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USE OF HYALURONIDASE ENZYME FOR HASTENING THE CERVICAL DILATATION IN SUCCESSFULLY DETORTED UTERINE TORSION AFFECTED BUFFALOES

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ABSTRACT

The successfully detorted uterine torsion buffaloes were either subjected to routine post-detorsion treatment (n=10) or in addition to routine treatment, intracervical hyaluronidase enzyme was administered (10,000 IU; 2.5 ml at each of 3, 6, 9, 12 o'clock position of cervix) immediately post-detorsion (n=10), followed by repeated administration at 6h interval (0, 6, 12, 18 h) till complete cervical dilatation or till 24 h post-detorsion. The time elapsed between successful uterine detorsion to complete cervical dilatation was reduced (P<0.05) in buffaloes subjected to enzyme treatment in comparison to their control counterparts (754.6±114.0 *vs.* 213.5±33.1 minutes). In buffaloes delivering dead fetus, following intracervical treatment, the interval between detorsion to complete cervical dilatation was reduced (P<0.05) to 240.8±44.0 minutes in comparison to their control counterparts (838.1±126.2 minutes). Moreover, following hyaluronidase treatment, the time interval from detorsion of a buffalo to complete cervical dilatation with respect to degree of uterine torsion was much lower (P<0.05) in treated compared to their control group counterparts having either <180° or ≥180° uterine torsion. The fertility parameters (number of days to first estrus and duration of service period) were improved (P>0.05) in hyaluronidase-treated buffaloes of both the groups (<18 h or ≥18 h duration of torsion) as compared to their control counterparts. Conclusion: Intracervical hyaluronidase treatment hastens the cervical dilatation in successfully detorted uterine torsion affected buffaloes with a subsequent potentially beneficial impact on fertility.

Keywords: *Bubalus bubalis*, buffaloes, cervix, fertility, hyaluronidase, uterine torsion

INTRODUCTION

As far as buffalo species is concerned, the torsion of uterus is of significant importance due to its higher incidence. At referral hospitals, uterine torsion contributes up to 67 to 83% of dystocia cases in buffaloes (Ghuman, 2010). The striking feature of uterine torsion in buffaloes is its association with advanced pregnancy and process of parturition. Usually, uterine torsion occurs

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before the onset or during the late first stage of parturition as indicated by partially or completely dilated cervix prior to or immediately after the correction of torsion (Prabhakar *et al*., 1994).

The occurrence of cervical ischemia following uterine torsion causes damage to cervical tissue as well as the visco-elastic properties of cervix responsible for dilatation of cervix (Singla *et al*., 1989; Breeveld-Dwarkasing *et al*., 2003). The duration of torsion has a significant role in likelihood that cervix will completely dilate subsequent to successful uterine detorsion. When buffaloes are subjected to detorsion process within <36 h, between 36 to 72 h and >72 h after occurrence of torsion, the possibility that cervix will dilate and there will be vaginal delivery is 83, 52 and 9%, respectively (Dhaliwal *et al*., 1991; Honparkhe *et al*., 2009). It was strongly recommended that following detorsion, if the fetus is dead, the buffalo should be immediately subjected to cervical dilatation approaches, otherwise leaving even soft or moderately soft cervix to dilate on its own will lead to hardening of cervical texture within 24 h, followed by its failure to dilate (Honparkhe *et al*., 2009).

In past, hormones like oxytocin were used for inducing cervical dilatation in cattle, however literature has not consistently proven its efficiency (Ghuman, 2010). Also, attempts were made for induction of parturition with cervical application of prostaglandin E_1 in cattle as prostaglandins play an important role in cervical ripening by inducing the synthesis of collagenase responsible for cervical tissue collagen breakdown (Yildiz, 2009). Further, hyaluronidase enzyme is known to reduce the cellular adhesions in cervix by depolymerization of cervical conjunctive components (collagen, hyaluronic acid and chondroitin), thus causing cervical softening and dilation (Li *et al*., 1994). Intracervical hyaluronidase (10,000 IU) in partially dilated cervix of uterine torsion affected buffaloes lead to complete dilatation in 87.5% buffaloes (Singh, 2018). The present study in uterine torsion affected buffaloes aimed at the use of hyaluronidase enzyme for decreasing the time interval between successful uterine detorsion and complete cervical dilatation as well as the subsequent fertility parameters.

MATERIALS AND METHODS

History and obstetrical treatment

The present study was carried out on twenty full term pregnant buffaloes presented for the treatment of uterine torsion at Teaching Veterinary Clinical Complex, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. The history of each uterine torsion affected buffalo with regard to parity (80% in 2^{nd} to 4^{th} parity) and duration of occurrence of uterine torsion (50%: <18 h; 50%: 18 to 36 h) as judged from milk resorption and sacrosciatic ligament relaxation was recorded. All the presented buffaloes had right-side post-cervical uterine torsion and 65% of these buffaloes had ≥180^{o} torsion. Subjecting all the buffaloes to Sharma's modified Schaffer's method of uterine detorsion lead to successful uterine detorsion either in one (35% buffaloes), two (55%) or ≥ three rolls (10%). Following successful uterine detorsion, the cervical status in the buffaloes was observed as either closed or partially dilated (<4 fingers or upto 7 cm diameter open).

Groups

Based upon the history and obstetrical observations, all the buffaloes were equally divided into control and hyaluronidase Treatment

groups. Immediately after detorsion, the Control group buffaloes, through per-vaginal route, were subjected to intracervical administration of 0.1 M sodium phosphate buffer using 21 G scalp vein (2.5 ml at each of 3, 6, 9, 12 o'clock position of cervix) at 6 h interval until complete dilatation of cervix or till 24 h post-detorsion. In hyaluronidase group, intracervical hyaluronidase [10,000 IU: 20 mg hyaluronidase powder (500 IU/mg MP biomedicals Australasia Pvt. Limited, Australia) dissolved in 10 ml 0.1 M sodium phosphate buffer] treatment was given as per the procedure adopted for Control group. In addition, immediately after detorsion, all the buffaloes of both the groups were administered (i.m.) 500 µg cloprostenol sodium and 40 mg dexamethasone along with the routine supportive therapy.

Observations

Per-vaginal examination was carried out to evaluate the status of cervical dilatation immediately after detorsion, and at regular interval of 6 h after application of intracervical treatment. The time taken for cervical dilatation followed by immediate per-vaginal delivery (calf viability: live 30%; calf sex: male 70%) in buffaloes of both the groups was recorded.

Statistical analysis

Using statistical program (SPSS, version 16.0), *t*-test was applied to assess the impact of hyaluronidase enzyme on the decrease in time interval between successful uterine detorsion and complete cervical dilatation in the buffaloes. The impact of hyaluronidase treatment with respect to duration (<18 h and ≥18 h) of uterine torsion was evaluated on fertility parameters in successfully detorted uterine torsion affected buffaloes. The data was presented as mean±SE. The minimum significant interaction was considered at 5% level.

RESULTS AND DISCUSSIONS

Hyaluronidase treatment vs. interval between uterine detorsion to complete cervical dilatation

The time interval from occurrence of uterine torsion to successful uterine detorsion was similar (P>0.05) in buffaloes randomly allocated to control and treatment group (Table 1). However, the subsequent time taken for complete cervical dilatation was reduced (P<0.05) in buffaloes subjected to intracervical hyaluronidase enzyme treatment immediately after successful uterine detorsion in comparison to their control counterparts (754.6±114.0 *vs.* 213.5±33.1 minutes; Table 1). Intracervical hyaluronidase administration studies in humans have also recommended the same as an effective, low risk method to induce cervical ripening, decrease the duration of labour and increase the possibility of vaginal delivery (82% *vs.* 51%), even for women with prior caesarean sections (Spallicci *et al*., 2007).

Further, in control and hyaluronidase treated buffaloes, the time interval between occurrence of uterine torsion to detorsion was similar (P>0.05) in buffaloes delivering either live (630.0±239.9 *vs.* 617.5±80.3 minutes, respectively) or dead (1261.8±137.4 *vs.* 1538.3±164.7 minutes, respectively; Table 1) fetus. However, in buffaloes delivering dead fetus, following intracervical treatment, the interval between detorsion to complete cervical dilatation was reduced (P<0.05) to 240.8±44.0 minutes in comparison to their control counterparts (838.1±126.2 minutes; Table 1). A similar pattern (P>0.05) was observed between both the groups for the buffaloes delivering live fetus (Table 1). This suggested the beneficial impact of

intracervical hyaluronidase treatment on complete cervical dilatation especially in buffaloes carrying dead fetus. In fact, *in utero* death of fetus causes damage to the endometrium and myometrium as well as the absence of fetal movements lead to cessation of uterine contractions responsible for cervical dilatation, thus delaying complete cervical dilatation (Ghuman, 2010). However intracervical hyaluronidase treatment may lead to depolymerization of conjunctive components of cervix, thus lowering cellular adhesions of cervical collagen, cervical softening and cervical dilation (Li *et al*., 1994).

Hyaluronidase treatment and uterine torsion duration (<18 h or ≥18 h) or degree (<180° or ≥180°) vs. interval between detorsion to complete cervical dilatation

The extent of hypoxic degeneration of cervical tissue due to cervical ischemia is dependent upon duration of uterine torsion (Singla *et al*., 1989). Therefore, the time taken from detorsion to complete cervical dilatation in control buffaloes with ≥18 h elapsed since the occurrence of uterine torsion was higher (P<0.05) compared to their counterparts with <18 h duration of torsion (1042.5±200.6 *vs.* 562.6±67.3 minutes, Table 2). However, following hyaluronidase treatment, compared to control counterparts, the time interval between detorsion to complete cervical dilatation was much lower (P>0.05) in buffaloes having duration of torsion either <18 h (172.5±49.6 *vs.* 562.6±67.3 minutes, respectively) or ≥18 h (240.8±44.1 *vs.* 1042.5±200.6 minutes, respectively; Table 2).

Furthermore, the time taken from uterine detorsion to complete cervical dilatation in control buffaloes with ≥180° uterine torsion was higher (P<0.05) compared to their counterparts with <180° uterine torsion (897.8±128.4 *vs.* 420.3±2.6 minutes, Table 2). This could be due to decreased blood supply to the cervix caused by twisting of blood vessels at the time of torsion (Singla *et al*., 1989). However, following hyaluronidase treatment, the time interval from detorsion of a buffalo to complete cervical dilatation with respect to degree of uterine torsion was much lower (P<0.05) in treated compared to their Control group counterparts having either <180° (101.2±20.0 *vs.* 420.3±2.6 minutes, respectively) or ≥180° (288.3±18.5 *vs.* 897.8±128.4 minutes, respectively) uterine torsion (Table 2). This could be the impact of hyaluronidase enzyme which causes hydrolysis of hyaluronic acid and promotes cervical relaxation either directly via tissue hydration by attracting water molecules or indirectly via regulation of inflammatory genes (Dowthwaite *et al*., 1999; Uchiyama *et al*., 2005).

Hyaluronidase treatment and uterine torsion duration (<18 h or ≥18 h) vs. fertility parameters of successfully detorted buffaloes with per-vaginal fetal delivery

The number of days to first estrus and the duration of service period following fetal delivery was higher (P<0.05 to >0.05) in successfully detorted uterine torsion affected buffaloes which previously had ≥18 h duration of uterine torsion compared to their counterparts with <18 h duration of torsion in both Control and Treatment groups (Table 3). The delay in treatment of uterine torsion as well as the degree and duration of uterine torsion was negatively correlated with subsequent conception rate due to torsion induced congestion, edema and hematoma in the uterine ligaments, mesovarium and ovaries (Ghuman, 2010). Nevertheless, these fertility parameters were improved (P>0.05) in hyaluronidase-treated

Table 1. Impact of intracervical hyaluronidase enzyme treatment on shortening the time interval between successful uterine detorsion and complete cervical dilatation (CCD) in uterine torsion affected buffaloes.

Group		Uterine torsion to detorsion, min	Uterine detorsion to CCD, min
Control, n=10		1135.5±141.9	754.6±114.0[a]
Hyaluronidase, n=10		1155.0±162.3	213.5±33.1[b]
Control	Live fetus, n=2	630.0±239.9	420.5±4.49
	Dead fetus, n=8	1261.8±137.4	838.1±126.2[a]
Hyaluronidase	Live, n=4	617.5±80.3[a]	172.5±49.6
	Dead, n=6	1538.3±164.7[b]	240.8±44.0[b]

P<0.05: [a *vs* b]within column of a respective parameter.

Table 2. Impact of intracervical hyaluronidase enzyme treatment and uterine torsion duration (<18 h or ≥18 h) or uterine torsion degree (<180° or ≥180°) on the time interval (minute) between uterine detorsion to complete cervical dilatation.

Group	<18h	≥18h
Control	562.6±67.3[c], n=6	1042.5±200.6[d], n=4
Hyaluronidase	172.5±49.6, n=4	240.8±44.1, n=6
	<180°	≥180°
Control	420.3±2.6[a,c], n=3	897.8±128.4[a,d], n=7
Hyaluronidase	101.2±20.0[b,c], n=4	288.3±18.5[b,d], n=6

P<0.05: [a *vs* b]within column of a respective parameter; [c *vs* d]within a row.

Table 3. Impact of intracervical hyaluronidase enzyme treatment and uterine torsion duration (<18h or ≥18h) on fertility parameters of successfully detorted buffaloes with per-vaginal fetal delivery.

Group		<18h	≥18h
Control, n=8	Days to first estrus	106.8±20.6	170.3±10.7
	Service period, days	134.4±33.5	196.5±24.5
Hyaluronidase, n=10	Days to first estrus	67.7±8.1[c]	153.9±17.6[d]
	Service period, days	73.0±9.7	149.7±30.9

[c *vs* d]P< 0.05.

buffaloes of both the groups (<18 h or ≥18 h duration) as compared to their control counterparts (Table 3). This could be due to hyaluronidase induced decrease in time interval from detorsion to complete cervical dilatation observed in the present study (Table 1), thus, shortening the period of parturition stress for these buffaloes. The longer stress duration is a well-known detrimental factor for the optimal functioning of reproductive axis in ruminants (Dobson *et al*., 2003). In fact, the bovines with short duration (<12 to 36 h) of uterine torsion subsequently had 70% fertility (Schönfelder *et al*., 2005).

In brief, the intracervical hyaluronidase enzyme administration helps in decreasing the time interval between successful uterine detorsion and complete cervical dilatation in a buffalo, with a subsequent potentially beneficial impact on fertility.

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