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Ovulation Rate and Embryo Yield of Dorper Sheep in Non-Breeding Season under Different Superovulatory Protocols

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Abstract: The response to superovulatory treatment and the embryo yield of Dorper ewes in a non-breeding season were evaluated. Average 65 kg, weight, 3 years old donor ewes were inserted CIDR for 12 days and divided into four superovulation treatment groups; Exp. I: 12 mL Folltropin+200 I.U. eCG (n = 25); Exp. II: 10 mL Folltropin+200 I.U. eCG (n = 20); Exp. III: 10 mL Folltropin+600 I.U. eCG (n = 25) and Exp. IV: 10 mL Folltropin+750 I.U. eCG (n = 10) with single shot of FSH at CIDR removal. Donors were superovulated using FSH-p (total of 20 mg) (Folltropin-V; Vetrepharm, Canada) applied in six decreasing doses of 3, 2.5, 2, 2, 1.5, 1 mL i.m. starting 60 h before CIDR withdrawal. Donors underwent intrauterine AI with fresh diluted semen (a minimum of 50×10^6 motile sperm/each uterine horn) 40 h after CIDR removal and embryos collected 6 days after insemination. The CL responses were found to be similar in all treatment groups (Exp. I: 11.7±1.18; II: 9.7±1.31; III: 8.4±1.76 and IV: 9.9±1.63). Superovulation response (CL>3) was found relatively but not significantly higher in lower dose of eCG treated Exp. I: 92% and II: 91%, respectively compared to III: 76% and IV: 75%. Transferable embryo numbers for a fresh MOET program were found slightly higher (P: 0.333) in donors in Exp. III with 7.8 ±1.2 than those recorded for donors in Exp. I (4.7±1.12), II (6.3±1.24) and IV (6.3±1.7). The significant difference ($p<0.05$) was observed in embryo recovery rates; Exp. II and III: 63%, Exp. I: 38% and IV: 43%. Stages of embryonic development were also affected by the different hormonal treatments used in the study Exp. I and IV giving the best results for a fresh MOET program. In conclusion, it was found that Dorper ewes treated with different superovulation protocols in out of season (May) *in vivo* fresh embryo MOET program yielded 9 embryos and the recovery rate was markedly higher than those of earlier findings. Study also showed that a single FSH injection can be used in place of decreasing dose of multiple FSH injections with similar results.

Key words: Embryo transfer, dorper sheep, embryo recovery rate, superovulation, non-breeding season

INTRODUCTION

In small ruminants, embryo transfer is a relevant tool for increasing the reproductive rate of selected donors (Cognie, 1999), the salvage of endangered native breeds (Solti *et al*., 2000) and the method of choice for health control in germplasm exchanges (Thibier and Guerin, 2000). Early investigators have recognized the requirement for synchronization of the estrus cycles of donor and recepients (Moore and Shelton, 1964). Progestogens either alone (Jabbar *et al*., 1994; Wheaton *et al*., 1990) or with gonadotropin at the end of progestogen pretreatment (Powell *et al*., 2006) have been used to induce fertile estrus in anestrous ewes. The Controlled Internal Drug Release dispenser (CIDR) is an intravaginal device constructed of a progesterone-impregnated medical silicone elastomer molded over a nylon core. The CIDR-G is suitable for treatment of parous ewes, lambs and goats. Plasma progesterone levels increase rapidly after insertion of CIDR, reach highest concentrations on day 3 and then gradually decrease. CIDR have been substituted successfully for sponges for estrous synchronization, superovulation, artificial insemination and embryo transfer. The CIDR-G provides a convenient means to deliver exogenous progesterone to sheep and goats and offers an alternative to the progestogen sponge for reproductive management.

On the other hand, there are three most widely used gonadotropin preparations for superovulation such as eCG, pituitary Follicle Stimulating Hormone (FSH-P) and Horse Anterior Pituitary extract (HAP). Many researchers have evaluated efficiacy of eCG and FSH on

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superovulatory response and embryo recovery rate and reported different findings (Walker *et al*., 1989; Pendelton *et al*., 1992; Blanco *et al*., 2003; Riesenberg *et al*., 2001).

The Dorper breed was derived from crossing Blackheaded Persian ewes with Dorset Horn rams in the early 1940s in South Africa and were introduced to Turkey in 2012 to improve the meat performance of local breeds. It is an adaptable sheep breed, capable of maintaining acceptable levels of production under varying conditions. Dorper is regarded as an early-maturing breed (Cloete *et al*., 2000) and ewe fertility is approximately 0.90 ewes per lambing (Manyuchi *et al*., 1991).

The objectives of this study were to evaluate the response to differring superovulatory treatments with embryo yield and embryo development in Dorper sheep in an intensive production system in South East Anatolia, Turkey.

MATERIALS AND METHODS

A total of 80 Dorper ewes at Zirve University Research Farm in Gaziantep, Turkey were ramdomly divided into four treatment groups. At an average weight of 65 kg, all donor ewes were inserted CIDR for 12 days and divided into four superovulation treatment groups as; Exp. I: 12 mL Folltropin+200 I.U. eCG (n = 25); Exp. II: 10 mL Folltropin+200 I.U. eCG (n = 20); Exp. III: 10 mL Folltropin+600 I.U. eCG (n = 25) and Exp. IV: 10 mL Folltropin+750 I.U. eCG (n = 10) with single shot of FSH at CIDR removal. Donors were superovulated using FSH-p (total of 20 mg) (Folltropin-V; Vetrepharm, Canada) applied in six decreasing doses of 3, 2.5, 2, 2, 1.5, 1 mL i.m. starting 60 h before CIDR withdrawal. Donors were sedated with an anesthetic cocktail containing 100 mg Ketamine (Vetalar, Boehringer Ingelheim Vetmedica, Inc.) and 0.12 mg Xylazine (Romphun, Bayer) and underwent intrauterine insemination with fresh diluted semen (a minimum of 50×10^6 motile sperm/each uterine horn) 40 h after sponge removal and embryos were collected 6 days after insemination.

The 6 days (after estrus (day 0) donors were submitted to a standard surgical embryo recovery procedure under general anesthesia, using uterine flushing methods described by Smith and Murphy, consisting of injection of the flushing media into the tip of the uterine horn near the utero-tubal junction through a shortened and blunt 18 g needle connected to a 10 cc syringe. The media was recovered into a Petri dish through a 10 g Foley catheter inserted at the base of the uterine horn. A gentle direct massage of the uterine horn was performed during the flushing and a total of 30 mL of flushing media (modified Dulbecco's phosphate buffer saline with 0.4% (w/v) BSA) was used for each uterine horn. Upon exposure of the genital tract at surgery the number of CL present in the ovaries were counted. The efficiency of recovery was calculated by the equation:

$$\frac{\text{Total embryos recovered}}{\text{Total CL counted}}\times 100$$

The SOV response was considered positive when the ewes showed 3 or more CL. Recovered ova/embryos were kept in culture medium (ViGro, Holding Plus, AB Technology, Pulman, WA USA) at room temperature and evaluated for stage of development and quality according to IETS (Stringfellow and Seidel, 1998) guidelines. Developmental stage codes were: M = Morula; CM = Compact Morula; EB = Early Blastocyst; B = Blastocyst; EXB = Expanded Blastocyst; HGB = Hatching Blastocyst, HB = Hatched blastocyst. Data were analyzed by analysis of variance and means were tested by Duncan multiple range test.

RESULTS AND DISCUSSION

Inequality of ovarian function occurs in various species. In this study, there was no significant difference observed between right and left ovaries in which superovulation induced by means of eCG and FSH combined protocols. Average number of corpora luteas in each ovary was 4-5. Positive SOV responses of donors in Exp. I and II were 92 and 91%, respectively while this response were decreased to 76 and 75% in Exp. III and IV, respectively for donors treated with higher dose of eCG. Ewes treated with 10 mL Folltropin and 600 I.U. eCG had a slightly higher ($p>0.05$) mean number of transferrable embryos than those treated with the other protocols (Table 1). The study was based on intrauterine laparoscopic ET using fresh embryos and therefore makes reference to transferable embryos as those which are suitable for such and not for transfer of frozen embryos. Transferable embryo numbers differred minimally among all experimental groups. The donors treated with 10 mL Folltropin and 600 I.U. eCG produced the most mean number of embryos (7.8±1.2) (P: 0.333) than those

Table 1: Superovulatory response of Dorper ewes treated with different hormonal protcols

Experiments	CL numbers in right ovary	CL numbers in left ovary	Total CL number	SOV response (%)
I	5.56±0.65	6.08±0.69	11.68±1.18	92.000
II	4.55±0.73	5.10±0.77	9.65±1.31	91.000
III	4.16±0.65	4.24±0.69	8.40±1.18	76.000
IV	4.84±0.90	5.08±0.95	9.92±1.63	75.000
p-value	0.493	0.316	0.274.000	0.183

Table 2: Embryo yield of donors treated with different superovulation protocols

Experiments	Transferable embryo numbers	Recovery rate (%)
I	4.74±1.22	37.67±7.29[b]
II	6.26±1.24	62.90±8.02[a]
III	7.84±1.24	63.50±8.02[a]
IV	6.30±1.70	42.49±11.06[b]
p-value	0.33	0.04

[a, b]Data bearing different surscript in a single column indicates significance where p<0.05

Table 3: Stages of embryo development in donors superovulated with different hormonal treatments given as means (%)

Stage of embryos	M	CM	EB	B (%)	EXB	HGB	HB
Exp. I	1.000[a]	6.900[a]	21.8[a]	30.700	23.800[a]	14.90	1.000
Exp. II	3.700[b]	15.300[b]	23.2[a]	32.200	15.300[b]	7.50	2.900
Exp. III	8.200[c]	11.900[b]	22.2[a]	30.100	11.200[b]	6.60	2.000
Exp. IV	0.000[d]	21.400[c]	0.0[b]	35.700	28.600[a]	14.30	0.000
p-values	0.006	0.046	0.0	0.899	0.023	0.15	0.363

[a-d]Data bearing different surscript in a single column indicates significance where p<0.05. Developmental stage codes were: M = Morula; CM = Compact Morula; EB = Early Blastocyst; B = Blastocyst; EXB = Expanded Blastocyst; HGB = Hatching Blastocyst, HB = Hatched Blastocyst

recorded for other group of donors (Exp. I: 4.7±1.12, II: 6.3±1.24 and IV: 6.3±1.7). While the embryo numbers were similar the recovery rates were significantly affected (p<0.05) by the different hormonal protocols. Donors in Exp. II and III both recorded recovery rates of 63% while donors in Exp. I and IV recovered 38 and 43%, respectively (Table 2).

The three most important criteria related to embryo morphology are age, stage and quality. Stages of embryo development differred significantly among the 4 treatment groups. Significantly higher (P: 0.006) number of morula stage embryos were obtained with Exp. III while single shot FSH (Exp. IV) resulted with higher (p<0.05) number of compact morula stage embryos. Exp. I, II and III had similar (20%) early blastocyst embryos and all experimental groups also had similar and the highest rate of embryos (30-35%) at blastocyst stage of development. However, Exp. I and III had the highest number of expanded blastocyst embryos 24 and 29%, respectively as well as hatching blastocyst at 15 and 14%, respectively. There were very minimal <3% hatched blastocyst embryos among all the experimental groups (Table 3).

Mean ovulation rate determined by counting CL at recovery was found similar in eCG and FSH combined treatment groups. Dorper ewes included in the out of season MOET program all responded to the superovulation treatments with an average number of 10 CL. The study results was similar to those (CL: 10.8±2.5) reported by Jasmi *et al.* (2010) who used a higher dose (50% more) of FSH in his research with the same breed. Same researcher found lower transferable embryos than the current study findings (1.8 vs. 6.3).

A positive SOV response was observed in the study donors and their ovulation rate was similar to that of low prolificacy breeds (Torres *et al.*, 1987; Wierzchos *et al.*, 1992; Chagas e Silva *et al.*, 2003). But generaly ovulation rates after SOV treatments are shown to be higher in prolific than in nonprolific breeds (Torres and Sevellec, 1987).

Embryo recovery rates were found satisfactory in Exp. II and III where 10 mL folltropin and moderate amount of eCG had been used, lower rates were experienced with higher doses of either eCG and/or FSH. This result might be associated with a high number of follicles at the time of flush. This was also the observation of Armstrong and Evans (1983) who reported on the development of unovulated follicles after various eCG treatments and those of Jabbour and Evans (1991) who reported higher periovulatory estradiol peak levels in eCG compared to FSH treated sheep which they suggested could interfere with ova capture by the fimbria or with the transport of ova through the oviduct.

Embryo transfer protocols and procedures in sheep have been successfully preformed by numerous researchers (Ishwar and Memon, 1996). Pregnancy rates after ET programs were reported by others for sheep have varied from 29-65% and numerous affecting factors were stated for the success of the programs such as the stage of development of the transferred embryos, synchronization protocol, out of season synchronization, age of the oocyte/embryo donor, embryo storage, method of embryo production (e.g., *in vivo* vs. *in vitro*) and culture conditions for *in vitro* produced embryos (Thompson *et al.*, 1995; Holm *et al.*, 1996; Ptak *et al.*, 1999; Dattena *et al.*, 2000). The efficacy of the stage of embryonic development was investigated by Gimenez Diaz (2013) and it was reported that expanded blastocyst stage was the most favorable for both pregnancy (75%; p>0.05) and embryo survival (100%; p<0.05). In the current study, the proportion of expanded blastocyst embryos were higher in single shot FSH treated group (Exp. IV) with 28.6% followed by the 12 mL Folltropin+200 I.U. eCG treated group (Exp. I) with 23.8% compared to the lower FSH and higher eCG treated animals in Exp. II and III.

Since, embryo age corresponds closely to stage of development, based on a large number of fresh *in vivo* ET programs carried out (Gimenez Diaz, 2013), it can be stated fairly conclusively that embryo stages ranging from late morula to expanded blastocyst result in comparably higher pregnancy rates, whereas following hatching, lower pregnancy rates can be expected (Hasler *et al.*, 1987).

Results in the study showed that superovulation protocols did not cause a significant difference in the number of later stage embryos yet had a marked effect on the production of earlier stage of embryos.

CONCLUSION

The study showed that in the out of season *in vivo* fresh embryo ET program, the ratio of recovered transferable embryos to the number of CL produced for the Dorper breed in all treatments were markedly higher that those of earlier findings and these recovery rates were significantly affected ($p<0.05$) by the different hormonal protocols used. Study results also indicated that superovulation in the Dorper breed using a single shot FSH treatment and a lesser dose of eCG lead to better ovarian response giving higher embryo yield. These specific treatments also produced the most viable embryos in regards to the stage of embryonic development namely; late morula to expanded blastocyst. However, all of the superovulation protocols used in the current study gave an acceptable result and could be selected for an out of season MOET program for the Dorper breed.

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