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Effects of Breed and Number of Embryos Transferred on the Efficacy of MOET in Sheep

Ayman Abdel-Aziz Swelum, Abdullah Nasser Al-Owaimer and Mohamed Ahmed Abouheif
Department of Animal Production, College of Food and Agriculture Sciences,
King Saud University, P.O. Box 2460, 11451 Riyadh, Saudi Arabia

Abstract: The present study aimed to evaluate the effect of sheep breed as well as number of embryos transferred on the success of MOET. Sixteen ewes were used as donor (Najdi = 11 and Naeimi = 5). Oestrus synchronization and multiple ovulation were performed using intra-vaginal progesterone sponge and eCG. Donors inseminated intrauterine using laparoscopic. Embryos were recovered and transferred surgically on day 8 after sponge withdrawal. Thirty five ewes (Najdi = 16 and Naeimi = 19) were used as recipients and divided into 4 groups: Najdi or Naeimi received one or two embryos. Pregnancy was diagnosed at day 35 post insemination and confirmed at day 60 using ultrasound. All recipients were followed up until lambing thereafter, gestation length, litter size and sex of lambs were recorded. The results revealed that the number of embryos recovered/responded donor was significantly ($p<0.05$) lower in Najdi (5.1±1.11) than Naeimi (7.6±0.98) sheep. The breed of ewes had a noticeable effects ($p<0.05$) on the percentages of pregnancy, lambing and embryos survival these said indices were lower ($p<0.05$) in recipient Najdi than Naeimi ewes when two embryos were transferred. On the other hand, the recipient Naeimi ewes that received one embryo had significant low ET success. Embryos survival rate in Najdi that had one-embryo transferred were higher ($p<0.05$) than those received two-embryos. In conclusion, the response of ewes for multiple ovulation using eCG was significantly ($p<0.05$) lower in Najdi (6.7±1.05) than Naeimi (9.8±1.17) ewes. Moreover, transfer of one embryo resulted in significant ($p<0.05$) higher pregnancy rate in Najdi (60%) than Naeimi (10%) sheep.

Key words: Multiple ovulation, embryo transfer, najdi, naeimi, sheep

INTRODUCTION

The natural reproductive potential of sheep breed is a limiting factor for the diffusion of genetic improvements. Multiple Ovulation and Embryo Transfer (MOET) highly increases the rate of the genetic gain through increases the number of lambs born from high merit ewes and shortens the generational interval by taking advantage of the great oocyte reserve in the ovary. The major constraint for MOET protocols is the wide variations in ovarian responses to gonadotrophin treatments (Cognie, 1999; Driancourt, 2001). Factors causing such high variability are classified either as extrinsic factors which depend on the multiple ovulation treatment protocol or intrinsic factors; namely, species, breed, age, nutrition, reproductive and lactation status of donors (Cognie *et al*., 2003; Gonzalez-Bulnes *et al*., 2004). The breed factor accounted for approximately 30% of the variability in the embryo yields obtained in response to FSH treatments (Vivanco *et al*., 1994). Most of the differences in multiple-ovulation response were related to the different prolificacy of the breeds used in MOET (Cahill and Dufour, 1979) with highly prolific breeds having a greater response to exogenous stimulation (Bindon *et al*., 1971; Smith, 1976; Piper *et al*., 1982). These differences were also found when comparing non-prolific breeds where an interaction between the type of gonadotrophin used and the breed has been described (Picazo *et al*., 1996).

There is an economic incentive on transferring multiple embryos in recipient sheep to reduce the number of recipient ewes and additional burden on their upkeep. There are conflicting reports available on embryo survival in sheep as it remained unaffected (Moore *et al*., 1960; Armstrong and Evans, 1983; Hinch *et al*., 1998) increased (Quirke and Hanrahan, 1977; Cseh and Seregi, 1993) or decreased (Mutiga, 1991). Transferring two embryos gives acceptable results and yields with increased number of twin pregnancies. More than 80% of recipients of two embryos carried pregnancies to term with approximately 66.7% giving twins at birth (Ishwar and Memon, 1996).

Corresponding Author: Ayman Abdel-Aziz Swelum, Department of Theriogenology, Faculty of Veterinary Medicine, Zagazig University, 44519 Sharkia Province, Egypt

Najdi and Naeimi are fat-tailed sheep and considered the breeds of choice in Saudi Arabia (Abdo *et al*., 1989) because of their unique characteristics such as resistance to many diseases and parasites, walk long distances, tolerating extreme temperatures and enduring adverse feeding conditions. The litter size in Najdi and Naeimi ewes are very small ranging from 1.11-1.38 and 1.04-1.08 per ewe lambing, respectively (Abouheif and Alsobayel, 1982; Said *et al*., 1999). Top Najdi sheep can sell for tens of thousands USD. Yet, studies concerning the multiple ovulation protocols, laparoscopic intrauterine insemination and embryo transferred in Najdi and Naeimi ewes were absent or scarce. To the knowledge, no studies have been reported in Saudi Arabia developing a protocol that could be easily applied in extensively managed flocks for embryo transfer. Therefore, the present study aimed to evaluate the effects of number of embryos transferred on the efficacy of MOET in both Najdi and Naeimi ewes.

MATERIALS AND METHODS

This study was conducted during the months of October to April at the Experimental Farm, Department of Animal Production, King Saud University, Riyadh (latitude 24°48'N and longitude 46°31'E), Saudi Arabia. During the course of this study, average daily temperature ranged between 14.4 and 21.2°C, relative humidity 21-47% and average rainfall was ranged between 1.7 and 24.2 mm month^{-1}. Ewes were maintained and group-housed in pens under a roof in an open-sided barn and fed on a commercial pellet (14.5% CP; 2.78 Mcal ME kg^{-1}DM) to meet daily energy and protein requirements.

Oestrus synchronization, multiple ovulation and insemination: Mature (3-5 years old) multiparous Najdi (n = 11) and Naeimi (n = 5) ewes with a mean body condition score of 3.5 were used as embryo donors. None of the ewes utilized in this trial had been previously subjected to a MOET program. Oestrus synchronization was carried out with the aid of intra-vaginal sponges containing 30 mg flurogestone acetate (FGA; Ova-Gest®, Bionich Animal Health) for 12 days thereafter, sponges were removed. On day 10 of progestagen treatment and after 24 h of sponge removal, ewes were injected intramuscularly with 1200 and 600 IU eCG (Folligon®, Intervet), respectively. Ewes were joined with rams for 18 h (36-54 h after sponge removal) in a ratio 3 ewes ram^{-1}. Additionally, laparoscopic intrauterine inseminations were performed with fresh diluted semen with Triladyl® (Minitube, Germany) 54 h after sponge removal (McKelvy *et al*., 1985; Brebion *et al*., 1992). An insemination dose of at least 100×10^6 motile sperms was equally deposited into the lumen of the mid portion of each uterine horn by an experienced laparoscopic AI operator (Vallet and Baril, 1990; Brebion *et al*., 1992). All rams used in this study had passed a breeding soundness evaluation test.

Embryo recovery and evaluation: All donor ewes were taken of feed for 24 h and water for 12 h prior to laparotomy on day 8 after intra-vaginal sponge withdrawal. After restraining the ewes on a surgical table in dorsal recumbency, the head was held down at a 45°C angle to the horizontal and the ventral abdomen was clipped free of wool or hair for surgical preparation. Thereafter, general anaesthesia was induced and a mid-ventral line incision (5-7 cm long) was performed cranial to the udder attachment to allow elevation of uterus and assess embryo recoveries as described in detail by Smith and Murphy (1987). Briefly, both uterine horn tips were canulated with an intravenous catheter (18 g) as close to the utero-tubal junction as possible and a Foley catheter (10 g) was introduced into the uterus and a volume of approximately 50 mL flushing media (Emcare™ Complete Ultra Flushing Solution) at 38°C was instilled for a washing of the cavities before aspirating and collecting the fluid which contains the embryos, the flushing medium was recovered in previously warmed sterile Petri dishes. Once embryo recovery was finished, surgical incision was sutured and antibiotics were administered.

The collected media was examined for the presence of oocytes and/or embryos by a stereomicroscope and on a thermal plate at 38°C. After identification, embryos were aspirated with a micropipette and placed in a small Petri dish containing a holding medium (Emcare™ Holding Solution) at a laboratory temperature. Embryo assessment was carried out based on morphological criteria and according to the guidelines of the International Embryo Transfer Society (Stringfellow and Seidel, 1998), only grade 1 embryos were counted and utilized in this trial. To observe the embryos from different angles, a micropipette was used. The integrity of the zona pellucida and its roundness was examined, cells must be clear and present regular boundaries. Opacity, if present, indicates degeneration. All these activities were performed under strictly sterile conditions.

Oestrus synchronization in recipient and embryo transfer: The selection of recipient ewes was based on criteria such as good history of mothering ability, good health conditions and any ewe with history of mastitis, reproductive disorders or dystocia was avoided. At the same time of oestrus synchronization in donor ewes, thirty-five recipient ewes (Najdi = 16 and Naeimi = 19) with a an average body condition score of 3.5 and 3-6 years of age were synchronized with intra-vaginal sponges

containing 30 mg flurogestone acetate for 12 days followed by the administration of 600 IU eCG i.m. at the time of sponge removal. This procedure was to ensure that both recipients and donors ewes reached the same day of the oestrus cycle at the time of embryo recovery and transfer. Oestrus was detected using vasectomised ram with a proved high libido desires.

Embryo transfer was done immediately after collection (day 8 after intra-vaginal sponge withdrawal) and in no case must embryos be >2 h in the holding medium. Recipient ewes were divided according to breed and number of embryos received into 4 groups: Najdi received one or two embryos and Naeimi received one or two embryos. Recipient ewes were sedated with xylazine and local anaesthetic was infiltrated at incision site. Transfer of embryos was accomplished by surgical procedures. Visual ovary examination of recipients was performed to ensure that ewes had at least one or two mature corpora lutea. A puncture was performed on the dorsal side of the uterine horn and in its upper third portion. The embryos were located in the uterine lumen by means of a micropipette.

Evaluation of MOET success: Pregnancy was diagnosed ultrasonography (Prosound 2, Aloka, Japan) at day 35 post insemination and confirmed at day 60 using multi-frequency abdominal sector probe of 3.5-5 MHz. Number and sex of foetuses were recorded and confirmed at lambing. Pregnancy and lambing percentages, embryo survival percent and gestation length were calculated. The pregnancy percent was defined as the number of pregnant ewes divided by the number of recipients (x100) and lambing percent as the number of ewes lambing divided by the number of recipients (x100). Embryo survival percent was defined as number of lambs born divided by the number of embryos transferred (x100).

Statistical analysis: The effect of the breed on number of corpus luteum and embryos recovered per treated or responded donor were analyzed using t-test. Moreover, the effect of the breed and number of embryos transferred on pregnancy, lambing, embryo survival percentages, litter size were all analyzed using Chi-square analysis. In addition, the effects of litter size on sex of lamb and gestation length were determined by Chi-square analysis. Data was analyzed using the SPSS 13.0 for Windows Statistical Software.

RESULTS

The responses of donor ewes to multiple ovulation are shown in Table 1 where 63.6% of Najdi and 100% of Naeimi ewes were responded to multiple ovulation protocol. All non-responded Najdi ewes had anovulatory luteinized follicles. The numbers of observed corpus luteum per responded donor was significantly lower ($p<0.05$) in Najdi (6.7±1.05) than Naeimi (9.8±1.17) donors. The embryos recovery rates were 76.6 and 77.6% for Najdi and Naeimi ewes, respectively. Number of embryos recovered per responded donor were significantly lower ($p<0.05$) in Najdi (5.1±1.11) than Naeimi (7.6±0.98) donors. The percentage of good embryos (grade 1 and 2) that transferred to recipients was 75% in Najdi and 73.7% in Naeimi.

Effects of breed and number of transferred embryos on the reproductive performance of Najdi and Naeimi ewes are showed in Table 2. The breed of ewes had a noticeable effects ($p<0.05$) on the pregnancy, lambing and embryos survival rates these said indices were lower ($p<0.05$) in Najdi than recipient Naeimi ewes when two embryos were transferred. On the other hand, the results showed clearly that very low number of the recipient Naeimi ewes that had one embryo transferred were responded to the ET treatment. Transferring one or two embryos to recipient Najdi ewes did not have significant effects ($p>0.05$) on the pregnancy and lambing rate whereas embryos survival rate in Najdi that had one-embryo transferred were higher ($p<0.05$) than those received two-embryos. Twin percent was higher ($p<0.05$) in Najdi (80%) than Naeimi ewes (54.5%) when two embryos were transferred. In addition, the results showed (Table 3) that there were no significant ($p>0.05$) effects for

Table 1: Responses of Najdi and Naeimi donor ewes to multiple ovulation protocol

	Breed of ewe		
Characters	Najdi	Naeimi	p-value
Number of donor ewes	11	5	-
Response to multiple ovulations (%)	63.6	100	0.195[a]
Corpus luteum/responded donor (±SE)	6.7±1.05	9.8±1.17	0.037[b]
Embryo recovery (%)	76.6	77.6	0.615[a]
Embryo/responded donor (±SE)	5.1±1.11	7.6±0.98	0.034[b]
Good embryo[c]/responded donor (±SE)	3.86±0.99	5.60±0.51	0.074[b]

[a]Calculated by χ^2-test; [b]Calculated by t-test; [c]Grade 1 and 2 embryos

Table 2: Effect of number of transferred embryos on the reproductive performance in Najdi and Naeimi ewes

	Number of transferred embryos per ewe					
	Najdi		Naeimi		p-value[1]	
Characters	One	Two	One	Two	B[2]	TE[3]
No. of recipient ewes	5	11.0	10	9.0	-	-
Pregnancy (%)	60[b]	54.5[b]	10[c]	88.9[a]	0.022	0.075
Lambing (%)	60[b]	54.5[b]	0[c]	88.9[a]	0.025	0.089
Embryo's survival						
Percentage (%)	60[a]	45.5[b]	0[c]	61.1[a]	0.034	0.065
No.	3	10.0	0	11.0	-	-
Litter size						
Single (%)	100[a]	20.0[c]	0[d]	45.5[b]	0.088	0.048
Twin (%)	0[c]	80.0[a]	0[c]	54.5[b]	0.042	0.006

[a-d]Means in same row bearing different superscripts differ ($p<0.05$); [1]Calculated by χ^2-test; [2]Breed effect; [3]Effect of number of transferred embryos

Table 3: Distribution of the sex of born lambs and gestation length in Najdi and Naeimi ewes

	Litter size					
	Najdi		Naeimi		p-value[1]	
Characters	Single	Twin	Single	Twin	B[2]	LT[3]
No. of born lambs	5	8	5	6	-	-
Male (%)	60	75[a]	60	66.7[a]	NS	NS
Female (%)	40	25[b]	40	33.3[b]	NS	NS
Gestation length (day)						
Male	151.7±1.8	150±0.4	149±0.6	150±0.6	NS	NS
Female	150.5±0.5	150±1.0	149±0.0	149.5±0.5	NS	NS

[a, b]Means in same column bearing different superscripts differ ($p<0.01$); [1]Calculated by χ^2-test; NS = $p>0.01$; [2]Breed effect; [3]Effect of litter size

breed and type of lambing on the sex of born lambs and the length of gestation period, the percentage of twinborn ram lambs was significantly higher ($p<0.05$) than those twinborn ewe lambs.

DISCUSSION

Related to intrinsic factors, breed was early identified as a factor that may contribute to the variability of the multiple ovulatory responses (Bindon *et al*., 1986) and as a general rule, prolific breeds show a better multiple ovulatory response (Dufour *et al*., 2000) than non-prolific breeds (Picazo *et al*., 1996). Differences in multiple ovulatory responses between Najdi and Naeimi ewes may be related to either a differential kinetic behaviour of the exogenous gonadotrophin or to a differential follicular dynamics and function in response to the hormonal treatment. Driancourt *et al*. (1986), Abdennebi *et al*. (1999) and Dufour *et al*. (2000) reported that the differences between breeds in multiple ovulation response were probably related to the wide variations in sensitivity of ovarian receptors to multiple ovulation treatment. Cognie *et al*. (2003), Gonzalez-Bulnes *et al*. (2004) and Ammoun *et al*. (2006), indicated that breed differences in multiple ovulation response were explained by breed differences in follicular dynamics in response to multiple ovulation treatment rather than to any differences in the dynamics of its absorption and clearance. The multiple ovulation responses in sheep and other species were related to the distribution of the follicular population present in the ovaries at gonadotrophin treatment in brief, a higher ovulatory response was associated to a higher number of small (2-3 mm) gonadotrophin-responsive follicles, able to grow to ovulatory sizes in response to the gonadotrophin administration (Veiga-Lopez *et al*., 2005).

The significant difference in embryo survival rates between Najdi and Naeimi ewes can be explained by the differences in genetic makeup. Several reports had been showed that embryo survival rates after ET in various sheep breeds remained unaffected (Armstrong and Evans, 1983; Hinch *et al*., 1998) increased (Cseh and Seregi, 1993) or decreased (Mutiga, 1991). Transferring one embryo to recipient Naeimi ewes resulted in very low survival rate in comparison with Najdi ewes, the most likely reason could be that one embryo was incapable of exerting a sufficient luteotrophic action on the corpus luteum, resulting in impaired luteal maintenance in recipient Naeimi ewes or impaired signals to the endometrium involved in the process of implantation. The improved embryo survival rates observed in Naeimi sheep after twin transfer suggested that there was some type of synergism between embryos in influencing each other's survival upon transfer. On the other hand, Moore *et al*. (1960) reported that transfers of less than two embryos did not affect the possibility of pregnancy. Additionally, Hinch *et al*. (1998) reported non-significant increases in the survival of embryos when two rather than one embryo was transferred. Ishwar and Memon (1996) reported that transferring two embryos gives acceptable results and yields with increased number of twin pregnancies (>80% of recipients of two embryos carried pregnancies to term with approximately 66.7% giving twins at birth). Conversely, Cseh and Seregi (1993) concluded that embryos survival rate was greater when one embryo per recipient ewe was transferred. In dairy goats, a higher rate of embryo survival was found after transfer of two embryos per recipient (Moore and Eppleston, 1979; Armstrong *et al*., 1983; Tervit *et al*., 1983).

The embryos survival rate in the study ranged from 45.5-61.1% which was within the range reported by Bolet (1986) in his review of embryo loss (20-70%). Hinch *et al*. (1998) reported that the overall survival level for embryos transferred directly was 43.2%. Additionally, Kelly *et al*. (1983) reported 58% embryo survival rate. The present result reveale that embryos transferred from one Najdi and other Naeimi donor gave no pregnancy. This may be caused by donor effect, defined as the variability observed in embryo survival rates (0-78%) for embryos of the same quality from different mothers (Heyman *et al*., 1987).

CONCLUSION

The efficacy of MOET was significantly affected by breed of sheep and number of embryos transferred. The response of ewes for multiple ovulation using eCG was significantly ($p<0.05$) lower in Najdi (6.7±1.05) than Naeimi (9.8±1.17) ewes. Moreover, transfer of one embryo in Naeimi sheep resulted in significant ($p<0.05$) low pregnancy rate (10%) in comparison with Najdi sheep (60%). However, further studies need to be carried out on the indigenous breeds of Saudi Arabia to investigate the response to multiple ovulation with different gonadotrophin preparations and administration protocols.

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