Original Article

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EFFECT OF CIDR PROTOCOL ON CONCEPTION RATE IN RFM AFFECTED AND NORMALLY CALVED BUFFALOES

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ABSTRACT

Delayed re-establishment of ovarian cyclicity after parturition leads to increased inter-calving interval in buffaloes. This interval is further extended by the occurrence of post-partum disorders especially retained fetal membranes (RFM) which in turn causes heavy economic loss to the farming community. A total of 16 buffaloes at 45 to 60 days post-partum period including 8 buffaloes treated for retained fetal membranes (RFM) and 8 normally calved (NC) were selected and dewormed orally with Albendazole and administered orally with 50 g mineral mixture for 15 days after deworming. All the buffaloes at 60 to 75 days post-partum were inserted with CIDR intravaginally and left *in situ* for 9 days. All the buffaloes received an intra-muscular injection of 500 µg $PGF_2\alpha$ at 24 h prior to CIDR withdrawal. At 48 (first AI) and 72 h (second AI) of CIDR removal, the buffaloes were artificially inseminated. Further, those buffaloes which failed to conceive and returned to oestrus (subsequent oestrus) following breeding at induced oestrus, were artificially inseminated twice at an interval of 24 h. Pregnancy diagnosis was done by rectal examination at 60 days post-insemination.

Retention rate of CIDR from insertion to withdrawal was 100%. The percentage of ovulatory response assessed by ultrasonography following oestrus induction in NC and RFM affected buffaloes was 100 and 87.50%. The first service, second service and overall conception rates were 25, 37.50 and 62.50 and 62.50, 25 and 87.50 in RFM and NC groups respectively.

In both the groups, the serum mean progesterone levels increased from the time of selection to the time of $PGF_2\alpha$ injection but at first AI, the serum progesterone levels reached below 0.5 ng/ml and at 10 days post AI, there was an elevated progesterone levels in both the groups. The serum oestradiol levels increased from the time selection of animals to the time of first AI and then declined at 10 days post AI. The mean serum oestradiol levels were higher in NC buffaloes from the time of selection of animal to first AI in all the groups when compared to RFM affected buffaloes. It is concluded that oestrus induction with CIDR plus $PGF_2\alpha$ with prior deworming and mineral mixture supplementation could be used in post-partum NC or RFM affected buffaloes to achieve maximum conception rate under field conditions.

Keywords: *Bubalus bubalis*, buffaloes, CIDR,

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RFM, oestrus induction, conception rate

INTRODUCTION

Retention of fetal membranes (RFM) is one of the major post-partum reproductive disorders affecting profitability of buffalo production since it delays uterine involution and resumption of ovarian activity (El-Malky *et al*., 2010). The incidence of RFM in buffaloes ranged from 10 to 15%. Furthermore, a wide variations were reported (2.89 to 12.23%) and the incidence gradually increases with parity, reached a maximum at the fifth parity (30%) and associated with malnutrition. RFM frequently results in a secondary bacterial infection and subsequently depress fertility, prolongs calving interval and reduces the milk production (Gohar *et al*., 2018) and involves higher costs of veterinary treatment.

Different treatment methods are employed to reduce the post-partum anestrus after normal or abnormal calving and subsequent inter-calving period in order to increase fertility in buffaloes. Administration of exogenous progesterone is considered as appropriate treatment for anestrus post-partum buffaloes (Ghallab *et al*., 2016) for the resumption of oestrus with normal oestrous cycle length (Rhodes *et al*., 2003). Progesterone (both exogenous and endogenous) suppress the oestrus and ovulation by inhibiting the release of luteinizing hormone and its withdrawal results in a gradual rise in plasma LH concentration culminating in a preovulatory LH surge which occurs approximately after 48 h (Ghallab *et al*., 2016).

Various research workers (Singh and Singh, 2006; Selvaraju *et al*., 2011) have satisfactorily induced oestrus and ovulation in post-partum cows using CIDR or norgestomet. However, fewer studies were conducted on the use of exogenous progesterone for inducing cyclicity in post-partum buffaloes and the results were not consistent. Further, the effects of RFM and efficiency of oestrus induction with CIDR treatment on conception in buffaloes which were affected and treated for RFM have not been reported in detail. Based on the above views, the present study was formulated to study the ovulatory response, blood progesterone and oestradiol 17β profiles and conception rate following CIDR treatment in buffaloes affected with and treated for retained fetal membranes (RFM).

MATERIALS AND METHODS

Selection of experimental animals

An experiment was conducted in 16 graded Murrah buffaloes, which were selected from Large Animal Gynaecology Unit of Teaching Veterinary Clinical Complex, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India. Out of which 8 buffaloes were previously treated for retained fetal membranes (RFM group) and the remaining 8 buffaloes had normal calving (NC group). All the buffaloes were dewormed orally with Albendazole suspension (VetCare Private Limited, India) at the dose rate of 10 mg/kg B.W., at 45 to 60 days post-partum and were treated orally with mineral mixture (MM) at the dose rate of 50 g per day per animal for 15 days from 45 to 60 days post-partum.

Oestrus induction with CIDR

At the end of mineral mixture treatment, all the buffaloes were inserted with Controlled Internal Drug Release Device containing 1.38

g progesterone (CIDR, EAZI-BREED, Pfizer Animal Health, India) intravaginally and left *in situ* for 9 days. All the buffaloes received an intramuscular injection of 500 µg $PGF_2\alpha$ (Cloprostenol Sodium, Pragma, Intas Pharmaceuticals Limited, India) at 24 h prior to CIDR withdrawal.

Oestrus detection and AI

Starting from 12 h of withdrawal of CIDR, all the buffaloes were monitored closely for the expression of the oestrus signs by critical observation of behavioural signs and by rectal examination of genital tract. The owners were also advised to keep the buffaloes under close observation for identification of oestrus signs in subsequent oestrous cycle, if it occurs. At 48 (first AI) and 72 h (second AI) of CIDR removal, all the buffaloes were artificially inseminated with good quality frozen thawed semen. Further, those buffaloes which failed to conceive and returned to oestrus (subsequent oestrus) following breeding at induced oestrus, were artificially inseminated twice at an interval of 24 h.

Ovarian changes and hormonal assay

Transrectal ultrasonography was performed in all experimental buffaloes to track the ovarian changes and also jugular venous blood was collected (i) at buffaloes selection (ii) insertion of CIDR in the vagina (iii) $PGF_2\alpha$ injection (iv) First AI (48 h after CIDR withdrawal) and (v) 10 days after first AI. The sera samples were stored in deep freezer (-20°C) and they were subjected for progesterone and oestradiol 17β estimation by RIA technique. The radioactivity in the sera samples was analysed in RIA method in PC-RIA.MAS, STRATEC (France).

Conception rate

Fertility rate was arrived (%) as number of buffaloes conceived at FTAI (First service conception rate) and subsequent oestrus (Second service conception rate) divided by number of buffaloes treated in each experimental group. Rectal examination at 60 days post-insemination confirmed the pregnancy.

Statistical analysis

The completely randomized design (CRD) method 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.

RESULTS AND DISCUSSIONS

The incidence of RFM in buffaloes cause longer delay in post-partum resumption of ovarian activity than normally calved buffaloes (El-Wishy, 2007). Hence, oestrus induction using CIDR plus $PGF_2\alpha$ with prior deworming and MM supplementation has been attempted in post-partum buffaloes which were affected and treated for RFM. The ovulatory response and conception rates were studied and these parameters were correlated with serum progesterone and oestradiol profiles in buffaloes under Indian condition especially in Tamil Nadu state which is the south most state of our country.

Cent percent retention rate was observed following CIDR treatment in buffaloes in RFM and NC groups of our current study. Similarly, Ghallab *et al*. (2016) in buffaloes found 100% retention rate with CIDR insertion. Retention rate

of 95.65 in buffaloes was reported by Ravikumar (2003). The 100% CIDR retention rate in the current investigation might be attributed to proper positioning of CIDR in the cranial vagina (Colazo *et al*., 2004). In this study, no complication during the period of CIDR *in situ* was recorded. Other investigators have published demerits such as trauma and inflammation of vaginal mucosa, and discomfort with the utilization of PRID and intravaginal P_4 sponges (Tregaskes *et al*., 1994; Colazo *et al*., 2004).

Sonography of the ovaries at the time of animal selection indicated the absence of corpus luteum in all the buffaloes. The selected buffaloes were at 45 to 60 days post-partum and at lactating stage. It could be the reason for the failure of ovarian activity in these buffaloes as suggested by Ravikumar (2003). At the time of CIDR insertion, 2 buffaloes in Group I and Group III had corpus luteum and proved the efficacy of deworming and mineral mixture supplementation in induction of oestrus in buffaloes as described by Pandey *et al.* (2007). At the time of first AI, all the buffaloes had matured follicle of 10 to 15 mm diameter as observed by Rahman *et al.* (2012) in buffaloes.

The 87.50 (RFM) and 100% (NC) ovulatory responses observed in this study after treatment with CIDR on day 10 post AI was in accordance with the report of Pursley *et al.* (1995) in buffaloes. However, Zaabel *et al.* (2009) found an ovulatory response as 90 to 100 and 85 to 100% in CIDR treated cows and buffaloes, respectively.

In this study, in RFM treated buffaloes had lower ovulatory response than NC buffaloes. The level of oestradiol 17β at the time of first AI was lower in RFM group than NC group. Sheldon *et al*. (2002) demonstrated that cows with uterine disease following RFM had smaller ovarian follicles and lower peripheral plasma oestrogen concentration. Konyves *et al.* (2009) concluded in their study that in cows with RFM, bacterial number was increased in the uterus that reduced the follicular activity in the ipsilateral ovary. Sheldon and Dobson (2004) stated that clinical or sub-clinical uterine bacterial infection following RFM disrupted not only the function of uterus but also the ovary and affected the control centers of hypothalamus and pituitary. These findings might explain the reduced ovulatory response in RFM affected groups than NC groups. Pandey *et al.* (2007) stated that minerals are the co-enzymes for the secretion of hormones of reproduction. Further they opined that the major mineral calcium has a significant role in steroid hormone synthesis. Calcium involves in delivery or mitochondrial utilization of cholesterol or converts pregnenolone into progesterone. Increased LH secretion by pituitary gland under GnRH stimulation is due to calcium dependent mechanism. Minimal phosphorus deficiency was found to be enough to alter the function of pituitary-ovarian axis without depicting any syndrome of deficiency (Selvaraju *et al*., 2011)

Oestrus induction with CIDR in the current experiment yielded 87.50 and 62.50% of overall pregnancy rates in NC and RFM affected buffaloes. It revealed that CIDR along with $PGF_2\alpha$ was effective in improving conception in postpartum riverine buffaloes. It was evident from our study that the occurrence of RFM might have damaged the endometrium and cyclical changes of ovaries that might have decreased the pregnancy rates following CIDR induction of oestrus as described earlier by Kimura *et al*. (2006). 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

Table 1. Induction of oestrus with CIDR in RFM affected and normally calved buffaloes.

S. No.	Group	Retention rate of CIDR	Ovulatory response	Conception rate								
				First service			Second service			Overall		
1	RFM	8 (100)	7 (87.50)	2/8 (25.00)			3/8 (37.50)			5/8 (62.50)		
2	NC	8 (100)	8 (100.00)	5/8 (62.50)			2/8 (25.00)			7/8 (87.50)		
S. No.	Group	Serum progesterone (ng/ml)					Serum oestradiol -17β (pg/ml)					
		At selection	At insertion of CIDR	At PGF2α injection	At first AI	10 days post AI	At selection	At insertion of CIDR	At PGF2α injection	At first AI	10 days post AI	
1	RFM	0.75aq±0.16	1.37bp±0.19	2.24cp±0.24	0.42a±0.18	4.47d±0.21	11.54ap±3.54	12.36ap±2.57	12.98ap±1.65	29.96cp±2.45	19.84b±4.77	
2	NC	0.41ap±0.14	1.57bp±0.17	2.43cp±0.26	0.41a±0.17	6.59d±0.20	17.60aq±4.66	18.65aq±4.32	18.94aq±3.49	41.75bq±1.94	19.35a±3.71	

Figure in the parentheses are in percentage. Mean values bearing superscripts between columns (a, b, c, d) with in a row of each parameter and among rows (p, q) with in a column of each parameter differ significantly (P≤0.05).

ovsynch protocol. The increased conception rate following CIDR treatment 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.

The mean serum levels of oestradiol-17β found in this study during different stages of treatment are depicted in Table. The mean serum oestradiol level at the time of selection of animals, ranged from 11.54±3.54 to 17.60±4.66 pg/ml. It indicated the anestrus stage of the buffaloes as described by Nath *et al.* (2003). Not much variation from the CIDR insertion to $PGF_2\alpha$ injection was observed in this study in serum oestradiol-17β levels. This was in agreement with the report of Singh and Singh (2006) in buffaloes. However, at the time of induced oestrus (AI), the serum oestradiol level got increased and proved the presence of matured Graafian follicle in the ovary. The observation made in this study was further supported by ultrasonographic examination of ovary at oestrus. All the buffaloes had matured follicle during the induced oestrus in this study. Altogether it indicated the efficacy of CIDR in induction of oestrus in buffaloes. In this experiment, at 10 days post AI, the mean serum oestradiol was 19.84±4.77 (RFM) and 19.35±3.71 pg/ml. Similar report was published by Singh and Singh (2006) in buffaloes.

The mean level of serum progesterone recorded at the time of animal selection indicated that all the buffaloes were in anestrus stage at the time of selection (Table). This was supported by the ultrasonographic evaluation of ovaries at the time of animal selection which revealed no developing or matured or regressing corpus luteum in any of the buffaloes. In this study, the serum progesterone level at the time of $PGF_2\alpha$ injection was increased when compared to insertion time. This was in agreement with the findings of Ravikumar (2003) in buffaloes. The elevated progesterone might be from CIDR which contains 1.38 g natural progesterone and the corpus luteum of the Group I and Group III as evinced by ultrasonography. The mean level of serum progesterone observed at the time of induced oestrus in this study was less than 0.5 ng/ml. This finding corroborates with the result of Rajkumar and Srivasatava (2008) in buffaloes. The reduction in the progesterone level below 0.5 ng/ml at the time of oestrus might be the reason for improved conception rates in oestrus induction programme in buffaloes in this study. Duchens *et al.* (1995) suggested that elevated progesterone level at oestrus might lead to asynchrony between the onset of oestrus and ovulation and consequently cause failure of conception.

In this study, in all the groups, at 10 days post AI, mean serum level of progesterone indicated the ovulatory response in NC and RFM groups following CIDR withdrawal as evinced by ultrasonography. The elevated progesterone level at 10 days post AI was in acceptance with report of Ravikumar (2003) in buffaloes. The increased progesterone concentration following AI might have caused embryonic development which in turn ultimately resulted in improved fertility of buffaloes in this study. It is concluded that the buffaloes affected with RFM or normally calved, the fertility rate could be improved with progesterone based oestrus induction programme along with prior deworming and mineral supplementation.

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