# INFLUENCE OF HORMONAL AND NON - HORMONAL THERAPIES ON FERTILITY AND SERUM MINERALS PROFILE OF CONCEIVING AND NON-CONCEIVING ANOESTRUS BUFFALOES

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**ABSTRACT**

A Study was carried out during breeding season on 47 anoestrus / suboestrus buffaloes to evaluate the therapeutic efficacy of hormonal and non-hormonal drugs in inducing fertile oestrus and its influence on serum macro-micro minerals profile. Fifteen true anoestrus buffaloes each were treated either with GnRH (Receptal 5.0 ml) i/m once (Gr-I) or Tono-Prepaline-Cyclomin-Lugols (TPCL) 4 times 4-5 days apart (Gr-II), while 10 suboestrus buffaloes were treated with $PGF_2\alpha$ (Juramate 2.0 ml) i/m once (Gr-III), keeping 7 animals as untreated control (Gr-IV). The oestrus induction response and conception rate at induced oestrus in 4 groups were 86.66 & 38.46, 80.00 & 50.00, 100.00 & 40.00, and 42.85 and 66.66 %, respectively, with the oestrus induction interval of 21.50±4.59, 27.73±4.62, 3.62±0.46 and 67.50±14.49 days. The overall serum mineral profile for hormonal and non-hormonal treated anoestrus buffaloes averaged: calcium 9.47±0.15 mg/dl, phosphorus 9.66±0.14 mg/dl and magnesium 3.48±0.09 mEq/L, respectively. The values of zinc, iron, copper, cobalt and manganese were 1.85±0.09, 3.54±0.09, 0.90±0.02, 0.16±0.01 and 0.13±0.01 ppm, respectively. The serum calcium level was significantly higher in all treatment groups as compared to control group. Inorganic phosphorus was significantly higher (P<0.01) in $PGF_2\alpha$ treated buffaloes as compared to GnRH treated and control group. The zinc content in $PGF_2\alpha$ treated group was significantly lower as compared to GnRH treated group. The values of iron for all treatment groups were significantly higher than the control. The copper and cobalt in GnRH and TPCL treated groups were significantly (P<0.05) higher as compared to control and $PGF_2$á treated groups. The conceived and non-conceived status did not influence significantly the serum profile of these elements in anoestrus buffaloes, though the values were higher in conceived buffaloes.

**KEY WORDS:** Anoestrus buffaloes, Hormonal/Non-hormonal therapy, Fertile oestrus interval, Conception rate, Mineral profile.

## INTRODUCTION

One of the major causes of anoestrus in dairy cattle and buffaloes is nutritional deficiency and mineral imbalance causing ovarian dysfunction (Hidiroglou, 1979). In nutritionally sound animals, gonadotrophin releasing hormone (GnRH) and prostaglandins ($PGF_2\alpha$) have good therapeutic actions to enhance early resumption of ovarian activity and to induce fertile oestrus (Sam Bruce *et al.*, 1988; Shah *et al.*, 2002). Non-hormonal remedials like Lugol's iodine alone or in combination with vitamin-mineral supplementation have been used with varying success in inducing oestrus and fertility in anoestrus buffaloes (Mathur *et al.,* 2005; Singh *et al*., 2006). However, the literature on comparative influence of these therapies on fertility and blood profile of minerals is scanty. Hence, the present study was planned to evaluate the comparative efficacy of Vitamin-minerals plus Lugols, GnRH and prostaglandin therapy on fertility and its influence on serum status of macro-micro minerals in anoestrus and suboestrus buffaloes.

## MATERIALS AND METHODS

This study was carried out from August 2007 to March 2008 under field conditions in Gujarat on 47 buffaloes confirmed to be anoestrus or suboestreus through gynaeco-clinical examinations made twice 10 days apart. All infertile animals were first dewormed using Albendazole 3000 mg (Helmiguard 3000, Vetcare India Ltd). The owners of the ear-marked animals were supplied with mineral mixtures (Amul brand) for supplementing to their animals @ 30 g/day/head. Problem breeders were then subjected to different therapeutic regimes as detailed below.

**Group-I : Nutritional Supplementation Therapy**

Fifteen true anoestrus buffaloes were subjected to Tono-Prepaline-Cyclomin plus Lugol's iodine (TPCL) treatment. This consisted of Lugol's iodine 0.5% solution 10 ml on os cervix or i/ut once, plus Inj. Protone 10 ml (2 g sodium salt of phosphoric acid, Vetnex-RFCL India Ltd) and Inj. Vitamin-A 6 ml (18 lacs IU Vitamin-A as palmitate, Virbac Co.) i/m, 4 injections each at 4-5 days intervals, and Cyclomin-7 (10 boli, Alved Pharma) one bolus every third day orally.

**Group-II: GnRH Therapy**

Fifteen true anoestrus buffaloes were treated with single dose of 0.02 mg GnRH, i.e Inj. Receptal (Buserelin acetate 0.0042 mg/ml, Intervet India Pvt. Ltd.) 5.0 ml i/m once.

**Group-III: Prostaglandins $F_2\alpha$ Therapy**

Ten buffaloes, with history of suboestrum and presence of CL on one of the ovaries, were subjected to single dose administration of $PGF_2\alpha$, i.e. Inj. Juramate (250 µg Cloprostenol/ml, Jurox Pvt. Ltd., Australia) 2.0 ml intramuscularly once.

**Group-IV: Untreated Control**

Seven anoestrus buffaloes were kept as untreated control, and were studied for their reproductive status and blood profile.

The animals detected in oestrus were inseminated at induced or natural oestrus by the trained lay inseminators, and pregnancy was confirmed per rectum 60 days later. Jugular blood samples were collected on the day of treatment, at induced oestrus and at 20-22 days post-AI. The serum samples were stored at -20°C till analyzed. The serum macro-minerals, viz. calcium (Arsenazol-III method), inorganic phosphorus (Molybdate UV method) and magnesium (Calmagite method) were estimated using standard procedures and kits procured from Coral Clinical Systems, Goa, India, with the help of Chemwell auto-blood analyzer (Awareness Technology, Germany). The blood sera samples (1 ml each) were wet digested with 5 ml volume of di-acid mixture (perchloric acid : nitric acid; 1:4) on a hot plate (Krishna and Ranjhan, 1980). Estimations of trace elements, viz. copper, cobalt, zinc, iron and manganese were done of wet digested samples on an Atomic Absorption Spectrophotometer (Model- 3110, Perkin Elmer). Oestrus induction response and conception rate at induced oestrus were calculated group-wise and compared by using chi-square test. The data on blood profile of minerals were analyzed by using completely randomized design and Duncan's new multiple range test (Snedecor and Cochran, 1986).

## RESULTS AND DISCUSSION

**Oestrus Induction Response and Conception**

The oestrus induction response for 15, 15 and 10 anoestrus/suboestrus buffaloes treated with GnRH, TPCL and $PGF_2\alpha$ was 86.66, 80.00 and 100.00 % as against 42.85 % in control group. The corresponding oestrus induction intervals were 21.50±4.59, 27.73±4.62 and 3.62±0.46 days vs 67.50±14.49 days in control, and conception rates at induced oestrus were 38.46, 50.00 and 40.00 % vs 66.66 % in control, respectively. The overall 80.85% buffaloes responded in 32.09±2.18 days with first service conception rate of 44.74%.

**Effect on Serum Calcium and Inorganic Phosphorus**

The overall average serum calcium and inorganic phosphorus concentration in anoestrus buffaloes under study was 9.47±0.15 and 9.66±0.14 mg/dl, respectively. The pre-treatment, oestrus/AI and 22 days post-AI values did not vary significantly, overall or, in any of the treatment groups, though it was apparently lower at pre-treatment stage (9.15±0.26 and 9.26±0.24 mg/dl). The serum calcium level was, however, significantly higher in all treatment groups, particularly TPCL treated group, as compared to control (9.22 ± 0.42 to 10.28 ± 0.24 vs 8.45 ± 0.27 mg/dl), while phosphorus was significantly higher ($P<0.01$) in $PGF_2\alpha$ treated buffaloes as compared to GnRH treated and control group (10.64±0.34 vs 9.25±0.25 and 8.75± 0.27 mg/dl). Neither GnRH and $PGF_2\alpha$ treatment nor conceived and non-conceived status influenced the serum calcium levels in anoestrus buffaloes, but the serum inorganic phosphorus was insignificantly higher in conceived as compared to non-conceived buffaloes (Table 1).

The TPCL treatment did not influence significantly the serum inorganic phosphorus concentration in animals under study. Although there was a gradual increase in both calcium and inorganic phosphorous levels in all the groups, and TPCL treated group in particular, from the day of administration to induced oestrus to day 20-22 post-AI, as has been reported by Singh *et al.* (2006). Sheshappa *et al*. (2002) also observed higher level of plasma calcium and inorganic phosphorus in GnRH plus $PGF_2\alpha$ treated cows as compared to control. Patel (2004), however, reported the mean plasma calcium level to be significantly lower ($P<0.05$) in $PGF_2\alpha$ treated HF cows as compared to GnRH treated cows. Dabas *et al.* (1987) and Biswas *et al.* (2005) observed supplementation of anoestrus cows and buffaloes with Nuvimin forte and Complemin forte for a month orally to restore the serum calcium and inorganic phosphorus concentrations to the level of normal cyclic animals. Kavani *et al.* (2007) found significantly higher plasma calcium level during luteal phase (12.24±0.82 vs 10.73±0.31 mg/dl, $P<0.05$) in conceiving than in non-conceiving buffaloes. Furthermore, Sheshappa *et al*. (2002) also observed higher level of plasma calcium in GnRH plus $PGF_2\alpha$ treated cows as compared to control.

Morrow (1969) attributed infertility in dairy heifers to phosphorus deficiency and stated that supplementation of phosphorus improved fertility. He opined that calcium deficiency did not affect reproductive performance of the cows, but the disturbance in plasma Ca:P ratio may prolong the interval to first ovulation postpartum. According to Martson *et al.* (1972) and Hidiroglou (1979) minerals play an important role in the regulation of reproduction and production in animals. Lower concentration of circulatory minerals results in impaired reproductive function leading to cessation of cyclic activity. Minerals like calcium, phosphorus and magnesium also influence the ability of animals to utilize other micro-minerals. The influence of these minerals on certain enzyme system may affect reproductive efficiency (Dhoble and Gupta, 1986), which might be reflected in lower blood level of them. In early studies, Webster (1932) found high correlation between the phosphorus supply and the breeding efficiency index in cattle. However, Rowlands *et al.* (1977) and Marinov (1978) did not find any correlation between serum inorganic phosphorus or calcium level and number of services required per conception in cows.

**Effect on Serum Magnesium**

The overall mean serum magnesium in anoestrus buffaloes under study was 3.48±0.09 mEq/L. The pre-treatment, oestrus/AI and 22 days post-AI values did not vary significantly, overall or, in any of the treatment groups. It was slightly higher in conceived as compared to non-conceived buffaloes (3.58±0.17 vs 3.43±0.10 mEq/L). Among GnRH and $PGF_2\alpha$ treated buffaloes, there was a gradual increasing trend of serum magnesium level during the three periods of blood sampling, but in TPCL treated group it was relatively higher as compared to other two groups and did not show rising trend, while in control group it was lower than any of the treatment groups (Table 2).

These findings are in close conformity with those of Ahlawat (2003). Patel (2004), however, reported significantly lower plasma magnesium level in conceived than non-conceived group of cows (2.83±0.05 vs 3.14±0.04 mEq/L). Moreover, the present findings to some extent coincided with the reports of Dabas *et al.* (1987) and Biswas *et al.* (2005), who also found restoration of the most serum minerals to normal level in anoestrus cows and buffaloes after one month of oral supplementation with Nuvimin forte and Complemin forte, respectively. However, no report was available to compare the present findings of hormone effect on magnesium status in anoestrus buffaloes.

**Effect on Serum Trace Minerals**

The overall mean serum concentration of zinc in anoestrus buffaloes under study was 1.85±0.09 ppm. The pre-treatment, oestrus/AI and 22 days post-AI values did not vary significantly, overall or, in any of the treatment groups, but it showed a gradual increasing trend in TPCL treated animals with the overall average of 2.08±0.15 ppm. Moreover, the overall zinc content in $PGF_2\alpha$ treated group was significantly lower, though at par with TPCL treated and control group, as compared to GnRH treated group. The serum zinc levels

did not vary between conceived and non-conceived groups, though the values in TPCL conceived buffaloes were apparently higher than the non-conceived buffaloes (Table 2). No reason, however, could be attributed to consistently higher concentration of zinc observed in GnRH treated group and lower in $PGF_2\alpha$ treated group.

The overall average serum iron profile at pre-treatment, at induced oestrus and at 20-22 days post-AI in anoestrus buffaloes was 3.55±0.14, 3.46±0.14 and 3.62±0.14 ppm, respectively, with a pooled mean of 3.54±0.09 ppm. The period effect was not significant, overall or, in any of the treatment groups. The pooled value of all treatment groups were, statistically at par and, significantly higher than the control group (3.49±0.14 to 3.92±0.16 vs 2.88±0.12 ppm). Moreover, the values showed gradual increasing trend from pre-treatment to oestrus to luteal phase. The overall serum copper at pre-treatment, at induced oestrus and at 20-22 days post-AI was identical with a pooled mean of 0.90±0.02 ppm. The pooled values of GnRH and TPCL treated groups were significantly higher as compared to control and $PGF_2\alpha$ groups, but did not differ significantly between conceived and non-conceived groups (Table 3).

The overall serum cobalt at pre-treatment, at induced oestrus and at 20-22 days post-AI averaged 0.15±0.01, 0.16±0.01 and 0.17±0.01 ppm, respectively, with a pooled mean of 0.16± 0.01 ppm. The overall pooled mean cobalt was significantly higher ($P<0.05$) in TPCL treated and GnRH treated groups as compared to $PGF_2\alpha$ treated and control groups. The values were also higher in conceived than the non-conceived buffaloes, overall and in most of the groups. The serum manganese profile at pre-treatment, at induced oestrus and at 20-22 days post-AI was identical (0.13±0.01 ppm). The values neither varied significantly between periods nor between groups, and similar was the situation for conceived and non-conceived buffaloes (0.11±0.01 vs 0.14±0.01 ppm, Table 4).

No comparable report was available to support or defit the present findings on the effect of different hormonal and non-hormonal therapies on serum trace minerals profile in anoestrus cows or buffaloes. Biswas *et al.* (2005), however, observed increase in serum manganese, copper and zinc concentrations, but not of iron, in postpartum anoestrus buffaloes supplemented with Complemin forte mineral mixture at 50 g + a tablet containing cobalt, iron and copper orally for a month. Patel *et al.* (2006) supplemented 20 gm of chelated minerals to anoestrus buffaloes and observed significant and gradual increase in the overall mean plasma copper, cobalt, zinc and manganese levels at day 15, 30, 45 and 60 of experiment over the 0 day. Kavani *et al.* (2007) did not find significant variation in the levels of Zn, Fe, Cu, Co and Mn among fertile and infertile cycles, however in conceiving buffaloes the weekly mean values of zinc, iron and copper were lower ($P<0.01$) at oestrus as compared to luteal phase.

Little-John and Lewis (1960) opined that manganese requirement for reproduction is enhanced if the dietary content of calcium and phosphorus are higher, depicting interrelationship of Ca-P-Mn. Alderman (1963) reported that cobalt plays a vital role in metabolism of copper, however copper levels in the blood alone may not be the true index of reproductive dysfunction (Wiener and Sales, 1976). Malecki (1973) found stronger manifestation of oestrus as well as lower incidence of irregular oestrous cycles and higher conception rate in cobalt treated cows. Sharma *et al.* (1999) observed significantly lower iron and zinc in anoestrus and suboestrus buffaloes. The imbalance of trace elements is believed to cause inactive ovaries and repeat breeding associated with decreased progesterone production by corpus luteum. The serum levels of trace minerals, except cobalt, in buffaloes under study were perhaps above the critical limits suggested by McDowell (1992), of course for cattle, and hence differences could not be observed in different groups or due to pregnancy status.

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