et al*., Lehloenya and Greyling 2010a).

Using superovulation protocols that contain progesterone for the synchronization of the animals reduce the superovulation response and the qualities of the obtained embryos. For this reason, this study was aimed to determine the effects of progesterone and progesterone-free superovulation protocols on embryo counts and quality of goats.

MATERIALS AND METHODS

The animal material of the study consisted of 22 goats and 3 bucks. The animals were healthy and fertile, between 2-3 years old. The animal materials were procured through purchase.

After recording all the goats' ear numbers, general clinical examinations (body temperature, respiration/pulse rate etc.) were done and results were recorded. The study was approved by the Ethics Committee of the Veterinary Faculty of Selcuk University with ethics committee approval number 2011/62. During the breeding season, the goats were divided into two groups of 11, and the following protocols were applied.

Group with progesterone (group 1 / control)

- Day 0: 20 mg progesterone-impregnated intravaginal sponge / flugestone acetate (Chronogest CR, Intervet, Holland) was placed in the goat.
- Day 9: morning 0.165 mg of PGF2α (Prostaglandin F2α) (Dalmazin, Vetas, Turkey) and morning/evening 50 mg FSH (Folltropin®, Bionics, Ireland) intramuscular (IM)
- Day 10: Morning / evening 30 mg FSH (IM),
- Day 11: Morning intravaginal sponge was removed and morning/evening 20 mg FSH (IM) was applied
- Day 12: Morning 0,008 mg GnRH (Gonadotropin Releasing Hormone) (Receptal®, Intervet, Holland) was administered as IM and rammed into groups after four hours.
- Day 19: On the 7th day after the first mating, the goats were operated for uterine flushing.

Group without progesterone (group 2 / experiment)

- In order to identify animals that are in oestrus, the goats were monitored twice a day for 30 minutes each. Superovulation began to be applied to the animals that were diagnosed with the estrus on the 4th day of the cycle (Day 0).
- Day 4: morning/evening 50 mg FSH (Folltropin®, Bionics, Ireland) intramuscular (IM),
- Day 5: Morning/evening 30 mg FSH (IM),
- Day 6: Morning 0.165 mg PGF2α (Dalmazin, Vetas, Turkey) (IM) and morning/evening 20 mg FSH (IM),
- 7. Day: 0.008 mg GnRH (Receptal®, Intervet, Holland) in the morning was administered as IM and rammed into groups after four hours.
- Day 14: On the 7th day after the first mating, the goats were operated for uterine washing.

Xylazine (Rompun® 2%, Bayer, Germany) was administered IM 0.22 mg/kg IM to provide sedation before general anesthesia. ketamine HCl (Ketasol 10%, Richter pharma, Austria) was administered at 2 mg/kg dose for general anesthesia. The area the operation is to be carried out (the part between the udder and the umbilicus) was cleaned. With paramedian incision the abdominal cavity was entered. Corpus luteum and follicles on ovaries were counted. Ovarium findings were noted. Two-way catheter [Foley catheter (Rusch, No. 10)] was used for uterine flushing. The Foley catheter was inserted into the lumen of the corpus uteri from without veins area and was forwarded the uterine horns, respectively. After insertion of a cannula (IV cannula, 18 G, Knives, Turkey) from the uterine-tubule junction, the stylet was removed and the washing medium [medium 199 with 1% fetal calf serum (FCS) added] was gradually given. Collected fluidkept in water bath at 37°C approximately 30-45 min for precipitated the embryos.

The collected liquid was scanned on the stereo-microscope and the embryos found were examined morphologically in an inverted microscope and classified according to the criteria determined by the IETS (IETS 2010). According to this, they have evaluated that grade 1 (good to excellent) and grade 2 (medium) embryos are transferable, grade 3 (poor) and grade 4 (degenerated or dead) embryos are non-transferable. Statistical analysis was performed using the Kikare test in the Minitab 11.12 program.

RESULTS

In the first group, one goat was excluded from the study due to the adhesion in the uterus during the operation. All 10 animals applied superovulation responded to the application. Early luteal regression was

detected in the 2 animals (20%). A total of 104 CLs was counted in animals' ovaries and 76 cells were collected after washing (RR: 73,08%). It was determined that 64 of the collected cells were embryos. 11 of the remaining cells were oocytes, and 1 was empty zona. The rate of fertilization in the animals in the experimental group was 85.52% (65/76). 53 of the collected embryos (82,81%) were transferable (Table 1 and Table 2).

One of the animals in the second group had inactivate ovaries (9.09%) and those were excluded from the study. Four and more than four CL were detected in the ovaries of three animals (27,3%). In The remaining 10 goats have been operated, 30 CL was counted, and then their uterus was washed and 11 cells were collected (RR: 36.67%). Eight of the collected cells were embryos and 3 of them were oocytes, and the fertilization rate was determined to 72.7%. Five of the 8 embryos obtained (62.5%) were seen to be in the transferable quality (Table 1 and Table 2).

Table 1: Number of Goats Responding to Superovulation, Early Luteal Regression (ELR) Number of Goats, CL, Embryo Transferable Embryo Counts.

	Group I	Group II
Number of goats treated with superovulation (n)	10	11
Number (n) of superovulation (CL≥4)	10	3
Number of Early Luteal Regression (n)	2	-
Total Number of CL (n)	104	30
Total Number of Cells Obtained (n)	75	11
Number of Embryos Obtained (n)	64	8
Number of Transferable Embryos (n)	53	5

There is no statistical difference between the two groups P>0.05.

Table 2: Superovulation, Early Luteal Regression, Embryo Collection, Fertilization and Transferable Embryo Rates.

	Group I	Group II
Number of goats treated with superovulation (n)	10	11
Superovulation Rate (%)	100 (10/10)	27,3 (3/10)
Early luteal regression rate (%)	20 (2/10)	-
Recovery rate (%)	73,08 (76/104)	36,67 (11/30)
Fertilization rate (%)	85,52 (65/76)	72,7 (8/11)
Transferable embryo rate (%)	82,81 (53/64)	62,5 (5/8)

There is no statistical difference between the two groups P>0.05.

There was no statistical difference between superovulation responses, embryo collection rates, embryo quality and fertilization rate of the animals in the first and the second group (P> 0.05). However, when the results were evaluated numerically, it was determined that outcomes from the group in which progastrin was used was much better than those in the unused group.

DISCUSSION

It is thought that superovulation protocols contained progesterone and used for the synchronization of the animals reduces the superovulation response and the qualities of the obtained embryos. For this reason, this study was aimed to determine the effects of progesterone and progesterone-free superovulation protocols on embryo counts and quality in goats.

In a study that was made in South Africa in May and after superovulation protocol with FSH applied for 3 days at intervals of 12 hours, superovulation rates were reported as 50% in Boer goats and 87.5% in native animals (Greyling *et al*., 2002).

In another study comparing the conventional protocol with the 0. day protocol, it was reported that a superovulation response of 85% on the 0. day protocol and 50% on the conventional protocol was obtained (Menchaca *et al*., 2007). In the study in which a comparison of the conventional method with the progesterone-free method, and superovulation response 3 ≥ CL is accepted, researchers reported that they achieved 100% superovulation response in both applications (Mayorga *et al*., 2011).

Superovulation response is influenced by reasons such as race, age, general condition, type of hormone, application form, climate, nutrition and environmental conditions (Mapletoft *et al*., 2002, Gonzalez-Bullnes *et al*., 2004, Agaoglu *et al*., 2012). The number of corpus luteum developing in goats is directly proportional to the number of follicles 2-6 mm diameter in ovarium during the first FSH injection. However, the number of embryos that can be collected and survived is related to the number of follicles 4-6 mm diameter. Exogenous FSH administration stimulates the growth of small follicles until the end of preovulatory stage and ovulation. However, some of these follicles may remain atretic or immature (Gonzalez-Bulnes *et al*., 2004). Due to differences between animals and many other factors. Superovulation response is unpredictable. The response, therefore, is the most important factor affecting success in embryo transfer studies.

The luteal phase of the cycle was considered to be insufficient in the group without progesterone administration. And accordingly, it was thought that the concentration of gonadotropin required for superovulation could not be reached. It is thought that if the application day is taken forward, the response of the superovulation due to the increased amount of stored LH could be improved.

In a study using two different goat breeds, embryo obtaining rates were reported as 80% and 94%, respectively (Greyling *et al*., 2002). In a study, embryo obtaining rates were reported to be 80% in the conventional method of superovulation protocol and 67% in the superovulation protocol without progesterone (Mayorga *et al*., 2011). Using exogenous progesterone for superovulation programs may have decrease high-quality embryo yield (Donalds *et al*., 1984). The superovulatory response is influenced by the ovarian status (follicular dynamics) at the beginning of the superovulation treatment. The stimulatory effect of gonadotropins depends on the presence of the developing dominant follicle and the number of follicles 3-4 mm in size on the ovary at the time of application (Callejas *et al*., 2008).

The phase of the estrus cycle at which the progesterone was added had major effects on the superovulation response. For instance, the superovulatory response can be negatively affected if the treatments are not started exactly at wave occurrence (Sawyer *et al*., 1995).

In a study in which superovulation was performed, 38.1% of the animals reported to have early luteal regression (Lehloenya and Greyling 2010b). In another study; hCG, GnRH, and the control group, daily

progesterone measurements were performed after the superovulation. In the hCG group, progesterone levels were normal, whereas 37.5% of animals in the GnRH group and 57.2% in the control group reported to have early luteal regression (Saharrea *et al*., 1998). In the study, early luteal regression (ELR) was not observed in animals in group 2, whereas ELR was see in 20% of animals in group 1.

In comparison with the conventional method (control) of the superovulation protocol without progesterone, transferable embryo rates were determined to be 97% and 96%, respectively (Mayorga *et al*., 2011). In another study, the transferable embryo rate as a result of superovulation was reported as 85.43% (Quana *et al*., 2010).

In the study using the day 0 protocol (Group 1), CIDR / PGF2α / FSH (Group 2) and CIDR / FSH (Group 3), the fertilization response was not occurred. Also, there was no statistical difference in the transferable embryo ratios between 2nd and 3rd groups (Lehloenya and Greyling 2010b. In the present study, 82.81% of goats in group 1 and 71.43% in the second group transferable embryos were obtained.

Suggestions

After the study, it was thought that the follicular wave couldn't catch at the right time in animals without progesterone, sufficient progesterone levels could not be reached and gonadotropin administration couldn't insufficient. Although progesterone administration has been reported to have a negative effect on embryo quality, it has been determined that using progesterone provides much better results on superovulation and embryo collection rates.

It has been concluded that the use of progesterone during superovulation. If progesterone is not to be used, changing the starting day of superovulation may increase the success of the application.

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