EFFECT OF HORMONAL AND NON-HORMONAL TREATMENT ON REPRODUCTIVE EFFICIENCY AND PLASMA PROGESTERONE, BIOCHEMICAL AND MACROMINERALS PROFILE IN POSTPARTUM ANOESTRUS BUFFALOES

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ABSTRACT

Fifty two postpartum anoestrus Surti buffaloes selected through glynaeco-clinical examinations in three village co-operative societies of Panchmahal district were randomly divided into four groups. Group-A (n=14) was treated with oral supplementation of Chelated mineral mixture @ 30 g/day/ animal for one month, Group-B (n=14) with Inj. inorganic phosphorus and Inj. vitamin AD3E 10 ml each, im at weekly interval for three consecutive week, Group-C (n=12) with Inj. GnRH (Receptal) 5.0 ml, im once and Group-D (n=12) served as untreated control. The effect of above treatments on reproductive efficiency and plasma profile of progesterone and various biochemical and macro-minerals was studied at pre-treatment, at induced oestrus and 22nd day post-AI. Significantly higher oestrus induction response (83.33%), the highest conception rate (80.00 %) and reproductive efficiency (66.66 %) were observed for group-C GnRH treated buffaloes followed by group-B and A, and the values were lowest in control group-D. The mean plasma progesterone (ng/ml) levels were significantly ($P<0.05$) higher at day 22nd post-AI in all the groups compared to those at pre-treatment and induced oestrus phase. The mean plasma protein level was significantly ($P<0.05$) higher at induced oestrus and 22nd day post-AI than at pre-treatment level in group-A (7.25 ± 0.22 and 7.31 ± 0.18 Vs 6.52 ± 0.31 g/dl). The mean total cholesterol levels (mg/dl) were non-significantly higher at induced oestrus and 22nd day post-AI than at pre-treatment level in all the groups. Plasma calcium levels were significantly ($P<0.05$) higher at induced oestrus and 22nd day post-AI than that at pretreatment level in group-B. Similarly the mean plasma inorganic phosphorus level was significantly higher at induced oestrus than the pre-treatment level in group-A. The mean plasma calcium and inorganic phosphorus levels at different stages showed non-significant differences in buffaloes of other groups. Results showed that, injection of GnRH had beneficial effect on reproductive performance of postpartum anoestrus buffaloes as compared to vitamin-mineral supplementation.

KEY WORDS: Postpartum Anoestrus Buffaloes, Treatment regimens, Reproductive efficiency, Plasma Progesterone, Biochemical profile, Macro-minerals.

INTRODUCTION

Early establishment of cyclic ovarian activity in postpartum buffaloes is desirable as it improves the reproductive efficiency. The nutritional, managemental and environmental factors have impact on fertility. Early postpartum phase exerts biological and physiological stress on the dam (Setia et al., 1992). Disturbances in mineral profile of diet are known to have an adverse effect on the physiological functions in general and reproduction in particular, since they act as co-factors, activators of enzymes or stabilizers of secondary molecular structures in domesticated animals (Valee and Wacker, 1976). Negative energy balance is a major cause of postpartum anoestrus in dairy animals. Gonadotrophin releasing hormone (GnRH) treatment has a good therapeutic action to enhance early resumption of ovarian activity in sucker buffalo-cows (Shah et al., 2002). Similarly, the supplementation of vitamins and minerals either orally or parentrally has been documented to

have varying effect on fertility and blood profile in postpartum anoestrus buffaloes. The data particularly on this line of investigation will help to rectify certain physiological abnormalities either by feeding, treatment or other managemental measures; hence this study was planned and executed under field conditions.

MATERIALS AND METHODS

This study was carried out on Surti buffaloes of three village co-operative societies of Panchmahal district in Gujarat during the months of January to June, 2011. Fifty two postpartum anoestrus buffaloes were selected through glynaeco-clinical examinations twice 10 days apart between day 80 and 90 postpartum. They were randomly divided into four groups-A,B,C, and D, and were treated from day 90th postpartum for induction of oestrus. Group-D (n=12) served as untreated control, while anoestrus buffaloes of group-A (n=14) were supplemented orally with Chelated mineral mixture (Manufactured by Panchamrut Dairy) @ 30 gm day/animal for 30 days. Buffaloes of group-B (n=14) were treated with im injection of Tonophosphan (Sodium salt of 4-dimethylamino-2-methylphenyl phosphoric acid 0.2g/ml, Intervet) and Vitacept (Vitamin AD3E, Concept Pharmaceuticals) 10 ml each per animal at weekly interval for 3 weeks. Anoestrus buffaloes of group-C (n=12) were treated with single Injection of GnRH, 5 ml, im (Buserelin acetate 0.0042 mg/ml, "Receptal", Intervet International GmbH, Germany). The animals detected in estrus were served by AI. The non-returned buffaloes were examined for pregnancy per-rectum 45 day post-AI. Oestrus induction response & induction interval, conception rate and service period were recorded and the reproductive efficiency was worked out using standard formula for all the treated as well as untreated control groups of buffaloes.

About 10 ml of jugular blood samples were collected in heparinized vials from selected animals on day 90th postpartum (just before treatment), on the day of estrus exhibition/AI and 22nd day post-estrus/AI. Plasma samples separated out were stored at -20°C till analysed. Plasma progesterone was estimated by RIA employing standard technique of Kubasik et al. (1984). The plasma biochemical constituents, viz., plasma total protein (Biuret method), total cholesterol (CHOD/PAP method), and macro-minerals, viz. calcium (Arsenazol-III method) and inorganic phosphorus (Molybdate UV method) were estimated on an autoanalyzer using standard assay kits procured from Crest Biosystems, Goa, India. Plasma profile of different treatment regimen at pre-treatment, oestrus and 22nd day post-AI were compared using CRD and CD test.

RESULTS AND DISCUSSION

Significantly ($P<0.05$) higher oestrus induction response and shorter oestrus induction interval was observed in buffaloes of GnRH treated group-C (83.33%, 20.90 ± 3.13 days) followed by Vit-min supplemented group-B (50.00%, 27.14 ± 3.74 days), chelated mineral supplemented group-A (42.85%, 40.83 ± 4.04 days) and control group-D (25.00%, 44.66 ± 5.72 days). The highest conception rate (80.00 %), shortest service period (123.60 ± 2.69 days) and highest reproductive efficiency (66.66 %) was observed for group-C buffaloes. The corresponding values for group-B were 71.42 %, 128.71 ± 1.93 days and 35.71 %, and for group-A 66.66 %, 137.83 ± 2.63 days and 28.56 %. Whereas, only 25.00 % (03/12) buffaloes of control group-D exhibited oestrus and two of them conceived with prolonged service period (151.00 ± 14.56 days) and reduced reproductive efficiency (16.66 %). Results showed that, injection of GnRH had beneficial effect over vit-min supplements on reproductive performance of postpartum anoestrus buffaloes.

The present findings of oestrus induction response and conception rate in Group-A and B were comparable with reports of Akhtar et al. (2004). Srivastava (2008) recorded oestrus induction response and conception rate as 50 and 80 % within 10 and 30 days following Tono-Prepaline therapy for 2 weeks. Butani (2008) also reported higher oestrus induction (82.08 %) and conception rate (69.10 %) with same line of treatment in anoestrus buffaloes. Singh et al. (2006) reported 47

per cent oestrus response after 30 days of supplevite-M oral therapy that led to 72 % conception rate in anoestrus buffaloes. Selvaraju et al. (2009) and Nidhi et al. (2010) reported higher oestrus induction and conception rate using herbal heat inducer and supplementation with area specific mineral mixture. The present findings of GnRH treatment are in agreement with the report of Khasatiya et al. (2004), and it partly corroborated with report of Dugwekar et al. (2006). On the contrary, Reddy et al. (1994) found lower oestrus induction response (50 %) with only 40 % conception using 5 ml Receptal.

The plasma progesterone profile of group-A was significantly ($P<0.05$) higher at 22nd day post-AI (3.65 ± 0.81 ng/ml) as compared to that of pre-treatment and induced oestrus phase (0.46 ± 0.12 & 0.44 ± 0.08 ng/ml). Similar trend was also observed for group-B, C and group-D, respectively (Table 1). The progesterone levels at oestrus and pre-treatment periods did not vary significantly ($P<0.05$) in any of the groups or even between groups. Significantly ($P<0.05$) higher plasma progesterone profile in responded buffaloes at 22nd day post-AI is due to pregnancy CL or active CL which had contributed higher circulatory progesterone. Significantly higher mean progesterone level in the conceived buffaloes than that of the non-responded buffaloes compared well with the findings of Sarvaiya et al. (1991) and Khasatiya et al. (2004). On the contrary to this, Sharma et al. (1999) reported 0.18 ± 0.07 ng/ml plasma progesterone in buffaloes at spontaneous oestrus.

The mean plasma protein levels were significantly ($P<0.05$) higher at induced oestrus and 22nd day post-AI than that of pre-treatment level in group-A treated with chelated minerals. However, the mean plasma protein at pre-treatment, oestrus and 22nd day post-AI of group-B, C and D showed non-significant variations (Table 1). These findings are in agreement with those of Dadarwal et al. (2009) and Kumar et al. (2010). The mean total protein levels of GnRH treated buffaloes were little higher at pre-treatment, oestrus and 22nd day post-AI than that of Chelated mineral mixture supplement and control group. Sheshappa et al. (2002) also reported similar protein profile in postpartum anoestrus cows treated with GnRH. Ahmed et al. (2010) reported comparatively lower total protein levels in delayed pubertal buffalo-heifers than the present findings. Protein deficiency retards the development of reproductive organs and was considered to be a factor responsible for failure or delay in onset of postpartum oestrus (Roberts, 1971).

The mean plasma total cholesterol levels were higher at induced oestrus and 22nd day post-AI than that of pre-treatment levels in all the treatment groups in the study. Plasma total cholesterol, being precursor of steroid hormones, is closely associated with physiological status of animal reproduction, resulted into better ovarian activity and pregnancy rate in different treatment groups, suggestive of increasing level of cholesterol concentration at oestrus and 22nd day post-AI than that of the pre-treatment level might help in improving gonadal steroidogenesis and ovarian rebound effect. The thyroid hormones and estrogen influence cholesterol synthesis, hence the variation observed between responded and non-responded buffaloes is conceivable. Present findings are in agreements with the report of Singh et al. (2004) and Butani (2008) who have also found significantly higher cholesterol levels in buffaloes on the day of oestrus as it was observed in the present study, whereas comparatively lower cholesterol levels were reported at oestrus by Dadarwal et al. (2009). The highest adrenal cholesterol concentration occurs at oestrus when females are under oestrogen dominance, which declines, later when the progesterone phase sets in. The higher cholesterol levels observed at oestrus in all the treatment group might be attributed to higher estrogen level at oestrus.

The comparison of mean plasma calcium concentrations at pre-treatment, induced oestrus and 22nd day post-AI for buffaloes of treatment group-A, B, C and untreated control group-D (Table 1) revealed significantly ($P<0.05$) higher plasma calcium level in the Tonophosphan plus Vitacept group-B at induced oestrus and 22nd day post-AI than the levels observed in pretreated buffaloes. However, the mean plasma calcium level was non-significantly higher at induced oestrus and 22nd

Table 1. Plasma progesterone and biochemical and macro-mineral profile at pre-treatment, induced oestrus and 22nd day post-AI in postpartum anoestrus buffaloes responded to different treatments and untreated control groups

Group	N	Progesterone (ng/ml)			Total Protein (g/dl)			Cholesterol (mg/dl)			Calcium (mg/dl)			Phosphorus (mg/dl)		
		Pre treatment	AI Oestrus / AI	22 day Post AI	Pre treatment	AI Oestrus/ AI	22 day Post AI	Pre treatment	AI Oestrus/ AI	22 day Post AI	Pre treatment	AI Oestrus/ AI	22 day Post AI	Pre treatment	AI Oestrus/ AI	22 day Post AI
A	5	0.46	0.44	3.65	6.52	7.23	7.31	149.66	158.95	149.02	8.30	9.80	9.55	3.77	7.19	6.35
		0.12	0.08	0.81	0.31	0.22	0.18	13.99	13.65	4.97	0.87	0.44	0.55	0.56	0.65	0.41
B	7	0.44	0.38	4.69	7.10	7.31	7.52	180.33	174.44	171.80	8.82	11.31	11.80	4.53	5.55	6.14
		0.05	0.11	1.44	0.17	0.12	0.21	14.87	7.73	5.59	0.66	0.5	0.56	0.78	0.73	0.54
C	10	0.62	0.36	2.97	7.86	7.43	7.15	149.70	170.70	157.20	9.52	10.50	9.73	5.20	5.52	6.08
		0.09	0.02	0.44	0.16	0.21	0.22	9.85	8.69	11.56	0.58	0.52	0.40	0.33	0.32	0.34
D	3	0.32	0.40	1.58	7.57	7.06	7.47	180.42	176.85	187.85	9.60	10.42	10.20	6.33	6.10	6.38
		0.09	0.28	0.65	0.13	0.27	0.44	20.30	4.41	3.69	0.67	0.56	0.52	0.57	0.56	0.60

A: Chelated mineral mixture, B: Inj. Inorganic phosphorus and Inj. vitamin AD3 & C: Inj. GnRH, and D: untreated control.
Means bearing different superscripts within the row differ significantly (P<0.05) for a trait.

day post AI than the pre-treatment levels in group A, C and D. All the treated groups of buffaloes had higher plasma calcium level at oestrus and 22nd day post-AI than the pre-treatment level. Similar findings were also reported by Singh et al. (2005). However, Biswas et al. (2005) and Singh et al. (2006) observed significantly higher serum calcium level in postpartum anoestrus buffaloes than the present study. The findings in the present study suggested that calcium level has no direct bearing on reproduction and also the calcium deficiency did not affect reproductive performance of the buffaloes. The alteration of Ca:P ratio which may affect ovarian function through its blocking action on pituitary gland, results in prolongation of first oestrus and ovulation, delayed uterine involution, increased incidence of dystocia, retention of placenta and prolapse of uterus (Kumar, 2003). Moreover, low calcium level in blood is also associated with anoestrus whereas, excess of the calcium can affect the reproductive status of animal by impairing absorption of phosphorus, manganese, zinc, copper and other elements from GI tract.

The mean plasma inorganic phosphorus level was significantly higher at induced oestrus than the pre-treatment level in group-A. The mean plasma inorganic phosphorus levels at pre-treatment, induced oestrus and 22nd day post-AI showed non-significant difference in group-B, C and control group-D (Table 1). All the treatment groups (A, B and C) have shown increase in the plasma inorganic phosphorus concentration than their pre-treatment levels. The Chelated mineral mixture supplement and Tonophosphan plus Vitacept treatment have resulted into increase in plasma inorganic phosphorus levels at induced oestrus. This might be due to treatment effect of postpartum anoestrus. The present findings of inorganic phosphorus levels at oestrus and 22nd day post-AI are comparable with the reports of Patel et al. (2009) and Tiwari et al. (2010) on similar line. Kumar et al. (2010) reported lower inorganic phosphorus level than that of the buffaloes responded to various treatment. Lower concentration of circulatory minerals results in impaired reproductive function leading to cessation of cyclic activity. Severe phosphorus deficiency may delay puberty and postpartum oestrus. Whereas, moderate deficiency may lead to repeat breeding condition and poor conception rate (Kumar, 2003). Minerals like calcium and phosphorus influence the ability of animals to utilize other micro-minerals. The influence of these minerals on enzyme system may affect reproductive efficiency which might be reflected in lower plasma phosphorus levels.

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