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Effect of Estrus Induction on Blood Biochemical and Mineral Profiles and Fertility Rate in Retained Fetal Membranes Affected and Normally Calved Riverine Buffaloes

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ABSTRACT

Background: Calving to conception interval is abnormally extended by the occurrence of retained fetal membranes (RFM) by altering the blood biochemical and mineral milieu in cows. Hence this experiment was conducted in RFM affected and normally calved (NC) buffaloes by inducing estrus with CIDR plus $PGF_2\alpha$ protocol to study the blood biochemical and mineral profiles and to correlate them with fertility rate.

Methods: Buffaloes (n=64) at 45-60 days postpartum including 32 treated for RFM and 32 NC from field were equally divided into groups I and II and groups III and IV, respectively. Buffaloes of groups I and III were initially dewormed and administered 35-50 g mineral mixture daily orally for 15 days in the concentrate feed. Then, buffaloes of all the groups were treated with CIDR plus $PGF_2\alpha$. After CIDR removal, all the buffaloes were artificially inseminated twice at 48 and 72 hrs. Blood was collected during different stages of treatment from all the buffaloes to assess the blood biochemical and mineral status. The animals returned to estrus following FTAI were again inseminated during subsequent estrus. Pregnancy diagnosis was done at 60 days post-AI and conception rates for induced estrus and overall of two cycles were calculated.

Result: In all the groups, blood glucose, total protein, triglycerides, cholesterol and phosphorus levels increased from the time of selection to 10 days post-AI. There was an altered calcium phosphorus ratio in RFM affected buffaloes (1:1) at the time of selection. CIDR plus $PGF_2\alpha$ protocol influenced the blood biochemical constituents and brought the calcium and phosphorus ratio as 2:1 and improved the fertility in riverine buffaloes. The overall conception rate was 62.50, 37.50, 87.50 and 75.00 per cent, in group I, II, III and IV buffaloes, respectively.

Key words: Blood biochemical constituents, Buffaloes, Estrus induction, Minerals.

INTRODUCTION

The buffalo (*Bubalus bubalis*) is one of the highest milk producing animals. Primary factor which affects the productivity of a buffalo is poor reproductive efficiency may be because of delayed puberty, age at first calving, prolonged calving intervals, higher number of services per conception, longer calving to conception interval, seasonal anestrus, uterine infections and various types of obstetrical ailments (Sachan *et al.,* 2019). Retention of fetal membranes (RFM) is one of the major postpartum reproductive disorders affecting profitability of buffalo production since it delays uterine involution and resumption of ovarian activity leading to postpartum anestrus (Selvaraju *et al.,* 2005). The incidence of postpartum anestrus in Indian buffaloes varies from 19 to 74% (Tomar *et al.,* 2002). Various research workers (Ravikumar, 2003; Jagadeesan *et al.,* 2006) have satisfactorily induced estrus and ovulation in postpartum anestrus cows using Controlled Internal Drug Release (CIDR) device. Velladurai *et al.* (2014) treated the crossbred normally calved (NC) and RFM affected cows with ovsynch protocol and concluded that ovsynch programme influenced the blood biochemical constituents and improved the conception. However, the effect of RFM and estrus induction with CIDR treatment in buffaloes which were affected and treated for RFM on the blood biochemical constituents and mineral profiles and their correlation with fertility have not been reported in detail. Hence, the present investigation was undertaken to assess the influence of estrus induction with CIDR plus prostaglandin $F_2\alpha$ ($PGF_2\alpha$) protocol on blood biochemical and mineral profiles and to correlate these parameters with fertility rate in NC and RFM affected riverine buffaloes.

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MATERIALS AND METHODS

An experiment was conducted in graded Murrah buffaloes

which were selected from Large Animal Gynaecology Unit, Veterinary College and Research Institute, Namakkal hospital and Veterinary Dispensaries which are located in and around Namakkal district from 2017-2019. Buffaloes (n=64) at 45-60 days postpartum including 32 buffaloes treated previously during immediate postpartum period for RFM and 32 NC were selected. They were equally divided into 4 experimental groups as group I and II (RFM groups) and group III and IV (NC groups), respectively. Buffaloes of groups I and III were dewormed orally with Albendazole suspension (Vetcare Private Limited, India; 10 mg/kg b.wt.) at 45-60 days postpartum. Further, they were treated orally with TANUVAS mineral mixture (30-50g/day/animal) for 15 days in the concentrate feed from 45-60 days postpartum. At 60-75 days postpartum, buffaloes of all the 4 groups were inserted with CIDR containing 1.38 g progesterone (CIDR, Eazi-Breed, Pfizer Animal Health, India) intravaginally and left *in situ* for 9 days. All buffaloes received an i.m. injection of 500 µg $PGF_2\alpha$ (Cloprostenol sodium, Pragma, Intas Pharmaceuticals Limited, India) at 24 hours prior to CIDR withdrawal. All the buffaloes were artificially inseminated twice at 48 and 72 hrs of CIDR removal, with good quality frozen thawed semen. Further, those buffaloes which failed to conceive and returned to estrus following breeding at induced estrus, were artificially inseminated twice at an interval of 24 hrs during subsequent estrus. Pregnancy was confirmed by per rectal examination at 60 days post-insemination. Pregnancy rate was calculated in percentage for induced estrus (first cycle), second cycle and overall of two cycles in all four groups. Blood was collected at the time of (i) selection of animals (ii) CIDR insertion (iii) $PGF_2\alpha$ injection (iv) first/fix timed AI and (V) at 10 days after first AI from all the animals. The serum glucose was estimated immediately and the sera samples were stored at -20°C until analysis of other blood biochemical parameters*, viz.,* total protein, triglycerides and total cholesterol and minerals*, viz.,* calcium and phosphorus.

The blood biochemical constituents were analyzed in UV-VIS double beam spectrophotometer (Systronics, Model 2202, India). Blood glucose level (mg/dl) was determined from the serum samples by the GOD/POD method (Glucose Oxsidase/Peroxidase). Total protein (gm/dl) was determined from the serum samples by the Biuret method. The triglycerides (mg/dl) level was determined by GPO (glycerol -3-phosphate oxidase) method. Serum total cholesterol (mg/dl) was determined by the method of Allain *et al.* (1974). Serum calcium (mg/dl) level was determined by Arsenazo-III method. Serum phosphorus (mg/dl) level was estimated by the UV Molybdate-End Point Assay. The completely randomized design (CRD) was followed for the experiment (Snedecor and Cochran, 1994) and the data collected were analysed using SPSS® 20.0 software package. Post hoc analysis was done by Tukey's honestly significance difference. The Fisher's exact test was used to analyze the data related to the conception rate.

RESULTS AND DISCUSSION

Conception rate

In the current investigation (Table 1), estrus induction with CIDR plus $PGF_2\alpha$ resulted in 81.25 and 50.00% overall conception rates in NC and RFM affected buffaloes, respectively. It indicated that CIDR plus $PGF_2\alpha$ was effective in augmenting fertility in postpartum buffaloes. In this study, the overall conception rate of first and second service ranged from 37.50 to 87.50%. Similar conception rates with CIDR plus $PGF_2\alpha$ were obtained by Zaabel *et al*. (2009) in buffaloes and Cevik *et al*. (2010) in cows. But andurkar and Kadu (1995) recorded 100% conception rate in CIDR plus $PGF_2\alpha$ treated buffaloes. However, Murugavel *et al*. (2009) reported only 27.30% conception rate in buffaloes following CIDR plus ovsynch protocol. In this experiment, the groups I and III buffaloes which were treated with combination of deworming, mineral mixture and CIDR protocol resulted in 62.50 and 87.50% overall conception rates, respectively, whereas groups II and IV, with CIDR protocol alone, had 37.50 and 75.00 % overall conception rates. This result indicated that either in NC buffaloes or in RFM affected buffaloes, alleviation of worm infestation and supplementation of mineral mixture for 15 days could further enhanced the fertility rate in buffaloes as described by Thavani *et al*. (2012).

In RFM affected groups (groups I and II) the overall first and second service conception rate obtained was 50.00% which was lower than the overall first and second service conception rate (81.25%) obtained in NC buffaloes. It was evident that RFM might have caused uterine damage and ovarian acyclicity which in turn might have reduced the conception rates following estrus induction as explained by Kimura *et al*. (2006).

Table 1: Conception rate following estrus induction with CIDR in RFM affected and NC buffaloes.

Treatment group		No of animals treated	Conception rate			p- value
			First service conception rate	Second service conception rate	Overall conception rate	
RFM Affected	Group I	16	4/16(25.00)	6/16 (37.50)	10/16 (62.50)	0.021326
	Group II	16	4/16 (25.00)	2/16 (12.50)	6/16 (37.50)	
Overall	32	8/32 (25.00)	8/32 (25.00)	16/32 (50.00)		
Normally Calved	Group III	16	10/16 (62.50)	4/16 (25.00)	14/16 (87.50)	
	Group IV	16	6/16 (37.50)	6/16 (37.50)	12/16 (75.00)	
Overall	32	16/32 (50.00)	10/32 (31.25)	26/32 (81.25)		

P≤0.05: Only group II had statistically significant difference with group III. No significant difference among other groups.

The overall conception rate of 75.00% in group IV proved the efficacy of CIDR plus $PGF_2\alpha$ in improving fertility in buffaloes. The increased conception rate might be due to the fixed time breeding of buffaloes (Zaabel *et al*., 2009) and altered secretion of oestrogen and progesterone (Singh *et al*., 2010) following CIDR withdrawal. Fisher's exact test revealed that only group II had statistically significant difference with group III. No significant difference was observed among other groups in conception rate.

Serum glucose

Blood glucose level exhibits a positive correlation with ovarian cyclicity in buffaloes (Singh *et al.,* 2010). In the present investigation (Table 2), the blood glucose level was lower in RFM affected buffaloes than NC buffaloes. Similarly, low blood glucose level was reported in RFM treated buffaloes when compared to NC buffaloes (El- Malky *et al.,* 2010; Thavani *et al*., 2012). In this investigation, from the time of selection of buffaloes to 10 days post AI, the mean blood glucose level was found to be low in RFM affected buffaloes when compared to NC buffaloes. This fact was reflected in conception rates between RFM affected and NC buffaloes. Velladurai *et al.* (2014) stated that blood glucose is a metabolic signal providing information for the central control of GnRH release and further reported that expression of estrus at first postpartum ovulation was more likely in cows which maintained a higher blood glucose level. The increasing concentration of blood glucose from animal selection to 10 days post-AI in the current experiment might be due to the increased energy metabolism to make the availability of glucose in the genital organs especially in the uterus as explained by Pandey *et al.* (2007).

Serum total protein

In this experiment, from the time of selection to 10 days post-AI, the mean serum total protein ranged from 8.09±0.23 to 8.56±0.21 mg/dl (Table 2). Similar finding was reported in buffaloes by others (Jagadeesan *et al.,* 2005; Jagadeesan *et al.,* 2006; Kumar *et al*., 2010). In this study, at the time of selection of animals, the total protein levels did not show any significant difference between RFM affected and NC buffaloes. Higher concentration of serum total protein in RFM affected buffaloes than in NC buffaloes were reported by other scientists (Pandey *et al*., 2007; El-Malky *et al.,* 2010). The increasing concentration of serum total protein from selection to 10 days post-AI might be due to the increased metabolism for the preparation of histotrophic nutrition in the uterus for the embryonic development (Cevik *et al.,* 2010).

Triglycerides

The mean serum triglycerides in this study ranged from 83.83±1.51 to 131.89±0.59 mg/dl (Table 3). Similar values were reported by Velladurai *et al*. (2014) in cows. However, lower level of serum triglycerides was reported by Singh *et al.* (2010). In this investigation, significantly low levels of mean serum triglycerides were observed in RFM affected groups than in NC groups. The reduction in mean serum triglycerides levels in this experiment might be due to disturbed lipid metabolism and increased tissue lipolytic enzymes in RFM affected buffaloes as demonstrated by Ravikumar, (2003). Induction of estrus in all the buffaloes increased the mean serum triglycerides from selection to 10 days post-AI. This might be due to the altered lipid metabolism towards the conception in buffaloes (Michal *et al*., 2006).

Serum total cholesterol

The mean serum total cholesterol ranged from 152.98±6.63 to 173.97±3.21 mg/dl (Table 3). Similar value was reported by Velladurai *et al*. (2014) in cows. Whereas Jagadeesan *et al.* (2006) found significantly low blood cholesterol concentration (119.89±3.32 mg/dl) in buffaloes before treating with CIDR. In this experiment, the mean serum total cholesterol level was lower in RFM affected buffaloes than NC buffaloes at the time of selection. Similar finding was reported by El-malky *et al*. (2010). The lowered cholesterol in RFM affected buffaloes might be due to the increase in breakdown of cholesterol (Velladurai *et al.,* 2014). In this study, there was an increase in serum total cholesterol level in buffaloes from the time of selection to 10 days post-AI and it might be due to persistent utilization of serum cholesterol for progesterone synthesis (Michal *et al*., 2006).

Table 2: Serum glucose and total protein levels before, during and after estrus induction in RFM affected and NC buffaloes.

Treatment	Glucose (mg/dl)				
groups	At selection	At insertion of CIDR	At $PGF_2\alpha$ injection	At first AI	10 days post-AI
Group I	$41.56^{ap} \pm 0.86$	$55.02^{c} \pm 1.82$	$54.24^{b} \pm 0.92$	$56.40^{dp} \pm 1.03$	$56.84^{dp} \pm 0.83$
Group II	$40.80^{ap} \pm 0.96$	$54.02^{b} \pm 0.46$	$55.24^{c} \pm 0.21$	$54.37^{dp} \pm 0.93$	$56.66^{ep} \pm 0.77$
Group III	$50.03^{aq} \pm 0.33$	$56.34^{b} \pm 0.72$	$56.36^{b} \pm 0.68$	$56.75^{cq} \pm 0.82$	$57.12^{dq} \pm 0.83$
Group IV	$52.00^{aq} \pm 0.93$	$55.94^{c} \pm 0.65$	$56.04^{d} \pm 0.68$	$54.37^{bq} \pm 0.84$	$57.28^{eq} \pm 0.98$
Total protein (g/dl)					
Group I	$8.16^{a} \pm 0.29$	$8.28^{bq} \pm 0.19$	$8.33^{c} \pm 0.22$	$8.43^{d} \pm 0.18$	$8.56^{cp} \pm 0.21$
Group II	$8.09^{a} \pm 0.23$	$8.19^{bp} \pm 0.23$	$8.32^{c} \pm 0.09$	$8.42^{d} \pm 0.22$	$8.43^{dq} \pm 0.18$
Group III	$8.17^{a} \pm 0.17$	$8.35^{bq} \pm 0.04$	$8.33^{b} \pm 0.10$	$8.46^{c} \pm 0.05$	$8.56^{dp} \pm 0.13$
Group IV	$8.12^{a} \pm 0.05$	$8.18^{ap} \pm 0.06$	$8.30^{b} \pm 0.14$	$8.42^{c} \pm 0.13$	$8.46^{cq} \pm 0.21$

Mean values bearing superscripts between columns (a, b, c, d, e) with in a row and among rows (p, q) with in a column differ significantly ($P \leq 0.05$).

Table 3: Serum triglycerides and total cholesterol levels before, during and after estrus induction in RFM affected and NC buffaloes.

Treatment	Triglycerides (mg/dl)				
groups	At selection	At insertion of CIDR	At $PGF_2\alpha$ injection	At first AI	10 days post AI
Group I	83.83^{ap}± 1.51	127.87^{bq} ± 0.88	128.99^{bq} ± 0.67	129.03^{cq} ± 0.83	129.84^{cq} ± 0.71
Group II	84.17^{ap}± 0.56	90.07^{bp} ± 0.58	100.25^{cp} ± 0.59	112.48^{dp} ± 0.39	118.36^{ep} ± 0.49
Group III	129.17^{aq} ± 0.56	131.89^{cq} ± 0.59	127.25^{bq} ± 01.64	127.48^{bq} ± 0.39	130.36^{cq} ± 0.49
Group IV	129.27^{aq} ± 0.49	129.87^{aq} ± 0.88	129.89^{aq}± 0.67	129.93^{aq} ± 0.83	129.85^{aq} ± 0.56
Total cholesterol (mg/dl)					
Group I	152.96^{ap}± 6.63	154.37^{ap} ± 3.50	167.41^{bp} ± 3.71	172.51^{c} ± 2.64	172.61^{c} ± 2.64
Group II	153.42^{ap} ± 4.48	154.25^{ap} ± 3.39	166.94^{bp} ± 4.51	172.94^{c} ± 2.38	172.98^{c}± 3.91
Group III	170.06^{aq} ± 5.41	172.41^{aq} ± 3.41	172.41^{aq} ± 2.54	173.54^{a} ± 2.31	173.97^{a} ± 3.21
Group IV	171.41^{aq} ± 4.31	172.04^{aq} ± 3.21	172.39^{aq} ± 3.21	173.10^{a} ± 2.45	173.21^{a} ± 3.41

Mean values bearing superscripts between columns (a, b, c, d, e) with in a row and among rows (p, q) with in a column differ significantly ($P \leq 0.05$).

Table 4: Serum calcium and phosphorus levels before, during and after estrus induction in RFM affected and NC buffaloes.

Treatment	Calcium (mg/dl)				
groups	At selection	At insertion of CIDR	At $PGF_2\alpha$ injection	At first AI	10 days post AI
Group I	6.47^{ap} ± 0.23	9.89^{bq} ± 0.19	10.52^{cp} ± 0.20	10.97^{c}± 0.20	10.14^{cp} ± 0.21
Group II	6.42^{ap} ± 0.56	6.75^{bp} ± 0.26	9.95^{bp} ± 0.22	10.80^{c} ± 0.19	10.14^{cp} ± 0.20
Group III	10.13^{aq} ± 0.28	10.72^{bq} ± 0.47	10.73^{bq} ± 0.25	11.02^{c} ± 0.19	10.97^{cq} ± 0.17
Group IV	9.90^{aq} ± 0.22	10.52^{bq} ± 0.27	10.75^{bq} ± 0.26	10.83^{b}± 0.20	10.74^{bq} ± 0.16
Phosphorus (mg/dl)					
Group I	5.06^{ap} ± 0.58	5.10^{bp} ± 0.06	5.19^{c} ± 0.15	5.24^{dp}± 0.11	5.36^{e}± 0.14
Group II	5.01^{ap} ± 0.08	5.08^{bp} ± 0.06	5.16^{c} ± 0.07	5.21^{dp} ± 0.15	5.43^{e} ± 0.12
Group III	4.00^{aq} ± 0.14	4.79^{bq} ± 0.11	5.25^{c} ± 0.16	5.40^{dq} ± 0.11	5.53^{e} ± 0.13
Group IV	4.06^{aq} ± 0.51	4.83^{bq} ± 0.08	5.29^{c} ± 0.15	5.37^{dq} ± 0.15	5.46^{e} ± 0.27

Mean values bearing superscripts between columns (a, b, c, d, e) with in a row and among rows (p, q) with in a column differ significantly ($P \leq 0.05$).

Calcium

The mean serum calcium level recorded in present study ranged from 6.42±0.56 to 10.97±0.17 mg/dl in NC and RFM affected buffaloes (Table 4). Similar values were reported in NC (Jagadeesan *et al.,* 2005) and RFM affected buffaloes (Pandey *et al*., 2007). In our study, the mean serum calcium level during all the stages of blood collection was lower in RFM affected groups than in NC buffaloes. This observation corroborated with the findings of Pandey *et al*. (2007) in buffaloes. It suggested that the lower calcium concentrations might be responsible for the occurrence of RFM in buffaloes and further reduction in fertility. The disturbances in the calcium metabolism and its utilization by the tissue result in atony of genital organs especially the uterus (Pandey *et al*., 2007). In the current experiment, the mean serum level of calcium increased in all the groups from the time of animal selection to 10 days post-AI. It indicated that estrus induction programme influenced the mineral metabolism of the buffaloes and increased the calcium availability to reproductive organs.

Phosphorus

The mean serum phosphorus level observed in this study ranged from 4.00±0.14 to 5.37±0.15 mg/dl in NC and RFM affected buffaloes (Table 4). In this study, higher levels of phosphorus in RFM affected buffaloes than in NC buffaloes were observed. Velladurai *et al.* (2014) stated that even marginal deficiency of phosphorus was sufficient to cause disturbances in pituitary-ovarian axis without manifesting the syndrome. The involvement of phosphorus in phospholipids and cAMP synthesis might be a key factor to its effect of reproduction (Ravikumar, 2003). In the current study alteration in calcium: phosphorus ratio was observed between NC (2:1) and RFM affected buffaloes (1:1). It clearly indicated the disturbance in calcium and phosphorus metabolism in RFM affected buffaloes as described by Pandey *et al*. (2007). The increased level of phosphorus from the time of selection to 10 days post-AI in all the experimental groups of the study showed that estrus induction programme with or without deworming and mineral mixture supplementation influenced the mineral metabolism in NC and RFM affected buffaloes and altered the calcium: phosphorus ratio at the time of AI as 2:1. It could be the reason for the achievement of higher fertility rate following estrus induction in reverine buffaloes of this study.

CONCLUSION

It is concluded that in RFM affected buffaloes estrus induction with CIDR plus $PGF_2\alpha$ changed the blood

biochemical milieu, corrected the calcium and phosphorus ratio and improved the conception rate.

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REFERENCES

Allain, C.C., Poon, L., Chan, S.G., Richmond W., Fu, P. (1974). Enzymatic determination of total serum cholesterol. Clinical Chemistry. 20: 470.

Andurkar, S.B. and Kadu, S.B. (1995). Induction of estrus and fertility with CIDR device and combination with $PGF_2\alpha$ in non-cycling buffaloes. Indian Journal of Animal Reproduction. 16: 81-84.

Cevik, M., Selcuk, M., Dogan, S. (2010). Comparison of pregnancy rates after timed artificial insemination in ovsynch, heatsynch and CIDR-based synchronization protocol in dairy cows. Kafkas üniversitesi veteriner fakültesi dergisi. 16: 85-89.

El-Malky, O., Youssef, M., Abdel-Aziz, N., Abd El- Salaam, A. (2010). Postpartum performance of buffaloes treated with GnRH to overcome the impact of RFM. Journal of American Science. 2: 225-233.

Jagatheesan, P.N.R, Selvaraju, M., Ramesh Saravanakumar, V., Chandrahasan, C. (2005). Effect of advanced pregnancy and early lactation on blood biochemical constituents in Murrah buffaloes. Indian Veterinary Journal. 82:401-403.

Jagatheesan, P.N.R, Selvaraju, M., Ramesh Saravanakumar, V., Chandrahasan, C. (2006). Blood biochemical Profile and oestrus induction to augment fertility in anoestrus Murrah buffaloes. Tamil Nadu Journal of Veterinary and Animal Sciences. 1: 10-12.

Kimura, K., Reinhardt, T.A., Goff, J.P. (2006). Parturition and hypocalcemia blunts calcium signals in immune cells of dairy cattle. Journal of Dairy Science. 89: 2588-2595.

Kumar, P., Sharma, M.C., Joshi, C. (2010). Effect of biochemical profile concurrent with micromineral deficiencies in buffaloes (*Bubalus bubalis*) of eastern Uttar Pradesh. Indian Journal of Animal Sciences. 77: 86-91.

Michal K., Edward, M., Hanna, M. (2006). Some hormonal and biochemical blood indices in cow with retained placenta and puerperal metritis. Bulletin of the Veterinary Institute Pulawy. 50: 89-92.

Murugavel, K., Antoine, D., Raju M.S., Lopez-Gatius, F. (2009). The effect of addition of equine chorionic gonadotropin to a progesterone based estrus synchronization protocol in buffaloes (*Bubalus bubalis*) under tropical conditions. Theriogenology. 71: 1120-1126.

Pandey, A.K., Shukla. S.P., Pandey S.K., Sharma, Y.K. (2007). Haemato-biochemical profile in relation to normal parturition buffaloes and buffaloes with retained fetal membranes. Buffalo Bulletin. 26: 46-49.

Ravikumar, K. (2003). Synchronization of ovulation using ovsynchand ovsynch plus CIDR and fertility in postpartum anestrus buffaloes. M.V.Sc., thesis submitted to Tamil Nadu Veterinary and Animal Sciences University, Chennai.

Sachan, V., Singh, V., Saxena, A. (2019). Study on biochemical changes during peri-parturient prolapse in Murrah buffaloes. Haryana Veterinarian. 58: 98-101.

Selvaraju, M., Veerapandian, C., Kathiresan, D., Chandrahasan, C. (2005). Incidence of bovine reproductive disorders. Indian Veterinary Journal. 82: 556.

Singh, J., Ghuman, S.P.S., Dadarwal, D., Honparkhe, M., Dhaliwal G.S., Jain, A.K. (2010). Estimations of blood plasma metabolites following melatonin implants treatment for initiation of ovarian cyclicity in true anestrus buffalo heifers. Indian Journal of Animal Sciences. 80: 229-231.

Snedecor, G.M. and Cochran, W.C. (1994). Statistical Methods, (9^{th} edn.) Oxford and IBM Publishing Company, Mumbai, India.

Thavani, W.W., Macmillan, K.L., Hansen P.J., Drost, M. (2012). Concepts for regulation of corpus luteum function by the conceptus and ovarian follicles to improve fertility. Theriogenology. 61: 149-154.

Tomar, K.P.S., Singh, P., Singh, R., Singh, S. (2002). Seasonal variations in reproductive problems of buffaloes under field condition. Indian Journal of Animal Reproduction. 23: 18-20.

Velladurai, C., Napolean, R.E., Selvaraju, M., Doraisamy, K.A. (2014). Effect of Ovsynch treatment on blood biochemical constituents and conception rate in retained fetal membranes affected and normally calved cows. Indian Journal of Animal Reproduction. 35: 21-24.

Zaabel, S.M., Hegab, A.O., Montasser A.E., El-Sheikh, H. (2009). Reproductive performance of anestrus buffaloes treated with CIDR. Animal Production. 6: 460-464.