



Fertility response on intrauterine administration of lochial extract in postpartum dairy buffaloes

V CHANDRA¹, S N SHUKLA², P R KUMAR³, O P SHRIVASTAVA⁴ and M A QUADRI⁵

Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh 482 001 India

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ABSTRACT

The present investigation aimed to assess the effect of intrauterine administration of lochial extract on fertility, uterine bacterial load, serum biochemical and hormonal profiles in postpartum buffaloes. Therefore, experiment was conducted in 30 postpartum buffaloes, randomly divided into 3 groups (10 each). Animals of G 1 received PBS (30 ml) however, in G 2, Lochial extract (30 ml) and in G 3, gentamicin (200 mg) were given as intrauterine infusion on day 15 postpartum. Fertility was ascertained in terms of uterine involution, induction of estrus and conception rate. Bacterial load in uterine contents, serum progesterone, calcium and phosphorus concentrations were also studied before and after the treatments. The uterine involution within 30 days postpartum was highest in both groups 2 and 3 (80% in each). The oestrus induction within 60 days postpartum was also highest in both groups 2 and 3 (60% in each). The conception rate at induced estrus was recorded higher (100%) in lochial extract group followed by gentamicin (83.33%) and control (75%) group. No significant differences were observed in the data of fertility responses. There was significant reduction in bacterial load after treatment in both the treated groups. However, no significant difference was recorded in serum progesterone, calcium and phosphorus neither within the group nor among the groups before and after treatments. Overall results of the present study revealed better and comparable fertility with low uterine bacterial load in postpartum buffaloes administered intrauterine lochial extract and gentamicin.

Key words: Bacterial load, Biochemical, Buffalo, Lochial extract, Postpartum progesterone

Buffaloes are very much prone to postpartum uterine disorders causing poor reproductive efficiency. Modulation of host response to uterine infections and stimulation of uterine defense mechanism is an alternative and attractive approach to various conventional therapeutics of clinical and subclinical uterine infection in buffaloes. The use of various antimicrobial agents like antibiotics and iodine preparations through intrauterine route may interfere natural uterine defense mechanism resulting several uterine disorders in postpartum buffaloes. The uterine lochial discharge found to have some immunological properties due to presence of several bacterial species viz. *E. coli*, *Streptococci* and *A. pyogens*; opsonising proteins; immunoglobulins (IgG2 and IgA) and PMNs cells (Watson *et al.* 1990, Noakes *et al.* 1991, Hakansson *et al.* 1993).

Present address: ¹Veterinary Officer (vetvikas.yadav567@gmail.com), Veterinary Hospital, Barhan District, Agra. ²Associate Professor (snshukla2@gmail.com), ⁴Professor and Head (vetopshri@gmail.com), Department of Veterinary Gynaecology and Obstetrics, ⁵Professor (afeefquadri@yahoo.co.in), Department of Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry. ³Assistant Professor (dr.pranjan007@gmail.com), Department of Veterinary Gynaecology and Obstetrics, Faculty of Veterinary and Animal Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh.

Due to evidence of such properties, it was thought that lochial secretion may enhance the uterine defense resulting low uterine disorders and thus enhance postpartum reproductive efficiency in buffaloes. Therefore, the present study was conducted to study the effect of intrauterine administration of lochial extract on the fertility, uterine bacterial load, serum biochemical and hormonal profiles in postpartum buffaloes.

MATERIALS AND METHODS

Animals: The present study was conducted in buffaloes of Livestock farm, College of Veterinary Science and Animal Husbandry and organized buffalo farms situated at Pariyat, Jabalpur. All the buffaloes were stall fed and maintained under standard managemental conditions. Thirty apparently healthy postpartum buffaloes having BCS between 2 to 4 in 5 points scale were selected for the study.

Preparation of lochial extract: The lochia was collected aseptically from the uterus of early postpartum buffaloes. The lochial extract was prepared by dissolving 10.0 ml of lochia into 25 ml of phosphate buffer saline (PBS) and sterilized by autoclaving. This solution was centrifuged at 500 rpm for 5 min then 30.0 ml of supernatant was collected. The freshly prepared lochial extract was used for intrauterine administration.

Table 1. Treatment schedule

Groups	Treatment	Dose	Route	Duration
Group 1	PBS	30.0 ml	I/U	Single
Group 2	Lochial extract	30.0 ml	I/U	Single
Group 3	Gentamicin	30.0 ml	I/U	Single

Preparation of gentamicin solution: 200mg gentamicin was dissolve in 30 ml phosphate buffered saline (PBS) for intrauterine infusion.

Treatment groups and schedule: The selected buffaloes were divided into 3 groups, each comprising ten buffaloes. The single intrauterine treatment was given to each animal on day 15 postpartum as given in Table 1.

Monitoring and observation of animals: All the experimental buffaloes were subjected to detection of estrus twice daily (morning and evening) by careful visual observations and use of breeding bull. Buffaloes were examined per rectally at weekly interval to monitor the ovarian and uterine changes for resumption of ovarian activities, uterine involution and induction of estrus. The nature of vaginal discharge was observed throughout the postpartum period for ascertaining the status of uterus. Natural service was done at estrus using breeding buffalo bull. All the served animals were observed for non-return to estrus and pregnancy diagnosis was done after 45 days of breeding.

Determination of bacterial load: The viable bacterial count was determined by standard plate count method in the uterine contents on day 15 postpartum (day, 0; before treatment) and day 30 postpartum (day, 15; after treatment).

Hormonal and biochemical assay: Five milliliter blood samples were collected aseptically from jugular vein on day 15 and day 30 after calving and then serum was separated and stored at -20°C till assay. The quantitative determination of serum progesterone concentration was made by ELISA method using commercial kits. The serum calcium and phosphorus estimations were done by fully automated analyzer (ammonium molybdate Method).

Statistical analysis: The generated data were analyzed statistically by Chi square test for uterine involution, oestrus induction and conception rate. Data of parameters such as bacterial load, hormonal and biochemical levels before and after the treatment within the group and between the groups were analyzed by independent sample T-Test and ANOVA respectively, using software SPSS version 16.0.

RESULTS AND DISCUSSION

The fertility response to treatments in postpartum buffaloes was studied in terms of uterine involution, onset of estrus within 60 days postpartum and conception at induced estrus (Table 2).

Uterine involution: Present study indicated higher percentage of uterine involution within 30 days postpartum in the buffaloes of both lochial extract and gentamicin treated group (80% each) as compared to control group (60%) however, did not differ significantly. This may be

due to significant reduction of bacterial load in uterine content after the treatment (Table 3). Some investigators reported shorter periods (<35 days) required for completion of uterine involution (Jainudeen *et al.* 1983, Usmani *et al.* 2001) while others reported longer periods (>40 days) in this species (Peiris *et al.* 1980, Bahga *et al.* 1988). This variation might be due to the failure of some buffaloes to eliminate postpartum bacterial infections of the uterus, which in turn, results in delayed involution. This assumption is supported by the reports of Ahmad *et al.* (1985) and Khan *et al.* (1985) who documented the presence of both pathogenic and nonpathogenic bacteria in uterine fluid samples of buffaloes that required more than 35 days to complete involution of the uterus. The variations in involution may also be due to difference in managemental practices among the farms.

Estrus induction: Estrus induction within 60 days postpartum varies between 40 to 60% among the buffaloes of treated and control groups. It was recorded higher in treated groups (60% each) than the control group (40%). The rate of oestrus induction did not differ significantly among the group. However, higher oestrus induction in treated groups may be due to faster uterine involution after the treatment (Table 2) as delayed uterine involution holdup resumption of ovarian activity (Kumar *et al.* 2014). Postpartum uterine infection (clinical or sub-clinical) suppress GnRH release and possibly LH secretion (Peter *et al.* 1990, Mateus *et al.* 2002), probably due to inflammatory response (Sheldon and Dobson 2004, Williams *et al.* 2007) thus, ovarian activity remains suppressed in uterine infections.

Conception rate: The conception at induced estrus was recorded higher (100%) in lochial extract treated group as compared to the gentamicin treated (83.33%) and control group (75%) but there is no significant variation among the groups. The lower conception rate in Gentamicin treated group may be due to its unknown effect in the uterus. However, conception up to 129 days postpartum was reported 57 and 63% in infected and normal postpartum buffaloes, respectively (Usmani *et al.* 2001).

Table 2. Fertility response to treatments in postpartum buffaloes

Particulars	Group 1	Group 2	Group 3
Animals (n)	10	10	10
Treatment	PBS	Lochial extract	Gentamicin
Animals with complete uterine involution at day 30 (%)	60	80	80
Animals exhibited estrus within 60 days postpartum (%)	40	60	60
Conception rate at induced estrus (%)	75 (3/4)	100.00 (6/6)	83.33 (5/6)

Bacterial load ($10^6/\text{ml}$) in uterine contents before and after treatment: The results of bacterial load ($10^6/\text{ml}$) in the uterine contents before (day-15) and after (day-30) treatment in postpartum buffaloes (Table 3) revealed that there is no significant reduction in bacterial load in control group however, highly significant reduction ($P<0.01$) in both the treatment groups on day 30 as compared to pre-treatment on day 15 postpartum. Further, bacterial load was found to be significantly low ($P<0.05$) in both the treated groups ($3.98\pm0.57\times10^6/\text{ml}$ and $4.63\pm0.98\times10^6/\text{ml}$) as compared to control group ($11.10\pm1.22\times10^6/\text{ml}$) post-treatment (day 30). The bacterial load in the postpartum uterus on day 30 was almost similar in the lochial extract and gentamicin treated group. Little information is available regarding the bacterial load on day 15 and day 30 of postpartum buffalo uterus. The most of investigators have studied the uterine bacterial load in the uterine infections such as endometritis before and after the treatments. Our findings are almost similar to the findings of Raju *et al.* (2006), who reported the bacterial colony count ($3.75\pm0.84\times10^6$) in healthy and ($79.58\pm8.10\times10^6$) endometritis affected buffaloes. The low bacterial load after treatment in lochial extract and gentamicin group is justifying the results of fertility response in the present study. The uterine lochial discharge found to have some immunological properties due to presence of several bacterial species viz. *E. coli*, *Streptococci* and *A. pyogens*; opsonising proteins; immunoglobulins (IgG2 and IgA) and PMNs cells (Watson *et al.* 1990, Noakes *et al.* 1991, Hakansson *et al.* 1993). Lochial extract used in this study may have some antigenic properties for enhancement of uterine defense resulting better fertility response in buffaloes.

Serum progesterone profile (ng/ml): Serum progesterone concentrations (ng/ml) ranges between 0.96 ± 0.28 to 1.20 ± 0.33 and 1.06 ± 0.27 to 1.09 ± 0.47 respectively, on day 15 and day 30 postpartum (Table 4) where no significant difference ($P>0.05$) was found neither within the group nor among the groups before and after the treatment. However, mean serum progesterone concentration was found $>1\text{ ng/ml}$ in all the groups, indicating presence of corpus luteum in majority of animals but values of progesterone concentrations in individual animals were not similar. The individual variation in the serum progesterone concentration may be due to different stages of the corpus luteum as per the stage of estrus cycle/postpartum ovarian activities. The basal value of progesterone (0.12 to 0.6 ng/ml) reached during calving suggesting complete luteolysis at parturition

Table 3. Bacterial load ($10^6/\text{ml}$) in uterine contents before and after treatment

Treatment groups	Day 15 PP	Day 30 PP
Group 1	$14.90\pm1.62\text{ap}$	$11.10\pm1.22\text{ap}$
Group 2	$12.70\pm1.55\text{ap}$	$3.98\pm0.57\text{bq}$
Group 3	$14.30\pm1.30\text{ap}$	$4.63\pm0.98\text{bq}$

Values differ significantly with different superscript (a, b) in rows ($P<0.01$) and (p, q) in column ($P<0.05$).

Table 4. Serum progesterone profile (ng/ml) before and after treatment

Treatment Groups	Day 15 PP	Day 30 PP
Group 1	$1.09\pm0.25\text{a}$	$1.06\pm0.27\text{a}$
Group 2	$0.96\pm0.28\text{a}$	$1.09\pm0.47\text{a}$
Group 3	$1.20\pm0.33\text{a}$	$1.07\pm0.36\text{a}$

with no significant changes during the postpartum period (Momongan *et al.* 1990, Savaiya *et al.* 1993, Tiwari *et al.* 1995). The decline of progesterone continued during the postpartum period to reach minimum between day 6 to 15 indicating complete regressions of CL of pregnancy (Bahga 1989). However, a wider range between 3 to 29 days was recorded to complete regression of CL after parturition (Pahwa and Pandey 1983). It is also reported that progesterone levels remains basal but a transient elevation may occur before resumption of cyclic activity (Sharma and Kaker 1990, Ghoneim *et al.* 1999, Shah *et al.* 2004). However, the time of luteolysis differs individually during postpartum periods in buffaloes reared under different managemental and climatic conditions. The problem of silent ovulation in buffaloes especially at early first postpartum oestrus and then presence of CL for next ten to twelve days may be the reason of little higher progesterone from basal level in the present study.

Serum calcium and phosphorus concentration (mg/dl): Mean values of both serum calcium and phosphorus concentrations (mg/dl) did not differ significantly neither within the group nor among the groups before and after the treatment (Table 5). In all the groups the serum calcium concentrations were found lower than normal value that may be due to high producer animals of this study which lead to drainage of calcium in milk. Ali *et al.* (1983) reported that the buffaloes in estrus cycle had greater plasma Ca concentration than silent estrus (8.00 ± 1.98 vs. $6.51\pm1.17\text{ mg/dl}$). However, the various investigators were reported calcium level in postpartum buffaloes 8.43 to 9.98 mg/dl (Kulkarni *et al.* 1984, Hussain *et al.* 2001). The low level of calcium in the present study may also be responsible for suboptimal reproductive efficiency mainly in terms of oestrus induction rate in present study.

The serum phosphorus concentrations were nearly similar to the normal value in all the groups before and after the treatment and comparable to the reports of Hussain *et al.* (2001) and Hagawane *et al.* (2009). However, the calcium and phosphorus level from late pregnancy to 60

Table 5. Serum calcium and phosphorus concentration (mg/dl) before and after treatment

Treatment groups	Serum calcium concentration		Serum phosphorus concentration	
	15 PP	30PP	15PP	30PP
Group 1	$5.82\pm0.18\text{a}$	$5.78\pm0.22\text{a}$	$4.12\pm0.24\text{a}$	$4.57\pm0.41\text{a}$
Group 2	$5.58\pm0.13\text{a}$	$5.43\pm0.19\text{a}$	$4.76\pm0.30\text{a}$	$4.98\pm0.23\text{a}$
Group 3	$5.59\pm0.18\text{a}$	$5.68\pm0.16\text{a}$	$4.30\pm0.36\text{a}$	$4.56\pm0.25\text{a}$

days post calving was found to be almost constant throughout the study and reported to be maintained by a body homeostasis system, indicating buffalo utilizes only a little of their endogenous mineral resources (Ciaramella *et al.* 2000).

The better and comparable fertility with low uterine bacterial load in postpartum buffaloes administered intrauterine lochial extract and gentamicin may be due to immunological properties of the lochia responsible for enhancing uterine defense mechanism and antimicrobial property of gentamicin, respectively in the postpartum uterus. The lochial extract may help in chemotaxis, thus enhance uterine defense mechanism through phagocytosis. However, no such study was conducted previously in buffaloes for minimizing postpartum estrus interval. The literature is scant regarding the use of lochial extract and its effect on uterine involution, conception rate and uterine bacterial load in postpartum buffaloes.

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