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Cervical histomorphology of successfully detorted uterine torsion affected buffaloes subjected to intracervical hyaluronidase or PgE_1 treatment

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The occurrence of uterine torsion mostly near the time of parturition in buffaloes is an emergency condition and the outcomes of delay in management of case include foetus and/or dam mortality, and the surviving dams may suffer from delayed uterine involution, reduced production, and compromised reproductive performance (Ghuman 2010). Nevertheless, following successful uterine detorsion, 18-50% buffaloes fail to exhibit complete cervical dilatation (Ghuman 2010). In fact, the visco-elastic properties of cervix responsible for its dilatation are disturbed following the torsion of uterus (Breeveld-Dwarkasing *et al*. 2003). Moreover, depending upon the degree and duration of torsion, there is variable amount of cervical ischemia leading to hypoxic degeneration of cervical epithelium, marked fragmentation of elastic fibers and irreparable coagulative necrosis of smooth cells in the cervical tissue (Singla *et al*. 1989). Furthermore, it was strongly recommended that following detorsion, if the fetus was dead, the dam should be immediately subjected to cervical dilatation approaches, otherwise leaving even soft or moderately soft cervix to dilate on its own will lead to complete hardening of cervix within 24 h, followed by its failure to dilate (Honparkhe *et al*. 2009).

Near the end of pregnancy period, the endogenous prostaglandins play an important role in cervical ripening by inducing or increasing the synthesis of collagenase responsible for cervical collagen breakdown (Soni and Rajput 2004). Moreover, the hyaluronidase enzyme reduces cervical cell adhesions by neutralizing hyaluronic acid which leads to softening of cervix and cervical dilatation (Sharma and Singh 1984). Considering the impact of delay in cervical dilatation subsequent to uterine detorsion in buffalo, the present study was planned in successfully detorted uterine torsion affected buffaloes for achieving complete cervical dilatation for per vaginal fetal delivery.

The present study was carried out on 24 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 between 36–72 h after the visible signs of occurrence of uterine torsion. A complete history of each uterine torsion affected buffalo with regards to age, parity, gestation period, feed and water intake as well as the duration of occurrence of uterine torsion as judged from milk resorption, sacrosciatic ligament relaxation and previous treatment was recorded for evaluating the diagnosis and prognosis of the case. The gynaeco-clinical examination of respective buffaloes confirmed the diagnosis as uterine torsion. In all the buffaloes, the direction of uterine torsion was right side and position was post-cervical. The degree of uterine torsion varied from 180º to >360º and visible signs of uterine torsion had started 36-72 h earlier in all the buffaloes. Following successful uterine detorsion, the buffaloes with incomplete cervical dilatation were randomly divided into three groups on the basis of treatment protocol administered immediately after detorsion. In group I (control group, n=8), buffaloes through per-vaginal route were intra-cervically administered with 10 ml (2.5 ml at each of 3, 6, 9, 12 o'clock position) of 0.1 M sodium phosphate buffer solution with the help of 21G scalp vein along with manual cervical massage. In group II (Hyaluronidase group, n=8), intracervical hyaluronidase injection (20 mg of hyaluronidase; 500 IU/mg, MP biomedicals Australasia Pvt. Limited, Australia; dissolved in 10 ml of 0.1 M sodium phosphate buffer, *p*H 5.3, with 0.15 M sodium chloride) was administered, instead of phosphate buffer, as in group I using same procedures. In group III (PgE_1 group, n=8), intracervical PgE_1 injection (Stock solution: 10 mg of PgE_1; Sigma Aldrich, St. Louis, MO; dissolved in ethanol to total volume of 2 ml (5 mg/ml); Working solution: 100 µl of stock solution diluted to 10 ml with 0.1M phosphate buffer) was administered, instead of phosphate buffer, as in group I using same procedures. In all the three groups, intracervical and manual massage procedures were repeated every 3 h until complete dilatation of cervix or till 15 h post-detorsion. In addition, immediately after detorsion, the buffaloes of all the groups were administered 500 µg cloprostenol sodium (synthetic

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analogue of Prostaglandin F2 alpha) and 40 mg dexamethasone through intramuscular route. The routine supportive therapy consisting of antibiotics, liver tonic, and intravenous calcium were administered as per requirement of the case. At the end of 18 h post-detorsion, if there was incomplete cervical dilatation in any of the groups, the buffaloes were subjected to caesarean operation.

The cervical biopsy sample of 4 mm size was collected with a sterile biopsy punch from the dorsal side of cervix and about 2 cm inside the external orifice under low epidural injection of 2% lignocaine hydrochloride and the samples were transferred to 10% neutral buffer formalin immediately for 48 h. The tissue samples were processed for paraffin embedding to examine further histomorphological changes. The first biopsy sample was collected immediately after detorsion before instituting any treatment and second after complete dilatation or after 18 h in case of non-responsive buffaloes. The biopsy samples were washed in running tap water overnight to remove excess fixative, and were subjected to ascending grades of alcohol (70%, 80%, 90% and absolute alcohol) and acetone for dehydration and benzene for clearing. The biopsy samples were infiltrated and embedded with paraffin wax. The sections of 4-5 µm thickness were subjected to Haematoxylin and Eosin (H&E; Luna 1968) for morphological studies and Masson's trichrome (Luna 1968) for collagen fibres. The stained slides were imaged using Nikon microscope (80i) with photographic unit. The H&E images were evaluated for histomorphological status viz. for epithelium, propria submucosa, blood vessels (hyperemia and haemorrhage) and infiltrating cells. The images at 400× magnification were used for counting of collagen fibres using Image J software to calculate total number of fibres per unit area.

The blood flow parameters in middle uterine artery of uterine torsion affected buffaloes were assessed before detorsion and 0.5 h after detorsion/intracervical treatment to establish their correlation with chances of cervical dilatation. Various doppler indices over the cardiac cycle like pulsatility index (PI), resistive index (RI), time-average peak velocity (TAP) and blood flow volume (BFV) were used for estimating the blood flow resistance in vessels distal to the point of examination (Dickey, 1997). Resistive index (RI) relates to negative relation with vascular perfusion and decreasing resistance increases vascular perfusion and vice versa (Elmetwally 2012). Pulsatility index (PI) relates to negative relation with vascular perfusion and increasing PI indicates constriction of vascular bed distal to the site of measurement, decreased tissue perfusion and vice versa (Elmetwally 2012). Time-average peak velocity (TAP, cm/s) is used to evaluate the blood flow in small vessels (Bollwein *et al*. 2002). Blood flow volume (BFV, ml/min) to the target tissue depends on the mean velocity and diameter of target blood vessel (Krueger *et al*. 2009). At least three wave form measurements were taken for each parameter and the mean of at least two to three cardiac cycle of each wave form were used to increase the accuracy of doppler indices. All information was recorded in an excel spreadsheet and the statistical analysis of blood flow parameters (RI, PI, BFV and TAP) and collagen separation was performed with Statistical Package for Social Sciences (SPSS, version 16.0) program. A t-test was applied to determine mean, standard error and level of significance. The data were presented as mean±SE. The minimum significant interaction was considered at 5% level.

The histomorphology of cervical biopsy in successfully detorted uterine torsion affected buffaloes before instituting an intracervical treatment revealed cervical mucosa with mucosal folds having primary and secondary branches (Fig. 1a-d). The lining epithelium was columnar type with occasional pseudo-stratified epithelium. The columnar cells had elongated nuclei located towards the basement membrane. The apical surface of these cells had either cilia or secretary blabs. At places goblet cells were observed. The propria submucosa had loosely arranged connective tissue fibres, few engorged capillaries and few infiltrating cells (neutrophils, eosinophils, and lymphocytes; Fig. 1a-d). At this stage, due to uterine torsion, the decrease in blood supply and few polymorphonuclear (PMN) cells might be the reason underlying non-dilatation of cervix. In fact, the pre-requisites for the cervical dilatation include heavy vascularization of cervical epithelium and multiple

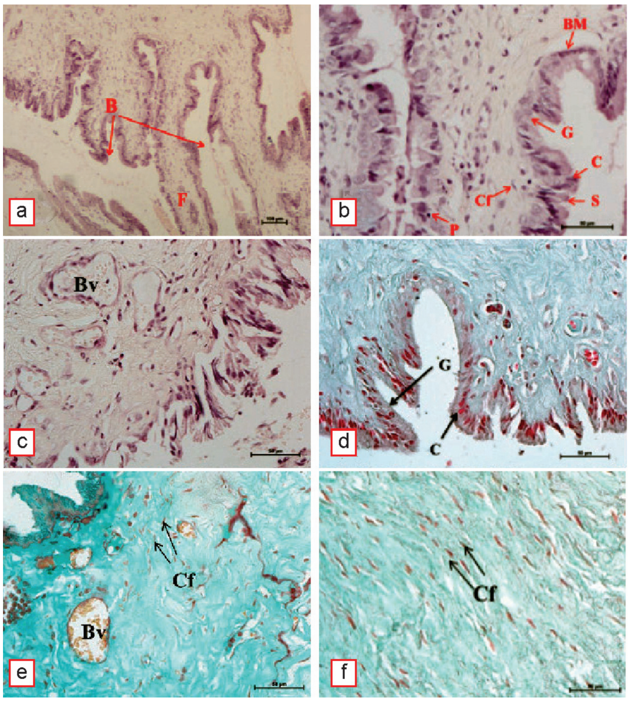

Fig. 1. Histomorphology of cervical biopsy in successfully detorted uterine torsion affected buffaloes before instituting an intracervical treatment. (a-c): Laminar epithelium consisted of columnar epithelium over basement membrane (BM), Mucosal folds (F). Lamina epithelia contained ciliated cells (C), secretory cells (S) and goblet cells (G). Propria submucosa contained collagen fibres (Cf), with slightly congested blood vessels (Bv) and polymorphonuclear cells (P). H&E; (d-f): Cervical epithelium lined with columnar cells (C) with darkly stained nuclei and lightly stained cytoplasm, few goblet cells (G) in between. Propria submucosa with closely placed Cf and slightly engorged Bv. MT.

infiltrations of PMN cells (Nagase and Woessner 1999). Furthermore, the collagen fibres as exhibited by elongated cells were closely arranged in parallel rows with little inter-fibre spaces (Fig. 1e-f). The quantitative assessment revealed the number of collagen fibres in pre-treatment stained sections of all the three groups in the range of 69.00±0.00 to 73.25±2.04 fibres per 77.5 mm^2, irrespective of the subsequent dilatation status of the cervix (P>0.05, Table 1). In an earlier study, these values were indicated to be related to a non-dilated cervix (Khandekar 2014). For cervical dilatation, the separation of collagen fibres and imbibition of fluids were reported as the primary requirements (Shi *et al.* 1999, Uchiyama *et al*. 2005).

Out of 24 buffaloes randomly allocated to a control and two treatment groups in the present clinical trial, following successful detorsion of uterus, 12 buffaloes (control-8, hyaluronidase-1 and PgE_1-3) failed to exhibit complete cervical dilatation till 18 h post-detorsion, therefore caesarean operation was carried out for fetal delivery (Table 1).

Table 1. Collagen content (fibres per 77.5 mm^2 area) in cervical biopsy samples collected from successfully detorted uterine torsion affected buffaloes before instituting an intracervical treatment and at the time of complete cervical dilatation or at 18 h post-detorsion in case of non-dilated cervix

Group	Cervical status	Before intracervical treatment	At cervical dilatation or at 18h post-detorsion
Control	Dilated, n=0	–	–
	Non-dilated, n=8	73.25±2.04^{a}	77.6±1.89^{a}
Hyaluronidase	Dilated, n=7	69.42±2.85^{a}	35.71±2.41^{b}
	Non-dilated, n=1	69.00±0.00^{a}	70.00±0.00^{b}
PgE_1	Dilated, n=5	71.00±3.48^{a}	37.80±3.79^{b}
	Non-dilated, n=3	69.60±2.48^{a}	66.30±2.67^{a}

^{a} *vs* ^{b}P<0.05, within a row.

The histomorphology of cervical biopsy in successfully detorted uterine torsion affected buffaloes at 18 h post-detorsion in case of non-dilated cervix revealed almost identical observations as recorded in biopsy samples collected before instituting any treatment. Moreover, the histomorphological findings at 18 h post-detorsion in buffaloes in case of non-dilated cervix were similar irrespective of control or treatment group. The lamina propria consisted of compactly arranged connective tissue fibres, slightly engorged blood vessels without appreciable haemorrhages and negligible PMN cells (Fig. 2a,c,e), however, the collagen fibres were closely placed (Fig. 2b,d,f). The quantitative assessment revealed the number of collagen fibres in 18 h post-detorsion biopsy samples similar to their pre-treatment values (p>0.05, Table 1). In a previous study, similar observations for non-dilated cervix with secondary constriction were reported (Honparkhe *et al.* 2009, Khandekar 2014). The reason for failing to dilate the cervix in these animals could be reduced recovery of blood flow in median uterine artery (Jaraquemada *et al.* 2007) or the less therapeutic efficiency of intracervical PgE_1 as compared to hyaluronidase.

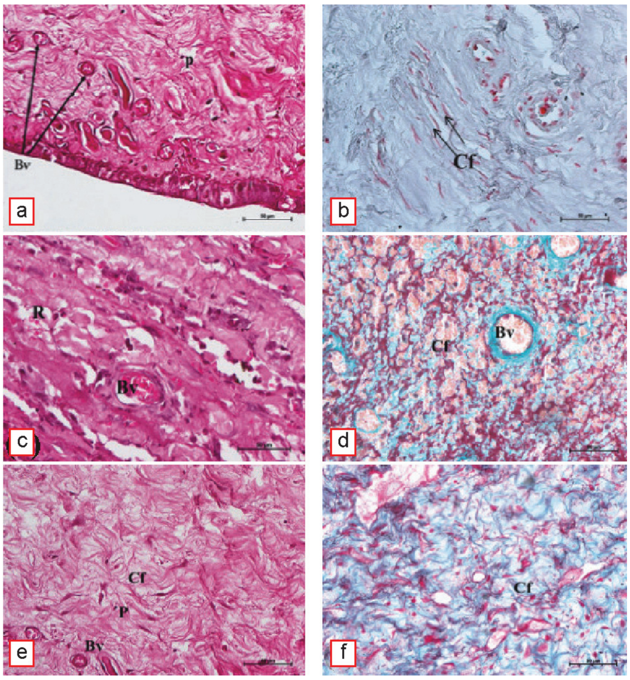

Fig. 2. Histo-morphology of cervical biopsy in successfully detorted uterine torsion affected buffaloes at 18 h post-detorsion in case of non-dilated cervix in control (a-b), hyaluronidase (c-d) and PgE_1 (e-f) group. (a, c, e): Propria submucosa with slightly engorged blood vessels (Bv), negligible hemorrhage and few polymorphonuclear cells (P) cells. H&E; (b, d, f): Propria submucosa with compactly arranged collagen fibres (Cf). MT.

Out of 24 buffaloes of the present study, following successful detorsion of uterus, 12 buffaloes (hyaluronidase-7 and PgE_1-5) were able to exhibit complete cervical dilatation within 18 h post-detorsion, thus ensuring per-vaginal fetal delivery (Table 1).

The histomorphology of cervical biopsy in successfully detorted uterine torsion affected buffaloes at the time of cervical dilatation were similar irrespective of hyaluronidase of PgE_1 treatment group (Fig. 3). The lamina propria consisted of loosely arranged collagen fibres with inter-collagen space filled by homogenous / watery substance (Fig. 3). The latter is known to promote dispersion or prevent aggregation of collagen fibrils, thus weakening the tensile strength of the cervical matrix (El-Maradny *et al.* 1997). An extensive presence of engorged blood vessels and hemorrhages along with abundant PMN cells in between the loosely arranged collagen fibres were observed as compared to pre-treatment biopsy findings (Fig. 3). The reduction in collagen fibres and increased PMN cells were observed during cervical dilation process (Junqueira *et al.* 1980, Winkler *et al.* 1999). Further, the quantitative assessment revealed the occurrence of cervical dilatation as the number of collagen fibres in post-treatment stained sections of hyaluronidase and PgE_1 group were 35.71±2.4 and 37.80±3.79 fibres per 77.5 mm^2, as compared to their

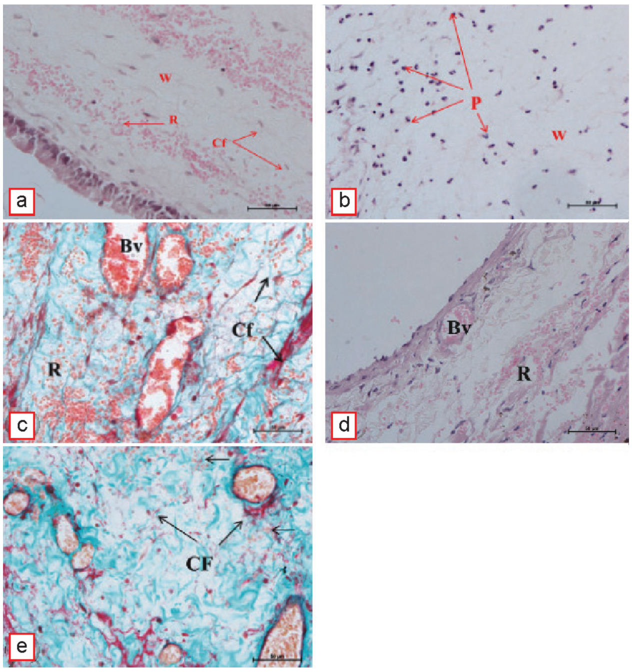

Fig. 3. Histo-morphology of cervical biopsy in successfully detorted uterine torsion affected buffaloes at the time of complete cervical dilatation in hyaluronidase (a-c) and PgE_1 (d-e) group. (a, b, d): Propria submucosa with extensive haemorrhages (R), engorged blood vessels (Bv), imbibed watery fluid (W), distantly placed collagen fibres (Cf) and abundant polymorphonuclear cells (P). H&E; (c, e): Propria submucosa with distantly placed Cf with imbibed fluids (W), extensive haemorrhages and congested Bv. MT.

respective pre-treatment values as 69.42±2.85 and 71.00±3.48, respectively ($P<0.05$, Table 1). An extensive loosening of collagen fibres in treated buffaloes could be due to action of hyaluronidase by depolymerization of conjunctive components of cervix (collagen, hyaluronic acid and chondroitin), thus reducing cellular adhesion of cervical collagen, causing softening and dilatation of the cervix (Kavanagah *et al.* 2009). In fact, hyaluronidase was suggested for promoting cervical relaxation either directly via tissue hydration by attracting water molecules or indirectly via regulation of inflammatory genes (Garfield *et al.* 1998, Dowthwaite *et al.* 1999, Uchiyama *et al.* 2005). This study exhibited a similarity in effectiveness of administration of hyaluronidase in buffaloes with the earlier reports in women (Spallicci *et al.* 2000, Kavanagh *et al.* 2009), mouse (Uchiyama *et al.* 2005), ewes (Perry 2010) and buffaloes (Malhotra 1990). Furthermore, PgE_1 acts on connective tissue stroma of cervix through disintegration and dissolution of collagen (El-Refaey *et al.* 1994). The positive attempts for induction of delivery with PgE_1 were made in women (EL-Sherbiny *et al.* 2001), goat (Alan and Tasal 2002) and cattle (Azawi *et al.* 2011).

The recording of doppler indices in the middle uterine artery (MUA) of buffaloes following detorsion of uterus signifies the recovery of blood perfusion to the uterus (Singh *et al.* 2019). In fact, the doppler indices of median uterine artery with respect to cervical dilatation status in successfully detorted uterine torsion affected buffaloes of present study suggested a differential blood supply status at 0.5 h after detorsion / intracervical treatment in buffaloes exhibiting complete cervical dilatation or failing to exhibit cervical dilatation by 18 h post-detorsion as compared to blood supply at the time of case presentation (-1 h; Table 2). At the time of case presentation, the doppler indices were similar in MUA of buffaloes which subsequently exhibited or failed to exhibit complete cervical dilatation ($P>0.05$, Table 2). However, at 0.5 h after detorsion / intracervical treatment, the doppler indices of MUA recovered substantially in buffaloes which ultimately had complete cervical dilatation ($P<0.05$, Table 2). This suggested recovery of blood flow in MUA towards the uterus and cervix in these cases and the role of improved blood flow in ensuring cervical dilatation as vascularization of cervix increases during dilation (Timmons *et al.* 2010). These findings also suggested the role of intracervical hyaluronidase enzyme or PgE_1 treatment in improving the blood supply in MUA as all the 12 buffaloes with complete cervical dilatation belonged to hyaluronidase enzyme or PgE_1 treatment group. Furthermore, increased cervical blood flow after PgE_1 treatment in female dog was

Table 2. Doppler indices of middle uterine artery with respect to cervical dilatation status in successfully detorted uterine torsion affected buffaloes (n=24) at the time of case presentation (–1 h) and at 0.5 h after detorsion / intracervical treatment

Doppler indices		Hours around detorsion / intracervical treatment			
		Complete cervical dilatation, n=12		No cervical dilatation, n=12	
		–1 h	0.5 h	–1 h	0.5 h
Ipsi MUA	TAP, cm/s	7.77±1.36	44.7±3.8^{a}	7.69±0.46	28.41±2.27^{b}
	BFV, ml/min	239±31	1602±134^{a}	255±10	408±30^{b}
	RI, Index	0.99±0.04	0.61±0.03^{a}	1.07±0.08	0.76±0.01^{b}
	PI, Index	7.8±0.71	1.49±0.2^{a}	8.5±0.28	5.9±0.3^{b}
Contra MUA	TAP, cm/s	40.81±2.72	65.78±6.41	46.14±2.33	52.51±4.89
	BFV, ml/min	1414±80	2674±174^{a}	1477±88	1979±114^{b}
	RI, Index	0.78±0.03	0.61±0.02	0.71±0.03	0.67±0.02
	PI, Index	3.18±0.35	1.13±0.07^{a}	3.43±00.3	2.28±0.22^{b}

^{a} *vs* ^{b}$P<0.05$, within a row. TAP, Time averaged peak velocity; BFV, Blood flow volume; RI, Resistive index; PI, Pulsatility index; Ipsi MUA, Ipsilateral middle uterine artery; Contra MUA, Contralateral middle uterine artery.

previously reported (Leffler and Amberson 1982).

In conclusion, intracervical hyaluronidase administration immediately after detorsion in successfully detorted uterine torsion affected buffaloes was more effective than PgE_1 to ensure complete cervical dilatation and per-vaginal fetal delivery.

SUMMARY

Twenty-four buffaloes presented between 36-72 h of occurrence of uterine torsion were successfully detorted and equally divided to intracervical hyaluronidase enzyme or prostaglandin E_1 (PgE_1) treatment or control group for investigating the treatment effectiveness for complete cervical dilatation. Intracervical treatment was administered immediately after detorsion, and cervical biopsy was collected immediately before instituting treatment and at time of cervical dilatation or at 18 h post detorsion in case of non-dilated cervix. The doppler indices of middle uterine artery were evaluated at an hour before detorsion and 0.5 h after detorsion. In control group, none of the buffaloes exhibited cervical dilatation, whereas, 87.5% buffaloes of hyaluronidase group and 62.5% of PgE_1 group exhibited cervical dilatation. Following intracervical treatment, lamina propria showed loosely arranged collagen fibres along with hemorrhages, polymorphonuclear (PMN) cells and inter-collagen space filled by homogenous/ watery substance in case of dilated cervix. In non-dilated cervix, the collagen fibres were tightly arranged with lesser number of PMN cells and negligible haemorrhages at 18 h after treatment. The doppler indices of the middle uterine artery revealed improvement ($P<0.05$) in blood supply towards cervix and uterus in buffaloes exhibiting complete cervical dilatation. In conclusion, intracervical hyaluronidase treatment in immediate post-detorsion period in uterine torsion affected buffaloes can be an effective strategy to ensure complete cervical dilatation and per-vaginal fetal delivery.

REFERENCES

Alan M and Tasal I. 2002. Efficacy of prostaglandin F2α and Misoprostol in the induction of parturition in goats. *Veterinary Record* **22**: 788–89.

Azawi O I, Naoman U T, Al-Kass Z M, Lazim E H and Fathi N G. 2011. Misoprostol treatment of dystocia due to incomplete dilatation of the cervix in a cow: A case report. *Iraqi Journal of Veterinary Sciences* **25**: 97–98.

Bollwein H, Baumgartner U and Stolla R. 2002.Transrectal Doppler sonography of uterine blood flow in cows during pregnancy. *Theriogenology* **57**: 2053–61.

Breeveld-Dwarkasing V N, Struijk P C, Lotgering F K, Eijskoot F, Kindahl H, Weijden G C and Taverne M A. 2003. Cervical dilatation related to uterine electromyographic activity and endocrinological changes during prostaglandin F (2alpha)-induced parturition in cows. *Biology of Reproduction* **68**: 536–42.

Dickey R P. 1997. Doppler ultrasound investigation of uterine and ovarian blood flow in infertility and early pregnancy. *Human Reproduction Update* **3**: 467–503.

Dowthwaite G P, Ward A C and Flanelly J. 1999. The effect of strain on hyaluronan metabolism and hyaluronan binding protein expression in embryonic fibrocartilage cells. *Matrix Biology* **18**: 523–32.

El-Maradny, Kanayama, N and Kobayashi H. 1997. The role of hyaluronic acid as a mediator and regulator of cervical ripening. *Human Reproduction* **12**: 1080–88.

Elmetwally M A A. 2012. 'Clinical applicability of non-invasive Doppler ultrasonography in small ruminants throughout pregnancy.' Thesis, University of Veterinary Medicine, Hannover, Germany.

El-Refae H, Calder L, Wheatley D N, Templeton A. 1994. Cervical priming with prostaglandin E1 analogues, misoprostol and gemeprost. *Lancet* **334**: 1207–09.

EL-Sherbiny M T, EL-Gharieb I H and Gewely H A. 2001. Vaginal misoprostol for induction of labour: 25 vs 50 microg dose regimen. *International Journal of Gynecology and Obstetrics* **72**: 3337–49.

Garfield R E, Saade G, Buhimschi C, Buhimschi I, Shi L, Shi S Q and Chwalisz K. 1998. Control and assessment of the uterus and cervix during pregnancy and labour. *Human Reproduction Update* **4**: 673–95.

Ghuman S P S. 2010. Uterine torsion in bovines: A review. *Indian Journal of Animal Sciences* **80**: 289–305.

Honparkhe M, Ghuman S P S, Kumar A, Sood N K, Gupta K and Ahuja C S. 2009. Cervical massage with sodium carboxymethyl cellulose for achieving complete cervical dilatation in successfully detorted uterine torsion affected buffaloes. *Indian Journal of Animal Sciences* **79**: 26–29.

Jaraquemada J M, Monaco G R and Barbosa N E. 2007. Lower uterine blood supply: extrauterine anastomotic system and its application in surgical devascularization techniques. *Acta Obstetricia et Gynecologica Scandinavica* **86**: 228–34.

Junqueira L C U, Zugaib M, Montes G S, Toledo O M S, Krisztan R M and Shigihara K M. 1980. Morphologic and histochemical evidence for the occurrence of collagenolysis and for the role of neutrophilic polymorphonuclear leukocytes during cervical dilation. *American Journal of Obstetrics and Gynaecology* **138**: 273–81.

Kavanagh J, Kelly A J and Thomas J, 2001. Hyaluronidase for cervical priming and induction of labour. The Cochrane Database of Systematic Reviews, The Cochrane Library.

Khandekar S. 2014. 'Studies on histo-morphological and hormonal alternations in buffaloes having incomplete cervical dilatation.' M.V.Sc. Thesis, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana.

Krueger L, Koerte J, Tsousis G, Herzog K, Flachowsky G and Bollwein H. 2009. Transrectal Doppler sonography of uterine blood flow during the first 12 weeks after parturition in healthy dairy cows. *Animal Reproduction Science* **114**: 23–31.

Leffler C W and Amberson J I. 1982. Prostaglandin E1 increases blood flow to the cervix uteri. *Prostaglandins Leukotrienes and Medicine* **8**(1): 27–30.

Luna L G. 1968. *Manual of Histologic Staining Methods of Armed Forces Institute of Pathology.* 3rd Edition. McGraw Hill Book Company, New York, USA.

Malhotra P. 1990. 'Some studies on the cervical changes in the uterine torsion cases with special reference to cervical filtration in buffaloes.' M.V.Sc. Thesis, Punjab Agriculture University, Ludhiana, India.

Nagase H and Woessner J F. 1999. Matrix metallo proteinases. *The Journal of Biological Chemistry* **274**(2): 1491–94.

Perry K, Haresign W, Wathes D C and Khalid M. 2010. Intracervical application of hyaluronan improves cervical relaxation in the ewe. *Theriogenology* **74**: 1685–90.

Sharma M and Singh V K. 1984. Cervix-hyalse alone and in combination with other drugs, *Journal of Obstetrics and Gynaecology of India* **34**: 1023–31.

Shi L, Shi S Q, Saade G R, Chwalisz K and Garfield R E. 1999. Changes in cervical resistance and collagen fluorescence during gestation in the rat. *Journal of Perinatal Medicine* **27**: 188–94.

Singh N, Ghuman S P S and Gandotra V K. 2019. Doppler indices of middle uterine artery as a tool for predicting the fetal status in cattle with uterine torsion and the recovery of uterine blood flow subsequent to detorsion. *Indian Journal of Animal Sciences* **89**(5): 491–94.

Singla V K, Roy K S, Sharma R D, Gandotra V K and Dhaliwal G S. 1989. *Histopathological studies of cervix and vagina in buffaloes suffering from uterine torsion.* Proceedings of VIIIth National symposium of Indian Society for the Study of Animal Reproduction, Anand, Gujarat, India.

Soni N and Rajput P. 2004. Comparative study between tablet Misoprostol (Prostaglandin E1) and Dinoprost gel (Prostaglandin E2) for the induction of labour. *Journal of Obstetrics and Gynaecology of India* **54**: 554–55.

Spallicci M D B, Chiea M A, Albuquerque P B, Singer J M, Bittar R E and Zugaib M. 2000. Ação da hialuron idasenamaturação do colouterinoemgestações a termo. *Revista De Ginecologia E D 'Obstetricia* **11**: 93–102.

Timmons B, Akins M and Mahendroo M. 2010. Cervical remodelling during pregnancy and parturition. *Trends in Endocrinology Metabolism* **21**(6): 353–61.

Uchiyama T, Sakuta T and Kanayama T. 2005. Regulation of hyaluronan synthases in mouse uterine cervix. *Biochemical and Biophysical Research Communications* **327**: 927–32.

Winkler M, Fischer D C, Ruck P, Marx T, Kaiserling E, Oberpichler A, Tschesche H and Rath W. 1999. Parturition at term: Parallel increases in interleukin-8 and proteinase concentrations and neutrophil count in the lower uterine segment. *Human Reproduction* **14**: 1096–1100.