**Effect of different doses of FSH on superovulation, production and quality of embryo in North Omani Cattle breed**

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**ABSTRACT**

The experiment was conducted to investigate the Ovarian response and embryo quality using different doses of follicle stimulating hormone (FSH-P) in North Omani Cattle breed (NOC) in order to determine the suitable dose. Eighteen donor NOC were divided into three groups (G1, G2, and G3) comprising of six cows in each group and treated with 20 (G1), 25 (G2) and 30 mg FSH (G3). Ovarian response was recorded as development of corpus luteum (CL), unovulated follicles (UOF), unfertilized ova (UFO), recovered embryo (RE) and transferable embryos (TE). Embryos were flushed seven days after the first AI and classified according to the developmental stage and quality. Results showed that, cows superovulated with FSH (G3) increased significantly \( P<0.05 \) number of CLs and number of RE (6.83 and 5.33 /flushed cow) compared with the G1 of FSH (3.17 and 2.00/flushed cow) and G2 (4.67 and 3.00/flushed cow). While using FSH in (G2) increased significantly \( P<0.05 \) the number of TE (excellent and good) quality, decreased average number of UOF and UFO (2.84, 3.33 and 0.67/flush) compared with FSH G3 (1.83, 5.67 and 1.50/flush). While no significant difference was observed in TE number between G1 and G2. G3 groups producing embryos at early stages of development (4 and 8 cells) and this was undesirable for embryo transfer program as this treatment (30 mg) produced more number of fair, poor and degenerated embryos compared with 20 and 25 mg FSH. Consequently, 25 mg FSH dose increased number of TE, decreased number of UOF and UFO, and that was the suitable dose for superovulation of NOC.

**Key words:** Embryo recovery, Evaluation, FSH, North Omani cattle, Superovulation.

**INTRODUCTION**

North Omani cattle had been classified as zebu cattle (*Bos Indicus*) breed (Mahgoub *et al.*, 2013). This breed is distributed in the northern parts of the Sultanate of Oman; Al-Batinah, Al-Dakhiliyah, Al-Dhahirah, Al-Sharqiyah and Muscat. They are raised under traditional systems in backyards and farms. They have the ability to resist heat and humidity during summer months. The indigenous cattle in Oman is described by Al Sinani (2015).

Commercial bovine ET is a well established, mature industry in its fourth decade of existence in many countries. The history of the development of the industry has been previously described (Betteridge, 1981; Betteridge, 2000). Among the reproductive biotechnologies that have been developed in the cattle industry, embryo transfer has been well accepted since its beginning as it was considered as a new tool for genetic improvement to increase production.

Embryo transfer was developed for researches on reproduction and extended the technology to farm animals (sheep, pigs and cattle) during the second half of the 20th century, convinced that embryo transfer could be of practical value to agriculture. Currently, almost a million bovine embryo transfers are performed worldwide each year according to the International Embryo Transfer Society (Thibier 2007) and in Europe only more than 100000 bovine ET are performed annually (S. Merton AETE 2009).

Superovulation of cattle is an efficient technique for obtaining progeny from genetically valuable females. The ovarian response of each female depends on the number of gonadotropin sensitive follicles present at the time that treatment is initiated (Driancourt, 2001). Synthetic gonadotropins are widely used for inducing superovulation in cattle. Some reports have shown many factors affecting superovulation in donor cows as the differences in nutrition, body condition score (BCS), species, and individual response to gonadotropins (Shea *et al.*, 1984), kinds of treatment (Staigmiller *et al.*, 1992), treatment timing (Goulding *et al.*, 1990), number of treatments (Bo *et al.*, 1994), effects of administration route, type and doses of gonadotropins on embryo quality in cattle (Marian *et al.*, 2015; Umut, *et al.*, 2012; Lopes da Costa *et al.*, 2001 ). Among these factors, the use of gonadotropins was one of the most effective methods to improve the efficiency of superovulation treatment. Whereas other reports have shown there is no effect of gonadotropins doses (FSH) on embryo quality (Barati *et al.*, 2006). There are several reports which indicate

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that Bos Indicus breeds have been shown more sensitivity to exogenous gonadotropins than Bos taurus (Randel, 1984) and lower doses of FSH for could be applied to Bos indicus cattle compared to Bos Taurus cattle (Lewis, 1992; Barros et al., 2003; Barati et al., 2006; Nilchuen et al., 2011). The development of practical approach to achieve enhanced responses to superovulation protocols still requires further research, especially in indigenous cattle breeds such as Omani cattle breeds.

Inducing superovulation is usually done by multiple injection of follicle stimulating hormone (FSH) in 12 hour intervals to achieve high ovulation rates. Induction of superovulation and the yield of maximum number of good quality embryos are most important factors for the success of embryo transfer.

Perusal of available literature revealed no report of applying superovulation and ET to northern Omani cattle breed. The objectives of this experiment were to evaluate the ovarian response and embryo quality using different doses of follicle stimulating hormone (FSH) for superovulation and to determine the suitable doses of FSH for North Omani cattle breed to achieve good quality embryos.

MATERIALS AND METHODS

This experiment was carried out at the Department of Animal Reproduction Research in Livestock Research Center (LRC) in Sultanate of Oman, belonging to Ministry of Agriculture during January –May 2015.

Animals: A total of 18 lactating North Omani cows from first to fourth lactations were used as donors during postpartum period at 70 ± 20 days. The cows were kept in similar conditions of handling and feeding and fed twice daily with a ration composed of Rhodes grass ad libitum and a concentrate-mineral mix according to body weigh and milk production and housed in a free-stall confinement facility.

Treatments: All animals were assigned randomly into three groups (6 cows in each) and superovulated with three levels of FSH 20, 25 and 30 mg; Pluset®, Barcelona, Spain).

Estrus synchronization and superovulation: All animals were estrus synchronized using Cloprostenol via intramuscular injection (IM) (2ml; Estrumate®). Oestrus signs were observed (visual observation of standing to be mounted by other cows in the herd, vaginal discharge) and confirmed by rectal palpation of fluctuant dominant follicles and uterine tone as described previously by Jousan et al. (2004). Method of superovulation applied in this experiment has been described by Jousan et al. (2004). Day 0 was considered as the day of the onset of standing estrus. On day 10 after the onset of standing estrus, all cows were superovulated with FSH twice daily, 12 h interval for consecutive days in decreasing doses from first day to last day, in G1 the donor injected with (G1) 20 ml as follow: 4ml AM – 4ml PM day, 3ml AM – 3ml PM day, 2ml AM –2ml PM day and 1ml AM – 1ml PM. For G2: 4.5ml AM – 4.5ml PM day, 3.5ml AM – 3.5ml PM day, 2.5ml AM –2.5ml PM day and 2ml AM – 2ml PM. For G3: 5.5ml AM – 5.5ml PM day, 4ml AM – 4ml PM day, 3.5ml AM –3.5ml PM day and 2ml AM – 2ml PM day. On day 3 of FSH injection, all animals were treated with Cloprostenol IM inj. (2ml; Estrumate®) to bring them on heat. At the first standing estrus, each animal was artificially inseminated (AI) two times with frozen-thawed semen of North Omani bull at 12 and 24 h after onset of estrus. All animals were treated with gonadotropin releasing hormone (GnRH) IM inj. (Buserelin acetate; Receptal®) at the first insemination.

Embryo recovery and evaluation: On day 7 after the artificial insemination of the donor cows, Superovulatory responses (number of corpora lutea (Figure 1), number of unovulated follicles (Figure 2)) were determined by rectal palpation and ultrasonography. To perform uterine flushing, a two-way Foley catheter was passed through the cervix into the uterine horn. The balloon was inflated with air and each uterine horn was flushed three or four times with (1000ml/cow) modified Dulbecco’s phosphate buffer saline (mD-PBS) containing 1 % fetal calf serum and 1ml antibiotic (gentamycin sulphate), and the embryos or ova were collected as described by Neto et al. (2005). The recovered lavage fluid was emptied in sterilized flask which placed in water bath at 37°C. Then, the fluid was filtered through Cup style bovine embryo filter using 0.22 micron nylon disk into 90 mm of Petri dishes. The recovered embryos were evaluated under a stereomicroscope and classified for different stages of development (i.e., oocyte, 2–8 cell, 8–16 cell, morula, compacted morula, early blastocyst, blastocyst, and expanded blastocyst) and classified for quality.
According to Reddy (1994) Excellent and good quality embryos were considered as transferable embryos.

Monitoring ovarian response: Ovarian structures were monitored by real-time ultrasonography per rectum using 7.5 MHz transducer (LS-300A; Tokyo Keiki Co.Ltd., Tokyo, Japan) and mapped on the day of flushing.

Results and Discussion

Results of 18 flushes (6 from each group) were used for the analysis. Number of corpora lutea, uniovulated follicles, unfertilized ova and total embryos per flush is depicted in Table (1).

The present results showed that, cows superovulated with 30 mg FSH group (G3) was significantly greater ($P<0.05$) in number of CLs, uniovulated follicles and number of unfertilized ova compared with the cows in G1 and G2 and were (3.17, 4.67 and 6.83 ) and (3.00 , 3.33 and 5.67/ flushed cow) and (0.00 , 0.67 and 1.50/ flushed cow) for (G1 , G2 and G3 ) respectively.

Total number of recovered embryos was significantly ($P<0.05$) higher with (30 mg) of FSH injection for superovulation compared with (20 and 25 mg) of FSH. Also, there were significant difference $P<0.05$ in recovery rate of embryos in G3 compared to G2 and G1, while no significant difference was observed between G1 and G2 $P>0.05$.

Statistical analysis: The statistical analysis was performed, using computer programme of SAS (2002) according to Snedecor and Cochran (1982). The significance differences were carried out using Duncan’s Multiple Range Test, (1955). All significant differences were set at a level of $P<0.05$.

Fig 2: Sonogram showing uniovulated follicules on the ovary of cow on day of flushing.

Fig 3: Photo of some embryos collected from donor cows of local Omani breed (A: Morula, B: compact Morula, C: Early blastocyst, D: blastocyst)
The results in Table 2 showed that the superovulation with (30 mg) of FSH injection increased significantly average number of recovered embryos at early blastocyst stages than the other groups, where as there is no significant difference $P > 0.05$ at morula stage between G3 and G2, while there is significant difference $P < 0.05$ at this stage between G3 and G1.

On the other hand, the average number of transferable embryos which is equal to (excellent and good) qualities was significantly higher for the cows treated with 25 mg FSH compared with the cows treated with 30 mg FSH, while the difference between number of transferable embryos of the cows treated with (20 and 25 mg) FSH was not significant ($P > 0.05$).

The induction of superovulation and the maximum number of good quality embryos are the most important factors for the success of embryo transfer, so the successful embryo transfer program is a program that produces many transferable embryos from proven cows and bulls to be transferred to a well prepared synchronized recipient to obtain a high pregnancy rate.

Results of the present study demonstrated that the superovulation with (25 mg) FSH yielded significantly higher number of transferable embryos and lower number of unovulated follicles than the cows treated with (30 mg) FSH. While did not differ significantly than (20 mg) FSH. Several authors had studied ovarian response for superovulation, Chagas et al., (1993) treated 57 cows with 36 and 32 mg FSH-p, found that the difference between treatments were not significant in the number of recovered embryos and number of transferable embryos. Similar results were reported by several authors (Agarwal et al., 1993. Basile et al., 1994, Arora et al., 1996., Adams, 1999., Kanitz et al, 2002., Gaur and Purohit 2007).

Some reports have shown in a number of breeds that administration of low and high doses of FSH for superovulation elicit similar superovulatory responses. Barati et al., (2006) reported that Sistani beef cattle, a native Bos indicus breed of Iran, had a similar superovulatory

### Table 2: Effect of different dose of FSH on embryonic stages and quality of embryos recovered from local Omani cows. (means ± SE).

<table>
<thead>
<tr>
<th>Items</th>
<th>FSH (20 mg)</th>
<th>FSH (25 mg)</th>
<th>FSH (30 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average number of recovered embryos/flushed cow</td>
<td>2.00 ± 0.45b</td>
<td>3.00 ± 0.26b</td>
<td>5.33 ± 0.88b</td>
</tr>
<tr>
<td>Number of recovered embryos with different embryonic stage/flushed cow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early blastocyst</td>
<td>0.50 ± 0.22b</td>
<td>0.17 ± 0.17b</td>
<td>1.00 ± 0.00b</td>
</tr>
<tr>
<td>Compact morula</td>
<td>0.67 ± 0.21</td>
<td>1.17 ± 0.31</td>
<td>0.83 ± 0.31</td>
</tr>
<tr>
<td>Morula</td>
<td>0.83 ± 0.48b</td>
<td>1.67 ± 0.33ab</td>
<td>2.00 ± 0.26b</td>
</tr>
<tr>
<td>8 cells</td>
<td>0.00 ± 0.00b</td>
<td>0.00 ± 0.00b</td>
<td>1.00 ± 0.37b</td>
</tr>
<tr>
<td>4 cells</td>
<td>0.00 ± 0.00b</td>
<td>0.00 ± 0.00b</td>
<td>0.33 ± 0.21b</td>
</tr>
<tr>
<td>Number of recovered embryos with different grades/flushed cow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excellent</td>
<td>1.00 ± 0.00</td>
<td>1.17 ± 0.17</td>
<td>0.83 ± 0.31</td>
</tr>
<tr>
<td>Good</td>
<td>1.00 ± 0.45</td>
<td>1.67 ± 0.21</td>
<td>1.00 ± 0.26</td>
</tr>
<tr>
<td>Fair</td>
<td>0.00 ± 0.00b</td>
<td>0.17 ± 0.17b</td>
<td>1.50 ± 0.22b</td>
</tr>
<tr>
<td>Poor</td>
<td>0.00 ± 0.00b</td>
<td>0.00 ± 0.00b</td>
<td>1.33 ± 0.49b</td>
</tr>
<tr>
<td>Degenerated</td>
<td>0.00 ± 0.00b</td>
<td>0.00 ± 0.00b</td>
<td>0.50 ± 0.22b</td>
</tr>
<tr>
<td>No of transferable embryos (TE)(Excellent + Good)</td>
<td>2.00 ± 0.45ab</td>
<td>2.84 ± 0.17a</td>
<td>1.83 ± 0.17b</td>
</tr>
<tr>
<td>Recovery rate of T.E (%)</td>
<td>100b</td>
<td>94.6a</td>
<td>34.33b</td>
</tr>
</tbody>
</table>

$^{abc}$ Means within the same raw with different superscripts differ significantly; $P < 0.05$. 

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response when treated with the doses of 120, 160 and 200 mg FSH. Baruselli et al. (2006) reported that Nelore cattle, a native Bos indicus breed of Brazil, treated with the doses of 100, 133 and 200 mg FSH, demonstrated the similar effect on superovulatory response. In Thailand, Sumretrpasong et al. (2008) found that the superovulatory response of Thai dairy cattle were indistinguishable when given in the different doses of 260 and 360 mg FSH. In addition, Leingcharoen et al. (2006) reported that Kao Lum Poon cows, a Thai native breed of Bos indicus, had a similar superovulatory response when treated with the doses of 150 and 200 mg FSH. Furthermore, Leingcharoen et al. (2010) demonstrated that doses of 160, 180 or 200 mg FSH did not show any differences in superovulatory response and embryo quality in Thai Black cattle (Bos Taurus X Bos indicus).

The study made by Barati et al. (2006) reported that Sistani beef cattle treated with a dose of 200 mg FSH, found that the mean number of total embryos was 8.2 and that of transferable embryos 4.3. Leingcharoen et al. (2006) reported that number of transferable embryos from Kao Lum Poon cattle treated with doses of 200 mg FSH was 1.82. In Thai Black cattle (Bos Taurus X Bos indicus), numbers of total embryos and transferable embryos from animals treated with doses of 200 mg FSH were 11.14 and 9.79, respectively (Leingcharoen et al., 2010).

Evaluation of superovulatory response in cattle is based on total number of corpora lutea plus total number of unovulated follicles. The present study in Omani cattle found that a superovulatory response were (6.17, 8.00 and 12.50) for cows treated with doses of (20, 25 and 30 mg) FSH respectively. While the mean number of total embryos were (2.00, 3.00 and 5.33) and transferable (excellent and good quality) embryos were (2.00, 2.84 and 1.83) for the cows treated with doses of (20 , 25 and 30 mg) FSH respectively, the differences between G2 and G3 were significant (P< 0.05).

In the present study, although, the dose of (30 mg) FSH injection for superovulation increased superovulatory response, but decreased number of transferable embryos, increased number of unovulated follicles, increased number of unfertilized ova and increased number of embryos at early stages of development compared with the cows treated with (20 and 25 mg) FSH and this undesirable for embryo transfer program.

In this respect, Ali et al. (2012) studied ovarian response to different doses of FSH (200, 240, 280, 320 and 360 mg. in three different genotypes of cattle – indigenous local, Pabana cattle and Friesian x Local cross. Ovarian response as corpus luteum (CL), recovered embryo (RE) and of transferable embryos (TE) count in Local were significant for 320, 280 and 280 mg respectively. In Pabna cattle CL, RE and TE count were found significant for 360, 320 and 320 mg respectively. In Friesian x Local cross CL, RE and TE count were found significant for 360, 320 and 320 mg respectively. The excellent quality embryos showed significantly the highest yield (1.80±0.20) in the 240 and 280 mg FSH levels in Local genotype. In Pabna cattle, the highest yield (2.00±0.32) was found at FSH level 320 mg. In Friesian x Local, the highest yield (2.20 ± 0.20) was found at FSH level 280 mg.

Nilchuen et al., (2011), reported that numbers of corpora lutea, total ova/embryos and transferable embryos did not differ between Kamphaeng Saen beef cattle both cow and heifer treated with a total of 200 and 250 mg (NIH-FSH-P1), they concluded that it is possible to use lower doses of FSH than that the recommended by the company for superovulation in local breed. Gonzalez et al. (1990), Hockley et al. (1992) and Mishra et al. (1990) reported that FSH binds to the limited numbers of receptors located on the granulose cells of the antral follicle in the ovary in order to activate the growth and development of follicles.

In conclusion, administration of (25 mg) FSH for superovulation in donor of local Omani breed decreased number of unovulated follicles, decreased number of unfertilized ova and increased number of transferable embryos and had beneficial effect on embryo quality compared with doses of (20 and 30 mg) FSH. It could be suggested that the dose of (25 mg) FSH for superovulation of the donor cows was the best and recommended for obtain higher number of good quality embryos for embryo transfer and realization of breeding program.

REFERENCES


