ULTRASONOGRAPHIC ELECTIVE SUPEROVULATION AND ITS RESPONSES IN CROSSBRED AND ZEBU CATTLE*

D.V. PATEL, N.N. CHAUDHARI, S.R. PATIL, J.G. BHATOL, S.D. BHALODIA AND C.P. DEVANAND

Sabarmati Ashram Gaushala
Bidaj Farm, PO- Lali, Kheda, Gujarat-387 120

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ABSTRACT

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In order to improve the total and viable embryo recovery rates, ultrasonography was used in cattle before induction of superovulation (SOV). The conventional SOV protocol followed in crossbred and indigenous donors at Sabarmati Asheram Gaushala was complemented by use of ultrasonography for selection of donors a day before stimulation. Donors with minimum 5 growing follicles were used for SOV. Dominant follicle (DF) if any was ablated 24 hours before SOV treatment. Total 54 donors were programmed from April 2010 to April 2011, out of which 49 were flushed with recovery of 362 (7.39) total embryos and 219 (4.47) viable embryo from 500 (10.20) ovulations; followed by 27 pregnancies from 114 embryo transfers. Data was analyzed for cross bred group (ablation vs non ablation) and indigenous group (ablation vs non ablation). Crossbred group with ablation showed highest average 12.26 ± 1.49 (mean ± SE) ovulations, 11.29 ± 1.66 total embryos and 6.18 ± 1.20 viable embryos. Indigenous group with ablation had an average of 10.13 ± 1.62 ovulations, 6.38 ± 1.59 total embryos and 5.63 ± 1.24 viable embryos. Selection of donor using ultrasonograpghy and selective ablation of dominant follicle improved the SOV responses.

Key words : Ultrasonography, ablation, superovulation, embryos.

INTRODUCTION

Ovarian response to the superovulation treatment vary between donors, within cycles of the same donor, stage of lactation, parity, etc. Limited availability of quality disease free donors is the major constraint in carrying out MOET programmes. Recent advances in understanding of the follicular waves and role of dominant follicle has now given an insight regarding the variations in number of follicles available before SOV programme.

The presence of dominant follicle of first follicular wave inhibits follicular responses to supervoulatory treatments. But ablation of dominant follicle before initiating SOV treatment has shown to increase follicular recruitment and ovulatory responses (Wolfsdorf *et al*, 1997). The population of small antral follicles at the time of follicle ablation is the most important factor affecting results as it is significantly correlated to the number of viable and transferable embryos produced (Durocher *et al*, 2006).

MATERIALS AND METHODS

Elite HF X S, HF X G, HF X K and J X S cross breds, HF and Jersey pure breds and Sahiwal, Gir and Red Sindhi indigenous donors in 2nd to 7th lactation were selected for the study. Crossbred and exotic animals were machine milked while indigenous animals were hand milked and allowed to suckle till two months after calving. Recipients were either lowest producers of farm, young lactating cows or crossbred heifers. All animals were maintained as per the standard management practices. Deworming, Vaccination and disease testing were done as per the standard health protocol.

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[2 and 4] Assistant Professor, [3] Head department of Animal Reproduction, PGIVAS, Akola.

Based on history of previous estrous cycle, donors and recipients were either synchronized using single PG or were selected after natural heat. Animals in standing estrus were checked per rectally for uterine tone and later on 9th to 11th day for development of CL. A day previous to initiation of SOV all donors were subjected to ultrasonograhy using B mode scanner (Aloka SSD 500) equipped with 7.5 MHz convex array vaginal probe. The dominant follicle/s if available was ablated as described by Bergfelt *et al*, 1994; number of even sized follicles available on both the ovaries was examined before starting of SOV treatment. If the total number of follicles were less than 5, the donor was excluded from the programme. Same donor was also repeated during the study period. SOV was done using either 200/400 mg (equally divided constant dose) of Folltropin V (Porcine Follicle Stimulating Hormone) and estrus was induced by injecting single dose of Prostaglandin $F_{2\alpha}$ (50 mg dinoprost tromethamine, Lutalyse) 48 hr. from first FSH injection. Artificial insemination (AI) was carried out three times at 12 hrs. interval during the estrus with the first AI starting at the onset of standing estrus. Flushing was carried on day 7 as per the standard procedure (Misra *et al.*, 1990) using 18 G Rusch catheter (Minitub, Germany) and DPBS media (IMV, France) added with 0.1% Bovine Serum Albumin (BSA, Fraction V, Sigma). All the recovered embryos were evaluated as per standards given by International Embryo Transfer Society Manual (IETS). Based on the availability of recipients, fresh embryos transfers were made in recipients and the surplus embryos were frozen using 1.5M ethylene glycol in cryologic CL 500 biofreezer or vitrified using vitrification kit obtained from Cook India.

Data were analysed using standard statistical methods given by Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

The summary of the results obtained from flushing, transfers and pregnancy rates along with techno economics is shown in Table. Total 54 donors were programmed from April 2010 to April 2011, out of which 49 were flushed with recovery of 362 (7.39) total embryos and 219 (4.47) viable embryo from 500 (10.20) ovulations; followed by 27 pregnancies from 114 embryo transfers. Overall 9.26% donors didn't respond to SOV which is higher than the results obtained by Mutharao *et al.* 2010 (5.17%).

Ablation of dominant follicle/s 24 hours before SOV treatment has shown positive effect on total ovulations, total embryo and viable embryos recovery. Total ovulations (mean ± SE) in indigenous animals was 10.13 ± 1.62 in the Ablation group as compared to 8.25 ± 1.37 in the Non ablation group showing non significant increase in the ablation group. In a similar study in indigenous animals by Mutharao *et al.* 2009, ovulations in ablation group were 15.08 ± 1.5 and 13.53 ± 1.80 in control group (non ablation), much higher than in the present study. Crossbred group showed a highly significant increase in ovulations with 12.26 ± 1.49 in ablation group and 5.80 ± 1.13 in non ablation group. The results are in agreement with the significant difference obtained by Shaw and Good, 2000 and Amiridis *et al.* 2006. As shown in table 1, 72% donors were having e" 10 ovulations in ablation group while only 33% donors were having e" 10 ovulations in non ablation group, thus showing significant effect of ultrasonographic selection of donors. In non ablation group one donor was flushed 4 times. Only once viable embryos were recovered.

Recovery rate was 78% in cross bred donors and 61% in indigenous donors. The difference is significant due to one indigenous donor (SW-39) where no embryo was recovered in two flushing with 7 and 16 ovulations. In indigenous donors TE was non significantly higher in ablation group (6.38 ± 1.59) than in non ablation group (5.36 ± 1.32). But the difference was significantly higher in crossbred donors in ablation group (11.29 ± 1.66) when compared to non ablation group (4.62 ± 1.33). TE recovery obtained post ablation of 2 largest follicle here is similar (11.0 ± 1.4) to the results obtained by Baracaldo *et al.* 2000. Out of total 25 donors flushed in ablation group, 18 donors produced more than 5 TE and only one indigenous donor did not produce any embryo. While out of 24 donors flushed in non ablation group, 11 donors produced more than 5 TE and three

donors did not produce any embryo. As emphasized by Durocher *et al* 2006, the practioner needs to differentiate between low responder (lower than average recovery) and lower potential (limited antral follicles) animals. In this study, both types of kind donors were selected keeping the minimum available follicle before SOV to be at least five. Low potential animals with exceptional high milk production were also selected as even a single pregnancy per flush was important for the breeding programme at the farm. Recovery rates were also affected by the persons flushing the donors.

Overall ablation group yielded 6.00 ± 0.89 viable embryos (highly significant difference), while non ablation group yielded only 2.88 ± 0.72 viable embryos. Average VE in indigenous donors were 5.63 ± 1.24 in ablation group and 3.64 ± 0.98 in non ablation group, nearly 2 viable embryos more (non significant difference) in ablation than non ablation group. Similarly In crossbred donors, ablation group yielded 6.18 ± 1.20 VE significantly higher than non ablation group which yielded nearly 4 embryos less per flush at 2.23 ± 1.03. Of the total embryos recovered, indigenous ablation group showed an excellent 88 % VE recovery, while crossbred non ablation group showed lowest of 48% VE of total embryos recovered. Mutharao *et al.* 2010, had also reported 94.77% fertility rates in indigenous animals. The poor performance in crossbred non ablation group might be due to high number of an ovulatory follicle at 2.20 ± 1.31. Also cross bred ablation donors produced nearly 55% of VE of the TE, which might be due to hyper responsiveness to SOV or due to untimely ovulations. This was shown by three donors producing more than 20 TE each out of which nearly 10 embryos in each donors were degenerated. Nearly 77% donors in crossbred non ablation group produced less than 5 VE and 46% didn't produce any viable embryo. As noted earlier, one donor was flushed 4 times but only once viable embryos was recovered.

Pregnancy rates following fresh embryo transfer was 24%. Embryo transfer from indigenous donors produced 30% pregnancies while crossbred donors produced 23 % pregnancies. The reasons for lower pregnancy rates may be 1) only 50% VE produced were grade I, while 34% were grade II and 16% were grade III embryos, 2) if more number of VE were produced and less number of recipients were available than Grade I embryos were used for freezing and remaining were used for fresh ET, 3) as on farm only fixed number of recipients were available, same recipients were used again and again 4) Effect of practitioner was seen as those in training transferred 50% embryos resulting in 10 % pregnancy rates. As reviewed by Bo *et al,* (2002) control of follicular wave dynamics in recipients using various technics like ovsynch, use of oestradiol and progesterone and use of eCG could improve upon the pregnancy rates.

Cost per flush was nearly same for both ablated and non ablated group, but cost per viable embryo produced showed significant difference between groups- Rs. 1150 in ablated and Rs. 2573 in non ablated group. Thus dominant follicle ablation and ultrasound scanning before initiation of SOV reduces the cost of viable embryos produced thereby reducing the cost per pregnancy by ET. Petrikovic and Svetlanska, (1991) Broadbent, (1992) and Hasler *et al.,* (1987) had emphasized the need to have the best embryos and recipients with good health and condition to achieve the best results.

Ultrasonography can be used as a tool for selection of donors before initiation of superovulation on the farm. More number of viable embryos can be obtained from fewer number of flushing thus reducing the cost of embryo produced.

TABLE : DETAILS OF FLUSHING, RECOVERY RATES, TRANSFERS AND TECHNO-ECONOMICS.

Attributes	Indigenous group		Cross bred group		Overall	
	Ablation	Non Ablation	Ablation	Non Ablation	Ablation	Non Ablation
No. of donors programmed	8	12	19	15	27	27
No. of animals flushed	8	11	17	13	25	24
Total ovulations (nos.)	10.13 ± 1.62 (81)	8.25 ± 1.37 (99)	12.26 ± 1.49[a] (233)	5.80 ± 1.13[a] (87)	11.63 ± 1.15[b] (314)	6.89 ± 0.89[b] (186)
No. of donors < 10	4	6	3	10	7	16
No. of donors ⩾10	4	5	14	3	18	8
Total anovulatory follicles (nos.)	1.00 ± 0.87 (8)	0.58 ± 0.35 (7)	1.00 ± 0.58 (19)	2.20 ± 1.31 (33)	1.00 ± 0.47 (27)	1.48 ± 0.75 (40)
Total embryos recovered (nos.)	6.38 ± 1.59 (51)	5.36 ± 1.32 (59)	11.29 ± 1.66[a] (192)	4.62 ± 1.33[a] (60)	9.72 ± 1.30[b] (243)	4.96 ± 0.92[b] (119)
No. of donors < 5	2	5	5	8	7	13
No. of donors ⩾5	6	6	12	5	18	11
Total fertilized ova (nos.)	6.13 ± 1.55 (49)	5.00 ± 1.31 (55)	9.24 ± 1.72[a] (157)	2.85 ± 1.22[a] (37)	8.24 ± 1.29[b] (206)	3.83 ± 0.90[b] (92)
Total viable embryos (nos.)	5.63 ± 1.24 (45)	3.64 ± 0.98 (40)	6.18 ± 1.20[c] (105)	2.23 ± 1.03[c] (29)	6.00 ± 0.89[b] (150)	2.88 ± 0.72[b] (69)
No. of donors < 5	3	5	8	10	11	15
No. of donors ⩾5	5	6	9	3	14	9
UFO	2	4	35	21	37	25
Degenerated	4	15	52	8	56	23
Embryos Frozen	9	5	33	9	42	14
Embryos (Recipients)	36 (24)	35 (24)	72 (45)	25 (21)	108 (69)	60 (45)
Pregnancy % (nos.)	29.17 (7)	37.50 (9)	24.44 (11)	19.05 (4)	26.09 (18)	20.00 (9)
Cost per flush (Rs.)	7382	6755	6719	8002	6898	7396
Cost per viable embryo (Rs.)	1312	1858	1088	3587	1150	2573

[a, b] -Values with same superscripts in a group indicates highly significant difference (P<0.005)

[c] -Values with same superscripts in a group indicates significant difference (P<0.05)

REFERENCES

1. Amiridis G.S., Tsiligianni T. and Vainas E. 2006. Follicle ablation improves the ovarian response and the number of collected embryos in superovulated cows during the Early stages of lactation. *Reprod. in Dom. Ani.,* **41:** 402-407.

2. Baracaldo M.I., Martinez M.F., Adams G.P. and Mapletoft R.J. 2000. Superovulatory response following transvaginal follicle ablation in cattle. *Theriogenology*, **53:** 1239-1250.

3. Bergfelt D.R., Lightfoot K.C. and Adams G.P. (1994) Ovarian synchronization following ultrasound-guided transvaginal follicle ablation in heifers. *Theriogenology,* **42:** 895-907.

4. Bo G.A., Baruselli P.S., Moreno D., Cutaia L., Caccia M., Tribulo R., Tribulo H and Mapletoft R.J. 2002. *Theriogenology,* **57:**53-72.

5. Broadbent, P.J.; (1992) Featured article: Management of bovine embryo transfer recipients. EmbryoTransfer Newsletter, Vol.10 No. 4:18-23.

6. Durocher J., Morin N. and Blondin P. 2006. Effect of hormonal stimulation on bovine follicular response and oocyte developmental competence in a commercial operation. *Theriogenology,* **65:** 102-115 Epub 2005 Nov 2.

7. Hasler, J.F., McCauley, A.D.,Lathrop, W.F. and Foote, R.H. (1987) Effect of donor-embryo-recipient interactions on pregnancy rate in a large-scale bovine embryo transfer program. *Theriogenology,* **27:**139-168.

8. International Embryo Transfer Society Manual. Chapter 9: Certification and identification of the embryo by Irma Robertson and Richard E.Nelson, 103-134.

9. Misra, A. K., Joshi, B.V., Agarwal, P.L., Kasiraj, R., Siviah, S., Rangareddy, N.S. and Siddique, M.U. (1990). Multiple ovulation and embryo transfer in Indian buffaloes (Bubalus Bubalis). *Theriogenology,* **33:** 1131-1142.

10. Mutharao M., Umamahesh Y., Venugopal Naidu K., Baburao K. and Misra A.K. (2009) Ovarian superstimulation after ablation and steroid induced follicular wave synchronization in zebu cattle. *Ind. J. of Ani.Sci.,* **79** :(3) 247-250.

11. Mutharao M., Umamahesh Y. and Misra A.K. (2010). Evaluation of ovarian response and embryo production pattern in ongole cows. *Ind. J. of Ani.Sci.,* **80** :(10) 973-975.

12. Otoi T., Koyama N., Yamamoto K. and Tachikawa S. 1998. Superovulatory response in beef cows following removal of the largest ovarian follicle. *Vet. Rec.,* **42:** 402-403.

13. Patel D.V., Dhanasekaran N., Nikhate R.V., Kunju M. and Kasiraj R. 2008. Techno-economics involved with the on farm production of pure jersey embryos in Ooty, Nilgiris. *Ind. J. of Ani.Sci.,* **78 :** (4) 422-424.

14. Petrikovic, P.and Svetlanska, M.(1991), Study of relations between nutritional value of rations and success in embryo transfers in heifers. *Archiv Fur Tierernahrung,* **41:** (5) 551-558.

15. Shaw D.W. and Good T.E 2000. Recovery rates and embryo quality following dominant follicle ablation in superovulated cattle. *Theriogenology,* **53 :** (8) 1521-1528.

16. Snedecor G.W. and Cochran W.G. (1989) Statistical methods. 8th edition. The Iowa state university press, Ames, Iowa, USA.

17. Wolfsdorf KE, Diaz T, Schmitt EJ, Thatcher MJ, Drost M, Thather WW. (1997). The dominant follicle exerts an interovarian inhibition on FSH-induced follicular development. *Theriogenology,* **48** : (3) 435-47.