

Resumption of postpartum reproductive cyclicity and pregnancy rates after treatment with CIDR plus PGF₂α in normally calved and retained fetal membranes affected water buffaloes

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ABSTRACT

An experiment was conducted to compare the estrus induction response, estrus pattern, endometrial regeneration and pregnancy rates following treatment with CIDR plus PGF₂α between normally calved (NC) and retained fetal membranes (RFM) affected water buffaloes. A total of 32 water buffaloes including 16 treated for RFM and 16 NC were selected and divided into 4 groups as I and II, and III and IV respectively. Buffaloes of group I and III were dewormed with Albendazole followed by the administration of mineral mixture in feed for 15 days. Buffaloes of all the groups were treated with CIDR intravaginally for 9 days and they received PGF₂α at 24 hours prior to CIDR withdrawal. After CIDR removal, the buffaloes of all the groups were artificially inseminated during induced estrus. Those buffaloes returned to estrus following breeding at induced estrus, were artificially inseminated twice at an interval of 24 hours. The estrus response following CIDR withdrawal was 100%. The RFM affected buffaloes had significantly longer interval to onset of estrus and shorter duration of estrus than NC buffaloes. More pronounced intensity of estrus following CIDR treatment was observed in NC buffaloes than in RFM affected buffaloes. The mean pregnancy rates obtained in NC and RFM affected buffaloes were 81.25% and 50%. Complete loss of epithelial layer, degenerative changes of endometrium and atrophic changes in the endometrial glands were observed in RFM affected buffaloes before estrus induction. After treatment, RFM affected buffaloes showed clear regeneration of endometrium and its glandular acini. It is concluded that estrus induction using CIDR plus PGF₂α might help to augment the fertility in NC and RFM affected buffaloes at field level.

Key words: Estrus induction; CIDR; retained fetal membranes; pregnancy rate; water buffaloes

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Introduction

Several international institutions have emphasized the potentiality of the water buffaloes (*Bubalus bubalis*) in the economy of a number of developing countries, due to its ability to produce and reproduce under the harsh environmental conditions compared to the dairy cattle (MARAI and HABEEB, 2010). Water buffaloes are considered one of the most important domestic ruminants in more than 40 countries; mostly in tropical and subtropical regions (GHALLAB and NOSEIR, 2016). Milk from water buffalo is preferred over the cow's milk due to its 100% more fat, high protein and low level of cholesterol. Due to its superior whitening property, buffalo milk is more suitable for the production of infant milk powder. Water buffaloes are well known for efficient conversion of low grade fibrous food into highly valuable milk. Hence, there is a large potential for expansion of dairy sectors particularly water buffalo industry in India (GANESH, 2013). Despite of these merits, water buffaloes are blamed for slow reproduction, long calving interval, delayed puberty, poor estrus expression and seasonality in breeding and calving (SELVARAJU et al., 2005).

In order to maintain the recommended calving interval, the water buffaloes need to conceive within 100 to 150 days postpartum. Postpartum anestrus is the period after calving during which dairy animals do not exhibit estrus. It is the most commonly prevalent, frustrating and challenging problem encountered in dairy animals. Field surveys on reproductive disorders have revealed that anestrus is the most common single cause of infertility in water buffaloes among which inactive or non-functional ovaries are important cause of anestrus (EL-WISHY, 2007). The incidence of true anestrus in water buffaloes of Indian subcontinent varies from 19 to 74% (KUMAR and KUMAR, 1995; TOMAR et al., 2002; SELVARAJU et al., 2005). Retention of fetal membranes (RFM) is one of the major postpartum reproductive disorders affecting profitability of water buffalo production since it delays uterine involution and resumption of ovarian activity leading to postpartum anestrus (EL-MALKY et al., 2010). EL-MALKY (2007)

demonstrated that retained fetal membranes were observed in 4.66% cases of buffaloes over 3 years of study.

Different methods are employed to reduce the postpartum anestrus after normal or abnormal calving and subsequent inter-calving period in order to increase fertility in water buffaloes. Administration of exogenous progesterone was considered as appropriate treatment for anestrus postpartum cows (YANIZ et al., 2004) and water buffaloes (PUROHIT et al., 2019) for the resumption of estrus with normal estrous cycle length. In order to maximize the response (resumption of follicular activity), hormones such as gonadotropin, estradiol and prostaglandin F₂α (PGF₂α) can be given at the end of progesterone treatment (PUROHIT et al., 2019). Administration of 30-50 gm mineral mixture orally daily for 15 days before the start of ovsynch treatment increased the conception rate by 25% in RFM affected crossbred cows (VELLADURAI et al., 2015). VELLADURAI (2013) reported deworming with albendazole at the dose rate of 10 mg/kg body weight increased the success rate of synchronization of ovulation in cows which were previously treated for RFM during immediate postpartum period.

Various research authors (MACMILLAN and PETERSON, 1993; LUCY et al., 2001) have satisfactorily induced estrus and ovulation in postpartum anestrus cows using Contolled Internal Drug Release Device (CIDR). However, fewer studies were conducted on the use of exogenous progesterone for inducing cyclicity in postpartum water buffaloes and the results were not consistent. Meager reports are available on the use of CIDR in water buffaloes with variable fertility rates (PUROHIT et al., 2019). Further, the effect of RFM on efficiency of estrus induction with CIDR plus PGF₂α treatment in water buffaloes which were affected and treated for RFM have not been reported in detail. Hence, an experiment was conducted to compare the estrus induction response, estrus pattern, endometrial regeneration and pregnancy rates following treatment with CIDR plus PGF₂α between normally calved and RFM affected water buffaloes.

Materials and methods

Selection of experimental animals. The committee for the approval of post graduate research programme of Tamil Nadu Veterinary and Animal Sciences University approved the experimental design in buffaloes. Healthy graded Murrah buffaloes aged 5½ to 10½ years, between second and fifth parity and owned by various farmers at field level in Namakkal District of Tamil Nadu State, India were subjected to thorough gynaecological examination. A total of 32 water buffaloes at 45-60 days postpartum period including 16 treated previously during immediate postpartum period for retained fetal membranes (RFM) and 16 normally calved (NC) were selected. They were randomly divided into 4 experimental groups as group I and II (RFM groups) and group III and IV (NC groups), respectively.

Deworming + Mineral mixture + CIDR+ PGF₂α (Group I & III). Buffaloes of group I and III were dewormed orally with Albendazole suspension (VetCare Private Limited, India) at the dose rate of 10 mg/kg body weight at 45-60 days postpartum. Further, they were treated orally with TANUVAS mineral mixture at the dose rate of 30-50 g per day per animal for 15 days in the concentrate feed from 45-60 days postpartum. The percentage of calcium, phosphorus, magnesium, iron, iodine, copper, manganese, cobalt, zinc, sulphur, fluorine and selenium in 1kg of TANUVAS mineral mixture was 23, 12, 6.5, 0.5, 0.026, 0.007, 0.12, 0.012, 0.38, 0.5, 0.07 and 0.03 respectively. At 60-75 days postpartum (i.e. at the end of mineral mixture treatment) buffaloes of these 2 groups 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 animals received an intramuscular injection of 500 µg PGF₂α (Cloprostenol sodium, Pragma, Intas Pharmaceuticals Limited, India) at 24 hours prior to CIDR withdrawal. The CIDR consisted of a 15 cm long silicone rubber elastomer impregnated with 1.38 g of progesterone and moulded over a 'T' shaped nylon spine. The perineal and vulval region of the water buffaloes were cleaned with 2% potassium permanganate solution and wiped

with absorbable cotton. Then, CIDR was inserted using the applicator supplied along with the device. The wings of the device were folded inward and were held in the folded position by the applicator. Squeezing of the plunger handle after introducing the applicator into anterior vagina expelled the device into correct position in the anterior vagina. When it was in place, the long tail of the device protruding from the vulva was cut to the required length. Removal of the device at the end of the treatment period was easily achieved by gentle and firm pulling of tail of the device.

Group II and IV (CIDR and PGF₂α alone). In the groups II and IV at 60-75 days postpartum, the buffaloes were treated intravaginally with CIDR for 9 days. All the buffaloes were given intramuscular injection of 500 µg PGF₂α (Cloprostenol Sodium) at 24 hours prior to CIDR withdrawal.

Estrus detection and Artificial insemination (AI). Starting from 12 hours of withdrawal of CIDR, all the buffaloes were monitored visually 2-3 hours interval for the expression of the estrus signs by critical observation of behavioural signs such as restlessness, reduction in milk yield, bellowing, micturition and teat engorgement before the milking time. The owners were also advised to keep the water buffaloes under regular observation for identification of estrus signs in subsequent estrus cycle, if it occurs. At 48 (first AI) and 72 hours (second AI) of CIDR removal, the buffaloes of all the four experimental groups were artificially inseminated with good quality frozen thawed semen. Further, those buffaloes which failed to conceive and returned to estrus (subsequent estrus) following breeding at induced estrus, were artificially inseminated twice at an interval of 24 hours.

Retention rate of CIDR. Percentage of retention of CIDR was calculated in each group as number of buffaloes which retained CIDR until withdrawal time divided by number of buffaloes inserted with CIDR.

Estrus response. Estrus response in percentage was calculated as number of buffaloes expressed estrus signs following CIDR removal divided by the number of buffaloes treated with CIDR in each group.

Onset, duration and intensity of induced estrus. Onset of estrus was calculated from the time of withdrawal of CIDR to the time of first appearance of estrus signs and was expressed in hours. Duration of estrus was estimated from the time of first appearance of estrus signs to the time of disappearance of estrus signs, in hours. The intensity of estrus was measured based on the score card described by SELVARAJU et al. (2009) in cows with slight modifications. The modifications included were peculiar estrus signs of buffaloes such as reduction in milk yield, bellowing, micturition and teat engorgement before the milking time.

Pregnancy rate. Pregnancy rate was calculated in percentage as number of animals became pregnant at induced estrus (first service conception rate) and subsequent estrus (second service conception rate) divided by number of animals treated in each experimental group. Pregnancy was confirmed by rectal examination at 60 days post-insemination.

Collection of blood samples. Blood samples were collected from jugular vein using 18G needle in the clotting activator vacutainer at (i) the time of selection of animals (ii) CIDR insertion (iii) PGF₂α injection (iv) First AI (48 hours after CIDR withdrawal) and (V) at 10 days after first AI in all the experimental animals. The serum samples were separated immediately and stored at -20°C until the analysis of progesterone and estradiol-17β.

Blood endocrine profiles. Serum samples were analysed in duplicate for progesterone and estradiol-17β by radioimmunoassay (RIA) technique. The ¹²⁵I labelled antigen, antibody-coated tubes and standards procured from Immunotech (Marseille, France) were used for the analysis. The radioactivity in the samples was measured in radioimmunoassay method in PC-RIA.MAS, STRATEC (France).

Endometrial biopsy. Endometrial biopsy was taken in all experimental animals (i) at the time of animal selection and (ii) at the time of first AI (48 hours after CIDR withdrawal) to study the endometrial changes before and after treatment. Uterine biopsy catheter (Albuchin's catheter) was used to obtain endometrial biopsy samples as per the method described by PRASAD and KRISHNA (2009). The closed, sterilized biopsy catheter

was introduced into the uterus adopting aseptic technique. The biopsy catheter was advanced into one of the uterine horns and the catheter was opened. The uterine wall was pressed with the thumb against the opening of the catheter. The catheter was closed, rotated and retracted slowly. Slight pressure was applied against catheter to prevent haemorrhage before retracting the instrument. A small piece of endometrial tissue (5 mm thickness) was removed from the cutting edge of the catheter into a vial containing Bouin's fluid and stored for 24 hours. The tissue was processed by routine paraffin technique and stained with haematoxylin and eosin.

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. The intensity of estrus and pregnancy rates were analysed by using Fisher's exact test. Post hoc analysis was done by Tukey's Honestly Significance Difference.

Results

Efficacy of estrus induction. All the water buffaloes (100%) retained CIDR from insertion to withdrawal. None of the water buffaloes had any complication during the period of CIDR treatment in this experiment. All the buffaloes exhibited estrus following withdrawal of CIDR. Therefore, treatment with CIDR resulted in 100% estrus response and it indicated that CIDR was highly effective to induce estrus in postpartum water buffaloes.

Pattern of induced estrus. The interval from withdrawal of CIDR to the expression of first estrus signs was calculated in hours and is presented in Table 1. There was no significant difference in the onset of estrus between group I and II and also between group III and IV water buffaloes. However, RFM affected buffaloes (group I and II) had significantly longer interval to onset of estrus when compared to NC buffaloes (groups III and IV). The results of the study indicated that the average duration of induced estrus (Table 1) in NC buffaloes were statistically (P<0.05) longer in

group III and IV than in RFM affected buffaloes in groups I and II. No significant difference was

observed between group I and II and group III and IV in the duration of induced estrus.

Table 1. Estrus response and onset, duration and intensity of estrus following CIDR treatment in RFM affected and NC water buffaloes

Treatment Groups		Estrus response	Onset of induced estrus (Mean ±SE)	Duration of estrus (Mean±SE)	Intensity of the estrus			
					Intense	Intermediate	Weak	P value
RFM	Group I	8 (100)	39.32 ^a ±2.20	27.88 ^a ±0.67	4 (50)	3 (37.5)	1 (12.5)	0.5631
	Group II	8 (100)	41.40 ^a ±2.33	26.75 ^a ±0.68	2 (25)	2 (25)	4 (50)	
Average		16 (100)	40.36 ^a ±2.21	27.31 ^a ±0.67	6 (37.5)	5 (31.2)	5 (31.25)	
NC	Group III	8 (100)	36.71 ^b ±2.65	29.38 ^b ±0.77	5 (62.5)	2 (25)	1 (12.5)	
	Group IV	8 (100)	38.14 ^b ±2.13	28.38 ^b ±0.66	3 (37.5)	4 (50)	1 (12.5)	
Average		16 (100)	37.42 ^b ±2.39	28.88 ^b ±0.71	8 (50)	6 (37.5)	2 (12.5)	

Figures in the parentheses are in percentage; Mean values bearing different superscripts (a, b) between rows with in a same column differ significantly (P≤0.05); RFM: Retained fetal membranes; NC: Normally calved; Group I and III: Deworming + Mineral mixture + CIDR+ PGF₂α ; Group II and IV: CIDR and PGF₂α alone.

The percentage of intense estrus intensity (Table 1) was higher in group III (62.50%) and group I (50%) followed by group IV (37.50%). The lowest percentage of intense estrus intensity was noted in group II (25%). The average percentage of intense estrus intensity in RFM affected buffaloes was less (37.50%) when compared to NC buffaloes (50%). Similar trend was noticed in intermediate estrus intensity also. However, percentage of average weak estrus intensity was higher in RFM affected buffaloes (31.25%) than normally calved (12.50%) buffaloes. The result of the present experiment showed that more pronounced intensity of estrus following CIDR treatment was observed in NC buffaloes than in RFM affected buffaloes. Further, deworming and supplementation of mineral mixture caused higher estrus expression rate in group I and III than the other two groups (group

II and IV) in which no deworming and mineral supplementation was done. The higher percentage of intermediate and weak estrus intensity was noticed in group IV (50%) and group II (50%), respectively. But statistical analysis revealed no significant difference (P = 0.5631) among the 4 groups in different types of estrus intensities.

Pregnancy rate. The conception rates obtained in this study following estrus induction in RFM affected and NC buffaloes are presented in Table 2. The result of current experiment revealed that estrus induction programme at 60 to 75 days postpartum in buffaloes yielded 81.25% and 50% average conception rate in NC and RFM affected buffaloes respectively. In this study, the highest conception rate of 87.50% recorded in group III followed by 62.50% in group I indicated the role of deworming and mineral mixture supplementation

in improving fertility in buffaloes. The lowest conception rate (37.50%) recorded in group II buffaloes indicated that RFM had deleterious effect on fertility in postpartum buffaloes. The conception rate of 75% achieved in group IV buffaloes proved

that CIDR alone can improve fertility in postpartum buffaloes considerably. However, statistical analysis of percentages of conception rate between groups revealed no significant difference (P-value =0.7678) among the 4 groups.

Table 2. Pregnancy rate (%) following estrus induction with CIDR in RFM affected and NC water buffaloes

Treatment Groups		Pregnancy rate			
		First service pregnancy rate	Second service pregnancy rate	Pregnancy rate	P value
RFM	Group I	2/8 (25.00)	3/8 (37.50)	5/8 (62.50)	0.7678
	Group II	2/8 (25.00)	1/8 (12.50)	3/8 (37.50)	
Average		4/16 (25.00)	4/16 (25.00)	8/16 (50.00)	
NC	Group III	5/8 (62.50)	2/8 (25.00)	7/8 (87.50)	
	Group IV	3/8 (37.50)	3/8 (37.50)	6/8 (75.00)	
Average		8/16 (50.00)	5/16 (31.25)	13/16 (81.25)	

Figures in the parentheses are in percentage; RFM: Retained fetal membranes; NC: Normally calved; Group I and III: Deworming + Mineral mixture + CIDR+ PGF₂α; Group II and IV: CIDR and PGF₂α alone.

Progesterone. The mean serum progesterone levels before, during and after estrus induction with CIDR in RFM affected and NC buffaloes are presented in Table 3. At the time of animal selection,

there was no significant difference between group I (0.75±0.16 ng/ml) and II (0.69±0.13 ng/ml) and group III (0.41±0.14 ng/ml) and IV (0.57±0.13 ng/ml). However, progesterone levels in group I and II

Table 3. Serum progesterone (ng/ml) levels following estrus induction in RFM affected and NC water buffaloes

Treatment groups	Progesterone (ng/ml)				
	At selection	At insertion of CIDR	At PGF ₂ α injection	At first AI	10 days post AI
Group I	0.75 ^{aq} ± 0.16	1.37 ^{bp} ± 0.19	2.24 ^{cp} ± 0.24	0.42 ^a ± 0.18	4.47 ^d ± 0.21
Group II	0.69 ^{bq} ± 0.13	0.88 ^{bq} ± 0.04	2.02 ^{cq} ± 0.15	0.33 ^a ± 0.12	5.82 ^d ± 0.21
Group III	0.41 ^{ap} ± 0.14	1.57 ^{bp} ± 0.17	2.43 ^{cp} ± 0.26	0.41 ^a ± 0.17	6.59 ^d ± 0.20
Group IV	0.57 ^{ap} ± 0.13	0.99 ^{bq} ± 0.16	2.06 ^{cq} ± 0.08	0.42 ^a ± 0.13	6.65 ^d ± 0.10

Mean values bearing superscripts between columns (a, b, c, d, e) with in a row and among rows (p, q, r, s) with in a column differ significantly (P≤0.05); RFM: Retained fetal membranes; NC: Normally calved; Group I and III: Deworming + Mineral mixture + CIDR+ PGF₂α; Group II and IV: CIDR and PGF₂α alone.

were significantly ($P < 0.05$) differed from group III and IV. At insertion of CIDR and PGF₂α injection, there was no significant difference between group I and III and also between group II and IV. However, the mean progesterone values in group I and III were significantly differed from group II and IV. At first AI and 10 days post AI there was no significant difference in serum progesterone levels among all the four groups. The progesterone levels between insertion and PGF₂α injection differed significantly in all the groups.

Estradiol - 17β. At the time of selection of animals, insertion of CIDR, PGF₂α injection and first AI, there was no significance difference between group I and II and group III and IV in the serum estradiol-17β (Table 4). However, group I and II were significantly differed from group III and IV. At 10 days post AI, there was no significant difference in serum estradiol levels among all four groups. In general, all experimental groups the serum estradiol levels increased from the time selection to the time of first AI and then declined at 10 days post AI.

Table 4. Serum estradiol (pg/ml) levels following estrus induction in RFM affected and NC water buffaloes

Treatment groups	Estradiol -17β (pg/ml)				
	At selection	At insertion of CIDR	At PGF ₂ α injection	At first AI	10 days post AI
Group I	11.54 ^{ap} ± 3.54	12.36 ^{ap} ± 2.57	12.98 ^{ap} ± 1.65	29.96 ^{cp} ± 2.45	19.84 ^b ± 4.77
Group II	12.76 ^{ap} ± 2.12	12.37 ^{ap} ± 3.04	12.44 ^{ap} ± 2.19	32.67 ^{cp} ± 4.33	17.63 ^b ± 4.01
Group III	17.60 ^{aq} ± 4.66	18.65 ^{aq} ± 4.32	18.94 ^{aq} ± 3.49	41.75 ^{bq} ± 1.94	19.35 ^a ± 3.71
Group IV	18.68 ^{aq} ± 4.06	18.96 ^{aq} ± 4.77	19.01 ^{aq} ± 3.06	37.63 ^{bq} ± 3.45	16.54 ^a ± 3.54

Mean values bearing superscripts between columns (a, b, c, d, e) with in a row and among rows (p, q, r, s) with in a column differ significantly ($P \leq 0.05$); RFM: Retained fetal membranes; NC: Normally calved; Group I & III: Deworming + Mineral mixture + CIDR+ PGF₂α; Group II and IV: CIDR and PGF₂α alone.

Histopathology of endometrium. In NC buffaloes (Fig. 1) endometrial lining epithelium was found to be normal with pseudo-stratified columnar cells. Further, subendometrial glandular acini were normal and lined with columnar epithelium. Few blood vessels were seen in the subendometrium. In RFM affected buffaloes (Fig. 2), prior to treatment, disassociated endometrial epithelial linings and subendometrial cellular degeneration were observed. The chronic inflammatory cellular infiltration associated with degenerative change around the endometrial glands was seen. In few cases, complete loss of epithelial cell linings was noticed. Subendometrial haemorrhages, cellular infiltration and stromal edema were also observed. Uterine glands were found to be dilated and filled

with desquamated cells. Cytoplasmic vacuolation in the glandular epithelium was also observed. After treatment, NC buffaloes showed (Fig. 3) the active proliferation of epithelial cells invaginating into subendometrium. The intact endometrium with hyperplasia of endometrial glands was observed. Inflammatory cellular infiltration and glandular acini formation were noticed. The histological changes in RFM affected buffaloes (Fig. 4) after treatment were highly impressive. Regeneration of lining epithelium of endometrium with scattered mononuclear cellular infiltration in subendometrium was noticed. Endometrium appeared normal with active glandular proliferation invaginating into endometrium with formation of more neovascularisation.

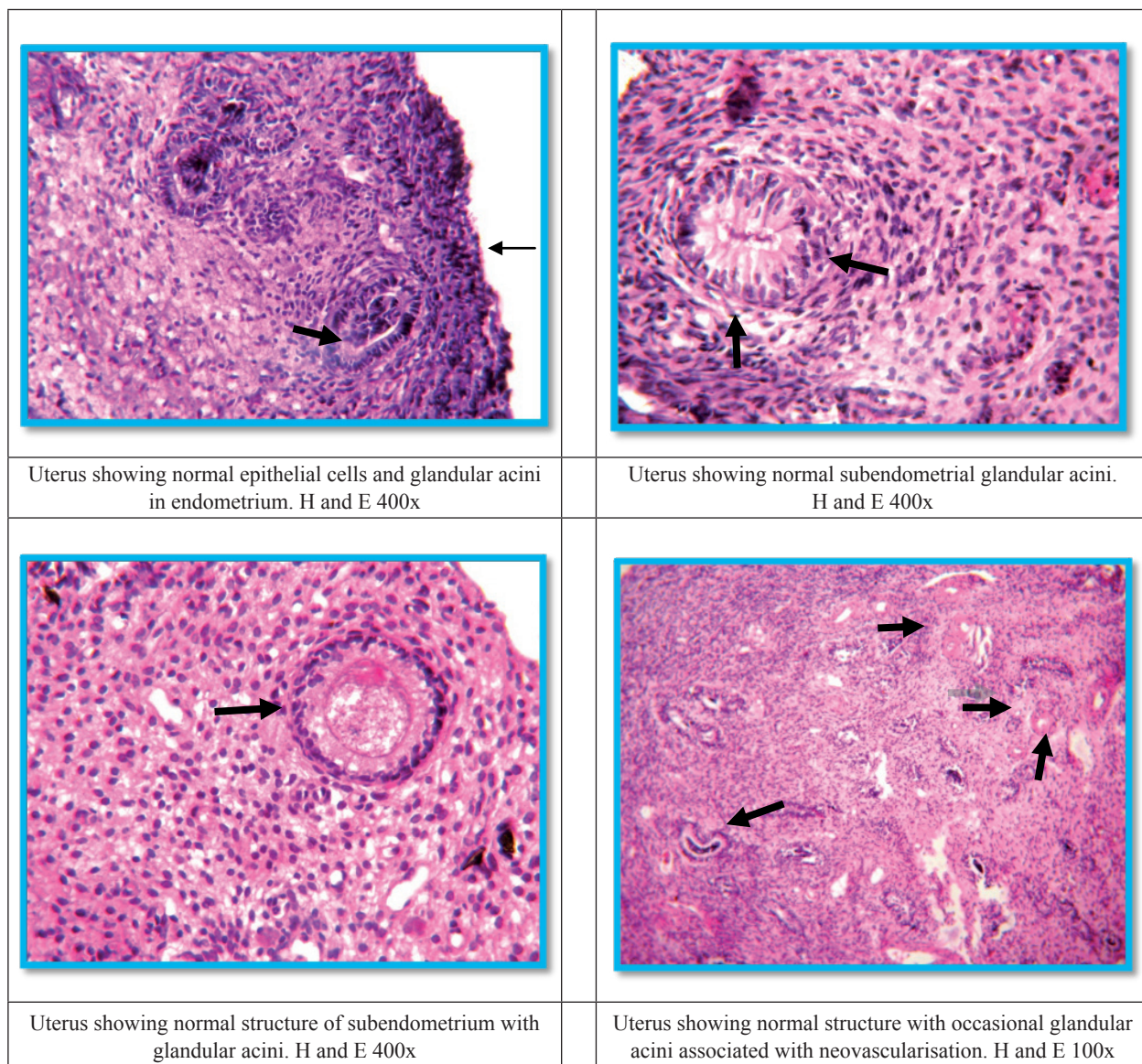


Fig. 1. Histopathology of endometrium in NC water buffaloes before treatment

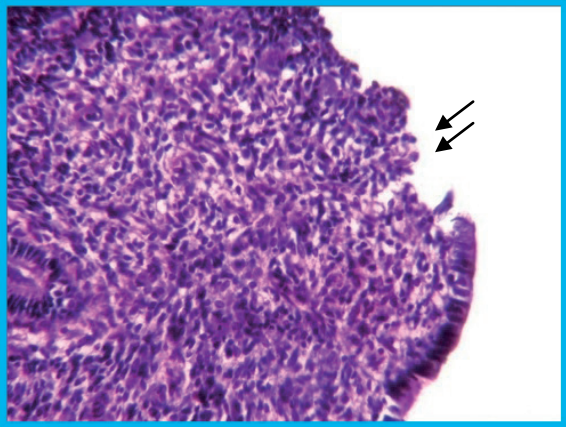
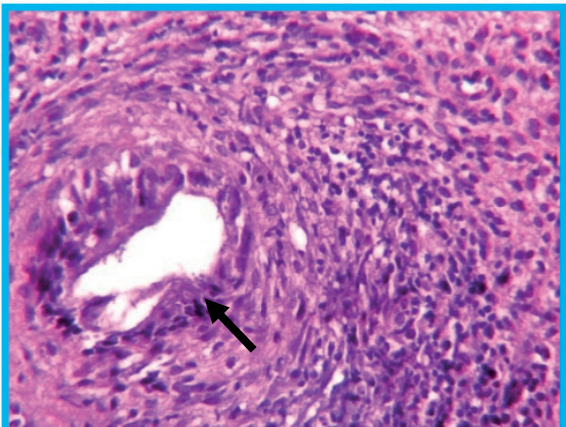
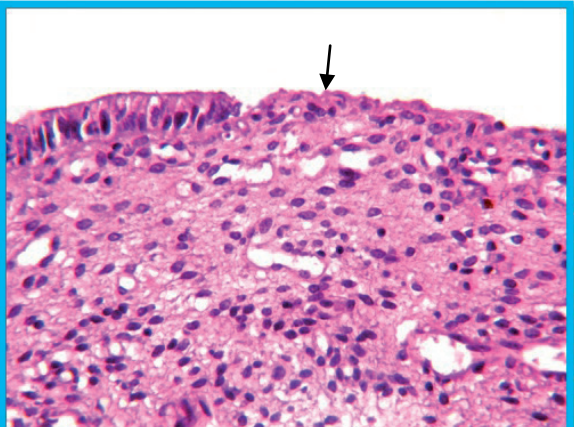
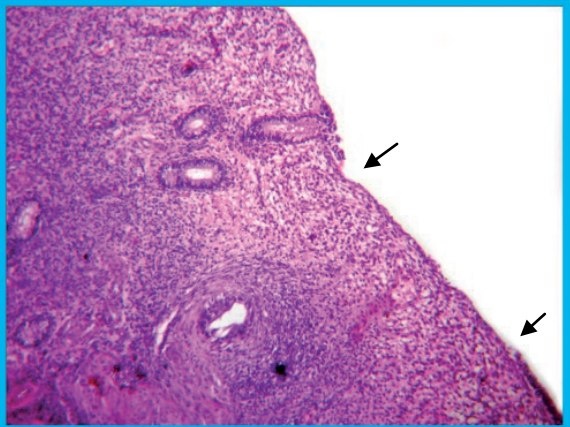
 A photomicrograph of a histological section of the endometrium stained with H&E. The epithelial lining is fragmented and discontinuous. Two black arrows point to the gaps in the epithelial layer.	 A photomicrograph of a histological section of the endometrium stained with H&E. The tissue shows signs of degeneration and is heavily infiltrated with chronic inflammatory cells. A black arrow points to a cluster of these cells surrounding an acinus.
<p>Uterus showing discontinued epithelial lining in endometrium. H and E 200x</p>	<p>Uterus showing degeneration and infiltration of chronic inflammatory cells around the acini. H and E 400x</p>
 A photomicrograph of a histological section of the endometrium stained with H&E. The epithelial lining is intact and shows a layer of proliferating epithelial cells. A black arrow points to this layer.	 A photomicrograph of a histological section of the endometrium stained with H&E. There is a complete loss of the epithelial cell lining. Two black arrows point to the exposed underlying connective tissue.
<p>Uterus showing intact endometrium with proliferating epithelium. H and E 400x</p>	<p>Uterus showing complete loss of epithelial cell lining. H and E 100x</p>

Fig. 2. Histopathology of endometrium in RFM affected water buffaloes before treatment

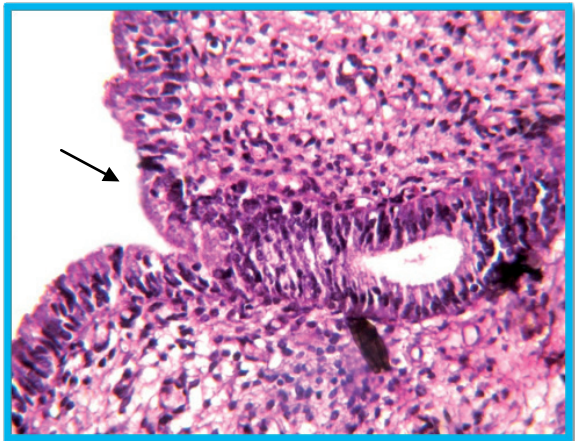
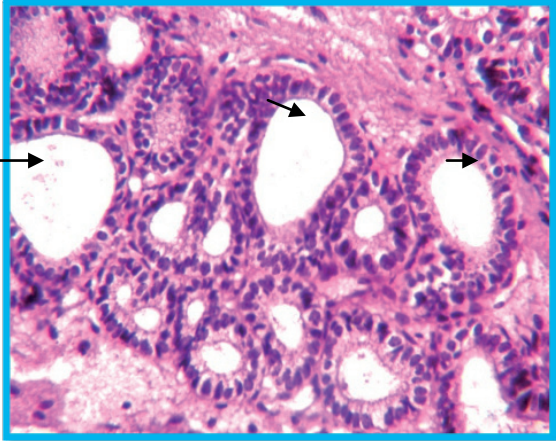
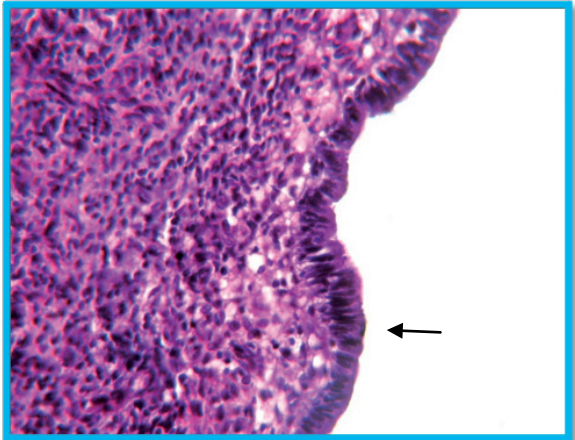
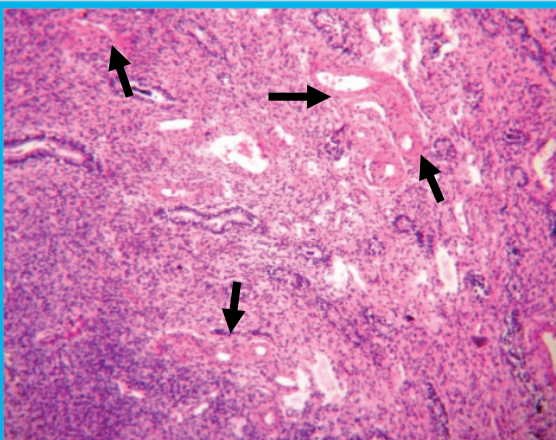
	
<p>Uterus showing active proliferation of epithelial cells and invagination into subendometrium. H and E 400x</p>	<p>Uterus showing single layer of epithelium lining the glandular acini in subendometrium. H and E 400x</p>
	
<p>Uterus showing intact endometrium with proliferating epithelium. H and E 400x</p>	<p>Uterus showing intense infiltration of inflammatory cells associated with neovascularisation and glandular acini formation. H and E 400x</p>

Fig. 3. Histopathology of endometrium in NC water buffaloes after treatment

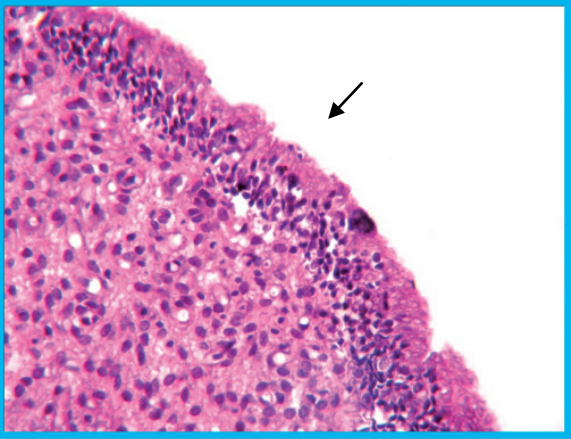
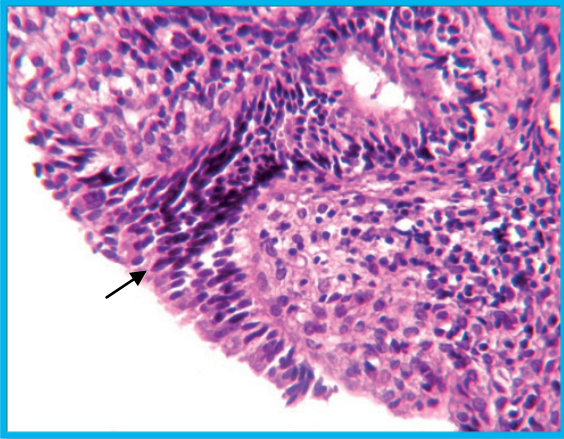
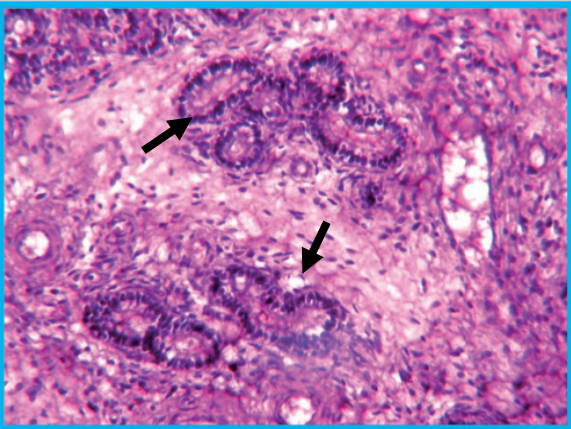
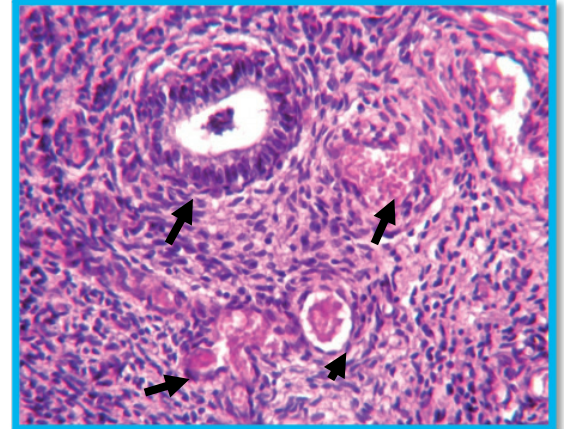
	
<p>Uterus showing active proliferation of epithelial cells in the endometrium with complete epithelial lining. H and E 400x</p>	<p>Uterus showing proliferation epithelial cells and its invagination into subendometrium. H and E 400x</p>
	
<p>Uterus showing active proliferation of glandular acini in subendometrium. H and E 400x</p>	<p>Uterine subendometrium showing glandular acini with prominent lining cells and neovascularisation. H and E 400x</p>

Fig. 4. Histopathology of endometrium in RFM affected water buffaloes after treatment

Discussion

To reduce the postpartum interval to conception, various hormonal strategies are available with more predictable success (BORGHESE, 2005). Induction of estrus with CIDR in postpartum normally calved cows/buffaloes produced considerable improvement in fertility (ZAABEL et al., 2009; RAMAKRISHNAN et al., 2012).

Effect of CIDR on resumption of cyclicity. In the present investigation, 100% retention rate was observed following CIDR treatment in buffaloes. Similar percentage of retention rate with CIDR was documented by BARUSELLI et al. (2002) in buffaloes. But, reduced retention 95.65% (RAVIKUMAR, 2003) and 89.60% (BARUSELLI et al., 2002) in buffaloes were reported. The 100% retention rate of CIDR in the present study might be attributed to proper placement of CIDR in the cranial vagina without allowing the protrusion of caudal end of the “T” portion of CIDR outside the vulva and cutting off long tail of the device protruding from the vagina at required length as recommended by COLAZO et al. (2004). In the present study, no complication during the period of CIDR treatment was noticed. Several authors reported disadvantages like vaginal trauma, vaginitis and discomfort with the use of PRID and vaginal sponges (TWAGIRAMUNGU et al., 1995). In this study, CIDR was used for estrus induction in buffaloes. Similarly, ZAABEL et al. (2009) recommended the use of CIDR for estrus synchronization in buffaloes. The duration of intravaginal CIDR treatment in the current experiment was 9 days. But, SINGH and SINGH (2006) and ZAABEL et al. (2009) treated the buffaloes with CIDR for 7 days.

In this experiment, 24 hours before withdrawal of CIDR, 500 µg of cloprostenol was injected intra-muscularly to all the buffaloes. Similar procedure was followed by ZAABEL et al. (2009) in buffaloes. However, SINGH and SINGH (2006) used eCG injection at the time of withdrawal of CIDR. SINGH (2003) opined that using CIDR in combination with intramuscular injection of PGF₂α was more effective than CIDR alone in terms of expression of estrus and conception rate. This can be explained by the fact that PGF₂α increases

pituitary responsiveness to GnRH in the postpartum cows (RANDEL et al., 1996). Hence, release of GnRH after CIDR removal effectively stimulates the pituitary gonadotropins with subsequent estrus induction in anestrus buffaloes (PUROHIT et al., 2019).

In this study, CIDR treatment resulted in 100% estrus response following their withdrawal. This was in accordance with the findings of ZAABEL et al. (2009) in buffaloes. But, estrus response of 90% (SINGH, 2003) was reported in buffaloes treated with CIDR. The 100% estrus response of the present study following withdrawal of CIDR might be due to the proper duration of CIDR treatment and elevation of progesterone for 9 days which was sufficient to sensitize the hypothalamo-hypophyseal and gonadal system of buffaloes for resumption of ovarian cyclicity as described by ZAABEL et al. (2009).

Onset of induced estrus. The single best practical parameter for basing the time of insemination in estrus induction programme is the onset of standing estrus (SELVARAJU et al., 2009). In the current study, the interval to onset of induced estrus ranged from 35 to 43 hours following withdrawal of CIDR in buffaloes. This was in agreement with the reports of RAVIKUMAR (2003) in postpartum buffaloes. However, onset of estrus following CIDR treatment was 48-96 (ZAABEL et al., 2009) and 72-96 hours (SINGH, 2003) in buffaloes in other studies. The earlier onset of estrus in this study when compared to other findings might be due to the early accelerated production of estradiol after CIDR withdrawal and proper observation of the buffaloes for detection of estrus (RAVIKUMAR, 2003) after CIDR removal.

In this experiment, the RFM affected buffaloes (group I and II) had delayed onset of estrus when compared to NC buffaloes (group III and IV). KONYVES et al. (2009) pointed out that RFM caused a delayed renewal of the ovarian activity and increased the interval between parturition and first ovulation. Further, they stated that in cows with RFM, bacterial number was increased in the uterus that reduced the follicular activity in cows. In another investigation, SHELDON et al. (2002) reported that cows affected with RFM had smaller ovarian follicles and lower peripheral plasma

estradiol concentration. Further, MATEUS et al. (2002) opined that in cows affected with RFM, the postpartum progesterone level was higher and decreased later in comparison with control group cows and it might be the cause for delayed folliculogenesis and ovulation. SHELDON et al. (2006) concluded that the sub-clinical or clinical uterine infections following RFM not only damaged the uterus but also suppressed the hypothalamic GnRH and pituitary gonadotrophin secretion and have localized effects on ovarian function. These findings might explain the reasons for delayed onset of estrus in RFM affected buffaloes in this study.

Duration of induced estrus. The duration of estrus was calculated from the first appearance of estrus signs to the time of disappearance of estrus signs. Similar procedure was followed by ZAABEL et al. (2009) in buffaloes. In the present study, the duration of estrus was slightly longer in NC buffaloes than RFM affected buffaloes. Cows affected RFM had smaller ovarian follicles and lower peripheral plasma estradiol concentration following estrus induction (SHELDON et al. 2002) which might cause for silent or sub-estrus in buffaloes. It was evinced by the fact that in this study, the serum estradiol levels at first AI in RFM affected buffaloes were lower than NC buffaloes. These could be the reasons for slightly reduced duration of induced estrus in RFM affected buffaloes in this study.

Intensity of estrus. In the present investigation, CIDR treatment in general caused good estrus expression rate in buffaloes. SARTORI et al. (2001) reported that norgestomet (progesterone) treatment increased the number of receptors in granulosa and thecal cells by their increasing the sensitivity of the follicle to elevated serum LH concentration which resulted in increased secretion of estradiol from the ovulatory follicle and in turn more pronounced intensity of estrus following its withdrawal. In estrus induction treatments, progesterone increased hypothalamus sensitivity of estrogen with subsequent increase in the intensity of estrus (DUTTA and DUGWEKAR, 1983). In the current experiment, more pronounced estrus intensity was noticed in NC buffaloes when compared to RFM

affected buffaloes. As stated by SHELDON et al. (2002) in cows, the RFM affected experimental buffaloes of this study had lower serum estradiol-17β levels at the time of first AI (estrus) than NC buffaloes. In this investigation, mineral mixture supplementation resulted in higher estrus expression rates in buffaloes. Minerals act as co-enzymes for the production of reproductive hormones especially in steroidogenesis (PANDEY et al., 2007). Calcium plays a vital role in steroidogenesis by influencing delivery or utilization of cholesterol by mitochondria or by stimulating the conversion of pregnenolone to progesterone. GnRH stimulation of the pituitary gland to increase LH secretion involved calcium dependent mechanism. Even marginal deficiency of phosphorus was sufficient to cause disturbances in pituitary-ovarian axis without manifesting deficiency syndrome (AGARWAL et al., 1998). These reasons might explain the increased rate of intense estrus intensity in mineral mixture treated groups than non treated group of buffaloes.

Pregnancy rate. In the current investigation, estrus induction with CIDR resulted in 81.25% and 50% average conception rates in NC and RFM affected buffaloes respectively. It indicated that CIDR plus PGF₂α combination was found to be effective in augmenting fertility in postpartum buffaloes. RAMAKRISHNAN et al. (2012) reported first service, second service and average conception rates as 33.30, 50 and 66 per cent in postpartum anestrus buffaloes respectively. In this experiment, the group I and III buffaloes which were treated with combination of deworming, mineral mixture and CIDR protocol resulted in 62.5% and 87.5% average conception rates, respectively. Whereas group II and IV, without deworming and mineral mixture supplementation, with CIDR protocol alone, had 75% and 37.5% average conception rates. This result indicated clearly that either in NC buffaloes or in RFM affected buffaloes, alleviation of worm infection and supplementation of mineral mixture could further enhanced the fertility rate in buffaloes as described by THATCHER et al. (1989).

In RFM affected groups (group I and II) the average first and second service conception rate obtained was 50% which was lower than the

average first and second service obtained in NC buffaloes. It was evident in this study 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 earlier workers (DRILLICH, 2006 and KIMURA et al., 2006).

The average conception rate of 75% obtained in group IV proved the efficacy of CIDR plus PGF₂α in improving fertility in postpartum buffaloes. 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. It is concluded that the buffaloes affected with RFM or normally calved, the fertility rate improved with estrus induction programme along with prior deworming and mineral supplementation which in turn reduced the postpartum interval to conception considerably.

Serum progesterone. The average level of serum progesterone recorded at the time of animal selection in this experiment varied from 0.41±0.14 to 0.75±0.16 ng/ml. It indicated that all the buffaloes were in anestrus stage at the time of selection. In this study, the serum progesterone level at the time of PGF₂α injection was increased when compared to insertion time. The elevated progesterone might be from CIDR which contains 1.38 g natural progesterone and the corpus luteum of the group I and III as evinced by rectal palpation. This was in accordance with the result of DUGWEKAR et al. (2008).

The average level of serum progesterone observed at the time of induced estrus in this study was less than 0.5 ng/ml. This finding corroborates with the result of RAVIKUMAR (2003) in buffaloes. The reduction in the progesterone level below 0.5 ng/ml at the time of estrus might be the reason for improved conception rates in estrus induction programme in water buffaloes in this study. DUCHENS et al. (1994) suggested that elevated progesterone level at estrus might lead to asynchrony between the onset of estrus and ovulation and consequently cause failure of conception.

In this study, in all the groups, at 10 days post AI, average serum level of progesterone ranged from 4.47±0.21 to 6.65±0.10 ng/ml. It indicated the 75-100% ovulatory response in all the groups following CIDR withdrawal as evinced by rectal palpation. The increased progesterone concentration following AI might have caused embryonic development which in turn ultimately resulted in improved fertility of buffaloes in this study.

Estradiol-17 β. The mean serum estradiol level at the time of selection of animals, ranged from 11.54±3.54 to 18.68±4.06 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 PGF₂α injection was observed in this study in serum estradiol-17β levels. However, at the time of induced estrus (AI), the serum estradiol level got increased and proved the presence of matured Graafian follicle in the ovary by rectal palpation. In this experiment, at 10 days post AI, the serum estradiol ranged from 16.54±03.544 to 19.84±4.77 pg/ml. Similar values were reported by SINGH and SINGH (2006) in buffaloes. The result of the study indicated the well defined pattern of secretion of estradiol following CIDR treatment in buffaloes.

Histopathology of endometrium. The endometrial biopsy was taken by Albuchin's biopsy catheter to study the endometrial changes before and after treatment in NC and RFM affected water buffaloes. RAJA et al. (2012) recommended that this technique can be used to diagnose sub-clinical endometritis in bovines. In NC buffaloes, the endometrium appeared normal prior to treatment and it was in accordance with the findings of MEE et al. (1990) in buffaloes and PRASAD and KRISHNA, (2009) in cows. But, the complete loss of epithelial layer, degeneration changes of endometrium, distorted endometrium and atrophic changes in the endometrial glands of RFM affected buffaloes before estrus induction indicated the damage caused by RFM in this study when compared to NC buffaloes. These results were in accordance with the findings of PRASAD and KRISHNA (2009) in cows and KAWASHIMA et al. (2010) in buffaloes. After treatment, RFM affected buffaloes showed clear regeneration

of endometrium and its glandular acini. It was evident that resumption of ovarian activity caused regeneration in the endometrium following estrus induction in RFM affected buffaloes. It proved that uterus had the powerful system of natural repair and recovery.

Conclusion

From the results of the study, it was evident in NC or RFM affected postpartum water buffaloes CIDR+PGF₂α the protocol improved the fertility rate. It was evident from the histopathology of endometrium that resumption of ovarian activity caused regeneration in the endometrium following estrus induction in RFM affected water buffaloes. Hence, it is concluded that estrus induction using CIDR plus PGF₂α might help to the augment fertility in NC and RFM affected water buffaloes at field level.

Conflict of interest

Authors have no conflict of interest to declare.

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SAŽETAK

Istraživanje je provedeno kako bi se usporedila indukcija estrusa, tijek estrusa, endometrijska regeneracija i stope gravidnosti nakon liječenja CIDR-om i PGF₂α između uredno oteljenih (NC) vodenih bivolica i onih sa zaostalom posteljicom (RFM). Ukupno 32 vodene bivoličice, 16 u skupini RFM i 16 u skupini NC, podijeljeno je u 4 skupine (skupine I i II, skupine III i IV). Bivolice u skupinama I i III dobivale su albendazol, dodan u mineralnu smjesu u hranu koju su životinje primale 15 dana. Bivolice u svim skupinama liječene su CIDR-om intravaginalno tijekom 9 dana, te su primile PGF₂α 24 sata prije nego što je obustavljena terapija CIDR-om. Nakon ukidanja CIDR-a bivoličice u svim skupinama umjetno su osjemenjene tijekom inducirano estrusa. Bivolice kod kojih se ponovno javio estrus, umjetno su osjemenjene dva puta u razmaku od 24 sata. Estrusni odgovor nakon ukidanja CIDR-a bio je 100 %-tan. Bivolice u skupini RFM imale su znatno dulji interval do pojave estrusa i kraće vrijeme estrusa nego bivoličice u skupini NC. U bivoličica u skupini NC uočen je veći intenzitet estrusa nakon primjene CIDR-a nego u bivoličica u skupini RFM. Prosječna stopa gravidnosti u bivoličica u skupini NC bila je 81,25 %, a u bivoličica u skupini RFM 50 %. U bivoličica u skupini RFM zapažen je potpun gubitak epitelnog sloja, degenerativne promjene endometrija te atrofične promjene u endometrijskim žlijezdama prije indukcije estrusa. Nakon liječenja bivoličice u skupini RFM pokazale su jasnu regeneraciju endometrija i njegovih žljezdanih acinusa. Zaključeno je da indukcija estrusa primjenom CIDR-a i PGF₂α može u terenskim uvjetima pomoći u povećanju plodnosti bivolica, i to onih uredno oteljenih i onih sa zaostalom posteljicom.

Ključne riječi: indukcija estrusa; CIDR; zaostala posteljica; stopa gravidnosti; vodene bivoličice
