

## EFFECT OF TWO DIFFERENT DOSES OF FSH-P (FOLLTROPIN-V) ON SUPEROVULATION IN BERARI (NAGPURI) BUFFALOES (*Bubalus bubalis*)

A.R. Ratnaparkhi<sup>1</sup>, C.H. Pawshe<sup>2</sup>, S.K. Sahatpure<sup>2</sup>, S.A. Chede<sup>2</sup>,  
A.P. Gawande<sup>2</sup>, M.S. Patil<sup>2</sup> and R.P. Kolhe<sup>2</sup>

### ABSTRACT

In the present study, superovulatory response was determined in eight healthy matured Berari (Nagpuri) buffaloes which were divided in two groups (A and B). The superovulatory treatment was started in donor buffaloes from the 10<sup>th</sup> day after induced estrus. Animals in Group A and Group B were administered, respectively, with 500 mg and 560 mg FSH-P in descending doses (100, 75, 50, 25 mg and 100, 80, 60, 40 mg twice daily) for four consecutive days. PGF<sub>2α</sub> (Lutalyse) 25 mg was injected to all the animals in both groups after 72 h of initiation of superovulatory treatment. Oestrus was recorded in all the buffaloes under study. Onset and length of superovulatory oestrus in Groups A and B were recorded to be 20.00±0.00 h, 36.50±5.50 h and 19.00±0.00 h, 38.75±3.32 h, respectively. Per rectal assessment of superovulatory response on the 6<sup>th</sup> day after the last FSH-P injection revealed the average number of corpora lutea and unovulatory follicles per donor buffalo in Group A and Group B to the tune of 3.00±0.71, 1.00±0.00 and 2.75±0.48, 1.50±0.29, respectively. No significant difference was recorded in terms of superovulatory response in either group at 5 percent level ( $P < 3.185$ ).

**Keywords:** buffalo, Berari (Nagpuri), superovulation

### INTRODUCTION

Indian buffaloes constitute more than 50 percent of the world buffalo population. Region specific breeds of buffaloes are still reared in rural India with Murrah as an exception. The Berari (Nagpuri) buffalo is a native breed of the Vidarbha region of Maharashtra. This breed is common in Akola, Buldhana, Yeotmal, Washim, Amravati and Nagpur districts. Infertility is the most common cause of reproductive failure especially in buffaloes. Silent estrus coupled with low conception rate, seasonality of breeding, higher optimal age at first calving, anoestrus and repeat breeding are some of the major constraints associated with buffalo reproduction. Although, artificial insemination (AI) technique has significantly enhanced timely conception in milch animals, problems of infertility and silent oestrus in buffaloes are still persistent. Oestrus synchronization and superovulation coupled with embryo transfer technology are proved to be promising tools in animal reproduction and has been found to be advantageous in national breeding

<sup>1</sup>Department of Veterinary Public Health KNP, College of Veterinary Science, Shirwal, Tal-Khandala, Dist-Satara, Maharashtra-412 801, India, E-mail: dranandratnaparkhi@gmail.com

<sup>2</sup>Department of Animal Reproduction, Post Graduate Institute of Veterinary and Animal Sciences, Krishinagar Akola-444104, Maharashtra, India

programme. The process of superovulation means the increased capacity of the dam to produce a greater number of ova by injecting gonadotrophins. This technique is highly useful for the improvement and conservation of native germplasm and increasing number of offsprings from donor females at faster rate (Misra *et al.*, 1990, Patel *et al.*, 2010).

The superovulation of donor females has been traditionally done by the single intramuscular injection of pregnant mare serum gonadotropine (PMSG) but, nowadays injections of pituitary extract containing follicle stimulating hormone (FSH-P) are used. FSH is usually given within a period of 4 to 5 days at the mid luteal stage of the oestrus cycle through a series of injections so that follicles are recruited immediately prior to the lysis of the existing corpus luteum. The results of superovulation in buffalo are disappointing when compared with those in cattle (Lindsell *et al.*, 1986). FSH-P preparations proved to be effective in superovulating cattle and produce no evidence of reduced embryo quality at high dosage (Wang *et al.*, 1988; Mapletoft *et al.*, 1988). Information about superovulation in Berari (Nagpuri) buffaloes is lacking in the available literature. Therefore, the present study was proposed with the objective of investigating the effect of two different doses of FSH-P (Folltropin -V) on superovulation in Berari buffaloes.

## MATERIALS AND METHODS

**Selection of animals:** Eight (n=8) normal, healthy, cyclic Berari (Nagpuri) buffaloes free from any pathological and reproductive disorder were selected from the experimental animals of the ICAR - Embryo Transfer Technology project being implemented at the Department of Gynaecology,

Dr. Panjabro Deshmukh Krishi Vidyapeeth, campus Akola. All the animals were maintained under uniform stall fed condition throughout the study.

**Synchronization and Superovulation:** All buffaloes showing regular cyclic activity for two consecutive cycles and those with an active corpus luteum were selected and oestrus was synchronized by administering 25 mg Lutalyse (prostaglandin F<sub>2</sub> alpha analogue) intramuscularly on day 0 and day 11. Synchronization of oestrus was determined on the basis of 1) efficacy of PGF<sub>2α</sub> for induction of synchronized oestrus, 2) time required for the onset of oestrus and 3) length of synchronized oestrus. Eight buffaloes, synchronized by injection PGF<sub>2α</sub> were divided into two groups (A and B). Folltropin-V (FSH-P) was used for superovulation. Superovulatory treatment commenced from the 10<sup>th</sup> day during the mid luteal stage of the oestrus cycle. The protocol for superovulation is shown in Table 1.

Experimental buffaloes were closely observed after treatment for induction of oestrus. The superovulatory response was estimated by per rectal palpation on day 6 after the last FSH-P injection.

**Data analysis:** The data collected were analyzed statistically and the difference in superovulatory response was tested using the student's t-test.

## RESULTS

**a) Oestrus response and the time required for onset of oestrus:**

The intramuscular injection of 25 mg Lutalyse was given to eight normal cyclic Berari (Nagpuri) buffaloes. Of the eight buffaloes, seven

(87.50 percent) exhibited oestrus, and on the 11<sup>th</sup> day the second intramuscular injection at same dose was again given. All buffaloes (100.00 percent) exhibited oestrus to the 2<sup>nd</sup> dose. The results of synchronization of oestrus are shown in Table 2.

#### **b) Length of oestrus:**

The length of synchronized oestrus after the first PGF<sub>2α</sub> injection ranged from 22 to 28 h with an average of  $20.80 \pm 3.07$  h. However, after the second injection of PGF<sub>2α</sub>, the length of oestrus was slightly higher (22-36 h) with an average of  $26.50 \pm 1.59$  h. The mean time required for induction of oestrus after the first and the second injection of PGF<sub>2α</sub> (Lutalyse) were  $36.75 \pm 6.10$  h and  $34.75 \pm 2.74$  h, respectively.

#### **c) Onset and duration of superovulatory oestrus:**

The time required for onset of oestrus after superovulatory treatment in Group A buffaloes was  $20.00 \pm 0.00$  h and that in Group B was  $19.00 \pm 0.00$  h. The aggregate average of onset of superovulatory oestrus in both the groups was  $19.50 \pm 0.18$  h. The calculated 't' value (2.00) for both groups elicited non-significant differences at 5 percent level of significance (3.182).

The length of oestrus during superovulatory treatment in Group A buffaloes was  $36.50 \pm 5.50$  h and that in Group B buffaloes was  $38.75 \pm 3.32$  h; however, the length of superovulatory oestrus in Group A was 26-46 h and while that in Group B was 29-44 h.

**d) Superovulatory response:** On the basis of number of palpable corpora lutea and unovulatory follicles on both the ovaries, the superovulatory response was graded manually. In the present studies, the number of corpora lutea in Group A

buffaloes treated with 500 mg FSH-P ranged from two to five with an average of  $3.00 \pm 0.71$ , whereas in Group B buffaloes treated with 560 mg FSH-P, the number ranged from two to four with an average of  $2.75 \pm 0.48$ . Calculated 't' value (0.245) elicited non-significant differences between the two groups (3.182) at 5% level. The number of unovulatory follicles in Group A buffaloes ranged from zero to one with an average of  $1.0 \pm 0.00$ , whereas the number in Group B buffaloes ranged from one to two with an average of  $1.5 \pm 0.26$ . No significance difference was recorded between the two groups. Numbers of corpora lutea were nonsignificantly higher with 500 mg FSH-P dose as compared to 560 mg FSH-P.

## **DISCUSSION**

In the present study, the time required for onset of oestrus was shorter; however, longer onset times have been recorded earlier by Pant and Singh (1980), Kamonpatana *et al.* (1979), Rajeshwaran *et al.* (1992) who recorded the time required for onset of oestrus to be  $50.33 \pm 9.46$  h,  $69.3 \pm 5.6$  h,  $71.33 \pm 6.38$  h, respectively. The length of oestrus was longer with the second PGF<sub>2α</sub> injection than with the first injection of PGF<sub>2α</sub>. The student's 't' test (1.81) elicited non-significant differences (2.365). The present findings are also in agreement with Rao *et al.* (1982), Kaikini and Pargaonkar (1969) and Patil (2000) who recorded the average duration of oestrus to be  $24.18 \pm 0.69$  h,  $28.61 \pm 0.51$  h and  $26.14 \pm 1.39$  h, respectively in Berari buffaloes. Patil (1997) also recorded the average duration of oestrus to be  $25.50 \pm 1.79$  h. In the studies of Chede (1990) and Chouhan *et al.* (1992) it was respectively  $28.61 \pm 2.62$  h and  $28.40 \pm 10.07$  h, which are higher as compared to present findings.

Table 1. Treatment schedule of superovulation in Berari (Nagpuri) buffaloes.

| Days of oestrus cycle | Time   | Donor buffalo treatment           |                                   |
|-----------------------|--|-----------------------------------|-----------------------------------|
|                       |  | Folltropin - V 500 mg (Group - A) | Folltropin - V 560 mg (Group - B) |
| 10 <sup>th</sup> day  | Morning  | 100 mg                            | 100 mg                            |
|                       | Evening  | 100 mg                            | 100 mg                            |
| 11 <sup>th</sup> day  | Morning  | 75 mg                             | 80 mg                             |
|                       | Evening  | 75 mg                             | 80 mg                             |
| 12 <sup>th</sup> day  | Morning  | 50 mg                             | 60 mg                             |
|                       | Evening  | 50 mg + PGF <sub>2α</sub> 25 mg   | 60 mg + PGF <sub>2α</sub> 25 mg   |
| 13 <sup>th</sup> day  | Morning  | 25 mg                             | 40 mg                             |
|                       | Evening  | 25 mg                             | 40 mg                             |
| 19 <sup>th</sup> day  | Per rectal estimation of superovulatory response |                                   |                                   |
|                       |  |                                   |                                   |

Table 2. Synchronization response and average time required for induction of oestrus in Berari (Nagpuri) buffaloes.

| PGF <sub>2α</sub> analogue used for synchronization | Treatment        | Response to the synchronization treatment |                            | Average time required for onset of oestrus (h) | Range for time required (h) |
|---|------------------|---|----------------------------|--|-----------------------------|
|   |                  | No. of buffaloes treated                  | No. of buffaloes responded |  |                             |
| Dinoprost (Lutalyse) 25 mg                          | First injection  | 8   | 7 (87.50 %)                | 36.75 ± 6.10                                   | 29-53                       |
|   | Second injection | 8   | 8 (100.00%)                | 34.75 ± 2.74                                   | 25-43                       |

The aggregate average of onset of superovulatory oestrus in both the groups was  $19.50 \pm 0.18$  h. The onset of superovulatory oestrus in both groups was shorter; however, some of researchers recorded average times required for onset of superovulatory oestrus in buffaloes ranging from 28 to 33 h (Taneja *et al.*, 1995; Mathroo and Meharsingh, 1997; Sartape, 1999). Non-significant differences between the two groups at 5 percent level of significance (3.182) were also recorded ('t' value -0.508) in terms of length of oestrus during superovulatory treatment. These findings are corroborative with the findings of Sartape (1999) and Yadav *et al.* (1985) who recorded the average duration of oestrus to be  $39.8 \pm 1.64$  h and  $41.3 \pm 1.25$  h, respectively.

No significant difference was recorded with superovulatory response and presence of unovulatory follicles when ovaries were palpated manually. Numbers of unovulatory follicles were non-significantly lower with the 500 mg dose schedule. These findings are in close agreement with the studies of Mathroo and Meharsingh (1997), Taneja *et al.* (1995), Singla and Madan (1990). These results are also in accordance with those of Drost *et al.* (1986) and Singh and Narayana (1997) who recorded the average number of unovulatory follicle to be 1.50 and  $1.4 \pm 0.21$ , respectively.

Motwani (1986), Misra *et al.* (1991) and Beg *et al.* (1997) recorded slightly higher average numbers of unovulatory follicles viz.  $2.2 \pm 0.82$ ;  $2.67 \pm 0.71$  and  $3.00 \pm 1.05$ , respectively. A comparatively better superovulatory response was obtained in buffaloes injected with 400 mg of FSH than those injected with 600 mg in the studies by Patel *et al.* (2010). A non-significant increase in number of unovulatory follicles in 600 mg ( $1.90 \pm 0.67$ ) compared to 400 mg ( $0.88 \pm 0.44$ ) of FSH dose was observed. Such variation in results could be attributed to differences in location, breed, hormonal treatments and individual response to the treatment.

## CONCLUSION

The response of synchronization of oestrus, time required for onset of oestrus and length of oestrus needs to be studied in a larger number of buffaloes in breeding and non-breeding seasons. Because of variable response to the two FSH-P treatment recorded in the individual buffaloes in the present studies, it is suggested that the trials on similar lines should be conducted in a larger number of donor buffaloes and in different seasons

Table 3. Onset and duration of oestrus in superovulated Berari (Nagpuri) buffaloes.

| Sr. No. | Groups                            | Number of animals | Average onset of oestrus (h) | Average length of oestrus (h) (with range in h) |
|---------|-----------------------------------|-------------------|------------------------------|---|
| 1       | Group-I FSH-P (500 mg treatment)  | 4                 | $20.00 \pm 0.00$             | $36.50 \pm 5.50$ (26-46)                        |
| 2       | Group-II FSH-P (560 mg treatment) | 4                 | $19.00 \pm 0.00$             | $38.75 \pm 3.32$ (29 - 44)                      |
|         | Aggregate average (h)             | 8                 | $19.50 \pm 0.18$             | $37.62 \pm 3.01$ (26-46)                        |

of the year.

## REFERENCES

- Beg, M.A., P.C. Sanwal and M.C. Yadav. 1997. Ovarian response and endocrine changes in buffalo superovulated at mid luteal and late luteal stage of the oestrus cycle. *Theriogenology*, **47**: 423-432.
- Chede, S.A. 1990. *Patterns of oestrus, oestrus behaviour and synchronization in buffaloes*. Ph.D. Thesis submitted to Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.
- Chouhan, K.R., R.A. Chaudhary, N.U. Khan, N.A. Chaudhary and M. Ahmed. 1992. Oestrus behaviour and fertility in normal cycling and oestrus synchronized buffaloes. *Indian J. Dairy Sci.*, **45**: 588-590.
- Drost, M., M. Wright and R.P. Elsdén. 1986. Intergeneric embryo transfer between water buffalo and domestic cattle. *Theriogenology*, **25**: 13-23.
- Kaikini, A.S and D.R. Pargaonkar. 1969. Nagpuri buffalo. *Indian Dairyman*, **21**: 47-48.
- Kamonpatana, M.A., P. Kunawongkrit, Bodhipaksha and Y. Luvita. 1979. Effect of PGF<sub>2α</sub> on serum prostaglandin levels in swamp buffalo (*Bubalus bubalis*) *J. Reprod. Ferti*, **56**: 445-448.
- Lindsell, G.E., B.D. Murphy and R.J. Mapletoft. 1986. Superovulatory and endocrine response in heifers treated with FSH-P at different stages of the estrus cycle. *Theriogenology*, **33**: 1131-1147.
- Mapletoft, R.J., A. Gonzalez and A.G. Lussier. 1988. Superovulation of buffalo heifers with Folltropin or FSH-P. *Theriogenology*, **29**: 274.
- Mathroo, J.S. and Meharsingh. 1997. Seasonal variation in superovulatory response and embryo recovery in buffalo (*Bubalus bubalis*). *Indian J. Anim. Reprod.*, **18**: 125.
- Misra, A.K., B.V. Joshi, P.L. Agrawala, R. Kasiraj, S. Sivaiah, N.S. Rangareddi and M.U. Siddiqui. 1990. Multiple ovulation and embryo transfer in Indian buffaloes (*Bubalus bubalis*). *Theriogenology*, **33**: 1131-1142.
- Misra, A.K., B.V. Joshi, P.L. Agrawala, R. Kasiraj, S. Sivaiah and N. S. Rangareddi. 1991. Improved super ovulatory regimen for buffalo (*Bubalus bubalis*). *Theriogenology*, **35**: 245.
- Motwani, K.T. 1986. Superovulation, non surgical recovery and embryo transfer in the water buffalo (*Bubalus bubalis*). *Indian J. Anim. Reprod.*, **7**: 100-101.
- Pant, H.C and G.D. Singh. 1980. Control of oestrus cycle in buffalo with a synthetic analogue of prostaglandin F<sub>2</sub> alpha (ICI 80,996). *Indian Vet. J.*, **57**: 870- 871.
- Patel, D.V., S.P. Singh, H.R. Shukla, C.P. Devanand and R. Kasiraj. 2010. Superovulatory response to FSH and embryo recovery rate in pandharpuri buffaloes (*Bubalus bubalis*). *Buffalo Bull.*, **29**(4): 244-249.
- Patil, G.R. 1997. *Oestrus response in post partum anestrus buffaloes treated with progesterone and prostaglandin during low breeding season*. M.V.Sc. Thesis submitted to Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.
- Patil, M.S. 2000. *Comparative efficacy of two different prostaglandin F<sub>2</sub> alpha analogues : Dinoprost (Lutalyse) and Luprostiol (Prosolvine) for inducing synchronized oestrus in Nagpuri buffaloes*. M.V.Sc. Thesis submitted to Dr. Panjabrao Deshmukh



- Krishi Vidyapeeth, Akola.
- Rajeshwaran, S., A.K. Misra and B.V. Joshi.1992.  
Synchronization of oestrus in Surti buffaloes.  
*Indian J. Anim. Reprod.*, **13**: 20-22.
- Rao, V.P., D.B. Reddy and T. Gopalkrishnan. 1982.  
Note on oestrus, oestrus cycle and time of  
ovulation in murrah buffaloes. *Indian J.  
Anim. Sci.*, **52**: 354-355.
- Sartape, B.P. 1999. *Superovulatory response with  
FSH-P in Nagpuri buffaloes synchronized  
by Norgestomet ear implant with  
supplementary Norgestomet oestradiol  
injection*. M.V.Sc. Thesis submitted to Dr.  
Panjabrao Deshmukh Krishi Vidyapeeth,  
Akola.
- Singh, A.K and K. Narayana.1997. Superovulation  
in Murrah buffaloes. *Indian J. Anim.  
Reprod.*, **18**: 77.
- Singla, S.K and M.L. Madan. 1990. Response  
of superovulation in buffaloes (*Bubalus  
bubalis*) with Super-ov and FSH-P.  
*Theriogenology*, **33**: 327.
- Taneja, M., S.M. Totey and A. Ali. 1995.  
Seasonal variation in follicular dynamics  
of superovulated Indian water buffalo.  
*Theriogenology*, **43**: 451-564.
- Wang, H., W.U.M., B.D. Murphy and R.J.  
Mapletoft. 1988. Superovulation of  
buffalo heifers with Folltropin: a dose trial.  
*Theriogenology*, **29**: 332.
- Yadav, M.C., K.E. Leslie and J.S. Walton. 1985.  
The onset and duration of ovulation in  
superovulated beef heifers. *Theriogenology*,  
**23**: 237.