Ecbolic and tocolytic agents in bovine reproduction

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Abstract

The use and efficacy of ecbolic and tocolytic agents to treat and manage reproductive conditions in the bovine have been controversial topics at times. This is in part due to our limited understanding and lack of research with regard to the pregnant and post partum uterus in the cattle. This article reviews the anatomy and physiology of the bovine uterus and research evaluating the use and efficacy of various ecbolic and tocolytic compounds.

Keywords: Bovine uterus, ecbolic, tocolytic

Introduction

Ecbolic agents have been administered in an attempt to treat and prevent many reproductive conditions in the periparturient cow which most commonly include retained fetal membranes, post-partum endometritis and metritis. Tocolytic agents have been used to inhibit uterine contractility in a variety of clinical situations which include delaying parturition and facilitating obstetrical manipulations. To further understand the uses and possible roles of ecbolics and tocolytics in bovine practice, one must first understand the anatomy and physiology of the uterus and the pharmacology of these agents.

Anatomy and physiology of the uterus

The uterine wall is composed of three layers. The innermost layer, the endometrium, lines the lumen of the organ and consists of columnar epithelium and underlying stromal tissues. The middle layer, the myometrium, which consists predominately of smooth muscle cells, also contains blood and lymph vessels, nerves,

immune cells, and connective tissue. The outer layer, the serosa, is a thin layer which covers most of the uterus and is composed of mesothelial cells. The individual smooth muscle cells of the myometrium are the physiologic units of uterine contraction. The muscle fibers of the outer longitudinal layer are arranged parallel and those of the inner circular layer are arranged concentrically around the long axis of the uterus. Advancing gestation is accompanied with hypertrophy and hyperplasia of the uterine smooth muscle. The increase in size can be up to three- to fivefold by the end of gestation.[1,2]

Smooth muscles are innervated by the sympathetic nervous system and have α and β receptors. The α receptors are responsible for muscular contractions while the β receptors are responsible for relaxation. The β_1 receptors are confined to the heart, adipose tissue, and small intestine. Stimulation of β_1 receptors leads to increased cardiac automaticity, positive chronotropic and inotropic effects, and elevated free fatty acids. The β_2 receptors are found in the smooth muscle of the uterus (myometrium), vascular smooth muscle, and bronchioles. Stimulation of β_2 receptors causes relaxation of the uterus, vasodilation, and bronchodilation. Activation of these β_2 receptors leads to an elevation of in cAMP, mediated through adenylate cyclase. These increased levels of cAMP prevent myosin light-chain kinase (MLCK) activity through both decreased phosphorylation and inhibition of release of stored intracellular calcium thus inhibiting uterine contraction.[1,2]

Uterine contractility

The contractile activity of the uterus is directly related to the electrical activity in the smooth muscle cells. This activity is characterized by cyclic depolarization and repolarization of the plasma membrane and action potentials. Contraction of the smooth

muscle cells occurs by the interaction of the myofilaments which are composed of myosin and actin. The sarcoplasmic reticulum also plays a key role as it is the site of calcium storage. In the resting stage, the intracellular calcium in the smooth muscle is low. Contraction of the smooth muscle is preceded by an increase in free intracellular calcium levels. Calcium ions bind with calmodulin which then activates myosin kinase. Myosin kinase in turns phosphorylates a myosin head. The phosphorylated myosin head then binds with an actin filament, thus inducing smooth muscle contraction.[2]

During pregnancy, the uterine smooth muscle is relatively quiescent, displaying weak, localized, and poorly coordinated contractions. In contrast, during parturition, the contractions are forceful, sustained, regular, and well-synchronized. Factors responsible for initiating the process of parturition include 1) an increase in the number of gap junctions which allows cell coupling and interactions, 2) decreased production of nitric oxide thereby inhibiting uterine relaxation, and 3) stretch of the myometrium which enhances contractility.[2]

Ecbolic agents (oxytocics)

Ecbolic agents or oxytocics are compounds that hasten uterine evacuation by stimulating uterine contractions of the myometrium. The best known and most widely used ecbolics are oxytocin and prostaglandins (PG). Ecbolics have been used for the treatment of retained placentas and for the treatment and prevention of metritis.

Oxytocin

In 1906, Sir Henry Dale reported that an extract of the neurohypophysis had oxytocic effects. The use of this extract to induce labor was first reported as early as 1911. The name given to this compound was oxytocin, derived from the Greek word

meaning "swift birth." Oxytocin is a neuropeptide hormone produced by magnocellular cells in the hypothalamus, transported to the posterior pituitary via axons, and stored and secreted by the posterior pituitary. Another major function of oxytocin in mammals is stimulation of milk letdown from the mammary glands. In the 1950s Du Vigneaud and others established the structure and synthesized oxytocin. Oxytocin was the first peptide to be synthesized.[3-5]

Oxytocin is a potent stimulus for uterine contractions. The action of oxytocin is mediated by binding to specific oxytocin receptors present in the uterus when the uterus is under the influence of estrogen. Thus oxytocin is considered to be effective in causing uterine contraction and uterine evacuation if administered within 48 to 72 hours postpartum. The binding of oxytocin to its receptors causes an increase in intracellular calcium which results in contraction of myometrial cells from the uterus. Oxytocin has three specific actions on the uterus which include 1) induction of myometrial contractions, 2) release of $PGF_{2\alpha}$ from the endometrium, and 3) release of PGE_2 from cervical mucosa. Most formulations of oxytocin contain 20 United States Pharmacopeia (USP) units/mL with package inserts recommending up to 100 USP units. This dosage recommendation seems high considering that 1.0 IU of oxytocin achieves physiologic levels comparable to those seen during milking. Thus, an oxytocin dosage of 10 IU is a supra-physiologic dosage.[6]

Prostaglandins

Prostaglandins were discovered in the early 1930s as substances present in human seminal plasma that induced, and sometimes relaxed, uterine muscle activity *in vitro*. Von Euler named these substances prostaglandins believing that they came from the

prostate gland. Although, it was later discovered that the seminal vesicles were the major source of prostaglandins; thus, prostaglandins is really a misnomer. In 1957, Bergstrom and Sjovall reported the first isolation of prostaglandins and determined their structures. By the 1960s, Sammuelsson and colleagues began describing the prostaglandin metabolic pathways. In 1971, Vane reported that aspirin inhibited PG production and later showed that non-steroidal anti-inflammatory drugs acted via PG inhibition. Bergstrom, Sammuelsson, and Vane were awarded the Nobel Prize in 1982 for their work with PGs.[5]

When biologically active PGs are released into the blood they are metabolized by enzymes in the liver, kidney, and particularly the lung. After one passage of $PGF_{2\alpha}$ through the lungs, over 90% of the PG is metabolized. This is one reason why most PGs have very short half-lives in blood (<1 min). In addition to the lung, the placenta also has very high concentrations of PG dehydrogenase. Thus, it is very unlikely the biologically active PGs can cross the placenta and affect the fetus. Prostaglandins are capable of causing muscle relaxation and muscle contraction depending upon the branch of the receptors (relaxant or stimulatory receptors). The relaxant receptors act via adenylate cyclase to elevate levels of intracellular cyclic adenosine monophosphate (cAMP) and phosphokinase A (PKA) activity. This in turns relaxes smooth muscle via elevated cAMP which induces sequestration of intracellular calcium. The stimulant receptors act via enhancing intracellular phosphokinase C (PKC) activity and intracellular calcium. The activity through this receptor stimulates smooth muscle contraction by elevating intracellular calcium levels. This discussion will focus on the stimulatory effects of PGs, specifically $PGF_{2\alpha}$, on the uterus.[5,6]

During the immediate postpartum period, serum concentrations of $PGF_{2\alpha}$ and its metabolites are elevated. These elevations are thought to facilitate uterine involution. Prostaglandin $F_{2\alpha}$ has many actions, including luteolysis, stimulation of myometrium, and constriction of blood vessels. In addition, PGs also have a relaxant effect on the cervix. Thus, $PGF_{2\alpha}$ is used to treat a variety of medical conditions. These conditions include 1) induction of parturition, 2) lysis of corpus luteum (CL) for cases of pyometra, 3) lysis of CL for management of the estrous cycle, 4) induction of abortion, and 5) evacuation of the uterus in cases of metritis or endometritis. Prostaglandins can be used to induce parturition and abortion by its uterotonic effect and by its luteolytic effect. Prostaglandins used in veterinary medicine include cloprostenol sodium, dinoprost tromethamine, fenprostalene, fluprostinol sodium, alfaprostol, and luprostiol.[6]

*Cloprostenol sodium***.** Cloprostenol (Estrumate®, Intervet/Schering-Plough Animal Health Corp., Summit, NJ, USA) is a powerful luteolytic agent and causes rapid regression of the CL and arrests its secretory activity. Cloprostenol is available in a concentration of 250 mcg/mL and is a PG analog that is administered by intramuscular injection for all indications in both beef and dairy cattle. Cloprostenol is used in beef or dairy cattle to induce luteolysis. It is recommended by the manufacturer for unobserved or undetected estrus in cows cycling normally, pyometra or chronic endometritis, expulsion of mummified fetus, luteal cysts, induced abortions after mismating and to schedule estrus and ovulation for controlled breeding.[7]

*Dinoprost tromethamine***.** Dinoprost tromethamine (Lutalyse®, Pfizer Animal Health, New York, NY) is the naturally-occurring $PGF_{2\alpha}$ as the tromethamine salt. Each mL contains 5mg of dinoprost and is luteolytic in cattle at 25 mg (5 mL) administered

intramuscularly. Dinoprost tromethamine is labeled for estrus synchronization, treatment of unobserved (silent) estrus and pyometra (chronic endometritis) in cattle; and for abortion of feedlot and other non-lactating cattle.[7]

Other PGs. Alfaprostol and luprostiol are prostaglandin analogs that are used mainly outside of the United States. These drugs have similar effects and uses as the other synthetic prostaglandins.

Xylazine

Xylazine produces uterine contraction by stimulation of α_2-adrenergic receptors in the uterus. The pregnant bovine uterus appears to be more sensitive to xylazine-induced contractility than the non-pregnant uterus, particularly after 270 days of gestation. This enhanced susceptibility may involve hormone-related changes in α-adrenergic receptor populations in myometrial tissue. In one study, administration of xylazine (10mg intravenously) significantly increased uterine motility during late gestation. Thus, the use of xylazine in the last month of gestation is contraindicated in cattle because of the increased tendency for induction of premature parturition. Xylazine has no practical uses as an ecbolic due to its sedative and muscle relaxant properties.[6,7]

Estrogen

Estrogen has been used in an attempt to initiate and/or strengthen myometrial contractions. However, the use of estrogen is controversial. Because estrogen levels normally decrease dramatically once the calf is expelled, it appears that normal uterine involution can proceed without the influence of estrogen in the normal cow. Studies have shown no beneficial effects on the prevention of metritis or reproductive performance, and that the use of estrogen may actually have a negative effect on subsequent fertility.[8]

It is believed that contractions induced by estrogen may force septic uterine contents not only through the cervix but also into the uterine tubes which results in severe bilateral salpingitis.[9] Research has shown that estrogen treatment postpartum has a negative impact on uterine motility where the normal uterine contractions changed to a sustained contraction or spasm.[8] In addition, one study demonstrated that the use of oxytocin in an estrogen-primed uterus did not increase the contraction frequency and thus did not enhance the myometrial effect of oxytocin. Thus, scientific evidence does not support the use of exogenous estrogens in the postpartum cow.

Use of ecbolics to treat retained fetal membranes and uterine infections

Two common problems that are encountered in periparturient dairy cattle and occasionally in beef cattle are retention of fetal membranes and metritis. Retained fetal membranes is one of the most important factors that predisposes cattle to uterine infections. Cattle that have retained fetal membranes are six times more likely to develop a uterine infection than are cows without retained fetal membranes. Primary retention of the fetal membranes results from lack of detachment from the maternal caruncles, whereas secondary retention is related to mechanical difficulty in expelling the already detached fetal membranes. Greater than three-fourths of cows expel the placenta by 6 hours post-partum with the majority of the remaining cows expelling the placenta before 12 hours postpartum. Because the incidence of retained fetal membranes and postpartum disease varies with parity, the definition of retained fetal membranes may also be age- or parity-dependent. Suggestions have been made to define retained fetal membranes from 8 to 48 hours; however, 12 hours is widely used to define retained fetal membranes.[9]

Detachment of the placenta in the cow involves separation of the cotyledon villi from the caruncular crypts without tearing of either fetal or maternal epithelia. For appropriate separation of the cotyledon villi from the caruncular crypts, proteolytic enzymes (collagenases) act to open the cotyledon thereby releasing the caruncle. Collagenase activity of cotyledon villi during delivery is increased in healthy cows and decreased in cows with retained fetal membranes. The cellular sources of collagenase and proteolytic enzymes responsible for placental release in the cow are unknown. However in laboratory animals and humans, myometrial cells, fibroblasts, and leukocytes have been identified as sources of collagenase in the uterus. By day 6 postpartum, the caruncle is disorganized; by day 15, caruncles are completely sloughed as a result of necrosis. Retained fetal membranes are detached by caruncle necrosis within 6 to 10 days and not later than 17 days postpartum. The surface of the endometrium is covered by new epithelium by day 26 to 30 postpartum. After placental detachment, uterine involution is completed in about 39 days in normal cows and 50 days in cows with retained fetal membranes. Lack of uterine motility is not considered a reason for primary retention because uterine motility is normal or above normal in cows with retained fetal membranes.[9]

Factors reportedly contributing to the development of retained fetal membranes include periparturient hypocalcemia, dystocia, abortion, twinning, stillbirth, and induction of parturition. There are several management approaches that have been used for cows with retained fetal membranes of which many are controversial and lack scientific evidence to support their use. These options include no intervention, manual removal of the fetal membranes, antimicrobial therapy, and hormone therapy. No

intervention in an otherwise healthy cow is a common practice that allows the fetal membranes to liquefy and necrose until they are passed. These cows should be monitored closely for any signs of septicemia or toxemia in which case systemic therapy is necessary. Manual removal of the fetal membranes was once practiced but has fallen from favor due to the likelihood of causing trauma to the uterine wall, the high incidence of leaving tags of the fetal membranes within the uterus, and iatrogenic contamination of the uterus. Intrauterine antibiotic therapy is beyond the scope of this discussion but will be discussed in a subsequent presentation. Hormone therapy is still one of the most common methods for managing cows with retained fetal membranes.[6,9,10]

The majority of cattle experience bacterial contamination of the uterus at the time of parturition. In the normal cow, the uterus is cleared of this bacterial contamination by four weeks postpartum. When these bacteria are not cleared by the cow's defense mechanisms, a uterine infection ensues. Numerous bacteria have been isolated from the cow's postpartum uterus, some of which may be incidental and not cause problems. Uterine infections are most commonly due to *Arcanobacterium pyogenes*. The gram negative anaerobes *Fusobacterium necrophorum* and *Bacteroides melaninogenicus* are frequently associated with *A. pyogenes*. Other organisms that may be associated with uterine disease in the cow include *Pseudomonas aeruginosa*, staphylococci, hemolytic streptococci, coliforms, etc. *Clostridium* sp. may occasionally infect the uterus and cause a severe gangrenous metritis or tetanus. Uterine infections are associated with retained fetal membranes, dystocia, and delivery of twins. Metritis is the result of severe inflammation involving all layers of the uterus – endometrial mucosa and submucosa, muscularis, and serosa. Metritis usually develops during the first week after calving and

is associated with dystocia, retained fetal membranes, and calving trauma. Affected cattle may be septic and present with fever, depression, and anorexia and a copious fetid vaginal discharge may also be present. Endometritis is characterized by inflammation of the endometrium extending no deeper than the stratum spongiosum. Cows with endometritis are usually not systemically ill, and bacteria are usually eliminated after a few estrous cycles. Pyometra is a collection of purulent exudate within the uterus with the persistence of the corpus luteum, and suspension of the estrous cycle. Pyometra usually develops in cows that have their first postpartum ovulation before bacterial contamination of the uterus has been eliminated. The corpus luteum that is associated with the infection persists because intrauterine fluid prevents luteolysis. Thus progesterone persists and suppressed uterine defense mechanisms.[9,10]

Several different hormones have been used in an attempt to manage retained fetal membranes and uterine infections with the ecbolic agents oxytocin and PG being the most common. Oxytocin appears to stimulate myometrial contraction by 1) direct activation of receptors on myometrial cells and 2) indirect stimulation of contraction through the release of stimulatory PGs from the endometrium. Circulating oxytocin binds to myometrial receptors which leads to rapid uterine contraction and an increase in $PGF_{2\alpha}$ levels. It is believed that $PGF_{2\alpha}$ stimulates the release of more oxytocin and also enhances the sensitivity of the myometrium to oxytocin.[3] As little as 2.5 IU of oxytocin intravenously will cause the proximal ends of the uterine horns to respond within 30 to 50 seconds when progesterone levels are low in a cycling cow, and this increase in myometrial activity persists for up to 80 minutes.[11] Studies such as this in cycling cows have supported the idea that the myometrium is only responsive to oxytocin when

estrogen is dominant; whether oxytocin is effective in cows with toxic metritis is unclear.[6] One study indicated that as little as 5 units of oxytocin intravenously can initiate a more intense rhythm of contraction in cows with retained fetal membranes.[12] Other studies refute this evidence and suggest that oxytocin was of no benefit to postpartum cows with retained fetal membranes; however, in these two studies a dose of 60 to 100 units of oxytocin was administered which causes a spasm of the uterus versus a progressive contraction.[13-15] It also appears that the traditional dose of oxytocin (40 units) when administered intravenously causes an initial tetanic spasm of the uterus.[16] Most of the studies demonstrating the positive effect of exogenous oxytocin have used the intravenous route of administration instead of the more commonly used intramuscular route of administration. However, one study did show that the myometrial response following administration of 20 to 30 units of oxytocin was similar following administration via intravenous, intramuscular, and subcutaneous routes of administration.[16] A day two to three protocol of repeated 20 unit (1.0 mL) oxytocin injections administered at least three hours apart or three doses evenly spaced between milkings, etc has been suggested.[6] Although the frequent administration with low dose oxytocin appears to be impractical in most situations, it would appear to induce a more physiologic response than current therapeutic protocols which use infrequent administration at supra-physiologic dosages which induce tetanic uterine spasms. The most physiologic uterotonic dose of oxytocin has not been determined.

Despite much research, the ability of exogenous PGs to have a direct effect on periparturient uterine activity in cattle has been a controversial issue among researchers and clinicians. Although a few studies indicate that $PGF_{2\alpha}$ may reduce the incidence of

retained fetal membranes, subsequent studies have failed to confirm these results and many report that exogenous PG has no effect. Many of these studies lack sufficient numbers of animals, lack control animals, and used concurrent medications which make interpretation of the results difficult. It appears that suboptimal uterine contraction is rarely the cause of retained fetal membranes in a nontoxic cow. Studies have shown that the presence of retained fetal membranes alone doubles the rate and increases the frequency of uterine contractions. In another study, cows that had evidence of uterine infection (fetid, sanguine-purulent lochia) at up to day 15 post partum had significantly higher concentrations of PGF metabolite (PGFM) than did cows that had a mucopurulent to purulent lochia.[17] In addition, studies indicate that a single intramuscular injection of PG before the formation of a functional corpus luteum will have no beneficial effect in the post-partum cow.[12,16] Even when the PG dose was doubled, there was still no increase in uterine tone. However, luteolytic doses of $PGF_{2\alpha}$ (25 mg) administered by rapid intravenous injection on day 2 postpartum did cause an increase in uterine contractions.[16] However, by day four postpartum the stimulatory effect was noticeably decreased. Intravenous administration of PG also has significant side effects (dyspnea, salivation, milk ejection, frequent urination) that make it impractical to use particularly in a toxic cow. Only when luteal tissue is present on the ovary is it widely accepted that exogenous administration of PG has a beneficial effect on the postpartum cow. Intramuscular injection of $PGF_{2\alpha}$ may not be uterotonic because the $PGF_{2\alpha}$ is metabolized almost entirely into PGFM upon a single passage through the lungs. Using PG to lyse the CL allows for removal of the immunosuppressive effects of luteal progesterone which may aid in the resolution of chronic postpartum endometritis.[6] Currently, there is no

scientific evidence that intramuscular or subcutaneous injections of either natural or synthetic $PGF_{2\alpha}$ aids in the expulsion of retained fetal membranes. In addition, administration of PG during the immediate postpartum period has not been shown to have an effect on the rate of uterine involution.[18]

Some studies indicate that the use of PG may improve overall reproductive performance in cows that are not affected by periparturient diseases. In addition, cows affected with dystocia, retained fetal membranes, or both that were treated with $PGF_{2\alpha}$ early post partum followed by a second treatment 14 days later experienced a higher conception rates to first service than non-treated cows.[19]

Conclusion

Although more research is needed on the postpartum cow uterus, there is no proven scientific evidence that supports the routine use of ecbolic agents as a treatment for the pathologic postpartum uterus. Based on scientific research, exogenous estrogen and PG at published doses appear unable to stimulate the appropriate rhythmic contractions necessary to empty the pathologic post partum uterus. There is some evidence that supports the use of exogenous oxytocin to stimulate uterine contractions that are similar to those contractions observed during stage II of labor. However, these studies used intravenous oxytocin rather than the more common intramuscular route of administration.

Tocolysis

Tocolysis is derived from Greek with “tokos” meaning childbirth and “lysis” meaning capable of dissolving. A tocolytic agent is a compound that is capable of inhibiting uterine contractions. Tocolytics were originally designed for use in human

medicine to interrupt premature labor and have, over time, been used more commonly in veterinary medicine. The use of tocolytics to inhibit uterine contraction has a number of potential clinical applications in cattle. Delaying parturition for controlled calving may be useful if parturition were occurring at a time that decreased fetal survival (nocturnal delivery). Obstetrical manipulations such as correction of malpresentation and malposition, repulsion and rotation of the fetus, correction of uterine torsion, ease of extraperitoneal lifting of uterus during cesarean section, and replacement of uterine torsion may be aided by the use of tocolytic agents. Some believe that these drugs may also be useful in the area of embryo transfer.

Tocolytic Agents

A variety of tocolytics have been used for the aforementioned applications to cause uterine relaxation. Ethyl alcohol, magnesium sulfate, progesterone, prostaglandin synthetase inhibitors, calcium channel blockers, epinephrine, and β sympathomimetics have all been used to induce uterine quiescence. However, the unpredictable efficacy and adverse side effects make some of these drugs less acceptable than others for the induction of uterine relaxation.[20]

Ethanol

In the mid 20th century, ethanol was a commonly used tocolytic agent in human patients to halt pre-term labor. It is believed to effectively inhibit the secretion of oxytocin and interfere with prostaglandin synthesis. Ethanol was given intravenously in humans at a rate to maintain a blood alcohol level of 0.9 to 1.6 mg/liter. Of course, side effects were observed which included nausea, vomiting, depression, intoxication of

mother and fetus, and acidosis. Research has since demonstrated that ethanol is not effective in delaying parturition.[21]

Magnesium sulfate

Magnesium sulfate has also been used as a tocolytic. Magnesium sulfate is a central nervous depressant which blocks neuromuscular transmission and lowers acetylcholine. In 1959, the tocolytic properties of magnesium sulfate were first described. The exact mechanism by which magnesium sulfate exerts its tocolytic effects is unknown. However, one possible mechanism may be its ability to block nerve transmission and/or by its actions as a calcium antagonist. Magnesium sulfate has been given intravenously as a 10% solution to delay parturition for 24 to 48 hours. Most human studies do not indicate a significant ability for magnesium sulfate to prolong pregnancy. The use of magnesium sulfate as a tocolytic is no longer recommended due to its lack of effect at preventing preterm deliveries and because of its association with a higher risk of perinatal death.[22]

Progesterone

The actions of progesterone on the pregnant myometrium include relaxation of myometrial smooth muscle, blocking the action of oxytocin, and inhibition of gap junctions. Progesterone decreases the concentration of myometrial oxytocin receptors which counteracts the effect of estrogens. Progesterone also inhibits PG production by the placenta. In human research, progesterone has been found useful for the maintenance of tocolysis to increase gestational age at delivery or as a preventative agent in women with high-risk pregnancies.[23] Progesterone has been shown to enhance the tocolytic effect of some of the beta sympathomimetics (ritodrine) when used in human patients.[24]

Progesterone prolongs gestation length when administered during advanced pregnancy and, as a result, chances of dystocia increase due to additional weight gain of the fetus.

Prostaglandin synthetase inhibitors

Prostaglandins are known to be important mediators in uterine contractility. At the time of parturition, there are increased concentrations of arachidonic acid, PG E_2 and $PGF_{2\alpha}$. Prostaglandins increase intracellular free calcium levels which may increase the frequency of uterine contractions. Thus, nonsteroidal anti-inflammatory drugs that inhibit PG synthesis in the uterus have been considered for use as tocolytics. Indomethacin, a product used in human medicine, has been studied, although with a small sample size, in human patients and was found to delay parturition for 48 hours. Indomethacin is relatively safe as far as the maternal side effects, but crosses the placenta and causes concerns regarding fetal pulmonary hypertension, gastrointestinal inflammation, and hemorrhage.[22] Flunixin meglumine (Banamine®, Intervet/Schering-Plough Animal Health), has been considered for use as a tocolytic agent in cattle based on its ability to block PG synthesis. Although the effects of flunixin meglumine have not been evaluated in a controlled study, the label actually warns against use of flunixin meglumine in late gestation as it is "known to have the potential to delay parturition through a tocolytic effect".[7] One study evaluated the effect of flunixin meglumine on uterine contractility on a small group of postpartum cows and concluded that flunixin meglumine inhibited PG production by more than 80% and decreased spontaneous uterine motility.[25] The effects of flunixin meglumine on uterine contractility have not been evaluated in the pregnant cow.

Calcium channel blockers

Calcium channel blockers have been used for their tocolytic effects in human medicine since the 1980's. Calcium channel blockers work to inhibit uterine contraction by blocking the influx of calcium into the cells of the myometrium through disruption of the voltage-operated calcium channels.[22] Some studies indicate that nifedipine is as effective as magnesium sulfate and beta agonists with fewer side effects. Limited research has shown that the calcium channel blocker, nifedipine, is capable of blocking xylazine-induced uterine contractions in goats. One study found that nifedipine at 80 mcg/kg given intravenously was able to delay parturition in sheep for six to seven hours.[26] Side effects associated with the use of calcium channel blockers for tocolysis include fluid retention and decreased cardiac output which can result in pulmonary edema.[22]

Epinephrine

Epinephrine is an adrenergic that has both α and β activity; therefore, it is capable of relaxing smooth muscle. The use of epinephrine in veterinary medicine has been primarily limited to emergency situations to treat anaphylactic shock or cardiac resuscitation and because of its vasoconstrictive properties as an additive to local anesthetics to decrease absorption and prolong effect.[7] However, epinephrine has also been administered to cattle at 10 cc per cow of the 1:1000 solution as a slow intravenous infusion to cause uterine relaxation and quiescence.[6,20] This use of epinephrine in cattle has been primarily to facilitate obstetrical procedures and conditions such as cesarean section, fetotomy, uterine prolapse, and uterine torsion. Uterine relaxation is almost immediate following intravenous administration. Side effects which may be seen with

rapid administration and overdose of epinephrine include severe increases in blood pressure, cardiac arrhythmias, pulmonary edema, and dyspnea.[20]

β sympathomimetics

As previously mentioned stimulation of β_1 receptors leads to increased cardiac automaticity, positive chronotropic and inotropic effects, and elevated free fatty acids while stimulation of β_2 receptors causes relaxation of the uterus, vasodilation, and bronchodilation. Although β sympathomimetic tocolytics may be β_2 selective, they do retain some β_1 activity which accounts for the side effects that may be observed.[22] An ideal β tocolytic would be completely β_2 selective. However, no such drug exists. Therefore, the benefit of uterine relaxation is often accompanied by side effects attributable to β_1 activity. Maternal side effects with β sympathomimetics are associated with cardiovascular complications which include tachycardia, arrhythmias, and ischemia. In humans, the most common complication is pulmonary edema which occurs in approximately 5% of patients.[22]

Clenbuterol and isoxsuprine have been widely used to induce uterine relaxation. Isoxsuprine was the first beta sympathomimetic used for tocolysis. One study demonstrated that isoxsuprine was able to induce tocolysis within 10 to 15 minutes of administration with a duration of one to 1.5 hours.[27] However, the lack of discrimination between β_1 and β_2 receptors and the resulting tachycardia have limited its use. Clenbuterol is a specific β_2-adrenergic agonist and thus has fewer side effects on extrauterine tissues than does isoxsuprine. Clenbuterol also has the longest duration of action (eight to 10 hours) of any of the β sympathomimetics.[28] Terbutaline is another specific β_2-adrenergic agonist that has been used as a tocolytic in humans. Limited

research has demonstrated the tocolytic effects of terbutaline in rats, sheep, and buffalo, and preliminary pharmacokinetic data suggests that it may be a useful tocolytic in cattle.[29] Terbutaline shares many of the side effects associated with other beta sympathomimetics which include tachycardia, cardiac arrhythmias, muscle fasciculations, hypotension, and hyperglycemia of both dam and fetus.[29] Although the beta sympathomimetic tocolytics do have some side effects, these side effects are considered to be a minor concern, particularly when using the more specific β_2-adrenergic agonist. Due to the more predictable efficacy and minor side effects, the beta sympathomimetics are generally considered to the best choice for tocolysis.[20]

However, it is important to remember that none of these drugs are approved for use in food animals in the United States. High doses of clenbuterol have been used as repartitioning agents to promote protein deposition while lowering fat deposition which improved carcass composition. This use of clenbuterol has been associated with acute poisonings in humans who consumed meat from clenbuterol-fed animals. In 1990 in Spain, 135 people had to be hospitalized after consuming tainted veal and liver. In 1994, another 140 people suffered from dizziness, heart palpitations, breathing difficulty, tremors, and headaches. In addition, clenbuterol was banned in the United States, Europe, and Canada in 1997 due to reports of aplastic anemia due to human intoxication with clenbuterol subsequent to its use as a repartitioning agent in cattle.[30] ***The use of clenbuterol in food-producing animals remains illegal in the United States.*** However, abuse of clenbuterol in show cattle in United States has been reported.[30]

Use of tocolytic agents

Tocolytic agents have been used historically to treat or to assist in the treatment of numerous conditions in cattle. These uses include 1) threatened abortion/preterm labor, 2) controlled calving/nocturnal delivery, 3) reduction in neonatal morbidity and mortality associated with dystocia, 4) aid in obstetrical operations such as cesarean section and fetotomy, 5) treatment of uterine prolapse, 6) treatment of uterine torsion, and 7) embryo technologies. Research on the use of tocolytics in ruminants has revealed several factors that may affect tocolysis. The parity of animal may be important to consider as heifers were found to respond faster to clenbuterol and have a longer duration of tocolysis when compared to cows.[31] The amount of cervical dilation and the position of the fetus may also have an affect on tocolyis. In animals where the cervix was fully dilated or fetal feet were found to be passing into cervical area, clenbuterol was only able to delay labor for a maximum of a few hours.[32] Another study evaluated the use of clenbuterol for postponing parturition at various stages in cows. This study found that cows treated during the second stage of labor postponed calving by two hours. In addition, cows treated during the first stage of labor calved 5.2 to 9.7 hours later than control animals.[33] Another study evaluated the use of clenbuterol in beef heifers. This study found that administration of clenbuterol during the first stage of labor (cervical dilation of 5 cm) was able to delay parturition by increasing the length of the first stage of labor with no adverse effects on the fetus or the dam.[28] One report suggests that the use of clenbuterol will reduce neonatal morbidity and mortality in dystocia, will aid in obstetrical operations such as cesarean section and fetotomy. The authors of this study also reported less requirement of epidural anesthesia when clenbuterol was administered versus controls, easier correction of malpresentation and malposition, correction of uterine torsion and

uterine prolapse, and no increase in the incidence of retained fetal membranes in bovine dytocias.[34] Another study evaluated the use of clenbuterol in seventeen cows undergoing cesarean section and concluded that the use of clenbuterol in these animals resulted in decreased uterine tone to the uterus which allowed for easier exteriorization and suturing of the uterus.[35,36]

Conflicting reports exist as to the usefulness of tocolytics with regard to embryo technologies. Some have speculated that relaxation of the uterus would improve embryo recovery when used in donor animals and increase pregnancy rates in the recipient animals. However, research and subjective evaluation have not shown any significant improvement in recovery rates of embryos or pregnancy rates in recipients.

Conclusions

It is important to remember that the much of the research on the efficacy and side effects of tocolytic drugs have been conducted in the field of human medicine. There are limited studies in ruminants which are burdened by the lack of critical evaluation and subjective interpretation of the studies' results. Among the β-sympathomimetic agents used in reproduction, only clenbuterol and isoxsuprine have been widely used in clinical management of obstetrical disorders apart from embryo biotechnology with encouraging therapeutic results. The efficacy of these drugs is mostly assessed clinically. However successful the beta sympathomimetics may be as tocolytics, their use is still extra-label and the use of clenbuterol is illegal. Thus more research regarding new tocolytic agents, efficacy, and the pharmacokinetics of these drugs needs to be studied in detail to ensure wide use with awareness of adverse effects of drug metabolites, if any.

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