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Prostaglandins *vis-à-vis* bovine embryonic mortality: a review

Jerome A*, Srivastava N

Division of Animal Reproduction, Indian Veterinary Research Institute, Izatnagar, 243122 UP, India

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ABSTRACT

Decline in fertility in bovines is attributed to various reproductive problems viz. anoestrus, repeat breeding, abortions and post parturient disorders. Among these, repeat breeding has been an important cause for reducing the animals' fertility and life-time productivity. Many researchers have reported embryonic mortality as a major cause of repeat breeding arising due to premature corpus luteum lysis. Prostaglandin $F_{2\alpha}$ released from the uterus causes alterations in luteal blood flow, induces luteal lysis, and hence reduces progesterone secretion from the bovine corpus luteum. Therefore various strategies have been tried to modulate prostaglandin $F_{2\alpha}$ synthesis and secretion in order to prolong the lifespan of CL. Administration of cyclooxygenase inhibitors which include non-steroidal anti-inflammatory drugs acting by competitive inhibition of key enzymes of prostaglandin synthesis is one such method. Feeding of diet rich in polyunsaturated fatty acids during critical period significantly reduces prostaglandin synthesis. Other drugs, which are potential candidates for reducing prostaglandin synthesis, include oxytocin receptor antagonist, recombinant bovine somatotropin, lysophosphatidic acid and prostaglandin F synthase inhibitors. To conclude, there is much scope of using various compounds to reduce prostaglandins synthesis during the critical period of pregnancy for improving the embryo survival rate.

1. Introduction

Poor reproductive efficiency in bovines is usually contributed by poor heat detection, anestrus, low conception rates and repeat breeding. Moreover, increase in milk production has been associated with decrease in fertility in bovines[1]. Repeat breeding is an important cause of fertility decline contributed by fertilization failure and embryonic mortality. Embryonic mortality accounts for the majority of reproductive failure in the cattle and buffalo as up to 40% of embryos are lost in cattle[2]. The majority of embryonic mortality occurs between day 8 and day 16 of pregnancy, the critical period, causing repeat breeding without affecting cycle length[2]. For a successful pregnancy, conceptus must signal its presence to maternal system and block the regression of corpus luteum (CL) in order to maintain luteal progesterone (P_4) production following successful mating and fertilization. This phenomenon was described as the maternal recognition of pregnancy (MRP)[3] which is essential for maintenance of CL and establishment of pregnancy in all farm animals. In cow, maternal recognition of pregnancy occurs between day 16 and day 19 of pregnancy facilitated by the secretion of a trophoblast interferon (IFN-tau), bovine trophoblast protein-1, secreted by the conceptus between day 16 and day 26 of pregnancy identified as an antiluteolytic agent in cattle[4]. Sometimes, this phenomenon is hampered by various intrinsic and extrinsic causes eventually leading to embryonic mortality in bovines.

Though there is no current estimate of economics of embryonic loss in India, it is estimated to be around 250 million pounds/year in UK alone[5] and 1.4 billion U.S. dollars in the USA[6] with the global cost of 1.28×10^{12} U.S. dollars[7]. Nonetheless, alleviating early embryonic mortality remains one of the high point in improving reproductive efficiency of fast growing dairy industry which has wider ramifications over population at large. This review throws light on the role of prostaglandins (PG) in embryonic mortality and ways to modulate PG synthesis and action.

*Corresponding author: Jerome A, Division of Animal Reproduction, Indian Veterinary Research Institute, Izatnagar, 243122, UP, India.
E-mail: jerome210982@gmail.com

2. Embryonic mortality vis-à-vis maternal recognition of pregnancy

Embryonic mortality is one of the important causes of poor reproductive efficiency in cattle and buffalo. Embryonic loss during this critical period may be due to utero-embryonic asynchrony[8-10], early increase in endometrial PG F2 alpha (PGF2 alpha) secretion[11], and delay in or insufficient production of IFN-tau by the conceptus[12], resulting in delay or failure to signal its presence to the mother for MRP and heat stress. Production of reactive oxygen species is an important cause of increased embryonic mortality in heat stressed embryos[13]. Negative energy balance and deficiencies in oocyte and embryo developmental competence is another important cause as it affects the follicular growth patterns and oocyte quality. Negative energy balance affects microenvironment of the growing and maturing female oocyte resulting in incompetent oocyte. Moreover, poor nutrition results in reduced progesterone and low insulin-like growth factor concentrations disrupting the uterine microenvironment, giving rise to the high incidence of embryonic mortality[14].

Heat stress between day 8 and day 17 of pregnancy may alter the uterine environment causing increase in secretion of PGF2 alpha and may also affect growth and secretory activity of the conceptus in cattle[15]. In other species, embryonic mortality increases following exposure of dam to elevated ambient temperatures[16]. When subjected to high ambient temperatures either *in vitro* or *in vivo*, fertilized eggs are damaged or die during the critical stage of implantation[1]. Heat stress decreases viability and developmental capacity of embryo and may account for the seasonal reduction in conception rate during summer. Similarly, buffaloes are more sensitive to direct solar radiation and high temperature than cattle. The heat stress primarily affects the early developing embryo and leads to embryonic death[17].

Reduced progesterone concentration attributes to 50% of embryonic mortalities in buffaloes. In buffaloes, progesterone levels on day 10 after artificial insemination (AI) were higher ($P<0.05$) in pregnant buffaloes compared to buffaloes that showed embryonic mortality[18]. Elevated uterine concentrations of PGF2 alpha have also been found associated with reduced embryo quality[19]. It has been observed that PGF2 alpha act directly on embryo and inhibits its development to expanded/hatched blastocyst stages[20]. Delay or insufficient production of IFN-tau by conceptus during critical period may lead to early embryonic mortality[12]. Maintenance of pregnancy is dependent on a successful blockage of endometrial PG production. Blocking luteolysis is dependent on the ability of the conceptus to send effective antiluteolytic signals and on the capacity of endometrium to respond to such signals, thus blocking PGF2 alpha production. Disturbances of these embryo-maternal interactions can result in embryonic loss.

During the critical period, concepti undergo a rapid increase in size but smaller concepti will not be able to synthesize enough bovine IFN-tau (bIFN-tau) to block luteolysis in multiparous animals. Therefore, pregnancies will be lost because of a transient inability of embryos to send antiluteolytic signals to endometrium. It has been estimated that 13%-15% of pregnancies are lost around day 14 to day 19 or thereabouts probably related to failure of the antiluteolytic IFN-tau secretary mechanisms[12]. Majority of embryonic mortality occurs prior to and around the period of MRP, hence many researches have been concentrated to develop strategies for the embryonic survival to improve pregnancy rates. Studies reveal the role of mutations in genes, viz., fibroblast growth factor 2 (*FGF2*), signal transducer and activator of transcription 5A (*STAT5A*), growth hormone (*GH*), prolactin (*PRL*), prolactin receptor (*PRLR*), osteopontin (*OPN*) and uterine milk protein (*UTMP*), showed significant decrease in fertilization and survival rate of embryos as these genes are involved in various pathways of fertilization and early embryonic survival[21]. Infections caused by bacterial, fungal, protozoan, and viral agents also contribute to embryonic and fetal mortality[22]. Other causes include deficiency of essential amino acids, production of advanced oxidized protein products and follicular determinants[10,23-24]. Decreasing the luteolytic response of the maternal unit has been identified as one of the antiluteolytic strategies for enhancing embryonic survival in bovine[25].

2.1. Interferon-tau

IFN-tau, also known as bovine trophoblast protein-1, has been identified as an antiluteolytic agent secreted by conceptus between day 16 and day 26 of pregnancy in cattle[4]. IFN-tau is expressed for a short period at a high concentration and has antiluteolytic, antiproliferative, antiviral and immunomodulatory effects. Bovine interferon-tau (molecular weight: 22 000-24 000 Da), consisting of 172 amino acids, is produced between day 16 and day 24 of gestation by trophoblast of conceptus. A 595 bp open reading frame of *bIFN-tau* gene codes for a 195 amino acid pre-protein of which the first 23 residues is a signal peptide that is cleaved to yield a mature protein of 172 amino acids[26]. bIFN-tau has multiple isoforms and is glycosylated with N-linked oligosaccharides. By various studies, 18 naturally occurring ovine IFN-tau variants and 12 bovine IFN-tau variants have been discovered by genomic and cDNA screening[27]. The ovine and bovine IFN-tau molecules have about 80% amino acid sequence homology, whereas there is about 50% homology between IFN-alpha and IFN-tau. Bovine and caprine *IFN-tau* genes show 85% and 96% homology with that of sheep IFN-tau[26]. IFN-tau shares 50% and 30% amino acid sequence identity with IFN-alpha and IFN-beta. The ovine and bovine IFN-tau molecules share about 80% amino acid sequence homology[26]. In sheep, IFN-tau has been reported to inhibit the expression of estrogen receptor in the uterine epithelium, preventing up-regulation of the oxytocin receptor by estradiol (E_2) and subsequent generation of luteolytic pulses of PGF2 alpha by oxytocin[28]. In contrast, IFN-tau at low doses has been shown to down regulate both cycloxygenase-2 (COX-2) and PGFS enzyme in bovine endometrial cells to inhibit PGF2 alpha synthesis, whereas it is stimulatory at higher doses[29]. It has also been

observed that IFN-tau attenuates PGF2 alpha secretion from epithelial cells and increased PGE_2 production in stromal cells, and it is modulated by steroid hormones[30]. In the presence of progesterone, rbIFN-tau has been shown to enhance the secretion of PGE_2 and thus increase the PGE_2/PGF2 lapha ratio[31].

Studies show that intrauterine infusion of highly enriched bovine trophoblast protein-1 complex exerts an antiluteolytic effect to extend CL life span in cyclic cows[32]. In response to IFN-tau, there is up-regulation of a large number of IFN-stimulated genes (ISG) in peripheral blood leukocytes in gravid uterus of ruminants. These genes include *Mx*1, *Mx*2, beta2-microglobulin, *ISG*-15, IFN regulatory factor-1, and IFN regulatory factor-2[33]. Recent studies reveal detection of low levels of ISG-15 is also indicative of embryonic loss in bovines[34].

3. PG and its roles

PG are members of the eicosanoid family of molecules. They are derived from open chain, 20 carbon polyunsaturated fatty acids, typically arachidonic acid. Arachdonic acid is primarily stored in an esterified state at the SN-2 position of cell membrane phospholipids. The biosynthesis of PG takes place in three steps: hydrolysis of arachdonic acid by phospholipases; oxidation and reduction of arachidonic acid to PGH_2 by endoperoxide synthases; and conversion of PGH_2 to biologically active end products by specific synthases[35]. It has been reported that endometrial epithelial cells are major source of PGF2 alpha, whereas stromal cells are major source of PGE2[30,36,37].

Oxytocin stimulates PGF2 alpha production only in bovine epithelial cells but not in stromal cells. Thus, to investigate function of the cultured uterine epithelial cells, the production of PGF2 alpha in response to oxytocin is used as a functional marker[38]. COX-2 enzymes play an important role in blastocyst hatching and implantation. However, when concentrations of substrates from COX activity that are produced locally at the uterus are increased, endometrial receptivity is affected[39]. PG stimulate inflammatory cells and stimulate uterine contractions, whereas thrombaxane A2 induces platelet aggregation and vasoconstriction. Therefore, a successful pregnancy is thought to be dependent on a very delicate equilibrium of the specific mediators generated by COX.

In vitro studies have shown negative effects of PGF2 alpha on embryonic survival in beef cows[40]. Addition of PGF2 alpha to the culture medium has been shown to inhibit *in vitro* development of bovine embryo[41]. In agreement, elevated uterine concentration of PGF2 alpha was negatively correlated with embryo quality[42]. Numerous factors such as heat stress, uterine manipulation, oxytocin administration, nutrition, mastitis, and plant toxins among others may contribute to early embryonic losses through premature increase of uterine luminal concentrations of PGF2 alpha in cow. Liquid chromatography-tandem mass spectrometry (LC-MS) studies revealed various PG-specific enzymes and receptors involved in PG generation in bovine uteri at different preimplantation pregnancy stages. Concentration of 6-keto PGF (1 alpha) (stable metabolite of PGI_2) was predominant as compared to PGF2 alpha>PGE_2>PGD_2. Moreover the highest PG concentration was measured on day 15 with 6-keto PGF (1 alpha, 6.4 ng/mL) followed by PGF2 $alpha_{2\alpha}$ (1.1 ng/mL) and PGE_2 (0.3 ng/mL). Study also revealed PGI_2 and PGF2 alpha receptors were abundantly expressed by the trophoblast in the uterine lumen[43].

3.1. Cyclooxygenases regulating PG synthesis

The production of endometrial PG is mainly governed by the rate limiting enzymes cyclooxygenase COX-1 and COX-2. These enzymes are responsible for the conversion of arachidonic acid into PGH_2, the common precursor of the various forms of PG including PGE_2 and $PGF_{2\alpha}$. The downstream enzymes, PGE Synthase (PGES) and PGF synthase (PGFS), catalyze the conversion of PGH_2 to PGE_2 or $PGF_{2\alpha}$, respectively. The expression and regulation of COX-1 and COX-2 are tissue and species specific. COX-2, rather than COX-1, is the primary isoenzyme involved in the endometrial production of PGs in bovine endometrium during the estrous cycle. COX-2 mRNA and protein are expressed at low levels between days 1 and 12 and at high levels between days 13 and 21 of the estrous cycle. The peak expression of COX-2 protein between days 16 and 18 of the estrous cycle also matches the expected time of luteolysis (days 16-17) in the cows [35].

During the bovine estrous cycle, luteolysis and recognition of pregnancy occur at the same period on a competitive basis. In ruminants, the presence of embryo prevents pulsatile $PGF_{2\alpha}$ secretion through the release of IFN-τ, but IFN-τ could also stimulate COX-2 expression and PGE_2 production *in vitro*[44]. It is presumed that the presence of viable embryo may switch on the COX-2 and PGES pathway and PGE_2 production and thus help in establishment of pregnancy, whereas in the absence of an embryonic signal, other factors may switch on the COX-2 and PGFS pathway for $PGF_{2\alpha}$ production and luteolysis [35].

Moreover synthesis of PG is also triggered by action of oxytocin on binding to endometrial oxytocin receptor, coupled to a G-protein, activates phospholipase C (PLC). The PLC cleaves membrane phosphatidylinositol biphosphate, yielding inositol triphosphate (IP_3) and diacyl glycerol (DAG). The IP_3 binds to specific receptors in the endoplasmic reticulum, resulting in release of Ca^{2+} from internal stores into the cytosolic compartments. The DAG activates PKC, leading to serine phosphorylation of cytosolic-calcium-dependant PLA_2 through a MAP Kinase-dependant pathway[45]. The IP_3-induced increase in cytosolic Ca^{2+} acts to further stimulate PLA_2 activity. Stimulated PLA_2 translocates to the membrane, where phospholipids are cleaved to yield arachidonic acids [46].

Oxytocin also stimulates COX-2 and PGFS in endometrial epithelial cells to induce PG production [47]. The initial released $PGF_{2\alpha}$ triggers the release of additional oxytocin from the CL by a positive feedback loop. The oxytocin

binds to endometrial oxytocin receptor to stimulate further $PGF_{2\alpha}$ secretion. Initially endometrial oxytocin receptor concentrations are low during the luteal phase of the cycle (day 5 to 17) but it increases and reaches to its peak at estrus[48].

3.2. Mechanism of action of PG

3.2.1. Luteolytic action of $PGF_{2\alpha}$

Luteolysis is defined as the loss of function and subsequent involution of the luteal structure. It involves functional and structural luteolysis. In cyclic cows, luteolysis is initiated by endometrial $PGF_{2\alpha}$ released between days 15 and 17 of the cycle [35]. In ruminants, the pulsatile release of oxytocin by CL and neurohyphophysis stimulates the production of uterine $PGF_{2\alpha}$ secretion.

In a number of farm animals, $PGF_{2\alpha}$ is recognized as the physiological luteolysin that is responsible for regression of CL at the end of a non-fertile cycle. $PGF_{2\alpha}$ acts on the CL by binding to a specific receptor belonging to the family of G-protein coupled receptors (G-PCR) localized mainly to large luteal cells, but also present on small luteal and endothelial cells of the CL[49]. Upon binding to its receptor, $PGF_{2\alpha}$ induces activation of membrane bound PLC which catalyzes the hydrolysis of phosphatidyl inositol 4,5 biphosphate to inositol triphosphate (IP_3) and diacyl glycerol[50]. $PGF_{2\alpha}$-increased-IP_3 levels stimulate mobilization of intracellular Ca^{2+}, and increased DAG stimulates the Ca^{2+} dependant PKC [51]. The antisteroidogenic effect of $PGF_{2\alpha}$ appears to be mediated by the PKC (Protein Kinase C) second messenger pathway. Decreased steroidogenesis by $PGF_{2\alpha}$ appears to be due to decreased transport of cholesterol to the inner mitochondrial membrane [52]. A mitrochondial protein, steroidogenic acute regulatory protein (stAR) play a pivotal role in the transfer of cholesterol to the inner mitochondrial membrane, which is rate-limiting step in steroidogenesis. $PGF_{2\alpha}$ stimulates endothelial cells of CL to produce endothelin-1 (ET-1) *in vivo*[53]. ET-1 has been reported to inhibit progesterone production from steroidogenic luteal cells, in addition to its effect on reduced blood flow to CL (on reduced blood flow or reduces blood flow). Nitric oxide (NO) seems to serve as a mediator of $PGF_{2\alpha}$ action in functional luteolysis and NO donor has been reported to inhibit progesterone secretion in luteal cells *in vitro* [54].

After the CL ceases to produce progesterone, it decreases in size, experiences a loss of cellular integrity, and then forms corpus albicans as a result of apoptosis of luteal cells. Apoptosis plays a central role in the regression of CL of several species including buffalo[55]. $PGF_{2\alpha}$ induces Fas-ligand expression on the luteal cells expressing Fas and induces luteal cell apoptosis by activating Fas pathway. The Fas and Fas-ligand system plays central role in luteal cell apoptosis in cattle. Fas expression is modified by factors such as cytokines, TNF-α, IFN-γ and progesterone. NO could be an important inducer of apoptosis in the bovine CL. reactive oxygen species and Bcl-2 family may be involved in apoptosis as mediators [56].

3.2.2. Steroid hormones and other factors in the regulation of PG synthesis

Estradiol and progesterone are responsible for regulating PG synthesis in the endometrium during the estrous cycle. Estradiol facilitates transcription of oxytocin receptor gene by increasing it more rapidly, but spontaneous up regulation of endometrial oxytocin receptor still occurs in the absence of estradiol[57]. Estradiol enhances PG production by elevating PG synthesis activity in human endometrial epithelial cells[58]. In contrast, estradiol has been reported to have no effect or inhibit PG production from endometrial strips and endometrial epithelial cells in cattle[47,59]. Asselin *et al*[36] observed that estradiol enhances oxytocin stimulated PG production in epithelial cells of bovine uterus. Estradiol is also important for the timing of luteolysis because removal of estradiol results in a prolonged cycle[60] and administration of estradiol in mid-cycle initiates luteolysis by increasing plasma PGFM (13, 14-dihydro-15-keto-PGF_2, expansion) concentration[17], presumably by raising endometrial OTR concentration[61]. The effect of estradiol is dependent on progesterone because it is only observed after the endometrium has been primed with progesterone for a certain period of time[62]. Estradiol can also affect the magnitude, timing, and pattern of $PGF_{2\alpha}$ pulses in response to oxytocin[63]. Estradiol facilitates transcription of oxytocin receptor gene by increasing it more rapidly, but spontaneous upregulation of endometrial oxytocin receptor still occurs in the absence of estradiol[57].

Moreover progesterone also stimulates $PGF_{2\alpha}$ secretion from endometrial epithelial cells and endometrial strips[59]. The observed stimulation of PG synthesis by progesterone in endometrial cells was not due to an increase in either COX-2 or PGFS mRNA expression[47]. Progesterone can regulate $PGF_{2\alpha}$ secretion in different ways. Prolonged exposure to progesterone promotes the endometrial accumulation of arachidonic acid and cyclooxygenase necessary for the synthesis of $PGF_{2\alpha}$. Progesterone also exerts a suppressive effect on $PGF_{2\alpha}$ secretion, which wanes after prolonged exposure. This suppressive effect is due to an inhibitory action on OTR gene expression during the early and mid-luteal phase of the estrous cycle[62]. Xiao *et al*[47] reported that estradiol in the presence of progesterone significantly reduced $PGF_{2\alpha}$ and PGE_2 secretion in bovine endometrial epithelial cells. Skarzynski *et al*[59] observed amplified effect of estradiol on progesterone stimulated $PGF_{2\alpha}$ secretion from endometrial strips. Progesterone has also been reported to suppress the ability of oxytocin to induce secretion of $PGF_{2\alpha}$ from bovine endometrial cells possibly by direct interference in the interaction of oxytocin with its own receptor[64]. As long as the endometrium is under the influence of progesterone, ovarian oxytocin does not affect $PGF_{2\alpha}$ secretion [59].

In ruminants, estradiol, progesterone and oxytocin regulate the uterine secretion of $PGF_{2\alpha}$ that causes luteolysis. Oxytocin stimulates the pulsatile release of $PGF_{2\alpha}$ via the endometrial oxytocin receptor, resulting in regression of the CL. Thus, the acquisition of responsiveness of oxytocin by the endometrial epithelium determines when endogenous secretion of $PGF_{2\alpha}$ will occur during the estrous cycle, and this appears to require the coordinated action of

progesterone and estradiol. Other factors modulating PG secretion are leukotrienes, nitric oxide (NO), angiogenic and vasoactive factors *viz*. vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), angiopoietin-1 and -2 (ANPT-1 and -2), endothelin-1 (EDN-1) and angiotensin II (Ang II) produced from the CL which directly and indirectly influence PG production and function thereby revealing the role during pregnancy in cattle[65-67].

3.2.3. Effect of IFN-τ on PG production

Interactions among the CL, endometrium and embryo are essential for the establishment of pregnancy. This is achieved by the action of progesterone acting on its receptor in the endometrium which stimulates the production of endometrial secretions. Moreover the secretion of IFN-τ which suppresses luteolytic pulses of $PGF_{2\alpha}$ which inhibits oxytocin receptor up-regulation directly and thereby inhibit PG synthesis. Danet-Desnoyers *et al*[30] observed the effect of bovine IFN-τ on secretion of the $PGF_{2\alpha}$ and PGE2 by endometrial epithelial and stromal cells *in-vitro*. They observed that treatment with bIFN-T attenuates basal and oxytocin stimulated $PGF_{2\alpha}$ secretion from epithelial cells, whereas secretion of $PGF_{2\alpha}$ and PGE2 from stromal cells is not altered by either IFN-T or oxytocin. Xiao *et al*[47] also reported that rbIFN-T attenuated $PGF_{2\alpha}$ and E_2 in epithelial cells by down regulating COX-2 mRNA; however they found enhanced $PGF_{2\alpha}$ and E_2 secretion in stromal cells by up regulating COX-2 mRNA. Furthermore, rbIFN-T decreased PGFS mRNA in both cell types and this was associated with the increase in PGE2/ $F_{2\alpha}$ ratio.

IFN-τ has been shown to down regulate both COX-2 and PGFS enzyme apart from down regulating OTR in bovine endometrial cells to inhibit $PGF_{2\alpha}$ synthesis[31]. Binelli *et al*[25] demonstrated that rbIFN-T suppress PDB induced $PGF_{2\alpha}$ production in BEND cells through inhibiting steady state level of COX-2 mRNA, protein expression and enzymatic activity of COX-2 and PLA_2. These interactions are facilitated by interferon and other hormones produced by CL and the conceptus contributing to successful pregnancy[68].

4. Modulation of uterine PG production

4.1. COX inhibitors

Among COX inhibitors potential candidates capable of inhibiting uterine $PGF_{2\alpha}$ release are non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs act by competitive inhibition of COX enzyme either non-selectively through both COX-1 and COX-2 isoforms i.e. flunixin, ibuprofen or specifically through COX-2 isoform i.e. celecoxib, rofecoxib, valdecoxib, and also through preferentially inhibiting COX-2 *viz*. meloxicam, nimusulide etc.

Research has shown that intensive parentral administration of flunixin is able to postpone luteolysis and prolong the estrous cycle in heifers[69]. Odensvik *et al*[70] studied the effects of feeding flunixin meglumine (FM) granules (2.2 mg/kg of body weight) on the length of the estrous cycle and maintenance of the CL. Heifers received FM orally twice, thrice, or four-times-daily for a 9 d period beginning on d 14 or 15 of the estrous cycle. Average estrous cycle length was increased in heifers receiving FM thrice daily from 19.8 to 22.5 d and four-times-daily from 19.5 to 26 d. However, single administration of flunixin meglumine in the form of granules has been found to inhibit the COX enzyme effectively. Studies by Rajkumar *et al*[71] with meloxicam as COX-2 inhibitor improved pregnancy rates in buffaloes post-AI. Guilbalt *et al*[72] reported administration of flunixin to cows twice daily during first 6 days postpartum inhibited the secretion of $PGF_{2\alpha}$, as indicated by a decrease in the concentration of 13, 14-dihydro-15-keto-PGF2 (PGFM) in the peripheral blood. Flunixin has been reported to accelerate uterine involution and shorten the calving to first estrus interval in cows with puerperal metritis[73]. COX-2 inhibitors like meloxicam and celecoxib are effective tocolytic agents and indicated in the treatment of preterm labour in humans[74]. Selective inhibitor of COX-2 (NS-398) has been reported to prevent the oxytocin induced $PGF_{2\alpha}$ production in bovine endometrial epithelial cells *in vitro*[44]. Studies by Geary[75] on use of flunixin meglumine in beef cattle revealed flunixin meglumine decreased ($P<0.01$) serum PGFM concentrations in ACTH-treated (Adeno cortico tropic hormone treated cows) cows proving their antiluteolytic role.

4.2. Polyunsaturated fatty acids (PUFA)

Feeding of PUFA during critical period can be used to inhibit secretion of endometrial $PGF_{2\alpha}$ occuring due to delay or insufficient IFN-τ secretion by embryo or decrease in response of endometrium to the IFN-τ, resulting in failure of MRP. *In vitro* studies revealed uterine tissues when incubated in medium only (M) or media supplemented with fatty acids *viz*. eicosapentaenoic (20:5omega3; EPA), docosahexaenoic acids (22:6omega3; DHA) or linoleic acid (C18:2omega6; LIN) revealed luteotrophic PGE2 release from 'pregnant' endometria was higher ($P=0.094$) than from 'non-pregnant' endometria, while $PGF_{2\alpha}$ concentrations were similar [76].

The luteolytic response of the uterus could be decreased by changing its lipid composition to increase the linoleic to arachidonic acid ratio. Abomasal infusions of yellow grease (high linoleic acid content) to lactating dairy cows resulted in release of less $PGF_{2\alpha}$ in response to oxytocin challenge given on day 15 of synchronized estrous cycle compared to cows receiving control infusion[77]. Beef cows fed with a diet containing formalin-treated cotton hulls rich in protected linoleic acid (escapes biohydrogenation) revealed higher conception rates to first inseminations (61 *vs*. 46%) as compared to control cows[78].

Polyunsaturated fatty acids like EPA or DHA inhibit secretion of $PGF_{2\alpha}$ from bovine endometrial cells *in vitro*[79]. EPA and DHA exert their regulatory effects as alternative substitutes that reduce the lipid pool of arachidonic acid in the endometrium[80]. Such strategies reduce the overall amount of arachdonic precursor for PG of the 2 series and increase the precursor pool of EPA and DHA for biosynthesis of 3-series PG[79]. High concentrations of EPA and DHA has been reported in Menhaden fish meal and supplementation of lactating dairy cows with Menhaden fish meal for 25

days reduced PGFM plasma concentrations in response to an estrogen injection followed by an oxytocin injection on day 15 of a synchronized estrous cycle[81]. Supplementation dietary fat rich in EPA or DHA enhanced embryo survival and pregnancy rate in dairy cattle[82]. Feeding fish oil enriched lipid (FO) supplement containing *n*-3 fatty acid such as EPA and DHA not only increased PR-mRNA expression, but also had differential cellular response with FO increasing PR in the SGE that may be beneficial for preparation of the uterus to maintain pregnancy. In addition, feeding FO reduced localization of endometrial ER_{α} (expansion) and PGH_2 and altered amount of substrate precursor available for PG synthesis. Feeding of cows with calcium salts of linoleic and trans-octadecenoic acids (LTFA) improved pregnancy rates[83]. Moreover, supplementation of calcium salts of polyunsaturated fatty acids during expected time of luteolyis improved pregnancy rates in Bos indicus beef cows [84].

4.3. Oxytocin receptor antagonist (OTA)

OTR gene is expressed in the myometrium, endometrium as well as in cervical epithelium. OTA is a peptide analogue that competitively inhibits the interaction of oxytocin to its membrane receptor. Futchs *et al*[85] reported the affinity and specificity of the antagonist [1-D(CH_2)$_5$, Tyr(ME)^2, Thr^4, TYR-NH92] ornithine vasotocin and its use in pregnant cows. The antagonist showed a high binding to endometrial and myometrial oxytocin binding sites and 40 fold molar excess of the antagonist inhibited the release of PG induced by oxytocin in the bovine endometrium prior to parturition. In addition, an effective inhibition of the uterotonic action of oxytocin was also documented. Inhibition by OTA of the myometrial, uterotonic effect of OTA in vivo has been demonstrated in rat, guinea pig and primates. Pierzynski *et al*[86] reported atosiban may improve receptivity during embryo transfer and may increase success rates of advanced infertility treatment procedures.

Active immunization of sheep against oxytocin has been reported to prolong the luteal phase of the estrous cycle, as judged by estrous behaviour and circulating progesterone concentrations[87]. Mann *et al*[88] investigated the effect of systemic administration of the oxytocin antagonist L-368899 on luteolytic $PGF_{2\alpha}$ release in ewes. They observed that treatment OTA caused a significant decrease in episodes of increased PGFM concentration during the period of luteolysis with a modest extension of estrous cycle length.

It has been observed that intra-arterial administration of oxytocin antagonist to goats (@ 0.2 μg/kg body weight) between days 12 and 20 of the cycle significantly delayed luteal regression beyond day 20 and suppressed the increase in PGFM concentration[89]. Skarzynski *et al*[59] used atosiban (10^{-7} M) to study the effect of oxytocin on PGFM production in presence of luteal cells in endometrial strips and reported no influence of oxytocin on PGFM production in presence of luteal cells i.e. progesterone. These findings suggest that treatment with OTA provides a approach by which early embryonic mortality may be reduced during the critical period. However no reports are available on these aspects in farm animals.

4.4. Recombinant bovine somatotropin (bST)

Recombinant bST, a commercially available product used to increase milk production was shown to increase pregnancy rates when given as part of timed-AI protocol in lactating dairy cows [90]. Santos *et al*[91] showed that bST increased pregnancy rate through reducing pregnancy loss. Badinga *et al*[92] demonstrated in a bovine endometrial cell line, that bovine GH and IFN-τ suppressed phorbol 12,13-dibutryate induced $PGF_{2\alpha}$ production; when added in combination, there was an additive effect in reducing $PGF_{2\alpha}$ secretion.

Bilby *et al*[80] observed differential responses in enzyme regulation (PGFS) to bST treatment depend on state of pregnancy. They observed that bST increased PGFS mRNA expression in cyclic cows, whereas it decreased PGFS mRNA expression in pregnant cows. Also bST increased PGES-mRNA expression in both pregnant and cyclic cows. Because bST decreased PGFS-mRNA and increased PGES -mRNA in pregnant cows, this may be potentially associated with more PGE_2 and less $PGF_{2\alpha}$ secretion creating a stronger antiluteolytic signal to maintain pregnancy.

4.5. Lysophosphatidic acid (LPA)

LPA is new biomolecule used in controlling PG secretion in domestic animals. LPA modulates PG synthesis in the bovine endometrium at the time of the early MRP through LPA-receptors (LPA-R) in the endometrium. Studies reveal LPA-R gene expression in the endometrium during early pregnancy show its role as an autocrine and/or paracrine cytokine in the bovine uterus. Moreover LPA-R gene expression is positively correlated with the expression of the enzyme responsible for luteotropic PGE_2 production (PGES) in endometrium. Thus, LPA has its role indirectly or directly in enhancing CL action via increase in the P4 synthesis and increase in the PGE_2/ $PGF_{2\alpha}$ ratio confirming LPA role in maintenance of CL. Moreover, studies with bovine epithelial and stromal endometrial cells cultured with LPA showed increased PGE2 production in stromal cells during the estrous cycle and early pregnancy, in addition to inhibiting $PGF_{2\alpha}$ production in epithelial cells. PGES mRNA expression was stimulated by LPA in stromal cells. These results show LPA may be a potent luteotropic agent by stimulating PGE_2 synthesis and decreasing $PGF_{2\alpha}$ synthesis during pregnancy[93,94].

4.6. PGF Synthase inhibitor

PGF synthase is an enzyme responsible for conversion of PGH2 to $PGF_{2\alpha}$. Bimatoprost, a potent PGF synthase-inhibitor has been found to inhibit $PGF_{2\alpha}$ in addition to having prostamide-like activity . Tolrestat and Stafil, which are inhibitors of aldose reductase, have been reported to inhibit PGF Synthase enzyme activity[95].

5. Conclusion

Though disturbance in uterine-conceptus synchrony

results in elevated levels of $PGF_{2\alpha}$ associated with embryonic mortality, this can be ameliorated by the use of various agents during the critical period of pregnancy to modulate PG synthesis and/or its luteolytic action. Application of outlined strategies will provide luteotrophic support in improving embryo survival rates in bovines.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

References

[1] Diskin MG, Parr MH, Morris DG. Embryo death in cattle: an update. *Reprod Fertil Dev* 2011, **24**(1): 244–251.

[2] Sreenan JM, Diskin MG. *The extent and timing of embryonic mortality in the cow. Embryonic mortality in farm animals*. Dordrech: Martinus Nijhoff; 1986, p. 1–11.

[3] Short RV. *Implantation and the maternal recognition of pregnancy*. Chichester: John Wiley & Sons; 1969, p. 2–26.

[4] Thatcher WW, Meyer MD, Danet-Desnoyers G. Maternal recognition of pregnancy. *J Reprod Fertil* 1995, **49**: 15–28.

[5] Peters AR, Ball PJH. *Reproduction in Cattle*. 2nd Edn. London: Blackwell Science Ltd; 1995, p. 89–104.

[6] Hansela W, Spaldinga RW, Larson LL, Laster DB, Wagner JF, Braun RK. Influence of human chorionic gonadotropin on pregnancy rates in lactating dairy and beef cows. *J Dairy Sci* 1976, **59**: 751–754.

[7] FAO. *Production Yearbook Food*. Rome: Food and Agriculture Organization of the United Nations; 1994, p. 336.

[8] Pope WF. Uterine asynchrony: a cause of embryonic loss. *Biol Reprod* 1988; **39**: 799–1003.

[9] Rhinehart JD, Starbuck-Clemmer MJ, Flores JA, Milvae RA, Yao J, Poole DH, et al. Low peripheral progesterone and late embryonic/early fetal loss in suckled beef and lactating dairy cows. *Theriogenology* 2009; **71**(3): 480–490.

[10]Pohler KG, Geary TW, Atkins JA, Perry GA, Jinks EM, Smith MF. Follicular determinants of pregnancy establishment and maintenance. *Cell Tissue Res* 2012; **349**(3): 649–664.

[11]Thatcher WW, Guzeloglu A, Mattos R, BinelliM, HansenTR, Pru JK. Uterine-conceptus interactions and reproductive failure in cattle. *Theriogenology* 2001, **56**: 1435–1450.

[12]Demmers KJ, Derecka K, Flint A. Trophoblast interferon and pregnancy. *Reproduction* 2001; **121**: 41–49.

[13]Sakatani M, Yamanaka K, Kobayashi S, Takahashi M. Heat shock-derived reactive oxygen species induce embryonic mortality in *in vitro* early stage bovine embryos. *J Reprod Dev* 2008; **54**(6): 496–501.

[14]Leroy JL, Opsomer G, Van Soom A, Goovaerts IG, Bols PE. Reduced fertility in high-yielding dairy cows: are the oocyte and embryo in danger? The importance of negative energy balance and altered corpus luteum function to the reduction of oocyte and embryo quality in high-yielding dairy cows. *Reprod Domest Anim* 2008; **43**(5): 612–622.

[15]Geisert RD, Zavy MT, Biggers GG. Effect of heat stress of conceptus and conceptus secretion in bovine. *Theriogenology* 1988; **29**: 1075–1082.

[16]Warnick AC, Hansen PJ. Comparison of ovulation, fertilization and embryonic survival in low-fertility beef cows compared to fertile females. *Theriogenology* 2010; **73**(9): 1306–1310.

[17]Thatcher WW, Collier RJ. *Current therapy in Theriogenology*. Philadelphia: W.B. Saunders; 1986, p. 301–309.

[18]Campanile G, Neglia G, Gasparrini B, Galiero G, Prandi A, Di Palo R, et al. Embryonic mortality in buffaloes synchronized and mated by AI during the seasonal decline in reproductive function. *Theriogenology* 2005; **63**(8): 2334–2340.

[19]Schrick FN, Inskeep EK, Butcher RL. Pregnancy rates for embryo transferred from early postpartum beef cows into recipients with normal estrous cycle. *Biol Reprod* 1993; **49**: 617–621.

[20]Fazio R, Schrick FN. Effect of elevated prostaglandin $F_{2\alpha}$. during *in vitro* culture of blastocyst stage bovine embryos. *Biol Reprod* 1997; **56** (Suppl. 1): 187.

[21] Khatib H, Huang W, Wang X, Tran AH, Bindrim AB, Schutzkus V, et al. Single gene and gene interaction effects on fertilization and embryonic survival rates in cattle. *J Dairy Sci* 2009; **92**: 2238–2247.

[22]Givens MD, Marley MS. Infectious causes of embryonic and fetal mortality. *Theriogenology* 2008; **70**(3): 270–285.

[23]Bonilla L, Luchini D, Devillard E, Hansen PJ. Methionine requirements for the preimplantation bovine embryo. *J Reprod Dev* 2010; **56**(5): 527–532.

[24]Celi P, Merlo M, Da Dalt L, Stefani A, Barbato O, Gabai G. Relationship between late embryonic mortality and the increase in plasma advanced oxidised protein products (AOPP) in dairy cows. *Reprod Fertil Dev* 2011; **23**(4): 527–533.

[25]Binelli M, Thatcher WW, Mattos R, Baruselli PS. Antiluteolytic strategies to improve fertility in cattle. *Theriogenology* 2001; **56**: 1451–1463.

[26] Roberts RM, Leaman DW, Cross JC. Role of interferons in maternal recognition of pregnancy in ruminants. *Proc Soc Exp Biol Med* 1992; **200**(1): 7–18.

[27]Alexenko AP, Ealy AD, Bixby JA, Roberts RM. A classification for the interferon-τ. *J Interferon Cytokine Res* 2000; **20**: 817–822.

[28]Bazer FW, Spencer TE, Ott TL. Interferon tau: a novel pregnancy recognition signal. *Am J Reprod Immunol* 1997; **37**: 412–440.

[29]Binelli M, Guzeloglu A, Badinga L, Arnold DR, Sirosis J, Hansen TR,Thatcher WW. Interferon-τ modulates phorbol ester induced production of prostaglandin and expression of cyclooxygenase-2 and phospholipase-A2 from bovine endometrial cells. *Biol Reprod* 2000; **63**: 417–424.

[30]Danet- Desnoyers G, Wetzels C, Thatcher WW. Natural and recombinant bovine interferon-τ regulate basal and oxytocin-induced secretion of prostaglandin $F_{2\alpha}$ and E_2 by epithelial and stromal cells in the endometrium. *Reprod Fertil Dev* 1994; **2**: 194–202.

[31]Xiao CW, Murphy BD, Sirosis J, Goff AK. Down-regulation of oxytocin-induced cyclooxygenase-2 and prostaglandin F Synthase expression by interferon- τ in bovine endometrial cells. *Biol Reprod* 1999; **60**: 656–663.

[32]Helmer SD, Hansen PJ, Anthony RV, Thatcher WW, Bazer FW, Roberts RM. Identification of bovine trophoblast protein-1, a secretory protein immunologically related to ovine trophoblast protein-1. *J Reprod Fertil* 1987; **79**(1): 83–91.

[33]Gifford CA, Racicot K, Clark DS, Austin KJ, Hansen TR, Lucy MC, et al. Regulation of interferon-stimulated genes in peripheral blood leukocytes in pregnant and bred, nonpregnant dairy cows. *J Dairy Sci* 2007; **90**(1): 274–280.

[34]Matsuyama S, Kojima T, Kato S, Kimura K. Relationship between quantity of IFNT estimated by IFN-stimulated gene expression in peripheral blood mononuclear cells and bovine embryonic mortality after AI or ET. *Reprod Biol Endocrinol* 2012; **22**; 21.

[35]Arosh JA, Parent J, Chapdelaine P, Sirosis J, Fortier MA. Expression of cyclooxygenase 1 and 2 and prostaglandin E synthase in bovine endometrial tissue during the estrous cycle. *Biol Reprod* 2002; **67**: 161-169.

[36]Asselin E, Goff AK, Bergeron H, Fortier MA. Influence of sex steroids on the production of prostaglandin $F_{2\alpha}$ and E_2 and reponse to oxytocin in cultured epithelial and stromal cells of the bovine endometrium. *Biol Reprod* 1996; **54**: 371-379.

[37]Fortier MA, Guibault LA, Grasso F. Specific properties of epithelial and stromal cells from the endometrium of cows. *J Reprod Fertil* 1988; **83**: 239-248.

[38]Takahashi H, Kosuke IGA, Sato T, Takahashi M, Okano A . Isolation and culture of bovine endometrial epithelial cells in a serum-free culture system. *J Reprod Dev* 2001; **47**(3): 181-187.

[39]Rubinstein M, Marazzi A, de Fried E. Low-dose aspirin treatment improves ovarian responsiveness, uterine and ovarian blood flow velocity, implantation and pregnancy rates in patients undergoing *in vitro* fertilization: a prospective, randomized, double-blind placebo controlled assay. *Fertil Steril* 1999; **71**: 825-829.

[40]Lemaster JW, Seals RC, Hopkins FM, Schrick FN. Effects of administration of oxytocin on embryonic survival in progesterone supplemented cattle. *Prostaglandins Other Lipid Mediat* 1999; **57**: 259-268.

[41]Scenna FN, Hockett ME, Towns TM, Saxton AM, Rohrbach NR, Wehrman ME, et al. Influence of a prostaglandin synthesis inhibitor administered at embryo transfer on pregnancy rates of recipient cows. *Prostaglandins Other Lipid Mediat* 2005; **78**(1): 38-45.

[42]Moon HS, Park SH, Lee JO, Kim KS, Joo BS. Treatment with piroxicam before embryo transfer increases the pregnancy rate after *in vitro* fertilization and embryo transfer. *Fertil Steril* 2004, **82**(4): 816-820.

[43]Ulbrich SE, Schulke K, Groebner AE, Reichenbach HD, Angioni C, Geisslinger G, et al. Quantitative characterization of prostaglandins in the uterus of early pregnant cattle. *Reproduction* 2009; **138**(2): 371-382.

[44]Asselin E, Drolet P, Fortier MA. Cellular mechanisms involved during oxytocin induced prostaglandin $F_{2\alpha}$ production in endometrial epithelial cells *in vitro*: Role of cyclooxygenase-2. *Endocrinology* 1997; **138**: 4798-4805.

[45]Lin LL, Wartmann M, Lin AY, Knopf JL, Seth A, Davis RJ. cPLA2 is phosphorylated and activated by MAP kinase. *Cell* 1993; **72**(2): 269-278.

[46]Clark JD, Lin LL, Kriz RW, Ramesha CS, Sultzman LA, Lin AY, et al. A novel arachidonic acid-selective cytosolic PLA2 contains a Ca(2+)-dependent translocation domain with homology to PKC and GAP. *Cell* 1991; **65**(6): 1043-1051.

[47]Xiao CW, Liu JM, Sirosis J, Goff AK. Regulation of cyclooxygenase-2 and prostaglandin F Synthase gene expression by steroid hormones and interferon-τ in bovine endometrial cells. *Endocrinology* 1998; **139**: 2293-2299.

[48]Wathes DC, Lamming GE. The oxytocin receptor, luteolysis and the maintenance of pregnancy. *J Reprod Fertil* 1995; **45**: 53-67.

[49]McCracken JA, Custer EE, Lamsa JC. Luteolysis: a neuroendocrine-mediated event. *Physiol Rev* 1999; **79**(2): 263-323.

[50]Davis JS, Weakland LL, Weiland DA, Farese RV, West LA. Prostaglandin F2 alpha stimulates phosphatidylinositol 4,5-bisphosphate hydrolysis and mobilizes intracellular Ca2+ in bovine luteal cells. *Proc Natl Acad Sci U S A* 1987; **84**(11): 3728-3732.

[51]Wiltbank MC, Guthrie PB, Mattson MP, Kater SB, Niswender GD Hormonal regulation of free intracellular calcium concentrations in small and large ovine luteal cells. *Biol Reprod* 1989; **41**(4): 771-778.

[52]Stocco CO, Chedrese J, Deis RP. Luteal expression of cytochrome P450 side-chain cleavage, steroidogenic acute regulatory protein, 3beta-hydroxysteroid dehydrogenase, and 20 alpha-hydroxysteroid dehydrogenase genes in late pregnant rats: effect of luteinizing hormone and RU486. *Biol Reprod* 2001; **65**(4): 1114-1119.

[53]Ohtani M, Kobayashi S, Miyamoto A, Hayashi K, Fukui Y. Real-time relationships between intraluteal and plasma concentrations of endothelin, oxytocin, and progesterone during prostaglandin F2alpha-induced luteolysis in the cow. *Biol Reprod* 1998; **58**(1): 103-108.

[54]Jaroszewski JJ, Skarzynski DJ, Hansel W. Nitric oxide as a local mediator of prostaglandin F2alpha-induced regression in bovine corpus luteum: an in vivo study. *Exp Biol Med* 2003; **228**(9): 1057-1062.

[55]Juengel JL, Garverick HA, Johnson AL, Youngquist RS, Smith MF. Apoptosis during luteal regression in cattle. *Endocrinology* 1993; **132**(1): 249-254.

[56]Sugino N, Okuda K. Species-related differences in the mechanism of apoptosis during structural luteolysis. *J Reprod Dev* 2007; **53**(5): 977-986.

[57]Leung ST, Wathes DC. Oestradiol regulation of oxytocin receptor expression in cyclic bovine endometrium. *J Reprod Fertil* 2000; **119**: 287-292.

[58]Schatz F, Markiewicz L, Barg P, Gurpide E. *In vitro* effects of ovarian steroids on prostaglandin F2 alpha output by human endometrium and endometrial epithelial cells. *J Clin Endocrinol Metab* 1985; **61**(2): 361-367.

[59]Skarzynski DJ, Bogacki M, Kotwica J. Involement of ovarian steroids in basal and oxytocin-stimulated prostaglandin (PG) $F_{2\alpha}$ secretion by the bovine endometrium *in vitro*. *Theriogenology* 1999; **52**: 385-397.

[60]Zhang J, Weston PG, Hixon JE. Influence of estradiol on thesecretion of oxytocin and prostaglandin F2 during luteolysis in the ewe. *Biol Reprod* 1991; **45**: 385-403.

[61]Hixon JE, Flint AP. Effects of a luteolytic dose of oestradiol benzoate on uterine oxytocin receptor concentrations, phosphoinositide turnover and prostaglandin F-2 alpha secretion in sheep. *J Reprod Fertil* 1987; **79**(2): 457-67.

[62]Lamming GE, Mann GE. Control of endometrial oxytocin receptors and prostaglandin F2 alpha production in cows by progesterone and oestradiol. *J Reprod Fertil* 1995; **103**(1): 69-73.

[63]Beard AP, Lamming GE. Oestradiol concentration and the development of the uterine oxytocin receptor and oxytocin-induced PGF_2 alpha release in ewes. *J Reprod Fertil* 1994; **100**(2): 469-475.

[64]Bogacki M, Silvia WJ, Rekawiecki R, Kotwica J. Direct inhibitory effect of progesterone on oxytocin induced secretion of prostaglandin $F_{2\alpha}$ from bovine endometrial tissue. *Biol Reprod* 2002; **67**: 184-188.

[65]Miyamoto A, Shirasuna K, Sasahara K. Local regulation of

corpus luteum development and regression in the cow: Impact of angiogenic and vasoactive factors. *Domest Anim Endocrinol* 2009; **37**(3): 159–169.

[66]Korzekwa AJ, Bah MM, Kurzynowski A, Lukasik K, Groblewska A, Skarzynski DJ. Leukotrienes modulate secretion of progesterone and prostaglandins during the estrous cycle and early pregnancy in cattle: an in vivo study. *Reproduction* 2010; **140**(5): 767–776.

[67]Shirasuna K. Nitric oxide and luteal blood flow in the luteolytic cascade in the cow. *J Reprod Dev* 2010; **56**(1): 9–14.

[68]Robinson RS, Mann GE, Lamming GE, Wathes DC. Expression of oxytocin, oestrogen and progesterone receptors in uterine biopsy samples throughout the oestrous cycle and early pregnancy in cows. *Reproduction* 2001; **122**: 965–979.

[69]Aiumlamai S, Odensvik K, Stabenfeldt G, Kindahl H. Regulation of prostaglandin biosynthesis with flunixin meglumine in the bovine species. *J Vet Med A* 1990; **37**: 16–22.

[70]Odensvik K, Gustafsson H, Kindahl H. The effect on luteolysis by intensive oral administration of flunixin granulues in heifers. *Anim Reprod Sci* 1998; **50**: 35–44.

[71]Rajkumar R, Singh SK, Agarwal SK, Mahmood S, Shankar U. Effect of selective COX-2 inhibitor on conception rate, progesterone and PGFM profile in buffalo (*Bubalus bubalis*). *J Appl Anim Res* 2010, **38**: 209–212.

[72]Guilbault LA, Thatcher WW, Drost M, Haibel GK. Inflence of a physiological infusion of prostaglandin Finto postpartumcows with partially suppressed endogenous production of prostaglandins. Uterine and ovarian morphological responses. *Theriogenology* 1987; **27**: 931–946.

[73]Amiridis GS, Leontides L, Tassos E, Kostoulas P, Fthenakis GC. Flunixin meglumine accelerates uterine involution and shortens the calving-to-first-oestrus interval in cows with puerperal metritis. *J Vet Pharmacol Ther* 2001; **24**(5): 365–367.

[74]Borna S, Saeidi FM. Celecoxib versus magnesium sulfate to arrest preterm labor: randomized trial. *J Obstet Gynaecol Res* 2007, **33**(5): 631–634.

[75]Geary TW. Effects of adrenocorticotropic hormone and flunixin meglumine on pregnancy retention in beef cows. *J Anim Sci* 2012, **90**(1): 207–211.

[76]Meier S, Ledgard AM, Sato TA, Peterson AJ, Mitchell MD. Polyunsaturated fatty acids differentially alter $PGF_{2\alpha}$) and PGE_2 release from bovinetrophoblast and endometrial tissues during short-term culture. *Anim Reprod Sci* 2009; **111**(2–4): 353–360.

[77]Oldick BS, Staples CR, Thatcher WW, Gyawu P. Abomasal infusion of glucose and fat effect on digestion, production, and ovarian and uterine functions of cows. *J Dairy Sci* 1997; **80**: 1315–1328.

[78]Wilkins JF, Hoffman WD, Larsson SK, Hamilton BA, Hennessy DW, Hillard MA. Protected lipid/protein supplement improves synchrony of estrous and conception rate in beef cows. 13th Intern Cong. *Anim Reprod* 1996; **3**: 19–29.

[79]Mattos R, Guzeloglu A, Badinga L, Staples CR, Thatcher WW. Polyunsaturated fatty acids and bovine interferon-tau modify phorbol ester induced secretion of prostaglandin $F_{2\alpha}$ and phospholipase A2 in bovine endometrial cells. *Biol Reprod* 2003; **69**: 780–787.

[80]Bilby TR, Guzeloglu A, MacLaren LA, Staples CR, Thatcher WW. Pregnancy, Bovine Somatotropin, and Dietary *n*-3 Fatty Acids in Lactating Dairy Cows: II. Endometrial Gene Expression Related to Maintenance of Pregnancy. *J Dairy Sci* 2006; **89**(9): 3375–3385.

[81]Thatcher WW, Binelli M, Burke J, Staples CR, Ambrose JD, Coelho S. Antiluteolytic signal between the conceptus and endometrium. *Theriogenology* 1997; **47**: 131–140.

[82]Cullens FM, Staples CR, Bilby TR, Silvestre F, Bartolome J, Sozzi A. Effect of timing of initiation of fat supplementation on milk production, plasma hormones and metabolites, and conception rate of Holstein cows in summer. *J Dairy Sci* 2004; **87**(Suppl. 1): 308.

[83]Juchem SO, Cerri RL, Villaseñor M, Galvão KN, Bruno RG, Rutigliano HM, et al. Supplementation with calcium salts of linoleic and trans-octadecenoic acids improves fertility of lactating dairy cows. *Reprod Domest Anim* 2010; **45**(1): 55–62.

[84]Lopes CN, Cooke RF, Reis MM, Peres RF, Vasconcelos JL. Strategic supplementation of calcium salts of polyunsaturated fatty acids to enhance reproductive performance of *Bos indicus* beef cows. *J Anim Sci* 2011, **89**(10): 3116–3124.

[85]Fuchs AR, Behrens O, Maschek H, Kupsch E, Einspanier A. Oxytocin and vasopressin receptors in human and uterine myomas during menstrual cycle and early pregnancy. *Hum Reprod Update* 1998; **4**(5): 594–604.

[86] Pierzynski P, Reinheimer TM, Kuczynski W. Oxytocin antagonists may improve infertility treatment. *Fertil Sterility* 2007; **88**(1): e19–e22.

[87]Sheldrick EL, Mitchell MD, Flint APF. Delayed luteal regression in ewes immunized against oxytocin. *J Reprod Fertil* 1980; **59**: 37–42.

[88]Mann GE, Lamming GE, Scholey D, Hunter M, Pettibone DJ. Attenuation of PGF2alpha release in ewes infused with the oxytocin antagonist L-368,899. *Domest Anim Endocrinol* 2003; **25**(3): 255–262.

[89]Homeida AM, Khalafalla AE. Effect of oxytocin antagonist injections on luteal regression in the goat. *Br J Pharmacol* 1987; **90**: 281–284.

[90]Moreira F, Orlandi C, Risco CA, Mattos R, Lopes F. Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. *J Dairy Sci* 2001; **84**(7): 1646–1659.

[91]Santos JEP, Thatcher WW, Chebel RC, Cerri RLA, Galvão KN. The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. *Anim Reprod Sci* 2004; **82**: 513–535.

[92]Badinga L, Guzeloglu A, Thatcher WW. Bovine somatotropin attenuates phorbol ester-induced prostaglandin $F_{2\alpha}$ production in bovine endometrial cells. *J Dairy Sci* 2002; **85**(3): 537–543.

[93]Woclawek-Potocka I, Brzezicka E, Skarzynski DJ. Lysophosphatic acid modulates prostaglandin secretion in the bovine endometrial cells differently on days 8–10 of the estrous cycle and early pregnancy. *J Reprod Dev* 2009; **55**(4): 393–399.

[94]Woclawek-Potocka I, Komiyama J, Saulnier-Blache JS, Brzezicka E, Bah MM, Okuda K, et al. Lysophosphatic acid modulates prostaglandin secretion in the bovine uterus. *Reproduction* 2009; **137**(1): 95–105.

[95]Koda N, Tsutsui Y, Niwa H, Ito S, Woodward DF, Watanabe K. Synthesis of prostaglandin F synthase and identification of bimatoprost as a potent inhibitor of the enzyme: new enzyme assay method using LC/ESI/MS. *Arch Biochem Biophys* 2004; **424**: 128–136.