ADHESIONS PREVENTION FOLLOWING REPEATED SUPEROVULATION AND SURGICAL EMBRYO RECOVERY IN BALADY GOATS

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SUMMARY

Repeated superovulation and surgical embryo collection with minimization of adhesions were performed in twenty adult Balady goats. Animals were treated with one third of the cattle ear implant (2 mg Norgestomate) and injected with 0.75 mg Norgestomate and 1.25 mg Oestradiol Valerate. The implant was left in situ for 12 days. For superovulation, the does were injected with 750 IU PMSG (group 1) or 1000 IU PMSG (group 2), in combination with PGF2\alpha (125 \mug g cloprostenol), 24 hours before implant removal. Ovarian response and ovulation were recorded. Repeated superovulation and surgical embryo recovery were performed 45 days and 90 days after the first embryo collection. In a trial to prevent post-operative adhesions, sodium carboxy methyl cellulose (1% solution, 14 ml/kg b.wt.) was used intraperitoneally. The results revealed that repeated superovulation influences the estrus parameters, ovulation rate and mean number of embryos recovered. Moreover, intraperitoneal administration of sodium carboxy methyl

cellulose minimized post-operative adhesions.

INTRODUCTION

Embryo transfer is a valuable tool used for introduction of new genetic properties into closed herds, multiplication of genetic information, treatment of reproductive problems of chronic infertile animal and to preserve the genetic pool of terminally ill animal (Amoah and Gelaye, 1997; Banos, et al., 1996; Riha, et al., 1994). Considerable progress in embryo transfer in sheep has been made, however, a limited number of studies have been reported in goats (Ishwar and Memon, 1996).

In caprine, the most available productive way of embryo recovery needs direct surgical manipulation of the reproductive tract through laparotomies. In this respect, the common problems associated with this technique, and limiting repeated collections, are the development of refractoriness to gonadotrophins and formation

of post-operative adhesions. These problems significantly and adversely affect further embryo recovery and reducing future female fertility (Holtz, 1996; Ishwar and Memon, 1996; Moll, et al., 1992; Riha, et al., 1994).

gonadotrophins, when of Ineffectiveness repeatedly administered to farm animals, have been reported by many authors (Kanagawa and Ishikawa, 1980; Lamerson and Lambeth, 1986; Ismail, 1991). Moreover, studies had been repeated time of limited published on superovulation in sheep (Boland and Gordon, 1982; Al-Kamali et al., 1985 and Torres and Sevellec, 1987) but no available literature could be traced in goats.

Numerous clinical trials have been developed to salvage post-operative adhesions (Ahmed, 1979; Arora, et al., 1994; Beauchamp, et al., 1984; Dargenio et al., 1986; Graebe et l., 1989). Administration of sodium carboxy methyl cellulose (SCMC) has been tried in rats, rabbits, ewes and ponies (Elkins, et al., 1984; Eric Mueller, et al., 1995; Diamond et al., 1988 a & 1988 b; Heidrick et al., 1994; Moil et al., 1991; Parra et al., 1991; Ortega-Moren, 1993; Gehlbach, et al., 1994; Ryan and Sax, 1995; Wurster et al., 1995). However, in goats the data about use of sodium carboxy methyl cellulose are very limited.

The present study was aimed to increase productivity of does by repeated collection through overcoming the development of refractoriness to the gonadotrophins and to evaluate the effect of intraperitoneal administration of sodium carboxy methyl

cellulose (SCMC) on subsequent episodes of post-operative adhesions.

MATERIALS AND METHODS:

Animals:

Twenty adult female Balady goats (1-4 years old, 18-25 kg body weight) were used in this study. The experimental work was conducted during September - November. The animals were assigned randomally into two equal groups for the first experiment and were kept under observations for three weeks for estrous detection.

Treatments and estrous detection:

All does were treated with one third of the dose used for cattle (ear implant 2 mg Norgestomate, Crestar-Intervit) and injected with 0.75 mg Norgestomate and 1.25 mg Oestradiol Valerate). The implant was left in situ for 12 days. The animals were injected with 750 IU PMSG (Folligon-Intervet) (group 1, n =10) or 1000 IU (group 2, n =10), in combination with PGF2 α 1 /2ml Estromate-Coopers = 125 µg cloprostenol), 24 hours before implant removal.

Estrous detection began 12 hours after implant removal and continued for 3 days. All does were exposed to one or two aproned males 4 times daily. Does seeking the buck or showing other signs of estrus were hand mated twice daily until signs of estrus disappeared.

Surgical embryo collection:

Surgical collection of the embryos was done from the superovulated does on the 5th, day of the

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estrous cycle (onset of heat = day 0). Food was withheld for 12 hours prior to surgery. Xylazine-Hcl (Rompun-Bayer) was injected intramuscularly (0.01 mg/kg b.wt.) for each doe. Epidural (lumbo-sacral) anaesthesia and linear infiltration analgesia along the intended line of incision were undertaken using xylocaine Hcl 2%. The abdomen was shaved and disinfected using diluted Povidone- iodine (Betadine-The Nile Co.). A ventral mid-line incision was made just cranial to the udder and extended forward for about 10 cm. The uterus was then completely withdrawn outside the abdominal cavity. The number of corpora lutea and follicles on each ovary was counted.

A blunt perforation was made through the uterine wall just above the external bifurcation. Foley catheter (8 or 10 FG) was inserted for a distance of 3-5 cm. towards the oviduct, and fixed in situ by inflation of its balloon. For rinsing the uterus, a blunt needle was inserted through the utero-tubal junction and each uterine horn was rinsed with 20 ml of flushing medium (modified Dulbeccoís phosphate buffered saline) then, collected in a collecting dish. Searching for the morphological identification and embryos, classification were performed on these collected embryos. The collected embryos were classified into excellent, good, fair and poor depending upon morphological symmetry, color, age, stage of individual blastomeres and the presence of vesicles (Takeda, 1986).

Adhesions minimization:

After embryo collection, the animals were assigned into 2 equal groups (n =10). In the first group (control) no intraperitoneal treatment was done. The linea-alba was closed, in a simple continuos pattern using poly-glycolic acid suture. The subcutaneous tissue was closed in a simple continuos pattern using chromic cat gut. The skin was closed in an interrupted horizontal mattress pattern using silk.

Sodium Carboxy Methyl Cellulose (SCMC 1%) was used in the second group. Preparation of this solution was done by boiling 200 ml of sterile water and adding 10 gm of SCMC while stirring. After dissolving of SCMC additional sterile water was added, while stirring, to bring the total volume to 1 liter. The solution was autoclaved at 121°C for 20 minutes. The pelvic and abdominal cavities were impacted with SCMC (14 ml / kg of b.wt) as described by Moll et al., (1992). A stab incision was made, prior to closure of the linea-alba, in all layers of the abdominal wall (2 cm lateral to the initial incision). Through this stab incision a 16-F Folly catheter was inserted. After closure of the abdominal wound, SCMC solution was infused through the Folly catheter.

Repeated superovulation and embryo recovery:

age, stage After 45 days (group 1, n = 10) and 90 days esence of (group 2, n = 10) from the first embryo collection, the does were treated in the same method described previously for synchronization and superovulated with 1000 IU PMSG. Surgical

interference through laparotomy for ovarian evaluation and embryo collection and for detection of degrees of uterine and ovarian adhesions was performed on day 5 after the beginning of estrus.

Uterine and ovarian adhesions were classified according to Moll, et al., (1992) into: Non (0) = genitalia showed no adhesions on gross examination; Slight (1) = adhesions between the uterine horns and body; Moderate (2) = adhesions extended to the oviducts and ovarian bursae and Severe (3) = adhesions involved the ovaries and the neighboring organs.

Statistical analysis:

All data were statistically analyzed by the least-squares analysis of variance using the General Linear Models Procedures (GLM) of the Statistical Analysis System (SAS, 1990).

RESULTS:

In the present study, all does treated with 750 or 1000 IU PMSG for the first time showed signs of estrus within 2 days after implant removal. The time clapsed from implant removal to onset of estrus was significantly (p<0.01) decreased in animals treated with 1000 IU PMSG compared to those treated with 750 IU (22.55± 6.99 Vs 29.60± 7.59 hr.). Moreover, estrous duration (Table 1) was significantly (p<0.01) prolonged in does treated with 1000 IU PMSG (39.20 ± 8.60 Vs 26.00 ± 8.43 hr.).

Repeated superovulation (1000 IU PMSG) 45 days later reduced the percentage of does in estrus to 80%. This repeated treatment did not only prolong (p<0.05) the time between implant removal and onset of estrus (22.55 \pm 6.99 Vs 27.20 \pm 7.73 hr.) but also has shortened (p<0.01) the estrous duration (39.20 \pm 8.60 Vs 29.45 \pm 7.85 hr.) compared to animals treated for the first time.

Table (1): Influence of dose of PMSG and repeated superovulation on estrous parameters.

Dose of PMSG (Number of animals) 750 IU PMSG (n =10)		1000 IU PMSG (n =10)		
superovulation	1st. treatment	1st. treatment	2nd treatment	
CONTRACTOR OF THE PROPERTY OF			(after 45 days)	(after 90 days)
Number of does in estrus (%)	10/10 ^(d) (100%)	10/10(d) (100%)	8/10 (c) (80%)	10/10 (d)(100%)
Time from implant removal to onset of estrus (hr.± SD)	29.60 ± 7.59(b)	22.55 ± 6.99(a)	27.20 ± 7.73(b)	24.80± 7.96(ab)
Duration of estrus (hr. ± SD)	26.00± 8,43(b)	39.20 ± 8.60(a)	29.45 ± 7.85(b)	37.60 ± 8.20 (ab)

Figures with different subscripts within rows were significantly different.

(a) Vs (b) at p < 0.01

(c) Vs (d) at p< 0.05

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All does treated after 90 days showed estrous signs similar to those treated for the first time. No significant variations were recorded in either the time from implant removal to estrous or the duration of estrus compared to those treated for the first time (Table 1).

Repeated superovulation after 45 days from the first embryo collection (1000 IU PMSG) resulted in a significant decrease in the mean number of corpora lutea and significant decrease in the ovulation rate. No significant changes were observed in the mean number of unovulatory

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Table (2): Influence of PMSG dose and repeated superovulation on ovarian response (Mean \pm SE)

Ovarian response/PMSG dose	No. of corpora lutea	No. of Follicles (<0.4 cm.)	Total response	Percentage of ovulation
750 IU PMSG	4.40 ±0.31(b)	1.70±0.47 ^(a)	6.10±0.59 ^(b)	70.97% ^(d)
1000 IU PMSG. 1st treatment	5.90±0.43(a)	3.30±0.47 ^(b)	9.20±0.32 ^(a)	64.13% ^(a)
100 IU PMSG, 2nd treatment (after 45 days)	3.88±0.66 ^(b)	2.86±0.34(a b)	6.74±0.62 ^(b) Islando establa	57.57% ^(c)
1000 IU PMSG, 2nd treatment (after 90 days)	4.64±0.82(b)	3.00±0.68 ^(b)	7.64±0.77(c)	60.73% ^(ac)
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Figures with different subscripts within columns were significantly different.

(a) Vs (b) at p < 0.01

(a) Vs (c) & (a) Vs (d) at p<0.05

Table 2 showed the influence of PMSG dose and repeated superovulation on ovarian response. It was noticed that the mean number of corpora lutea was significantly (p<0.01) higher in animals treated with 1000 IU PMSG (5.90 ± 0.43 Vs 4.40 ± 0.31). Moreover, the mean number of unovulatory follicles (<0.4 in diameter) was significantly higher in those treated with 1000 IU. The ovulation rate (number of corpora lutea \ total ovarian response) was significantly higher (70.97%) with the small dose (750 IU) than that with a higher one (64.13%).

follicles at that time. On the other hand, repeated superovulation after 90 days resulted in a non significant increase in the mean number of unovulatory follicles and ovulation rate compared to those treated after 45 days (Table 2).

Although the mean number of recovered embryos was higher in animals treated with 1000 IU. Which was significantly higher the small dose (750 IU) than that the (64.13%).

Although the mean number of recovered embryos was higher in animals treated with 1000 IU. PMSG in the first treatment and the recovery rate was higher in those treated with 750 IU PMSG these variations were statistically not significant. The mean number of fertilized embryos and the mean number of transferable did not vary with the dose of PMSG (Table 3).

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Comparing between the first treatment (1000 IU PMSG) and repeated superovulation (after 45 days and 90 days), there was a significant decrease in the mean number of recovered embryos. While the fertilized embryos showed significant decrease in the mean number only with repeated superovulation after 45 days. Moreover, no significant variations were recorded in the mean values of transferable embryos between the first treatment and repeated superovulation (Table 3).

As shown in Table (4), the coefficients of correlation between the number of corpora lutea and embryo parameters revealed a significant (r = 0.31; p<0.05) correlation between the number of corpora lutea and recovery rate but no significant correlation was recorded between transferable embryos and fertilized ones. The number of unovulatory follicles (<0.4 cm in diameter) present during the day of embryo recovery was significantly negatively correlated with embryo recovery(r = -0.53, p<0.01), transferable embryos (r = -0.47, p<0.01 and fertilized embryos (r = -0.48, p<0.01).

Table (3): Influence of PMSG dose and repeated superovulation on embryos parameters (Mean \pm SE).

Treatment	First tre	eatment	Repeated superovulation (1000 IU PMSG)		
PMSG dose	750 IU PMSG	1000 IU PMSG	(after 45 days)	(after 90 days)	
No. of embryo recovered	2.80±0.47 (ab)	3.50±0.42 (a)	1.50±0.46 (a)	2.00±0.39 (b)	
Recovery rate (No. of embryos recovered / No. of corpora lutea x 100)	63.64% ^(a) (28/44)	59.32% ^(a) (35/59)	38.71% ^(b) (12/31)	43.48% ^(b) (20/46)	
No. of fertilized embryos	2.20±0.47 (ab)	2.60±0.48 (a)	1.00±0.38 (b)	1.40±0.40 (ab)	
Fertilization rate (No. of fertilized embryos/No. of embryos re covered x 100)	78.57% ^(a) (22/28)	74.29% ^(a) (26/35)	66.67% ^(a) (8/12)	70.00% ^(a) (14/20)	
No. of transferable embryos	2.1±0.42 (a)	2.20±0.44 (a)	1.00±0.38 (a)	1.2±0.36 (a)	
Transferable rate (No. of transf. embryos/No. of embryos recovered x 100)	71.43% ^(a) (20/28)	62.86% (ab) (22/35)	50.00% ^(b) (6/12)	60.00% ^(ab) (12/20)	
Average quality score of transferable embryos	1.85	2.00	2.33	2.08	

Figures with different subscripts within rows were significantly different. (a) Vs (b) at p < 0.05

Table (4): The coefficients of correlation between the number of corpora lutea and unovulatory follicles on the day of embryo recovery and subsequent embryos yield.

Ovarian structures	Recoverd embryos	Transferable embryos	Fertilized embryos
No. of corpora lutea	0.31*	0.02	0.09
No. of unovulatory follicles	-0.53**	-0.47**	-0.48**

* P<0.05

** P <0.01

Table (5): Degree of adhesions in treated groups after surgical embryo collection.

	Degree of adhesions					
Groups	No.	None (0)	Slight (1)	Moderate (2)	Severe (3)	Mean score ± SE
Control	8	0/8 ^(a) (0%)	2/8 ^(a) (25%)	3/8 ^(a) (37.5%)	3/8 ^(a) (37.5%)	2.13±0.10 ^{(a}
SCMC (1%)	10	3/10 ^(b) (30%)	4/10 ^(a) (40%)	2/10 ^(a) (20%)	1/10 ^(b) (10%)	1.10±0.12 ^{(b}

Figures with different subscripts within columns were significantly different.

(a) Vs (b) at p < 0.05

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Regarding the formation of post-operative adhesions, it was observed that all control animals had suffered from slight (25%), moderate (37.5%) and severe (37.5%) degree of adhesions (Table 5). Thirty percentage of does treated intraperitoneal 1% solution of SCMC showed no degrees of adhesions while forty percentage showed a slight form of adhesions. Moreover, the moderate and severe forms of adhesions were detected only in 20% and 10% of animals respectively. Comparing between control and SCMC treated groups, the mean score value showed a significant (p<0.01) reduction in the post-operative surgical adhesions in treated group (Table 5).

Regarding the relationship between different forms of adhesions and embryo recovery: fertilization and transferable rates (Table 6 & Fig. 1), it was found that the increase in adhesion scores decreases the recovery rate. In this respect. the recovery rate was (0%) with severe form of adhesions. In animals with slight adhesions, no effects on the fertilization rate was observed. This rate was slightly decreased in does with moderate adhesions than those without any forms of adhesions (60% Vs 66.67%). The transferable rate showed gradual decrease from the first degree of adhesions (non) to the fourth (severe) (66.67%. 61.54%, 40.0% and 0.00% respectively. On the other hand, the average quality score showed a gradual increase with increases of adhesion scores.

Table (6): Effects of degree of adhesions on embryo parameters.

Degrees of adhesions	Number of does	Recovery rate	Fertilizat ion rate	ble rate	Average quality score
None	3	9/14 ^(a) (64.28%)	6/9 ^(a) (66.67%)	6/9 ^(a) (66.67%)	1.66
Slight	6	13/22 ^(a) (59.09%)	10/13 ^(a) (76.92%)	8/13 ^(a) (61.54%)	1.90
Moderate	5	10/29 ^(b) (34.48%)	6/10 ^(a) (60.00%)	4/10 ^(a) (40.00%)	2.75
Sever	4	0/12 ^(c) (0.00%)			

Figures with different subscripts within columns were significantly different at p<0.05.

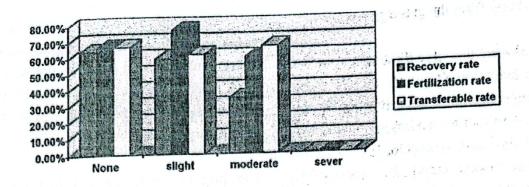


Fig. (1): Effects of different degree of adhesions on embryo parameters.

DISCUSSION

There are two main factors limiting repeated collection of embryos in small ruminants; the development of refractoriness to gonadotrophins and the formation of post-operative adhesions resulting from previous embryo collection (Torres and Sevellec, 1987; Tervit et al. 1991).

In the present study, estrous induction was 100% in does treated with short time progestagen in combination with $PGF_{2\alpha}$ (and superovulated with 1000 or 750 IU PMSG. This result was more or less similar to that reported by Fitzgerald et al., (1985). The increase in PMSG dose resulted in shortening the time interval from Norgestomate implant removal to the appearance of estrus, accordingly an increase in the duration of heat was observed as previously recorded in ewes

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(Mansour, 1993) and Damascus goats (Mansour and Gabr, 1997). These findings might be due to the marked increase in the preovulatory oestradiol concentrations. The early build up of high level of this hormone in superovulated animals could be responsible for the rapid onset of estrus and elongation of its duration (Yadov et al., 1986).

It was recorded that repeated superovulation after 45 days from the first embryo collection reduced the number of does in estrus to 80%, increase the time interval between progesterone removal and onset of estrus and reduced estrous duration. These results are in agreement with that reported by Al-Kamali et al., (1985) who observed that a reduction in the incidence of estrus at repeated superovulation after 55-76 days. As well as, the formation of antibodies to PMSG which triggers the injected PMSG leading to delaying in the

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oestradiol secretion from the growing follicles.

Comparing between the first treatment trial and repeated superovulation (45 days and 90 days), the repeated superovulation after 45 days showed significant variations. This finding coincided with that of Boland and Gordon, (1982) who concluded that ewes could be successfully superovulated if a period between treatments greater than two months.

The present study revealed that the total ovarian response, including the corpora lutea and unovulatory follicles, was significantly higher in does superovulated with 1000 IU PMSG than those treated with 750 IU. These results are on the contrary of that noticed by Mansour, (1993) who reported that, in ewes, 750 IU PMSG gave good ovarian response and embryo recovery. Regarding the percentage of ovulation, it was higher (70.97%) in does treated with 750 IU. In this respect, Armstrong et al., (1982) and Moore, (1982) mentioned that a higher dose of PMSG was slightly more effective in inducing ovulation accompanied with excessive stimulation of large number of follicles. These follicles mostly luteinized and did not ovulate.

In the present work, repeated superovulation after 45 days significantly reduce the mean value of corpora lutea, total response and the ovulation rate while the follicular growth significantly not affected. These results were more or less similar to that of Torres and Sevellec, (1987). They recorded that, compared with the first treatment, repeated superovulation at interval of 45-55 days significantly reduced the mean number of corpora

lutea. On the other hand, Al-Kamali et al., (1985) mentioned that, in ewes, no significant difference in the ovulation rate between the first treatment and a second one after 55-76 days. He added that, the only significant reduction was in the number of ovulating ewes.

The present study showed that there was no significant variations in the recovery, fertilization and transferable rates between the two doses of PMSG. In Rahmani ewes the mean number of recovered eggs, fertilized eggs and transferable embryo were markedly higher in animals superovulated with 750 IU than those treated with 1000 IU of PMSG (Mansour, 1993).

The mean number of recovered eggs per doe was significantly decreased following the second treatment even after 90 days. These findings were similar to that recorded, in ewes, by Boland and Gordon, (1982); Al-Kamali et al., (1985) and Torres and Sevellec, (1987). Moreover, the mean number of fertilized eggs and fertilization rate were significantly decreased repeated superovulation after 45 days with no difference after 90 days. These results could be attributed to the development of post-operative lesions (Torres and Sevellec, 1987). However, Boland and Gordon, (1982) reported that the fertilization rate was increased after that treatment, while, Al-Kamali et al., (1985) recorded that it was not affected.

In the present trial, a significant correlation between the number of corpora lutea (on the day of recovery) and the number of recovered eggs was recorded. This might be due to the increase in

progesterone production which improves the egg recovery. in this respect, Wubishet et al., (1991) mentioned that the accuracy of predicting the recovered embryos of number by concentration of plasma progesterone was 86%. Moreover, Petr et al., (1992) declared that the number of good embryos was significantly correlated to the frequency and mean basal concentration of progesterone. For solving the last problem, the use of laparoscopy for embryo collection and transfer might be helpful. However, the recovery rate compared with surgical collection could be decreased (Amoah and Gelaye, 1997; Ishwar and Memon, 1996; Meinecketillmann and Meinecke, 1986 Vallet, et al., 1991),

Regarding adhesions prevention, the mean score values showed a significant reduction in the post-operative surgical adhesions in the treated group (SCMC group) than in the control one. These results were in agreement with that reported previously by Elkins, et al., (1984); Graebe, et al., (1989); Diamond et al., (1988 a & 1988 b); Heidrick et al., (1994); Parra et al., (1991); Ryan and Sax, (1995) and Wurster et al., (1995). The mechanism by which SCMC was able to reduce adhesion re-formation is uncertain, but may be related to (hydroflotation) or (siliconizing) effects (Elkins, et al., 1984 Diamond et al., 1988 and Eric Mueller et al., 1995). On the other hand, Eric Mueller, et al., (1995); Moll, et al., (1992); Ortega-Moren, (1993) and Gehlbach et al., (1994) reported that, SCMC solution reduced adhesions formation but the statistical achieve improvement did not significant compared with the control group.

However, Arora et al., (1994) mentioned that gentle tissue handling, irrigation and good surgical skills remain the most prerequisite for adhesions prevention.

In conclusion, the present work was a trial to increase the productivity of female goats by repeated surgical embryo collection through overcoming the development of refractoriness to gonadotrophins. Moreover, the intraperitoneal administration of sodium carboxy methyl cellulose (SCMC) appeared to be effective on subsequent episodes of post-operative adhesions.

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