Original Research

Effect of PUFA Enriched Diet on Superovulatory Response, Embryo Quality, Embryo Recovery and Conception Rate in Cattle

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Abstract

The present study was designed to observe the effect of supplementation of PUFA enriched diet on superovulatory response, embryo quality, embryo recovery and conception in cattle. Donor (n=6) and crossbred recipient cows (n=24) were selected and divided into four groups viz; control (no treatment), T-1 (treatment given to recipient only), T-2 (treatment given to donor only) and T-3 (treatment given to both recipient and donor). The superovulatory response, mean ovulation rate ,mean embryo/ova recovered, recovery rate and average transferable embryos was found to be 75% v/s 100%, 12.25 ± 2.70 v/s 12 ± 0.71,3.5 ± 1.54 v/s 7.5 ± 1.60, 28.57% v/s 62.5 % and 50 % v/s 80 % in non-treated (control + T-1) and treated (T-2 + T-3) groups respectively. On the basis of our results, it can be concluded that flax seeds supplementation is effective in increasing above measured parameters following embryo transfer.

Key words: Conception and Ovulation, PUFA, Superovulation

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Introduction

Artificial insemination (AI), synchronization of estrous, multiple ovulation and embryo transfer (MOET), and use of various other assisted reproductive technologies may be of great importance for rapid multiplication, propagation and development of animal species (Mondal *et al.*, 2014). To improve genetic makeup of animal herds and to increase high pedigreed animal numbers quickly, embryo transfer has become the most effective tool with scientists (Weldegebriel, 2015). Poor super ovulatory response, improper storage and higher embryo mortality leading to lower conception rate in recipient animals, are some of the major problems limiting large scale field application of this technology. Management of reproductive functions by supplementation of fat source in cattle is fairly a recent development. Feeding of fat supplement affect reproduction by affecting the dominant follicle's size, decreasing the interval to first postpartum ovulation in cattle and buffaloes, increasing the luteal phase progesterone concentration, manipulating uterine prostaglandin synthesis, improving embryo quality and developmental competence (Santos *et al*., 2008).The supplementation of flaxseed improved conception rates , embryo number and quality and decreased pregnancy losses in dairy cows (Thangavelu, 2007; Staples *et al*., 1998).

Materials and Methods

Superovulation and Synchronization of Animals

The present study was conducted on Sahiwal and crossbred cattle at Instructional Dairy Farm, Nagla, G. B. Pant University of Agriculture and Technology, Pantnagar-263145, Udham Singh Nagar (Uttarakhand). Normal cyclic high producer Sahiwal cows (n=6) as donor and crossbred cattle (n=24) as recipient were selected on the basis of their breeding records and per-rectal examination of their genital organs. Donors were divided into two groups i.e. control (n=3) and treatment (n=3). Recipients were divided into 4 groups of 6 animals i.e. 2 group of control and 2 group of treatment. All the donor animals were super ovulated by using FSH-P (folltropin-V 400mg NIH-FSH-P1, Canada) @ 320 mg/animal as total dose given at 12 hour interval in 8 divided doses in constant dose schedule i.e. 40mg each in morning and evening by IM route starting from day 9 (Day 0 = day of estrus) of donor estrous cycle and continued for 4 days. The super estrus in donor cows was induced with IM injection of cloprostenol @ 500 µg i/m (VETMATE™, VETCARE) with 5th dose of FSH-P. Recipient cows were synchronized using double injection of cloprostenol@ 500µg IM, at 10 day interval. The superovulated donor cows were inseminated 3 times at 12 hours interval using good quality frozen semen of pedigree Sahiwal bull.

Embryo Collection and Transfer

The embryos were collected on 7th day of super ovulatory estrus by nonsurgical method. The embryos were then given five washings in the holding medium and were evaluated under stereo zoom microscope at 400x magnification for their developmental stages as well as quality. Using sterile condition, the transferable embryos were loaded in mini straw as per standard protocol and were transferred at tip of uterine horn by ET gun epsilateral to ovary bearing corpus luteum.

Ultrasonographic Examination

Monitoring of follicular development and super ovulatory response was performed by B-mode ultra-sonographic (DIGI 600, PRO VET, SS medical, India) scanning of ovaries. Pregnancy diagnosis was done on 45th day of embryo transfer.

Statistical Analysis

The data obtained during experimentation were analysed for mean, standard error, and coefficient of correlation. Differences between means were compared using two sample t-test and One-way ANOVA and completely randomized design (CRD).

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Results and Discussion

Super ovulatory Response and Embryo Recovery

A total of 08 flushings were taken from 06 Sahiwal donor cows. In non-treated (control + T-1) group out of four, three animals responded to FSH treatment while in treated (T-2 + T-3) group all four animals responded. A total number of 97 ovulation were counted by per-rectal palpation, 49 in non-treated (control + T-1) and 48 in treated (T-2 + T-3) group and confirmed by ultrasonographic examination of both ovaries. The total embryos/ova recovery were 14 in non-treated (control + T-1) and 30 in treated (T-2 + T-3) group. The mean ovulation rate (number of C.L. palpated), was 3.5 ± 1.54 and 7.5 ± 1.60 in non-treated (control + T-1) and treated (T-2 + T-3) group respectively while embryo recovery rate was 28.57% and 62.5 % respectively. There was no significant difference found between non-treated and treated groups for super ovulatory Response and embryo recovery but value of these parameters were found to be higher in treated group.

The overall superovulatory response was 87.5 % which is similar to Malik (2017) as 90%, Rawat (2004) as 89.47% and Ferrira *et al.* (2014) as 85.7%, but it was higher than Betteridge *et al.* (1980) as 76.5% and Prasad (2000) as 78.95%. One animal did not respond to FSH treatment in which only two palpable CL was found. Baruselli *et al.* (2006) observed that 20-30% donors were unresponsive to superovulatory protocol. Previous studies have also concluded that usually 33 % animal did not respond to superovulatory regimen even after following standard superovulatory protocol (Misra and Pant, 2006) (Table 1 and 2).

Table 1: Superovulatory response in cows following Folltropin-V treatment (n=6)

Attributes	Treated (T-2 + T-3)	Non-Treated (Control + T-1)
Animal Responded	4	3
Animal failed to respond	0	1
Total flushing	4	4
Percentage	100%	75%

Table 2: Embryo/Ova recovery rate in cows following Folltropin-V treatment (n=6)

Attributes	Non-Treated (Control + T-1)	Treated (T-2 + T-3)
Number of CL	49	48
Mean of No. of CL	12.25 ± 2.70	12 ± 0.71
Embryo recovery	14	30
Mean embryo recovery	3.5 ± 1.54	7.5 ± 1.60
Recovery rate	28.57%	62.50%

The results of the superovulatory treatment used in the present experiment indicate that there were more number of C.L. i.e. mean ovulation rate (~11) as higher than reported by Beg *et al.* (1996). However, overall ovulation rate (12.13 ± 1.83) was similar to number of corpus lutea found in Nguni cows 11.3 ± 1.41 (Maqhashu *et al.*, 2015). Ovulation rate was higher than 9.5 CL (Mikkola and Taponen, 2017), 8.8 CL (Karaivanov *et al.*, 1990) and 7.1 ± 4.44 CL (Posadas *et al.* 1991) but it was lower than 16.1 CL (Hay *et*

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al., 2010). The overall embryo recovery rate of 45.36% in present study was nearly similar to 39.27% (Sharma, 2004) and 35.57% (Prasad, 2000). It was lower than 65.63 % (Malik, 2017), 69.7% (Acosta *et al.*, 2016) and higher than 31.76% (Sheetal, 2017), 25% (Maithani, 2017). Mean Embryo/ova recovery was 5.5 ± 1.64 which was similar to 5.4 ± 0.8 (Kohram *et al.*, 1998), but lower than 6.2 ± 4.7 (Posadas *et al.*, 1991), 6.4 (Hay *et al.*,1990) and higher than 4.8 (Betteridge *et al.*,1980).

In one animal in which palpable CL were 11, only one embryo could be recovered with recovery rate of 9.01%. The lower embryo recovery rate in relation to the number of ovulation may be due to inability to trap ova from enlarged superovulatory ovary (Ullah *et al.*, 1992), difficulties in locating hatched blastocyst (Alexiev *at al.*, 1998) and premature entry of ova/embryo into the uterus, resulting into their expulsion (Karaivanov *et al.*, 1990).

Embryonic Development and Quality

A total 44 embryo/ova, 14 in non-treated (control + T-1) and 30 in treated (T-2 + T-3) group were recovered from Sahiwal donor cows with a mean of 5.5 ± 1.64 embryos/ova per animal per flush. A total of 31 good quality embryos of transferable stage were recovered. A total number of unfertilized, 4-16 cells, morula, blastocyst, degenerated and hatching embryo/empty zona recovered were 0, 4, 13, 11, 2 and 0 respectively in treated (T-2 + T-3) group. On pooling the data, mean total unfertilized, 4-16 cells, morula, blastocyst, degenerated and hatching embryo/empty zona recovered were 0, 1 ± 0.41, 2.75 ± 0.93, 3.25 ± 1.20, 0.5 ± 0.20 and 0 respectively. The percentage of recovery of unfertilized, 4-16 cells, morula, blastocyst, degenerated and hatching embryo/empty zona were 0%, 13.33%, 36.67%, 43.33%, 6.67% and 0% respectively are presented in Table 3 and 4.

Table 3: Stages of embryo recovered from Sahiwal (Non-treated, control + T-1) donor cows

S. No.	Embryo Stages	Number	Mean	Percentage (%)
1	Blastocyst	2	0.5 ± 0.20	14.28
2	Morula	5	1.25 ± 0.90	35.71
3	4-16 cells	5	1.25 ± 0.53	35.71
4	Degenerated	2	0.5 ± 0.35	14.28
5	Total embryos/ova	14	3.5 ± 1.54	

Table 4: Stages of embryo recovered from Sahiwal (treated, T-2 + T-3) donor cows

S. No.	Embryo Stages	Number	Mean	Percentage (%)
1	Blastocyst	13	3.25 ± 1.20	43.33
2	Morula	11	2.75 ± 0.93	36.67
3	4-16 cells	4	1 ± 0.41	13.33
4	Degenerated	2	0.5 ± 0.20	6.67
5	Total embryos/ova	30	7.5 ± 1.60	

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The number of blastocyst and morula increased in treated group than non-treated group while others embryonic stages had no difference. The overall blastocyst and morula recovery was similar to that of Sheetal (2017) who reported 2.17 ± 0.51 and 2.00 ± 0.64 mean recovery of blastocyst and morula respectively. The stages of embryo recovered from Nguni cow code I and code II, 2.5 ± 1.0 and 1.3 ± 0.59 respectively were slightly similar whereas overall unfertilized ova and degenerated embryo 5.5 ± 1.05 and 3.7 ± 1.00 respectively were higher (Maqhashu *et al.*, 2015). 4-16 cell embryo stage recovered 25.71 % (Prasad, 2000) was slightly similar with respect to non-treated but higher with respect to treated in present study. The overall recovery percentage of morula was 45.71 % (Prasad, 2000) which was higher than the present study. The total mean of blastocyst and grade 1 embryo were 4 ± 0.94 and 3.6 ± 0.1 respectively (Maithani, 2017; Shaw and Good, 1999). The mean total good quality embryos were similar to 4.5 ± 3.9 embryos (Takahashi *et al.*, 2013) but lower than 5.7 ± 0.2 (Shaw and Good, 1999), 6.5 ± 1.7 (Carvalho *et al.*, 2014) and 7.1 (Mikkola and Taponen, 2017) while it was higher than mean total of 1.37 ± 0.43 viable embryos (Rawat, 2004) and 1.00 ± 0.63 (Sharma, 2004).

Totey *et al*., 1996 reported that high dose of FSH can also cause hypersecretion of progesterone 2-4 days post estrus. Embryos are normally in oviduct during this period and do not enter the uterus until day 4. However, abnormally high level of progesterone may alter normal estrogen and progesterone ratio and eventually force the embryos prematurely into the uterus. The uterine environment, being hostile to embryo prior to day 4, may halt cleavage and causes degeneration of embryo.

Conclusion

Superovulatory response was 100% v/s 75% and recovery rate was 62.5 % v/s 28.57 % in treated v/s non treated donor groups. Conception could occur only in the recipients in which donor as well as recipients were supplemented with flax seed in their diet. On the basis of our results, it can be concluded that flax seeds supplementation is effective in increasing the superovulatory response, embryo quality, embryo recovery thus it may be beneficial in improving conception rate following embryo transfer.

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