

Some blood biochemical indices and fertility following treatment of anestrus in Murrah buffaloes*

K.K. RATHOUR¹, R.K. PANDIT², R.G. AGRAWAL^{3†}, M.A. QUADRI⁴ AND O.P. SHRIVASTAVA⁵

Department of Animal Reproduction, Gynaecology and Obstetrics
College of Veterinary Science & Animal Husbandry, Jabalpur - 482 001 (MP)

Received: August 31, 2004

Accepted: August 3, 2005

ABSTRACT

The efficacy of some of the hormonal and non-hormonal drugs for the treatment of anestrus, their effect on certain blood biochemical indices and conception rate in 36 Murrah buffaloes were studied. The overall mean values of calcium, inorganic phosphorus, total protein were 6.09 ± 0.55 mg per cent / 3.18 ± 0.31 mg per cent / 5.75 ± 0.23 g per cent, respectively, in the anestrus animals. The values elevated significantly ($P < 0.01$) in the post treated animals at the time of expression of estrus to 9.20 ± 0.42 mg per cent, 5.28 ± 0.25 mg per cent / 7.46 ± 0.17 g per cent, respectively. The animals expressing estrus following different treatments were 66.66 per cent within the duration of 9.60 ± 2.44 days. The overall conception rate was 65.33 per cent. The maximum number of expressed animals resulted in estrus in PMSG group (83.33%) and 60.00 per cent concentration. The maximum number of animals conceived in GnRH and progesterone treated groups (75.00 per cent), whereas estrus expression in these groups was only 66.66 per cent. In the Lugol's iodine treated group 50.00 per cent animals expressed estrus and 66.66 per cent of them conceived.

Key words: Anestrus, biochemical indices, treatment, fertility, Murrah buffalo

Anestrus is the most prevalent, frustrating and challenging problem encountered in buffaloes. Lack of minerals, especially calcium and phosphorus and total protein upset the proper functioning of reproductive organs. Hence, the present study was undertaken to study the efficacy of some of the hormonal and non-hormonal drugs for the treatment of anestrus, their effect on certain blood biochemical indices and conception rate in Murrah buffaloes.

MATERIALS AND METHODS

Total 36 anestrus healthy Murrah buffaloes aging between 5 to 10 years and having quiescent ovaries were randomly distributed in equal numbers to 6 groups. The following treatment regimen was administered to the different group of animals.

Group I : 5 ml gonadotropin releasing hormone (0.021 mg buserelin)¹, single dose, intra-muscularly.

Group II : 15 ml phosphorus² and 20 ml multivitamins³, intra-muscularly, for three days.

Group III : 1 ml progesterone (250 mg hydroxy progesterone caproate)⁴, intra-muscularly.

Group IV : 40ml Lugol's iodine solution, single dose, intra-uterine.

Group V : 5 ml pregnant mare serum gonadotropin⁵ 1000 IU, single dose, intra-muscularly.

Group VI : Untreated control.

The buffaloes were stall-fed and were let loose daily at least for 30 minutes during morning and evening hours in the paddock with good water splashing following milking. Clean drinking water was made available. The estrus behavior of all the buffaloes was observed in the presence of breeding bull. The blood samples were collected before and after the treatment following expression of estrus. About 12 ml of blood was used for serum separation. The serum samples were stored in deep freezer at -20°C temperature. Estimation of serum calcium,

*Part of M.V.Sc. thesis

¹Student, M.V.Sc. & A.H. Dept. of Anim. Reprod., Gynae. & Obstet.

²Dean, College of Vety. Science, Mhow

³Prof., Dept. of Anim. Reprod., Gynae. & Obstet.

⁴Assoc. Prof., Dept. of Anim. Biochem.

¹GnRH analogue, Receptal, Intervet Pharmaceuticals.

²Sodium salt of 4-dimethylamino-2 methylphenyl phosphinic acid, Tonophosphan, Intervet Pharmaceuticals.

³Multi vitamins, Hivit, Ranbaxy.

⁴Duraprogen, Unichem.

⁵Non-pituitary gonadotropin, Folligon, Intervet Pharmaceuticals.

[†]Corresponding author

inorganic phosphorus and total protein was done using kits* and fertility response to the different treatment regimen was evaluated.

RESULTS AND DISCUSSION

The overall mean values of serum calcium was 6.09 ± 0.55 mg per cent in the anestrus animals. It got elevated significantly ($P < 0.01$) in the post-treated animals at the time of expression of estrus to 9.20 ± 0.42 mg per cent. The level of calcium in different treatment groups did not show a significant difference (Table 1). The role of calcium in steroid biosynthesis is well documented on testes, adrenal glands and ovaries. The GnRH induced Lh release is a calcium dependent mechanism. Venkateswarlu *et al.* (1994) reported plasma concentration of calcium in cyclic and non-cyclic rural buffaloes as 10.32 ± 0.95 and 8.01 ± 0.83 mg/dl. Similarly, Amanullah *et al.* (1997) studied the serum calcium level in estrus and post-partum anestrus buffaloes as 10.68 ± 0.35 and 8.88 ± 0.35 mg per cent. The overall mean values of serum inorganic phosphorus were 3.18 ± 0.31 mg per cent in the anestrus animals in the present study. The values elevated significantly ($P < 0.01$) in the post-treated animals at the time of expression of estrus to 5.28 ± 0.25 mg per cent. The level of inorganic phosphorus in different treatment groups did not show a significant difference. One of the classical manifestation of phosphorus deficiency on reproduction is alteration of estrus. It also induces lowered conception rate, decreased ovarian activity, increased incidence of cystic follicles and generally depressed fertility. As found in the present study, Dabas *et al.* (1987) also reported a significantly ($P < 0.05$) lower concentration (4.0 ± 0.31 mg/dl) of inorganic phosphorus in anestrus buffaloes as compared to cyclic animals (6.2 ± 0.25 mg/dl). Umesh *et al.* (1995) reported serum concentration of inorganic phosphorus in cyclic (day of estrus) and post-partum anestrus buffaloes as 5.369 ± 0.207 and 2.030 ± 0.099 mg/dl, respectively.

In the present study, the overall mean value of serum total protein was 5.75 ± 0.23 g per cent in the anestrus animals. The values elevated significantly ($P < 0.01$) in the post-treated animals at the time of expression of estrus to 7.46 ± 0.17 g per cent. Deficiency of protein (hypoproteinemia) retards the development of sex organs and body growth in young animals and affects the

* Span Diagnostics Ltd., Sachin, Surat, Gujrat

* Beacon Diagnostics Pvt. Ltd., Navsari, Gujrat

Table 1. Serum calcium, inorganic phosphorus and total protein values during anestrus and at estrus in different treatment groups in Murrah buffaloes (Mean \pm SE)

Group No.	Treatment regimens	Calcium (mg %)		Inorganic phosphorus (mg %)		Total protein (g %)	
		During anestrus	At estrus	During anestrus	At estrus	During anestrus	At estrus
I	GnRH	6.33 ± 0.52	$9.69 \pm 0.28^{**}$	3.03 ± 0.36	$5.13 \pm 0.16^{**}$	5.79 ± 0.21	$7.75 \pm 0.10^{**}$
II	Phospho-vit. comp.	6.20 ± 0.67	$8.77 \pm 0.20^{**}$	3.31 ± 0.30	$5.42 \pm 0.20^{**}$	5.78 ± 0.28	$7.27 \pm 0.20^{**}$
III	Progesterone	6.05 ± 0.55	$9.46 \pm 0.46^{**}$	3.21 ± 0.33	$5.33 \pm 0.21^{**}$	5.71 ± 0.20	$7.13 \pm 0.16^{**}$
IV	Lugols' iodine	6.16 ± 0.42	$9.52 \pm 0.60^{**}$	3.21 ± 0.30	$5.22 \pm 0.29^{**}$	5.64 ± 0.19	$7.69 \pm 0.15^{**}$
V	PMSG	5.71 ± 0.37	$8.57 \pm 0.54^{**}$	3.17 ± 0.25	$5.28 \pm 0.25^{**}$	5.84 ± 0.27	$7.45 \pm 0.23^{**}$
	Overall mean	6.09 ± 0.55	$9.20 \pm 0.42^{**}$	3.18 ± 0.31	$5.28 \pm 0.25^{**}$	5.75 ± 0.23	$7.46 \pm 0.17^{**}$

Table 2. Treatment response for estrus induction and conception in true anestrus Murrah buffaloes

Group No.	Treatment	Animals expressing estrus (%)	Duration for estrus induction (Mean±SE days)	Conception rate in the animals responding in the treatment (%)
I	GnRH	66.66	9.50±1.93	75.00
II	Phospho-vit.comp.	66.66	11.0±3.41	50.00
III	Progesterone	66.66	10.25±1.55	75.00
IV	Lugol's iodine	50.00	7.67±1.44	66.66
V	PMSG	83.33	9.60±3.87	60.00
	Overall mean	66.66	9.60±2.44	65.33

subsequent reproductive performance. Kabir *et al.* (2001) concluded that the total serum protein was low in acyclic buffaloes (7.92 ± 0.11 g/dl) as compared to cyclic ones (8.46 ± 0.11 g/dl) which is in agreement to the present study also. The animals expressing estrus following different treatments were 66.66 per cent within the duration of 9.60 ± 2.44 days with overall conception rate 65.33 per cent (Table 2). The maximum number of animals expressed estrus in PMSG treated group (83.33 per cent), however, the breeding of such animals resulted in only 60.00 per cent conceiving. The PMSG has been used by the clinicians with a view to stimulate the follicular growth in the ovaries producing endogenous estrogen which exerts positive feed back on the anterior pituitary function and in turn induces estrus. Tiwari and Gupta (1995) noted that 1500 I.U. dose of PMSG treated anestrus in buffaloes with PMSG with the dose rate of 2000 I.U. and found that the estrus was expressed in 70 per cent animals within an average period of 34.86 ± 0.94 days of treatment.

In the present study the maximum number of animals conceived in GnRH treated group (75.00 per cent), whereas estrus expression in this group was only 66.66 per cent. The GnRH plays a key role in regulation of reproductive processes in animals. As found in the present study Pattabiraman *et al.* (1986) also reported that out of 15 anestrus buffaloes treated with a single intra-muscular injection of 5 ml Receptal, 8 animals showed estrus, 6 ovulated and 3 conceived. Nautiyal *et al.* (1997) treated 6 anestrus buffalo heifers with 1.5 ml GnRH and 5 heifers treated with 2.5 ml GnRH. The buffaloes exhibited estrus within 83.02 ± 10.04 hr and 63.30 ± 17.22 hr, respectively. Singh *et al.* (2003) treated

20 anestrus buffaloes with 5 ml of Receptal intra-muscularly and reported that the estrus was established in 80 per cent animals with the average period of 16.06 ± 0.65 days of treatment and of these 70.00 per cent buffaloes were ovulated with the conception rate of 62.5 per cent.

The response of progesterone in the treatment of anestrus in the Murrah buffaloes was expression of estrus (66.66 per cent) and conception (75.00 per cent) in the present study. The role of progesterone in regulation of estrous cycle involves to prolong the luteal phase of the cycle or to establish artificial luteal phase. Markandeya and Patil (2003) treated six anestrus buffaloes with 250 mg progesterone, intra-muscularly, and reported that progesterone therapy was successful in induction of post-partum estrus in five out of six (83.33 per cent) buffaloes and all the heats were ovulatory of which three animals conceived. In the Lugol's iodine treated group of the present study 50.00 per cent animals expressed estrus and 66.66 per cent of them conceived. The action of Lugol's iodine in the induction of estrus was thought to be due to either stimulatory effect on the hypothalamus or by the release of uterine luteolytic factor acting via the utero-ovarian and utero-pituitary-ovarian pathway. Porwal *et al.* (1976) observed that after utero-ovarian massage and painting of Lugol's iodine on os, 46.66 per cent buffaloes manifested heat within 8 days after treatment out of which 92.85 per cent conceived. Reddy *et al.* (1994) reported that the application of paint consisting one per cent Lugol's iodine solution to the os cervix initiated estrus behaviour in 10 out of 20 buffaloes with 70 per cent conception rate.





REFERENCES

- Amanullah, M., Tandle, K., Honnappagol, S.B., Sonwane, S.D., Kartikesh, S.M., Das, B.C. and Jagjiwariram (1997). Serum cholesterol, calcium, phosphorus and total protein in relation to estrus and anestrus in non-descript buffaloes. *Indian J. Dairy Sci.*, **50**: 410-412.
- Dabas, Y.P.S., Singh, S.P. and Saxena, O.P. (1987). Serum concentration of certain minerals in anestrus cows and buffaloes. *Indian J. Anim. Reprod.*, **8**: 98-101.
- Henry, R.J.D.C. and Winkelman, J.W. (1974). *Clinical Chemistry, Principles and Techniques*. 2nd edn., Harper and Row.
- Kabir, K.K., Varshney, J.P., Rawal, C.V.S. and Ansari, M.R. (2001). Studies on serum progesterone and certain blood biochemical indices in cyclic and acyclic non-descript rural buffaloes. *Indian Vet. J.*, **78**: 1116-1118.
- Markandeya, N.M. and Patil, A.D. (2003). Studies on hormonal therapies for induction of post-partum estrus in buffaloes. *Intas Polivet*, **4**: 167-169.
- Nautiyal, H., Shankar, U. and Agrawal, S.K. (1997). Effect of gonadotrophin releasing hormone (GnRH) on induction of ovarian cyclicity in pubertal anestrus buffalo heifers. *Indian J. Anim. Reprod.*, **18**: 13-14.
- Pattabiraman, S.R., Veereapandian, C. and Quayam, S.A. (1986). Effect of Receptal treatment in anoestrus and early post-partum cows and buffaloes. *Indian Vet. J.*, **63**: 409-413.
- Porwal, M.L., Saxena, N.K., Shrivastava, A.M. and Karandikar, G.W. (1976). Efficacy of different medicaments on anestrus buffaloes. *Indian Vet. J.*, **53**: 435-437.
- Reddy, K.R.C., Rao, A.S., Reddy, V.S.C., Yadagiri, B., Sharma, G.P., Reddy, M.R. and Reddy, C.E. (1994). Efficacy of certain non-hormonal and hormonal drugs on estrus induction in post-partum anestrus buffaloes. *Indian J. Anim. Reprod.*, **15**: 127-130.
- Singh, S., Wani, N.A. and Maurya, S.N. (2003). Use of different hormones for the treatment of post-partum anestrus in buffaloes under field conditions. *Indian J. Anim. Sci.*, **73**: 894-889.
- Tiwari, R.P. and Gupta, S.K. (1995). Response of subestrus and true anestrus buffaloes to treatment under field conditions. *Indian J. Anim. Reprod.*, **16**: 101-102.
- Umesh, K.R., Sudhir, V., Chandra, R., Rao, A.S.S., Reddy, V.S., Reddy, G.V.N. and Reddy, C.C. (1995). Studies on certain blood biochemical constituents of rural buffaloes during cyclic and post-partum anestrus periods. *Indian Vet. J.*, **72**: 469-471.
- Venkateswarlu, M., Reddy, V.S.C., Sharma, G.P., Reddy, V.S., Yadagiri, B. and Reddy, C.E. (1994). Calcium and phosphorus level in cyclic and true anestrus buffaloes in flurosis endemic areas. *Indian J. Anim. Reprod.*, **13**: 135-137.

ISSAR AWARDS

R.D. SHARMA AWARD



- 
 The award is for the best presentation in Obstetrics by a young scientist below 35 years of age.
- 
 The applications along with five copies of the article with certificate in proof of age may be submitted to the General Secretary, ISSAR.
- 
 He should also send the abstract and full text of the article to the Organizing Secretary of the convention.
- 
 Application form may be obtained from General Secretary, ISSAR.