Review Article

INCOMPLETE CERVICAL DILATATION IN ANIMALS – AN UPDATE

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Abstract: Cervical dilatation is a key event for successful vaginal delivery of young ones that occur just before parturition. Failure of cervical dilatation due to alterations in cervical ripening mechanism or insufficient uterine contraction poses problems in delivery of fetus. Cervical ripening is a multifactorial process which is an outcome of hormonal regulation, inflammatory process and enzymatic breakdown of collagen. Whenever there are alterations in above mechanisms, animal may be affected with cervical dilatation failure. For the management of cervical dilatation failure, a number of hormones have been used but they have shown limited success and in field conditions; caesarean section is only available best tool for management. In this article we focused on the process of cervical dilatation and management of incomplete cervical dilatation at field conditions.

Keywords: Incomplete cervical dilatation, cervical ripening, dystocia, Ring womb, Prostaglandin, hyaluronic acid.

Introduction

Incomplete cervical dilatation is an important cause of maternal dystocia among farm animal species with maximum incidence in sheep and goat (Noakes *et al*., 2001). In cattle, incomplete cervical dilatation occurs as important etiology in various forms of dystocia like uterine torsion, twin pregnancy etc. In cows, the cervix is relatively more cartilogenous than in the other farm animal species and severe dystocia can result if it is not dilated properly at the time of parturition (Sloss *et al*., 1980). In cows, out of all cases of dystocia 17% are of cervical origin (Wehrend *et al*.,2003) while in beef cattle it is 38% (Sloss *et al*.,1967). In sheep and women, cervical non dilatation is root cause for maternal type of dystocia and an indication for caeseran section. Although cervix functions as an important physical barrier to

protect the fetus during pregnancy, it has to be sufficiently dilated at the time of parturition for easy vaginal delivery. For this, cervical tissue undergoes a number of changes several days before and during the first stage of parturition. A number of factors and mechanisms are responsible to control these changes and when one or more of these changes fails to occur, animal experience cervical dilatation failure.

Cervix

The cervix is a fibrous organ composed of predominantly of connective tissue with only small amounts of smooth muscle bundles running longitudinally and transversely (Word *et al*., 2007). Cervical tissue is made up of 3 layers as that of genital tract, a mucosal layer which consist of epithelium and underlying stroma, a muscular layer which consist of an inner circular and outer longitudinal layer and a outermost serosal layer (Burkitt *et al*., 2002). Cervical stroma is made up of ground substance consist of hyaluronic acid, chondroitin 4-sulphate, chondroitin 6-sulfate, dermatan sulfate, heparin sulfate, heparan sulfate and keratin sulfate associated with proteins like collagen, elastin and reticulin (Fitzpatrick *et al*.,1977). Cellular element composed of mast cells, fibroblasts and wandering cells. Among the fibers, collagen appears to be numerous, dense closely packed, randomly oriented and embedded in high molecular weight Proteoglycans complex while elastin is present in only small amount (Ward *et al.,* 1968). There are different types of collagen fibrils within cervix of non pregnant and early pregnant cow such as type I, III or IV. Collagen fibrils strongly bind to anionic group of GAGs that act as cement or binding substances (Fitzpatrick *et al*., 1977). Functional properties of cervix depend upon the biochemical interactions of molecules present in the extracellular matrix of cervical connective tissue. This interaction is regulated by several endocrine factors that mediate dynamics of cervical remodeling (Fitzpatrick *et al*., 1977). At parturition, changes start to occur in collagen and GAGs concentration. Danforth *et al*. (1974) detected a decrease in collagen per gram of dry matter in women cervical tissue. It is also evident in various studies in sheep (Ward *et al*., 1968) and rat (Harkness *et al*., 1959), that there is a fall in collagen concentration in the last third of pregnancy but there is no decrease at parturition. In contrast to these, collagen fibrils undergo reorganization just before parturition to make the cervix easily distensible. There are also changes in GAGs concentration at parturition as they increase markedly in the cervix during late pregnancy in human, sheep, guinea pig, rabbit, and rat (Osmers *et al*., 1993).

The reorganization of fibrils is known as cervical ripening that can be divided into two stages- first is gradual cervical ripening which starts during last month of pregnancy. The

second is final cervical ripening which happen during the parturition process (Winkler *et al*., 1999b; Malmstrom *et al*., 2007). Gradual cervical ripening is utmost prerequisite for a successful and final cervical dilatation. Matrix Matello Proteinases (MMPs) secreted by stromal cells, fibroblast and smooth muscle cells plays an important role during this period (Breeveld-Dwarkasing *et al*., 2003). Inflammatory cells also form an additional source of MMPs during final ripening (Stygar *et al*., 2002). These are secreted as zymogens and become activated after proteolytic cleavage (Hulboy *et al*., 1997). Different types of MMPs are involved in gradual ripening i.e. MMPs-1(Fibroblastcollagenase), MMP-2 (Gelatinase-A), MMP-8(leukocyte collagenase), MMP-9(Gelatinase-B) (Malmstrom *et al*., 2007; Osmers *et al*., 1992). MMP-1,2 & 8 cleave fibrillar collagen types I, II, III while MMP-9 cleaves collagen types IV and V(Nagase *et al*., 1999; Hulboy *et al*.,1997). The digestion process results in to changes in collagen organization pattern from densely packed, large bundles of fibrils with little intervening extracellular matrix material (Bryant *et al*., 1968) to smaller bundles, more dispersed, and randomly oriented. However, collagen is not completely digested during gradual ripening (Breeveld-Dwarkasing *et al*., 2003). During this process, activity of MMPs is tightly regulated by some inhibiting factors like β2 macroglobulin and tissue inhibitors of Matello Proteinases (TIMPs) (Nagase *et al*., 2006).

Concomitant with the changes in collagen structure, there is also marked increase in the glycosaminoglycan (GAG) content of the cervix in human and rat (Downing and Sherwood 1986). In particular, the GAG hyaluronan (HA) increases markedly in the cervix during late pregnancy in human, sheep, guinea pig, rabbit, and rat (Anderson *et al*., 1991; Rajabi *et al*., 1992). At the onset of labor, HA is the predominant GAG in the cervix. High hyaluronan content of cervix attracts water molecules and increase hydration that ultimately lead to dispersion of collagen fibers. At the end of first stage there is increase space between collagen fibrils, collagen denaturation and hydration due to high content of hyaluronan (Breeveld-Dwarkasing *et al*., 2003; Maillot *et al*.1976). These changes bring about changes in tensile property of cervix that become more extensible (Kelly *et al*., 2005) at the time of final dilatation.

Final cervical ripening is very similar to inflammatory process and characterized by presence of proinflammatory cytokines, granulocytes such as neutrophils and eosinophil's (Tornblom *et al*., 2005; Wehrend *et al*., 2004). The placenta and extraplacental membranes (gestational tissues) has been said to be sources of a large number of cytokines, chemokines and related factors (Bowen et al., 2002a,b). Recently cDNA array studies carried out by Keelan *et al*.

(2003) in human showed cytokine expression in the amnion and choriodeciduata and identified clusters of inflammation-associated genes upregulated with labour at term and preterm. But the pattern of cytokine expression has been said to be different between term and preterm labour that most robustly occur at the time of preterm deliveries with intrauterine infection (Keelan *et al*., 2003). Role of cytokines in cervical dilatation has been described in both spontaneous and induced parturition in several small laboratory animals and in humans. Erik van Engelen *et al*. (2009) studied the cytokine expression in bovine cervical tissue at different day of pregnancy. They reported that there is an increase in the expression of IL-8, IL-1β and IL-10 at parturition at term, compared to day 185 pregnancy but the expression of TNF-α decreased while the expression of IL-6 remained unaltered.

 Both proinflammatory and antinflammatory cytokines were expressed in gestational tissue. Among the pro inflammatory cytokines are IL-1β, IL-6, TNF-α , granulocyte colony stimulating factor (G-CSF) and leukemia inhibitory factor (LIF) are found to be increased in gestational tissues during term-labour (Keelan *et al*., 2003). This enhanced expression of cytokines is mainly responsible for recruitment of large numbers of leukocytes in gestational tissues. Among the proinflammatory cytokines, IL-8 plays a very important role in cervical softening. IL-8 synthesis and secretion is greatly increased in term versus non-pregnant cervix and concentrations of IL-8 in the cervical and lower uterine segment increased with cervical ripening (Sennstrom et al., 1998; Winkler *et al*., 1998). The increase in IL-8 during cervical ripening correlates with increases in leukocyte infiltration and concentrations of MMPs in the tissue (Osmers *et al*., 1995a; Winkler *et al*., 1999a,). Moreover It has been demonstrated in various animal models that IL-8 can softening the cervix after intracervical application (Chwalisz *et al*., 1994) or (El Maraddny *et al*., 1994) vaginal administration. In addition to proinflammatory cytokines, antinflammatory cytokines IL-10 & IL-4 also involved in cervical ripening process. Although theses cytokines typically have been said to suppress LPS induced release of TNF-α and production of PGE$_2$ by gestational tissue (Sato *et al*., 2003, Bry and Hallman, 1992) but in term gestational tissues these have been reported stimulate the release of prostaglandins and inflammatory cytokines (Keelan and Mitchell, 1999). This has led to the hypothesis that term labour is associated with a withdrawal or reversal of anti-inflammatory agents as part of an evolutionary adaptation to accelerate inflammatory processes necessary for successful labour and delivery (Simpson, Keelan and Mitchell, 1998).

Etiological factors for cervical dilatation failure

Uterus associated disorders- It is well known that Myometrial contraction play an important role in dilatation of a ripened cervix (Lindgren, 1973). A weak myometrial contraction may be a factor for incomplete cervical dilatation that may be either because of primary uterine inertia or secondary uterine inertia. In primary uterine inertia, the causal factors may be hypocalcaemia, hypomagnesaemia, old age, debility, lack of exercise and preterm calving and possibly hyposelenaemia (Mee, 2004), failure of sufficient hormones secretion that control uterine contraction like estrogen, relaxin and prostaglandin or their imbalance. In secondary uterine inertia, it may be because of prolonged dystocia, malposition and twin calving (Noakes *et al*., 2009). Among the hormonal factors, it has been seen that increased progesterone activity at the time of parturition inhibited myometrial contractility and myometrial gap junction formation (Da Fonseca *et al*., 2003) and stimulated uterine inducible nitric oxide synthase (iNOS) that quiten uterus. Higher progesterone is also involved in down regulation of prostaglandin production, inhibition of development of calcium channels and Oxytocin receptors (Da Fonseca *et al*., 2003). Among the metabolic factors, fat mobilization can reduce magnesium availability and calcium mobilization leading to uterine inertia and cervical dilatation failure (Anon, 2006). Also in some cases of abortion and uterine torsion, cervix fails to dilate but exact mechanism is not known.

Failure of cervix to ripen: This is main factor responsible for cervical non-dilatation in farm animals. Exact mechanism which causes failure of cervical ripening is still unknown. Alteration in hormonal concentration including steroids, prostaglandin and relaxin at term may be a factor in poor cervical ripening. In a study in mice it is found that increased concentration of progesterone with deficiency of 5 α-reductase (Mahendroo *et al*., 2012) in the cervix, increases tissue inhibitor of matrix metalloproteinase 1 (TIMP-1) (Leppert *et al*., 1992) and hence inhibits collagenolysis. It is also found that in 5 α –reductase deficient mice, there is decrease expression of HAS and hence decrease HA and there is a failure of cervical ripening process (Kelly *et al.,* 2005). Cervical fibrosis, a condition affecting many pluriparious animals that might have cervical tear in previous parturitions will experience poor cervical ripening process at the term due to overgrowth of collagen fibers that will be poorly digested.

Clinical signs: History plays an important in diagnosing the case which is evident by non-progressing prolonged first stage labor. During natural parturition, generally cervix is not palpable per rectally because it became continuous with vagina but, in cervical non-dilatation,

it is palpable through per rectal examination (Purohit *et al*., 2011). On per vaginum examination, 1 or 2 fingure may be inserted but whole hand cannot be passed through cervix. Parts of fetus or fetal membrane can be palpated sometimes (Jackson *et al*., 2002**).**

Management of incomplete cervical dilatation

The patients are examined to ensure that signs of imminent birth including ligament relaxation and colostrum in udder are present. Manual attempts by inserting 1 or 2 fingure (or the hand with fingers pointed in conical shape) can be made to dilate the cervix but bovine cervix consist of many annular rings, it is not often possible to dilate the cervix (Purohit *et al*., 2011). If the fetus is present in the birth canal, gentle traction over long period sometime can dilate cervix. If the cervix is closed and fetus is live and fetal membranes are intact, then patients may be left for 30 minutes and reexamined. When the legs of a putrefied dead fetus are present in the birth canal and the fetus cannot come out be-cause of incompletely dilated cervix, partial cervicotomy is suggested instead of caeseran section (Purohit *et al*., 2011). In this technique cuts are made at 1 or more places in cervical rim to dilate the cervix at the point. But there is a potential risk of sever hemorrhage in ventral quadrant and uncontrolled tearing may occur as fetus pass through. To avoid this problem, caeseran section is more safe procedure in bovine obstetrics. Some clinician suggests use of potent analgesic like velethamide bromide. The effect is supposed to be mediate by its neurotropic (anticholinergic) and papaverine like action on cervical smooth muscle (Sharma *et al*., 1990).

Use of relaxin: Relaxin induces cervical dilatation by remodeling of connective tissue in several mammalian species, especially in pigs. But in ruminants' peptide homologous to the relaxin has been not found that is present in C.L, uterus and placenta of other species (Hartung *et al*., 1995). Yet several groups have suggested that there is a very similar physiology for preparation of birth canal in cattle. This evidence is supported by presence of relaxin like factor (RLF) expression that is very similar in temporal and spatial pattern of expression to other species. The effect of porcine relaxin on cervical tissue of late pregnant heifers is very suggestive that effect is mediated by presence of receptors. Relaxin injected intramuscularly causes cervical dilatation in late pregnant heifers but not in sheep. In contrast to these results intravenous infusion of relaxin in late pregnant heifers doesn't results in earlier calving ,although a decrease concentration of progesterone has been shown in these heifers. Thus the timing and method of administration known to play an important role in relaxin induced cervical remodeling in ruminants

Use of β-adrenergic agonist -Some β: adrenergic drugs have been used for cervical dilatation by virtue that they cause relaxation of entire genital tract. In this, Isoxuprine at the doses of 200-300 mg IV or 0.3 mg IV Clenbuterol have been suggested. But their results are not promising always. Moreover they also delay the parturition process.

Use of hyaluronan: It has been shown in various studies on human and animal that hyaluronan content of cervix increases at term to facilitate cervical ripening by attracting water molecules, increasing collagenase activity, decreasing collagen concentration, affecting function of PMN cells and relaxation of cervical smooth muscle. Increased hyaluronan synthase expression and the subsequent increase in HA is a distinct feature of cervical ripening and dilatation (Kelly *et al*., 2005) in mouse at the term. The function of HA is influenced by HA size and HA binding proteins. In the mouse cervix HA is predominantly high molecular weight (HMW HA) before birth and low molecular weight (LMW HA) shortly after birth (Ruscheinsky *et al*., 2008). Perry (2010) also demonstrated increase expression of 2 types of HA in ovine at estrus in cervical tissue tissue. In normal tissue, HMW HA is the predominant form and regulates cell behavior (Alaniz *et al*., 2009) While LMW HA is angiogenic (Joddar *et al*., 2006) and up regulates the expression of inflammatory genes (Turley *et al*., 2002). These include chemokines, cytokines (IL-8, IL-12 and TNF-α) and NO synthase (Haslinger *et al*., 2001). Both of these actions of LMW HA are key components for cellular remodeling. There are two main routes by which HA could promote cervical relaxation either directly via tissue hydration (Dowthwaite *et al*., 1999) by attracting water molecules or indirectly via regulation of inflammatory genes (Garfield *et al*., 1998; Uchiyama *et al*., 2005). The accumulation of HA and water in interstium between the collagen may promote dispersion or prevent aggregation of collagen fibrils, thus weakening the tensile strength of the matrix (Maradny *et al*., 1997). Perry (2010) also demonstrated intracervical application of hyaluronan improves cervical relaxation in ewes. These results show similarities with El Maradny *et al*.(1997), who studied the effect of hyaluronan on the cervix of both pregnant and non-pregnant rabbit and found that hyaluronan administration is efficient in cervical ripening. HA may also induce cervical smooth muscle relaxation through binding with CD44 receptors that known to induce important changes in actin cytoskeleton rearrangement and the reorientation of the cells, suggesting a strong link between HA, F-actin and possible cervical relaxation (Leethongdee *et al*., 2007).

Use of estradiol: As it is obvious that high estrogen is very essential for cervical ripening process. It induces collagenase activity and involved in collagen remodeling. It stimulates

Oxytocin receptor (OTR) expression in endometrium as well as in cervical epithelial cells and hence causes release of prostaglandins. It is also responsible for powerful Myometrial contraction. Thus, it can be used only in a case where cervix is partially dilated.

Prostaglandins : Role of prostaglandins in cervical softening may be suspected by virtue of that their increased levels in the uterus, cervix and fetal membranes have been observed in cattle (Fuchs *et al*., 2002) and ewes (Challis *et al*., 2000) during the time parturition. Moreover, it has been well proved that an analogue of prostaglandin, PGE2 has capacity to change in GAGs content of cervix (Kershaw *et al.,* 2009) in sheep during estrus cycle. Analogue of PG has been extensively used for induction of labor in women; mainly PGE2 and PGE1 analogue have been used. Knowing the results of PGE2 analogue in women, their use are also evaluated in animal. Local application of PGE2 were widely attempted in clinical obstetrics for induction of cervical softening (keirse *et al.,* 1993). The cervix of ewes (Stys *et al*., 1981; ledgert *et al*., 1983) & heifers (Duchens *et al*., 1993; lavoir *et al*., 1996) undergo softening after endocervical administration of PGE2. PGE2 has both proinflammatory and anti-inflammatory action (Kelly, 1996). Proinflammatory actions are involved in synergistic action with IL-8 that attracts and degranulate the invading neutrophils while anti-inflammatory action is involved in maintenance of pregnancy (Kelly, 1996). Leethongdee *et al.* (2007) investigated usefulness of misoprostol (PGE1analogue) as a cervical relaxant for ewes with 1mg of misoprostol dissolved in 0.5ml of 30% gelatin. But action seems to be ineffective because there is only intrauterine penetration at 54Hrs post treatment in cyclic ewes. But in cattle, the results are goods with misoprostol as compared to ewes. Azavi *et al.,* (2011) reported first successful use of misoprostol in a local breed of cows suffering from incomplete cervical dilatation with 1 mg (5 tablets) of misoprostol (Cytotec, Searle Pharmaceuticals Ltd, UK) inserted in partially dilated cervical canal. Use of Dinoprostone (PGE2 analogue) is good as compared to misoprostol in ewes. Rickords & White (1988) investigated usefulness of exogenous Dinoprostone with a single 10 mg dose of Dinoprostone via a lipid based vaginal suppository to anestrous ewes of various ages. About 67% ewes displayed cervical dilatation 3Hrs post treatment. Carbol *et al* (1987) have also shown that the cervix can undergoes structural changes similar to those preceding normal delivery with use of Dinoprostone in rat.

Use of combination of Denaverine hydrochloride & Carbetocin: These are newly investigated drugs that were used very firstly in Simmental breeds of cows for painless delivery (Zobel *et al*., 2014) with promising results in relaxation of birth canal. Denaverine

hydrochloride (DH) is a neurotropic –musculotropic spasmolytic agent with analgesic properties. DH has anesthetic like action on birth canal soft tissue and increases the influences of Carbetocin. Carbetocin is a uterotone drug which increases frequency of uterine contraction and strengthen them during parturition. In one study Robert *et al.(*2014) found that application of DH and CT facilitated cervical dilatation in cows. It also improve welfare by decreasing the number of animals suffering from difficult calving, decreasing the number of animals requiring episiotomy and reducing numbers with post calving birth canal lesions.

Conclusion

The cervical ripening process is poorly understood in animals. The studies available are the results of experiments carried out in either laboratory animals or in women that cannot be entirely comparable with animals. The etiological factors leading to poor cervical dilatation during parturition are not exactly known. The current therapies available are not satisfactory or their role is not fully evaluated. Till date, caesarean section is a best tool for cervical non dilatation in cattle as well as in sheep.

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