INFLUENCE OF CONTROLLED BREEDING TECHNIQUES ON FERTILITY AND PLASMA MACRO-MINERALS PROFILE IN CONCEIVING AND NON-CONCEIVING ANOESTRUS AND REPEAT BREEDING BUFFALOES

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ABSTRACT

The study covered 50 postpartum buffaloes, 20 true anoestrus , 20 repeat breeders and 10 normal cyclic ones under field conditions with a view to evaluate oestrus response, conception rate and plasma profile of macro-minerals following use of different hormonal treatments. Ten anoestrus buffaloes each treated with CIDR and Ovsynch protocols with FTAI resulted in induction of ovulatory oestrus in all 10 and 8 buffaloes with conception in 4 and 3 animals at induced oestrus, respectively. Ten repeat breeding buffaloes with mature CL of mid-cycle treated with i/m injection of 25 mg $PGF_2\alpha$ (Inj. Lutalyse) with FTAI caused ovulatory oestrus in 9 animals and conception in 4 buffaloes at induced estrus, while other ten repeat breeding buffaloes inseminated with simultaneous i/m Inj. of GnRH 20 μg (Inj. Receptal) resulted in conception in 3 animals. Among 10 normal cyclic control buffaloes, the first service conception was found in 3. The mean values of plasma calcium, phosphorus and magnesium in normal cyclic control group were 9.47 ± 0.31, 6.17 ± 0.18 and 2.96 ± 0.05 mg/dl, respectively, and only the phosphorus was significantly higher in conceived than non-conceived buffaloes. There was no influence of periods of blood sampling (day 0 & 7 of treatment, Day of AI and day 21 post-AI) or conceived and non-conceived groups of CIDR and Ovsynch protocols on the three minerals. The plasma profile of none of the three minerals was influenced by days of sampling or pregnancy status in repeat breeder buffaloes under Mid-cycle $PGF_2\alpha$ and AI + GnRH treatment groups and the values were close to those of normal cyclic group. It was thus concluded that the hormonal therapies improved conception rates in both anoestrus and repeat breeding buffaloes under field conditions, particularly with CIDR and Mid cycle PG injection, though they did not influence the plasma minerals profile significantly.

KEY WORDS : Buffalo, Anoestrus, Repeat breeder, Treatment protocols, Fertile oestrus response, Plasma macro-minerals profile

INTRODUCTION

The anoestrus and repeat breeding are the most prevalent twin reproductive disorders in dairy animals, for which several hormonal preparations and protocols are being practiced by the field vets, but with inconsistent results. Hormonal therapies have good therapeutic values to enhance reproductive efficiency in infertile animals with good nutritional status (Patel *et al.*, 2005**;** Singh *et al*., 2008). The variable results reported following hormonal treatments may be largely due to nutritional status, faulty management, ovarian changes, endocrine events and even uterine infection. However, the literature on the influence of these therapies on blood profile of macro-minerals is scanty (Patel *et al*., 2005; Sharma and Dhami, 2008). Hence, this study was planned under field conditions to evaluate the comparative efficacy of CIDR and Ovsynch protocol in anoestrus; and mid cycle PG and AI + GnRH protocol in repeat breeding buffaloes for fertility enhancement and their influence on plasma macro-minerals profile.

MATERIALS AND METHODS

This study was undertaken during breeding season from September 2012 to February 2013 on 50 pluriparous 90 to 180 days postpartum buffaloes selected from 5 villages under the milk shed area of AMUL, Anand, Gujarat. The buffaloes were initially screened gynaeco-clinically for their reproductive status as cyclic, anoestrus or repeat breeders and the detailed history and rectal palpation findings were recorded. Problem breeders were confirmed by rectal palpation twice 10 days apart. The buffaloes of average body condition score (BCS) selected included 20 true anoestrus, 20 repeat breeding and 10 normal cyclic/fertile (control) ones, which were subjected to different therapeutic regimes. Buffaloes in spontaneous or induced oestrus were inseminated using good quality frozen-thawed semen. All selected buffaloes were initially dewormed with 3 g fenbebdazole orally and injecting Ivermectin 70 mg s/c. Owners of the ear-marked animals were also supplied with mineral mixtures (Amul brand).

Therapeutic Management:

Twenty true anoestrus buffaloes of average BCS (2.75 to 3.50) were randomly sub-grouped and 10 each were subjected to either standard CIDR or Ovsynch protocol with FTAI on day 9 and 10, respectively (Naikoo *et al.,* 2010). Amongst 20 repeat breeding buffaloes of average BCS without visible and palpable genital abnormalities, 10 buffaloes having palpable mid-cycle CL were treated with i/m injection of $PGF_2\alpha$ 25 mg (Inj. Lutalyse, 5 ml, Pfizer) and FTAI was done twice at 72 and 96 hours later, while another 10 repeat breeding buffaloes with clear standing oestrus were inseminated and were simultaneously administered with i/m Inj. of GnRH 20 μg (Inj. Receptal, 5 ml). Ten buffaloes detected in oestrus spontaneously within 90 days postpartum and inseminated without any treatment served as normal cyclic/fertile control. The signs of oestrus and rectal palpation findings were recorded for each animal initially and at AI/FTAI. Animals in all the groups once inseminated were followed for recurrence of oestrus and pregnancy was confirmed per rectum 60 days post-AI in non-return cases.

Jugular blood samples were collected in heparinized vacutainers four times from representative numbers (n=8) of true anoestrus animals, i.e., on day 0 - just before treatment, day 7 - at the time of $PGF_2\alpha$ administration, day 9 (CIDR)/10 (Ovsynch), i.e. induced oestrus/AI and on day 21 post-AI. Amongst repeat breeding and normal cyclic buffaloes, blood samples were collected from representative numbers (n=8) depending upon treatment protocol, i.e., on day of mid cycle $PGF_2\alpha$ injection, at the time of spontaneous/ induced oestrus (AI) and on day 21 post-AI. The samples were centrifuged at 3000 rpm for 15 minutes, and plasma separated out was stored deep frozen at -20°C with a drop of merthiolate (0.1%) until analyzed. Plasma profile of calcium, inorganic phosphorus and magnesium was estimated by using standard procedure and assay kits procured from Crest Bio-systems, Goa, with the help of Chemistry Analyzer (Mindray, BS 120). The data were analyzed suitably employing online SAS system of analysis.

RESULTS AND DISCUSSION

Out of 10 anoestrus buffaloes each treated with CIDR and Ovsynch protocols with FTAI, induction of ovulatory oestrus occurred in all 10 (100%) and 8 (80%) buffaloes with conception in 4 (40%) and 3 (30%) animals at induced oestrus, respectively. Ten repeat breeding buffaloes with mature CL of mid-cycle treated with i/m injection of 25 mg $PGF_2\alpha$ (Inj. Lutalyse) with FTAI resulted in induction of ovulatory oestrus in 9 (90%) animals and conception in 4 (40%) buffaloes, while other ten repeat breeding buffaloes inseminated at standing oestrus with simultaneous i/m Inj. of GnRH 20 μg (Inj. Receptal) showed conception in 3 (30%) animals. Among 10 normal cyclic (control) buffaloes also, the first service conception was found in 3 (30%) buffaloes. These findings are in accordance with or even better than those documented in several earlier reports (Totewad *et al.,*

2007; Sharma and Dhami, 2008; Butani *et al.,* 2009; Naikoo *et al.,* 2010; Pawshe *et al.,* 2011; Ali *et al.,* 2012).

The results on plasma minerals profile recorded on different days of treatment/breeding in anoestrus and repeat breeding buffaloes under various treatment protocols, and on day of AI and day 21 post-AI in normal cyclic group are presented in Tables 1 to 3.

Influence of CBT on Plasma Calcium:

The overall mean values of plasma calcium obtained for anoestrus buffaloes under CIDR and Ovsynch protocols and in normal cyclic buffaloes were statistically at par in all three groups. It neither revealed significant variation between periods of the treatment nor between conceived and non-conceived groups, except the pooled value was significantly higher in non-conceived than conceived buffaloes (10.48 ± 0.29 vs. 9.50 ±0.13 mg/dl, $P<0.01$) under CIDR treatment protocol (Table 1). Parmar *et al.* (2012) observed non-significantly higher mean plasma calcium concentration at induced oestrus as compared to values at 22nd day post-AI and pre-treatment for GnRH treated anoestrus buffaloes. The influence of oestrus induction protocols like, CIDR and Ovsynch on macro-minerals profile could not be seen in the literature reviewed. However, comparable findings in terms of values and trend were reported earlier by Paul *et al.* (2000) and Sharma and Dhami (2008). In contrast, Ahmed *et al.* (2010) and Kumar *et al.* (2010) reported the higher mean plasma calcium level in cycling than anoestrus buffaloes.

Among repeat breeding buffaloes under mid cycle $PGF_2\alpha$ and AI + GnRH protocols also, the plasma calcium profile did not reveal significant variations between days/periods and between conceived and non-conceived groups, except that it was higher in non-conceived than conceived buffaloes on day of AI in AI + GnRH protocol, and supported the observations of Sharma and Dhami (2008). Parmar *et al.* (2013) obtained lower levels of calcium in GnRH treated Gir cows as compared to untreated control. Patel *et al.* (2005) reported the mean plasma calcium level to be significantly lower in $PGF_2\alpha$ treated repeat breeder HF cows as compared to untreated control group, which is contrary to the present findings. Patel (2008) reported the mean values of plasma calcium for postpartum buffaloes under $PGF_2\alpha$ treatment and control group to be 9.98 ± 0.04 and 9.17 ± 0.04 mg/dl ($P<0.05$), that is somewhat similar to the trend in present findings. Butani *et al.* (2011) also reported serum calcium and phophorus levels to be non-significantly lower in repat breeding than normal cycling buffaloes.

Influence of CBT on Plasma Inorganic Phosphorus:

The plasma inorganic phosphorus levels also did not show any significant variation between periods of the treatment with CIDR, while in Ovsynch treatment protocol it was significantly higher on the day of induced oestrus/AI than other days/periods. Similarly, pregnancy status also did not influence the plasma inorganic phosphorus profile in anoestrus buffaloes (Table 2). Bhaskaran and Abdulla Khan (1981) documented that the marginal deficiency of phosphorus is enough to cause disturbances in pituitary-ovarian axis, without manifesting specific systemic deficiency symptoms. Butani *et al.* (2009) and Parmar *et al.* (2012) did not find appreciable variation in the mean plasma inorganic phosphorus levels on the day of GnRH and/or PG treatment, at induced oestrus and on day 22 post-AI in anoestrus or suboestrus buffaloes. The present insignificant differences observed in phosphorus profile between different phases of the cycle and even conceived and non-conceived groups corroborated with the earlier reports of Paul *et al.* (2000). On the contrary, higher mean plasma phosphorus level has been documented in cycling animals than anoestrus buffaloes by some workers (Paul *et al.,* 2000; Patel *et al.,* 2007; Ahmed *et al.*, 2010).

The mean values of plasma inorganic phosphorus in normal cyclic and repeat breeding buffaloes under mid cycle $PGF_2\alpha$ and AI + GnRH protocols were nearly the same. The levels neither showed

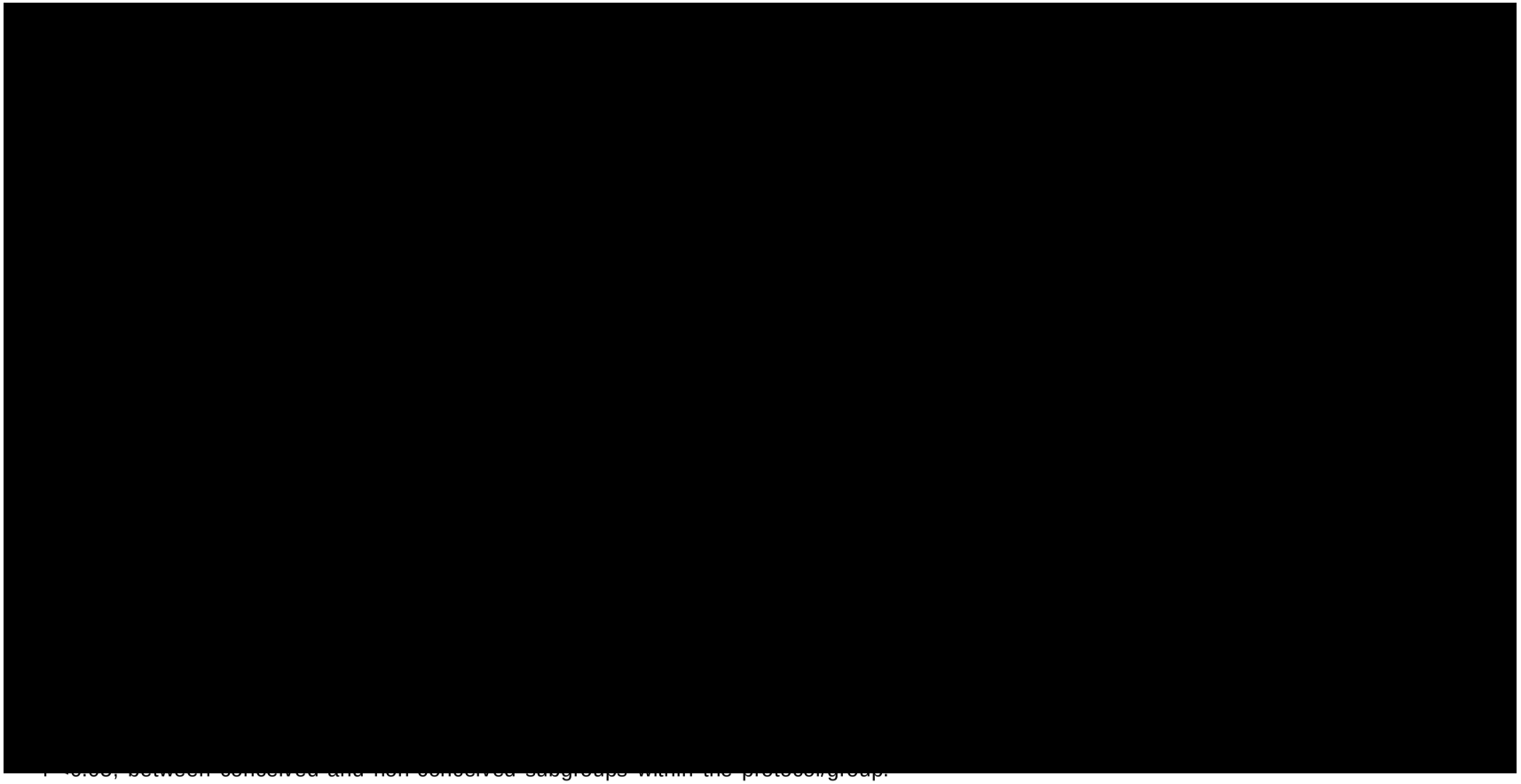
Table 1 : Plasma calcium concentration (mg/dl) in anoestrus and repeat breeding buffaloes on different days of treatment and AI under various treatment protocols

*P<0.05, **P<0.01 between conceived and non-conceived subgroups within the protocol/group.

D-0 = Day of starting the treatment, D-7 = Administration of PG

D-9/10 = Fixed time AI (Day 9 in CIDR; Day 10 in Ovsynch, Day 3 or 4 in Mid cycle PG), D-21 = Day 21 post-AI.

Table 2 : Plasma inorganic phosphorus concentration (mg/dl) in anoestrus and repeat breeding buffaloes on different days of treatment and AI under various treatment protocols



Means bearing uncommon superscripts within the row differ significantly (P < 0.05).

D-0 = Day of starting the treatment, D-7 = Administration of PG

D-9/10 = Fixed time AI (Day 9 in CIDR; Day 10 in Ovsynch, Day 3 or 4 in Mid cycle PG), D-21= Day 21 post-AI.

Table 3 : Plasma magnesium concentration (mg/dl) in anoestrus and repeat breeding buffaloes on different days of treatment and AI under various treatment protocols

D-0 = Day of starting the treatment, D-7 = Administration of PG

D-9/10 = Fixed time AI (Day 9 in CIDR; Day 10 in Ovsynch, Day 3 or 4 in Mid cycle PG), D-21 = Day 21 post-AI.

any significant variation between periods nor between pregnancy status, except that it was significantly greater in conceived than non-conceived group of normal cyclic buffaloes (Table 2). Patel *et al.* (2005) and Patel (2008) however reported the mean plasma phosphorus level to be significantly higher ($P<0.05$) in $PGF_2\alpha$ treated repeat breeder HF cows and buffaloes as compared to untreated control groups. Butani *et al.* (2011) observed non-significantly lower serum calcium and phophorus levels in repeat breeding as compared to normal cycling buffaloes. Parmar *et al.* (2013) observed lower levels of phosphorus in GnRH treated repeat breeding Gir cows as compared to untreated control, without significant differences between conceived and non-conceived groups.

Influence of CBT on Plasma Magnesium:

The levels of plasma magnesium did not vary significantly between periods of treatment and between conceived and non-conceived groups of CIDR and Ovsynch treated anoestrus animals (Table 3). Reports on the influence of oestrus induction protocols, viz., CIDR and Ovsynch on plasma magnesium profile could not be seen in the literature reviewed. The present findings in respect of magnesium levels and trend between anoestrus and normal cyclic or conceived and non-conceived groups however corroborated with the reports of Paul *et al.* (2000) and Butani *et al.* (2011). However, Newer *et al.* (1999) reported the significant difference in the mean plasma magnesium levels between cyclic and anoestrus buffaloes.

The mean values of plasma magnesium obtained in normal cyclic group and repeat breeding buffaloes under mid cycle $PGF_2\alpha$ and AI + GnRH protocols were statistically the same. No significant differences were observed in plasma magnesium profile between pregnancy status and between periods, except in mid cycle $PGF_2\alpha$ treatment protocol, wherein the levels increased gradually and significantly from day of treatment to day of AI and day 21 post-AI (Table 3). Parmar *et al.* (2013) recorded insignificantly lower mean plasma magnesium levels in GnRH treated repeat breeding cows as compared to untreated control and the levels between conceived and non-conceived groups were also identical. Butani *et al.* (2011) reported significantly lower serum magnesium concentration in repeat breeding as compared to normal cycling buffaloes, which is also in contrast to the present findings.

Thus, it can be inferred that the hormonal therapies used improved conception rates in both anoestrus and repeat breeding buffaloes under field conditions, particularly with CIDR and Mid cycle PG injection beyond normal cyclic control group, though they did not influence the plasma minerals profile significantly.

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