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# SUPEROVULATORY RESPONSE AND EMBRYO TRANSFER IN NAIMI SHEEP IN SAUDI ARABIA

By

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## SUMMARY

This trial examined the possibility of achieving successful embryo production for embryo transfer in Naimi sheep. The specific objectives were to determine the superovulatory response, the fertility to inntra-uterine AI of superovulated ewes and the mean embryo production per treated ewe. Some embryos were transferred into recipient ewes (Naimi cross) to observe if successful lamb production with embryo transfer could be achieved.

## INTRODUCTION

The Naimi sheep is one of the breeds corresponding to the Awassi fat tailed type and it is the most important local breed of sheep in Saudi Arabia. Reproductive performance of the Naimi sheep in Saudi Arabia is poorly documented, however some information exists for similar Awassi type sheep in other parts of the Middle East.

The use of reproductive technologies within the genetic improvement schemes for the Naimi sheep in Saudi Arabia has been limited due to both, the lack of sufficient information on their reproductive physiology and the relatively low efficiency of the reproductive technologies applied in this bread of sheep.

Previous attempts in the AI Jouf Centre to superovulate the Naimi sheep and produce embryos for transfer using progestogen sponges and PMSG were of low superovulatory response and low fertility and the embryo production was non successful.

This trial examined the possibility of achieving successful embryo production for embryo transfer applying to the Naimi sheep the strategies developed by Greany et al (7) for the large scale commercial application of embryo transfer in Texel and Finnish Landrace sheep. The specific objectives were to determine. the

superovulatory response, the fertil3ity of superovulated ewes to intra-uterine AI and the mean embryo production per treated ewe. In addition, some embryos were transfered to observe if successful lamb production with embryo transfer could be expected.

## MATERIAL AND METHODS

Five mixed age Naimi sheep located at the FAO Range and Animal Development Research Centre, Sakaka, AI Jouf, Saudi Arabia were used as donors near the end of the breeding season (November).

The ewes received intravaginal progesterone pessaries (EAZI-BREED CIDR 0.33gm of natural progesterone. Inter Ag. Hamilton, New Zealand) on day zero (day of pessary insertion) at 8 AM, the pressaries were replaced with new one day 10 and removed on day 14 at 8 PM.

FSH injections (Folltropin V. Vertrephram A* Asia Pty, ltd Vic. Australia) started on day 12 at 8 AM and continued at 12 hr intrevals until the particular donor was in heat by the vasectomized teaser rams (which were introduced at time of CIDR removal. Ewes marked by the ram did not receive further injections. Inspection of ewes for estrus was done 3 times per day (8 AM, 12 Noon and 8 PM). The number of injections of FSH varied for each ewe according to its time interval from CIDR removal to onset estrus. The doses were of FSH from first to the 6th injection (at CIDR removal) was 2.6,2.6,1.6,1.6,1.2 and 1.2mls of Folltropin. The dose of FSH after CIDR removal and up to onset of estrus was 1.0ml of Folltropin. All ewes received 160 i.u. PMSG (0.8ml. Pregneeol, Heriot Ag Vet. Pty. Ltd, Melbourne, Australia) at time of CIDR removal in addition to the corresponding FSH injection.

Ewes marked by the easer rams were isolated from the rest of the group and fastened until insmination time that was performed in average 14 hours post estrus detection. The ewes were inseminated by laparoscopic intra-uterine technique in both uterine horns, using a total dose of 0.25 of fresh diluted semen per ewe.

The ewes were subjected to ventral laporatory for embryo flushing six days post insemination. The ewes were starved for 24 hours prior embryo flushing. Surgery was performed under sedation of the ewes with Acetopromazine (Ceva Laboratories, Inc. Overl an Park Ks0 at a dose of 1 mg/Kg body weight and infiltration with local anaesthetic (lidocaine 2% solution, Astra Pharmaceutical products, Inc. Worcester, MA) at 10 mg/Kg body weight. The embryo were flushed using FG foley catheters and modified Dulbeco's containing 4% BSA, sodium pyruvate (36 mg/litre) and glucose (1gm/litre) supplements.

Embryo transfer was performed using the same protocol for sedation and local anaesthesia as per donors. Ovulation side was determined by laparoscopic inspection and the ipsilateral uterine horn was exteriorised through a small

incision. The uterine horn was punctured using a blunt needle and the embryos (zembryos per and both in the same uterine horn) were transferred using 20 µl UNOPETTES (Mini Tub, GmbH Germany). The reproductive tract was rinsed with warm saline solution and restored into the abdominal cavity. The wound was than sutured, the ewe injected with antibiotics and observed for 2 hours before returning to their feeding pens.

The variables recorded included: number of ewes showing estrus post CIDR removal, time interval CIDR removal to onset of estrus, number of corpora follicles of 5 mm or more in diameter, number of embryos recovered per flush, number of non fertilized oocytes recovered per flush, proportion of pregnant ewes receiving embryos and embryo survival rate.

The embryos recovered were graded as follows: Grade 1 embryos were the embryos whose development was corresponding to the expected stage of development at time of flushing and not showing any sign of abnormalities. Grade 2 embryos were also embryos at the expected stage of developement at flushhing but showing some loose blastomeres or dark areas, grade 3 embryos were the ones not at the expected stage of development and/or showing large extent of abnormalities.

Since there was no different treatments and/or comparison and due to the small population of ewes available for testing the superovulatory protocol, the data was processed simply by calculating the descriptive statistics for each variable recorded and for the fertility rate expressed as the proportion of embryos recovered over total structers recovered (embryos plus non fertility oocytes) and for the rate of good transferable embryos (Grade 1 plus Grade 2 embryos over total embryos) obtained.

## RESULTS

The results are summarized in Table 1.

Table (1): (Mean, ± SE) of the mean and coefficient of variation for the variable analyzed in the study

| Variables | Number of ewes | Mean ±SE | Coefficient Of variation (%) |
|---|---|---|---|
| Percent of ewes showing heat post CIDR removal (%) | 5 | 100.0±0.0 | 0.0 |
| Interval CIDR removal to onset of estrus (hours) | 5 | 38.4±5.87 | 34.23 |
| Interval onset of estrus to insemination (hours) | 5 | 14.4±2.4 | 37.26 |
| Total number of ovulations (corpora lutea) per ewe. | 5 | 7.0±1.41 | 45.17 |
| Proporation of ewes showing non ovulated follicles 5mm diameter or larger at surgery time (%) | 5 | 20.0±0.0 | 0.0 |
| Total number of embryos ecovered per ewe | 3 | 5.66±0.33 | 10.18 |
| Total number of non fertilized oocytes recovered per ewe | 3 | 0.67±0.66 | 173.2 |
| Total recoveries per ewe (fertilized plus non fertilized). | 3 | 6.33±0.88 | 24.11 |
| Recovery rate (%) | 3 | 79.62±0.15 | 32.97 |
| Fertility rate (%) | 3 | 91.66±0.08 | 15.74 |
| Total embryos of transferable quality (grade 1 and 2) produced per ewe. | 3 | 5.33±0.33 | 10.82 |
| Percent of embryos of good transferable quality (%) | 3 | 94.44±0.05 | 10.18 |

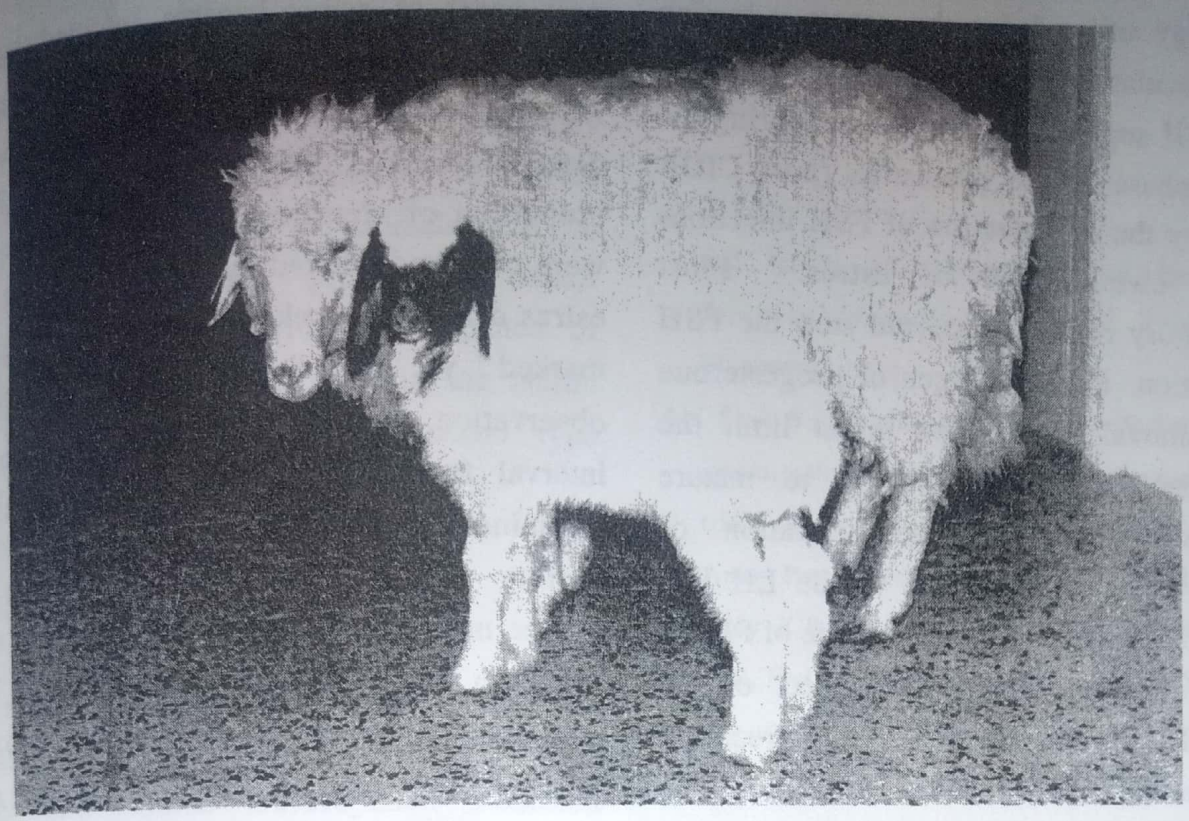

Fig. (1): The first ewe lamb produced and its foster (recipient) mother.

## DISCUSSION

The results of this trial show, even when the sample size was small, that multiple ovulation and embryo transfer (MOET) can be successfully achieved in the Naimi sheep opening the possibilities for its use in multiplication programs and genetic improvement schemes.

Overall most of the variables measured shoow a high level of performance with good response to synchronization , good ovulatory response, high fertility and embryo quality, comparable or better than MOET performances in other breeds of sheep (8,9). The MOET strategy developed by Greaney et al (7) for the Texel and Finnish Landrac ewes and applied in this trial proved effective also for Naimi sheep which had not responded to our previous attempts either with PMSG alone or with FSH alone. The combination of FSH and PMSG was reported previously as having beneficial effect in superovulation but not necessarily high fertility and recovery rates (10), the strategy used in this trial was not only the combination of FSH and PMSG but also the continuation of the FSH injections until onset of estrus and the timing of the PMSG injection at CIRD removal instead of at same time than the first FSH injection as practiced by others (10). This constitute the fundamental differencess of this approach which has been demonstrated to be highly successful in European breeds in Australia and New Zealand (7).

The strategy utilized for the estrus induction and superovulatory regime ensures that there is enough FSH support for the follicles during the follicular phase in the pro-estrus (post CIDR removal) by the continuation of FSH injections until the ewe's onset of estrus. Most superovulatory regimes reported stop the FSH administration at progestogen or progesterone pessary removal (7,8) when might limit the development of the follicles up to mature preovulatory stage. The administration of PMSG at CIDR removal due to the LH like activity of PMSG and the long half life of PMSG in the circulation mimics the effect of the pulsatile LH during the follicular phase required for oocyte and follicular maturation.

The fact that only one ewe showed some (four) non ovulated follicles of 5mm diameter or more at time of embryo flushing demonstrates that the LH surge was not affected in most of the ewes and the ovulatory process was normal. The reported negative effects of PMSG administration on the endogenous levels of FSH (11) were counteracted by the application of exogenous FSH until the follicles were mature and the ewes showed estrus.

The presence of non ovulated follicles in one of the ewes did not affect the ovulation of the other follicles in the same particular ewes but reduced the quality of the embryos obtained in comparison with the other ewes.

All insemination were done over detected estrus (ewes marked by the teaser ram), unfortunately the ewe inseminated at 24 hours post onset of estrus became infected so no evaluation of fertility was possible for that ewe. All ewes inseminated at 12 hours after being detected marked by the ram showed high proportion of fertilized oocytes, all the ewes were observed marked by the ram at the 8 PM estrus detection cheek and since they were not marked yet at the previous 12 Noon observation, they have been marked at the interval from 12 Noon to 8 PM and were inseminated at 8 AM next morning. Because the fertility was high (92%) we think that the regime must have synchronized ovulation time reduction the high variablity observed in this type of sheep in the length of estrus (1,3) and hence in the ovulation time. If we assume a 24 hours average length of estrus and ovulation time at end of onset of estrus, the ewes were inseminated approximately between 4 to 12 hours before ovulation.

It is observed for all the parameters that a high variability between individuals exist as reported for MOET in sheep elswhere.

The embryos produced had 50% , rate of survival (3 out of 6 embryos transferred resulted in lambs born) however all 3 recipient ewes (from Naimi cross breed) become pregnant. Perhaps the failure to produce twins is due to the low twining rate observed in the Naimi sheep populations rather than the inability to survive of the embryos transferred.

This trial confirms that the new strategy for MOET in sheep developed for the Texel and Finnish Landrace sheep (7) is also successful

for the Naimi sheep and perhaps could be extended as strategy for other ruminant species. New trials involving larger could be extended as strategy for other ruminant species. New trials involving larger number of animals will be implemented but this preliminary result establishes a protocol with good chance of success for future MOET work in the Naimi sheep and perhaps for other Awassi type animals.

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