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Multiple ovulation and embryo transfer during the breeding season in Angora goats: A comparison of fresh and vitrified-thawed embryo transfer

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Abstract

This study aimed to assess the superovulation response and pregnancy rates of fresh and vitrified-thawed embryos after transfer in Angora goats with comparing transfer at the beginning (BS) and end (ES) of the breeding season. Nine Angora goats were used as donors in both periods. Donor goats were synchronized and superovulated with the FSH and mated with five fertile bucks. At 156 hr following mating, embryos were collected surgically. Recipient Angora goats were divided into two groups at the beginning (fresh, n = 15; vitrified-thawed, n = 15) and end (fresh, n = 8; vitrified-thawed, n = 8) of the breeding season. Fresh or vitrified-thawed grade I embryos (early blastocyst/blastocyst) were transferred surgically to synchronized recipients. On the 30th, 60th and 90th day of transfer, goats were examined by ultrasonography. The number of corpora lutea (CL), total oocyte/embryo and transferable embryos obtained in BS was found to be statistically higher than ES. On the 30th day of transfer, pregnancy rates were 73.30% and 75.00% in the fresh transfer groups in both BS and ES periods; while, rates of 20.00% and 37.50% were found in the vitrified-thawed group, respectively. The embryo survival rates of fresh transfers were 55.55% and 31.25% at BS and ES, respectively. The number of CL, total oocyte/embryo and transferable embryos in the BS was higher than ES following superovulation. Also, fresh embryo transfer can be successfully carried out during the breeding season in Angora goats. Moreover, although pregnancies were obtained following the transfer of vitrified-thawed embryos, they did not sustain on the 60th and 90th days. So, further studies are needed for the vitrified-thawed embryos.

Introduction

Assisted reproductive techniques such as artificial insemination and multiple ovulation and embryo transfer (MOET) programs are utilized in sheep and goats in order to transfer the genetic resources of animals with high yield characteristics to future generations and to further eliminate reproductive deficiencies.[1-3] In recent years, MOET has taken on an important role in farm animal breeding and reproduction studies worldwide. This method allows the development of appropriate freezing procedures for the generation of genetically superior strains and protection of gene resources. Furthermore, MOET programs aim to shorten the breeding time and to increase embryo transfer and marketing.[4,5] The MOET programs in goats and sheep include stages such as estrus synchronization of donor and recipient animals, superovulation of donor animals via gonadotropins in the last days of synchronization, flushing the uterus 6–7 days following the mating and collection and evaluation of embryos.[3] After evaluation, embryos are transferred fresh or after having been freeze-thawed. The efficiency of this method has been evaluated in goats.[4] However, there remains a need for the development of embryo production programs due to differences in the rate of superovulation response and fertilization in goats.[2,3]

Superovulation is the most important step of the MOET program. Nevertheless, the response to superovulation is affected by many factors such as the use of exogenous gonadotropin, gonadotropin quality, breed, age, seasonal effect, nutrition, treatment protocol and reproductive status at the time of superovulation treatment initiation.[6,7]

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Baril and Vallet have reported that the breeding season has no effect on the mean number of ovulations in the goats; but, the embryo quality was better in the breeding season.[8] Also, although the ovulation rate was similar in and outside of the breeding season, the fertilization rate and the number of obtained embryos were higher in the breeding season.[3]

Embryos obtained after MOET programs are transferred to the recipient either fresh or having been freeze-thawed. There are two major methods to freeze the embryo including conventional slow freezing and vitrification. Initially, slow freezing was used; however, the devices used in this method are relatively expensive and the process takes a long time to complete. In recent years, the embryo vitrification method has been widely used in embryo freezing. This method does not require a special device and the embryo can be rapidly frozen.[9-11] Although vitrification has been reported as an effective and easy protocol for goat embryos cryopreservation, differences in survival rates of embryos after vitrification have been also stated by Gibbons *et al*.[12]

The aim of the present study was to evaluate the superovulation response and pregnancy rates of fresh and vitrified-thawed embryos after laparotomic transfer at the beginning and end of the breeding season in Angora goats.

Materials and Methods

Animals. Materials and methods used in this study were approved by the Ethics Committee of the Ankara University (2012-14-89; Ankara, Turkey) and the experiments were performed at the Education and Research Farm, Faculty of Veterinary Medicine, Ankara University, in Central Anatolia (40°06'08.50'' N, 32°37'18.65'' E) at 850 m above sea level. Fifty-five Angora goat does and five Angora goat bucks were used and housed in a straw-bedded semi-open fold. They were fed with corn silage, clover, straw, concentrated feed and vitamin-mineral supplement and water was provided *ad libitum*. All does were between 3- and 7-year-old and the mean (±SD) body weight was 38.50 ± 2.50 kg. All bucks were between 2- and 5-year-old and the mean (± SD) body weight was 46.80 ± 3.40 kg. There was no difference between the live weights and body condition scores of the recipient animals used in the study.

Experimental groups. The study was carried out in two separate periods including the beginning of the breeding season (BS; September) and the end of the breeding season (ES; December). In both periods, the same synchronization protocols were applied to the donors and recipients and same superovulation protocols were applied to donors. To obtain embryos, nine goats and five bucks were used in both periods. At BS, the recipient goats were divided into two groups. Fresh embryos were transferred to the first group (n = 15) and vitrified-thawed embryos were transferred to the second group (n = 15). At ES, the goats were again separated into two groups. Likewise, fresh embryos were transferred to the first group (n = 8) and vitrified-thawed embryos were transferred to the second group (n = 8).

Synchronization protocol. Donor and recipient goats were synchronized with traditional protocol at BS and ES. The donor and recipient animals were administered intra-vaginally with 0.33 g of progesterone source (Eazi-Breed CIDR®; Zoetis Animal Health, Florham Park, USA) on day zero. On day 9, the animals were injected with prostaglandin $PGF_2\alpha$ (Dalmazin®; Vetas, Istanbul, Turkey) once, with an intra-muscular dose of 150 µg per goat. On the 11th day, the progesterone source was removed. A GnRH analog (Receptal®; Intervet, Boxmeer, The Netherlands) was administered once daily at a dose of 10.00 µg per goat intra-muscularly 24 hr after removal of the progesterone source (12th day) and then, the donor goats were mated with fertile bucks 6 - 16 hr after the GnRH injection.

Superovulation protocol. Donor goats were super-ovulated with FSH (Bioniche Animal Health, Belleville, Canada) during synchronization protocol. The FSH was injected intra-muscularly as six decreasing doses at 12-hr intervals subsequent 9th-day along with the synchro-nization protocol. A total of 200 mg of FSH was injected intra-muscularly at 50:50 mg on the first day, 30:30 mg on the second day and 20:20 mg on the third day.

Collection of embryos. The same surgical team conducted all surgical procedures. Embryos were collected by surgical method 156 hr following mating. For this purpose, donor animals were withheld from feed and water for 12 hr pre-operatively. Approximately 15 min before the anesthesia, 0.10 - 1.00 mg kg^{-1} subcutaneous dose of atropine sulfate (Vetas, Istanbul, Turkey) was injected for pre-medication. Later on, 0.50 mg kg^{-1} diazepam (Deva Pharma, Istanbul, Turkey) and 2.00 mg kg^{-1} of ketamine HCl (Egevet, Izmir, Turkey) were injected via intravenous route for anesthesia. Following the mid-lateral incision, the ovary and uterus have been exposed in the incision area. Later, ovaries were examined and the presence of follicles, cysts or corpus lutea (CL) was recorded. After that, each uterine horn was flushed with a flushing medium (20.00 mL of mD-PBS; Sigma-Aldrich, St. Louis, USA) plus 3.00 mg mL^{-1} bovine serum albumin (Sigma-Aldrich) using a catheter (1.30 × 130 mm) inserted near the uterotubal junction. The embryos were recovered into a 90.00-mm Petri dish using a 2-way No. 10 Foley catheter (Rusch Teleflex, Morrisville, USA) inserted in the base of the uterine horns. To avoid intra-abdominal adhesions, 1.00 L of 2.50% heparin solution (Mustafa Nevzat Drug Co., Istanbul, Turkey) was used during flushing. The $PGF_2\alpha$ was injected intra-muscularly at a dose of 150 µg per goat to prevent possible post-operative pregnancies and post-operatively, 15.00 mg kg^{-1} of

amoxicillin trihydrate (Vilsan, Ankara, Turkey) was administered intra-muscularly for five days.

Evaluation and vitrifying/thawing of embryos. Embryos were morphologically evaluated under a stereomicroscope (M205 C; Leica Microsystems, Wetzlar, Germany) and classified according to the criteria recommended by the International Embryo Technology Society.[13] After classification, only grade I embryos including blastocyst (Bl) and early blastocyst (EBl) were used and transferred to embryo culture solution. In the fresh embryo transfer groups, embryos were directly transferred from this solution. In vitrified-thawed embryo transfer groups, the embryos were vitrified and thawed using the protocol described by Vajta and Kuwayama.[11]

Embryo transfer. The fresh and vitrified-thawed transfers were performed at BS and ES in the same year. Recipient animals were anesthetized with the same protocol used in donor animals. After the incision, the ovaries and uterus were evaluated and examined in terms of the presence of follicle, cyst or corpus luteum. Each recipient received one or two grade I embryo (EBl/Bl) ipsilateral to the ovary containing one or more corpora lutea by laparotomy.

Pregnancy examinations. At the 30th day after embryo transfer, transrectal ultrasonography was performed for pregnancy determination. Transrectal ultrasonography was performed with a real-time ultrasonic device with B mode, linear, 6.50 MHz transducer (Falco 100; Pie Medical, Maastricht The Netherlands) in the standing position and feces were cleaned out before the probe insertion into the rectum. Pregnancy examinations repeated at the 60th and 90th day of the transfer by transabdominal ultrasonography. The transabdominal examination was performed with B mode, linear, 5.00 MHz transducer real-time ultrasonography from the right side above the udder. This region was completely screened according to whether pregnancy-related findings such as doughnut or c-shaped caruncles were obtained or not.

Statistical analysis. The success of the obtained embryos for each application was evaluated statistically using Chi-square and Fischer exact tests. The McNemar's test was used to compare the quality of embryos obtained from the same donors at BS and ES. All statistical analyzes were evaluated with a minimum 5.00% margin of error. Data on the qualitative variables were presented in the form of a percentage value and the data of the quantitative variables were presented as the mean ± standard deviation in tables. Statistical analyses were performed using SPSS (version 14.01; SPSS Inc., Chicago, USA).

Results

In both periods (BS and ES), the estrus response was determined as 100% in the donor goats subjected to the synchronization protocol. The onset of estrus and estrus durations from the time of Eazi-Breed CIDR® removal were 23.70 ± 2.50 versus 30.10 ± 4.40 hr and 25.30 ± 5.00 versus 17.10 ± 2.80 hr in BS and ES, respectively ($p > 0.05$).

Superovulation response was obtained in all nine goats used at BS (100%) and in eight of the goats (88.80%) at ES ($p > 0.05$). The CL, oocyte/embryo, transferable embryo, degenerated embryo, unfertilized oocyte (UFO) counts, recovery rates and fertilization rates obtained after flushing of the uterus at BS and ES are given in Table 1. The CL, oocytes/embryos and the number of transferable embryos at BS were higher than ES ($p < 0.05$). After evaluation of embryos, 51 of 103 embryos at BS and 39 of 63 embryos at ES were identified as grade I.

Fifty-one grade I embryos were obtained at BS. Eighteen grade I embryos were freshly transferred to 15 recipients and 17 grade I embryos were vitrified-thawed transferred to 15 recipients at BS. At ES, 39 grade I embryos were obtained. Of these, 16 were freshly transferred to eight recipients and 16 were vitrified-thawed transferred to eight recipients. The number of transferred embryos obtained, pregnancies, litter size and embryo survival rates at 30, 60 and 90 days after transfer in both fresh transfer and vitrified-thawed transfer groups at BS and ES are presented in Table 2. At BS, 11 goats in fresh transfer group and three goats in vitrified-thawed transfer group were detected as pregnant on the 30th day following the transfer. However, four and three pregnancies were not sustained in fresh and vitrified-thawed transfer groups on the 60th and 90th days of pregnancy following transfer, respectively. At ES, six goats in fresh transfer group and three goats in vitrified-thawed transfer group were detected as pregnant on the 30th day following the transfer. However, three pregnancies were not sustained in both groups on the 60th and 90th days of pregnancy following transfer, respectively. Especially, at BS and ES, pregnancy was maintained following vitrified-thawed transfer; but, pregnancy did not sustain until birth.

Table 1. Ovarian responses in donor goats subjected to superovulation protocol and evaluation of collected cells from donor goats.

Breeding season	Superovulation response (%)	Number of corpus lutea	Number of cells	Recovery rate (%)	Number of embryos	No. degenerated embryos	Number of UFO	Fertilization rate (%)
BS	100	153 (17.00 ± 4.90[a])	108 (12.00 ± 3.40[a])	70.58	103 (11.40 ± 3.90[a])	9 (2.50 ± 1.00)	5 (1.70 ± 0.55)	95.37
ES	88.80	104 (11.50 ± 8.60[b])	72 (8.00 ± 6.90[b])	69.23	63 (7.00 ± 6.40[b])	7 (2.00 ± 0.70)	9 (1.60 ± 1.00)	87.50

BS: Beginning of the breeding season, ES: End of the breeding season, and UFO: Unfertilized oocyte.
[ab] Values with different superscript letters in the same column are significantly different ($p < 0.05$).

Table 2. Obtained pregnancies, number of born kids and embryo survival rates following fresh or vitrified-thawed embryo transfer during the breeding season in Angora goats.

Breeding season	Transfer method	No. recipients	No. transferred embryos	Day of pregnancy			Pregnancy rate (%)	No. Litter	Embryo survival rate (%)
				30	60	90			
BS	Fresh	15	18	11 (73.30%)^{aA}	8 (53.30%)^{b}	7(46.60%)^{b}	46.66	10^{A}	55.55^{A}
	Vitrified-thawed	15	17	3 (20.00%)^{aB}	1 (6.60%)^{b}	0	0	0	0
ES	Fresh	8	16	6 (75.00%)^{aA}	3 (37.50%)^{b}	3(37.50%)^{b}	37.50	5^{B}	31.25^{B}
	Vitrified-thawed	8	16	3 (37.50%)^{B}	0	0	0	0	0

BS: Beginning of the breeding season, and ES: End of the breeding season.
$^{ab, AB}$ different lowercase and uppercase superscript letters indicate significant differences in each row and column, respectively ($p < 0.05$).

Discussion

In the studies carried out in estrous synchronization protocols included in MOET programs, there is a difference between the times elapsed until the onset and duration of estrus after removal of the device containing progesterone. In a study conducted by Pampukidou *et al*., the duration from the removal of progesterone source to the onset of estrus was 29.30 ± 3.20 hr and the duration of estrus was 24.20 ± 3.40 hr during the breeding season.[14] In a study investigating the effect of season on superovulation in the Boer goats, the onset of the estrus was determined as 24.90 ± 4.80 hr and the duration of estrus as 24.00 ± 5.70 hr during the breeding season. Out of the breeding season, the time of estrus onset was 30.50 ± 9.10 hr and the duration of estrus was 18.20 ± 3.70 hr.[4] In this study, the onset of estrus was 23.70 ± 2.50 hr and the duration of estrus was 25.30 ± 5.00 hr at BS in Angora goats. At ES, the onset and duration of estrus were 30.10 ± 4.40 hr and 17.60 ± 2.80 hr, respectively. The results obtained here are similar to those reported by Lehloenya.[4] Also, Lehloenya[4] has reported that the season is effective on the onset and duration of estrus; but, it did not affect the estrous response. The results of this study also showed that BS and ES did not affect the estrus response.

The most important factor affecting the success of MOET programs in sheep and goats is the differences in superovulation response.[1,2] Studies with different gonadotropins yielded different results in superovulation response.[15] Many superovulation studies in goats[14,16,17] have reported that the response to FSH is better than that of PMSG. In the present study, a high superovulation response was obtained with FSH at BS and ES. In the light of these results, FSH can be successfully used for superovulation in the Angora goats. In addition to the superovulation responses obtained from MOET programs, the number of corpus luteum, embryo recovery rate and transferable embryo ratios of the animals are also important. Greyling *et al.*[16] have found that the mean numbers of CL in Boer and indigenous feral goats during breeding season after superovulation are 18 and 15, respectively. Embryo recovery rates were determined as 94.00% and 80.00%, respectively. Selvaraju *et al*. have found that the mean number of CL per goat after superovulation is 8.30 ± 1.75.[18] Sánchez-Dávila *et al*. have investigated the effects of three decreasing doses of FSH application on superovulation and embryo quality.[19] The mean numbers of CL in the goats treated with 80, 145 and 215 mg of FSH were determined as 4.00 ± 1.50, 13.40 ± 3.70 and 11.60 ± 2.60, respectively. In another study, the mean CL counts in the breeding season and anestrus were 17.50 ± 6.30 and 21.30 ± 5.90 in superovulation applications with FSH, respectively.[4] Ağaoğlu *et al*. have investigated the effects of repeated superovulation and surgical methods on embryo production in some indigenous goat breeds.[20] As a result of 1st, 2nd and 3rd superovulatory applications in Angora goats, the mean CL values were 8.21 ± 5.20; 8.85 ± 5.30 and 8.00 ± 6.96, respectively. In the present study, the total number of CL, total cell and transferable embryos in BS were higher than those of ES. However, there was no difference in recovery and fertilization rates. The reason for this was thought to depend on the decrease in hormonal activity and the slowing of the follicle development at ES. Moreover, decreased oocyte quality and the number of follicles selected from the follicle pool in ES may have caused this condition. Also, breed, age, general condition, applied hormone, application form, season, climate, nutrition and the environment affect the success of superovulation.[21,22] It is considered that the important factors affecting success in embryo transfer studies are the inabilities to predict the response to superovulation.

Fertilization rates in superovulation protocols are also important in goats. In a study, the fertilization rate was reported to be 77.00% in the conventional method and 83.00% for the day-zero protocol for goats in the breeding season.[23] Fertilization rates were not statistically different at BS and ES in this study. However, Lehloenya *et al*. have reported that the rate of fertilization in repeated superovulation studies is 99.40% in the first uterine flushing and 50.00% in the second flushing and the number of transferable embryos falls in parallel with the decrease in fertilization rate.[24] The fertilization rate of oocytes in the animals with high superovulation response can be decreased, sperm transport and oocyte quality can be differentiated and ovulation can spread over a wide range of time.[2,25]

In vivo-produced goat embryos can be obtained by flushing the uterine horns 6 - 8 days after mating by laparotomic, laparoscopic or trans-cervical methods.[26,27]

Embryo recovery rates range from 50.00 to 80.00% according to the methods of Flores-Foxworth.[28] Lehloenya and Greyling[7] have found that the rate of embryo recovery by laparotomy is 57.00% - 69.00% during the breeding season. Greyling *et al*. have reported an 80.00 - 94.00% embryo recovery rate after laparotomy in MOET.[16] In the present study, the embryo recovery rates by laparotomy were 70.58% at BS and 69.23% at ES. However, the obtained results were lower than the rates reported in the studies of Greyling *et al*. The reason for this reduced rate could be the individual differences and operating conditions of donors during the embryo collection procedures performed by laparotomy.[16]

In goats, embryos obtained after the MOET program can be transferred freshly or freeze-thawed by laparotomic or laparoscopic methods. There are differences in pregnancy outcomes and embryo survival rates obtained as a result of both methods. In a study comparing post-transfer pregnancy rates in Boer goats after fresh, freeze-thawed by slow freezing or vitrified-thawed transfer, the pregnancy rates were 85.70, 50.00 and 37.50% and embryo survival rates were 35.70, 25.00 and 31.30%, respectively.[7] In two different goat breeds, fresh embryos were transferred laparoscopically and the pregnancy rates at 5 weeks after transfer were 60.00% in Boer goats and 67.00% in indigenous goats.[16] Shin *et al*. have compared the pregnancy rates after laparotomic and laparoscopic transfers of embryos to the goats.[29] The pregnancy rate after the laparotomic transfer was 26.80% on the 30th post-transfer day; whereas, it was 46.10% in the laparoscopic transfer. In the present study, the pregnancy rates obtained after laparotomic transfer of fresh and vitrified-thawed embryos were 73.30% and 20.00% at BS and 75.00% and 37.50% at ES on the 30th day of the transfer, respectively. When laparotomic transfer findings with fresh embryos were compared with similar studies, pregnancy rates were significantly higher in this study.[7,14,16,29] The cause of this increase may be due to the individual characteristics of recipient animals after transfer as well as maintenance, feeding and management differences. Nevertheless, the rates of pregnancy and embryo survival rates from vitrified-thawed transferred embryos at BS and ES were found to be lower than the rates reported by other researchers. We propose that this trend can be a result of the vitrification protocol.

As a result, FSH can be successfully used for super-ovulation in the Angora goats. Also, MOET can be successfully carried out during the breeding season, because there is no difference in the superovulation response, corpus luteum counts and transferable embryo rates at BS and ES. Also, transfer of fresh embryo can be used successfully in Angora goats. However, further studies are needed considering the low rate of survivability in the transfer of vitrified-thawed embryos.

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Conflict of interest

The authors have no conflict of interest in the content of the article.

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