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Endocrine Causes of Early Embryonic Death: An Overview

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ABSTRACT

Embryonic mortality denotes the death of fertilized ova and embryos upto end of implantation. Early embryonic mortality accounts for majority of reproductive failures with a mortality rate upto 40% of fertilized eggs. PGF₂α secreted by endometrial cells by activation of specific receptors by oxytocin of luteal origin causes demise of corpus luteum. In bovines embryonic trophoblast secretes interferon, which constitutes major signals for maternal recognition of pregnancy having antiluteolytic effect and luteoprotective agents like PGE₂ around day 8-9. Abnormal luteal function can be due to short luteal phase or abnormal luteal phase. Lack of exposure of the uterus to progesterone and estrogen prior to first postpartum ovulation decreases the concentration of progesterone and up regulation of oxytocin receptors which causes premature secretion of PGF₂ α from uterus resulting in short luteal phase in most of cows. Milk, plasma and saliva progesterone concentrations, PAG test and ultrasonography can be used as diagnostic tool to know the embryonic mortality. Various treatment regimens including use of hCG, GnRH, PMSG, interferon and supplementing Omega-3 fatty acids can be tried.

Key words: Early embryonic death, maternal recognition of pregnancy, interferon, PGF₂α

INTRODUCTION

The success of dairy enterprise is dependent on the milk production of dairy cattle and buffaloes (Bajaj *et al.*, 2006a). Production is directly related to the reproductive phenomenon. The reproductive efficiency is affected by very well known factors like fertilization failure and embryonic mortality the later being more significant (Bajaj *et al.*, 2006b). Embryonic death/mortality denote the death of fertilized ova and embryos upto the end of implantation (Jainudeen and Hafez, 2000). Mortality is more common during the early than the late embryonic period, i.e., from day 8th to 16th at the hatching of blastocysts and initiation of elongation and commencement of implantation without affecting cycle lengths. Early embryonic mortality is a major source of embryonic and economic loss with mortality rate upto 40% in animal production through repeat breeding and increased cost of artificial insemination (Sreenan and Diskin, 1986; Zavy, 1994; Bajaj, 2001), extended calving intervals and prolonged dry period resulting in reduced life time milk production

(Roche *et al.*, 1981) and reduced net calf crop (Maurer and Chenault, 1983). Earlier, it was believed that the bovine conceptus was resorbed, but transrectal ultrasound examination (Kastelic *et al.*, 1991) had demonstrated that the conceptus and its breakdown products apparently is eliminated by expulsion through the cervix, which either goes unnoticed or appears as a vulval discharge of clear mucus.

When, the interestrus or the interovulatory intervals are extended in bred animals, it usually indicates embryonic loss that occurred around the period of Corpus Luteum (CL) maintenance (Van Cleef *et al.*, 1991; Humblot, 2001). Measurement of progesterone in blood suggested that embryonic death at the time of CL maintenance delayed luteolysis and extended interestrus interval (Humblot, 2001). Therefore, when embryonic death precedes luteolysis, luteal regression is delayed by at least 3 days after the end of pregnancy (Kastelic *et al.*, 1991). However, when luteolysis precedes and probably causes embryonic death, return to estrus are dependent upon the stage of follicle development. Humblot (2001) suggested that luteolysis and return to estrus prior to day 24 might be linked with early embryonic death (early embryonic loss) but if the CL is maintained and returns to estrus are delayed beyond day 24 and 24-49 (late embryonic loss). Pregnancy losses detected after day 50 known as fetal losses.

The cost of losses to farmers has been estimated as high as 250 million year⁻¹ in the UK alone (Peters and Ball, 1995) and \$1.4 billion in the USA (Hensela *et al.*, 1976). Although, it is clearly not possible to extrapolate these figures to the whole world as national agricultural economics vary so widely, it can be seen that global cost of embryo mortality in a total cattle population (*Bos taurus* and *Bos indicus*) of approximately 1.28×10^{12} (FAO, 1994) is a large amount clearly justifying the relatively modest sums invested in research into its cause.

EMBRYO AND NORMAL EMBRYONIC DEVELOPMENT

An embryo is a product of fertilization characterized by growth and differentiation leading to the establishment of different organ systems that make up the individual.

Fertilization in the bovines occurs at ampullo-isthamus junction of the fallopian tube (Uterine tube) and the embryonic period can be divided into zygote, cleavage, morula, blastocyst, implantation and postimplantation. The zygote being the first structure formed as a result of successful fertilization cleaves mitotically into 2, 4, 8 and 16 cell stages. The cleaved embryo enters the uterine horn at the morula (mass of cells within the zona pellucida) stage about 4-5 days after fertilization. The morula then develops into blastocyst (having distinct blastocoele, trophoblast and embryonic disc) at days 6-7. The zona pellucida ruptures, resulting in hatching of the embryo after 9-10 days. The hatched blastocyst begins a process of elongation from about day 12-13, which is accompanied by the secretion of embryonic interferons. Early attachment (apposition) of the conceptus to the endometrium takes place from about day 19 and actual adhesion occurs by day 21-22.

Maternal recognition of pregnancy: Short (1969) firstly coined the term maternal recognition of pregnancy which means that the luteal function is maintained in early pregnancy and that the normal luteolytic mechanism is inhibited. In bovines mononuclear cells of trophoblast secrete specific proteins (bovine trophoblastic proteins, later recognized as Interferon tau) (Thatcher *et al.*, 2001) having anti-luteolytic effect (Danet-Denosyres *et al.*, 1994) and luteal protective agents like PGE₂ around day 8-9 (Bazer *et al.*, 1994). Interferon-tau (IFN_τ) mediates its antiluteolytic effect by inhibiting expression of endometrial oxytocin receptors and by transduction

mechanism after oxytocin-receptor binding on the endometrial cells thus inhibiting the episodic release of $\text{PGF}_2\alpha$ (Demmers *et al.*, 2001). Whenever, development of embryo is compromised or underdevelopment of trophoblast, premature luteolysis occurs.

The ovine and bovine IFN_γ molecules have about 80% amino acid sequence homology whereas there is about 50% homology between IFN_α and IFN_γ (Roberts *et al.*, 1992). Both ovine and bovine IFN_γ are secreted by the conceptus coincident with the blocking of luteolysis (Roberts, 1989).

Hernandez-Ledezma *et al.* (1992) indicated that bovine trophoblastic protein-1 (bTP-1/ IFN_γ) production begins at the expanded blastocyst stage on day 8-9 just prior to the rupture of zona pellucida and hatching. They also suggested that embryonic mortality between day 14 and 19 is caused by luteal failure and occurs because certain embryos develop more slowly than normal and do not produce enough IFN_γ to prevent luteolysis and maintain the pregnancy.

Intrauterine infusion of recombinant ovine or bovine IFN_γ in on-pregnant cows extended oestrus cycle by abolishing oxytocin induced $\text{PGF}_2\alpha$ secretion on day 17 and hence proved to be more effective in preventing embryonic mortality than IFN_α (Meyer *et al.*, 1995).

Martal *et al.* (1997) have reported many deleterious cytokines such as TNF_α , IFN_γ , IL-2 and beneficial cytokines such as TGF_β , LIF, CSF-1, GM-CSF, IL-1, IL-3, IL-6, IL-10 and IFN_γ are involved in embryo survival in ruminants and other species.

Kerblar *et al.* (1994) reported that an increased concentration of progesterone in cattle during early luteal phase is associated with enhanced embryonic production of the anti-luteolytic signal, IFN_γ . The CL that is maintained during maternal recognition of pregnancy is capable to convert $\text{PGF}_2\alpha$ to its metabolite (PGFM) (Silva *et al.*, 2000).

According to Spencer and Bazer (2004) establishment and maintenance of pregnancy results from signaling by the conceptus and requires progesterone produced by the Corpus Luteum (CL). Trophoblastic hormones in most of the mammals maintain progesterone production by acting directly or indirectly to maintain the CL. In domestic animals (ruminants and pigs), trophoblastic hormones maintain progesterone production by acting on the endometrium to prevent uterine release of luteolytic $\text{PGF}_2\alpha$.

DEATH OF THE EMBRYO AND TIME OF OCCURRENCE

Embryo death before day 13 of gestation results in return to estrus at the normal interestrus period. Death after day 13 extends the interestrus period beyond the generally accepted figure of 18-24 days.

Linares (1981) concluded that Early Embryonic Mortality (EEM) rather than fertilization failure was the major cause of repeat breeding in heifers as long as estrus detection and insemination had been properly performed. EEM is more important than fertilization failure in parous females in their relative contributions to reduced reproductive efficiency (Maurer and Chenault, 1983). According to Maurer and Chenault (1983), the critical period of embryo demise is day 7 of gestation when the morula develops into a blastocyst.

Markette *et al.* (1985) studied the incidence of embryonic loss following embryo transfer during the first week of development and concluded that between 50 and 75% of the embryos of poor quality are eliminated during the first 3 weeks.

Sreenan and Diskin (1986) reported that 20-25% of inseminations fail during the embryonic period, i.e., between day 1 and 42, most of the losses have occurred before day 25, the most substantial losses occurring between day 8 and 13 (8-9%) and days 14 and (13-15%).

Dunne *et al.* (2000) carried out studies on embryo and fetal loss in beef heifers between day 14, 30 of gestation and full term by measuring embryo survival rates which were 68, 76 and

71.8%, respectively. This led to the conclusion that most embryo losses in heifers occurs before day 14 after insemination.

CAUSES OF EARLY EMBRYO DEATH

Survival of embryo is affected by nutrition, temperature and heat stress, time of insemination, genital infections (Bajaj *et al.*, 2006c), uterine environment and asynchrony (Bajaj, 2001), maternal age, genetic factors, immunological and endocrine factors. Endocrine factors/causes play an important role in embryonic death (Bajaj, 2001). To understand the influence of endocrine causes on embryo survival and mortality one should be familiar with the structural composition and functioning of corpus luteum and interaction between different reproductive hormones affecting luteal lifespan.

Cells of corpus luteum and their function: On the basis of morphology and biochemical properties corpus luteum is composed of two distinct steroidogenic cell types (Hoyer and Niswender, 1985):

- **Small luteal cells (follicular origin):** These cells derived from theca interna of preovulatory follicles (Priedkalns *et al.*, 1968)
- **Large luteal cells (granulosa cell):** These cells derive from granulosa cells (O'shea, 1987). Small luteal cells are able to differentiate into large luteal cells as the cycle progresses (Hansel and Dowd, 1986). Farin *et al.* (1988) postulated that conversion of small luteal cells to large luteal cells occurs only during the early part of oestrus cycle (Fig. 1)
- **Stem cells of corpus luteum:** These different cell types contribute to circulating progesterone (P_4) in different manner. Luteinizing Hormone (LH) is the major luteotropin in domestic ruminants (Niswender *et al.*, 1985) and cattle and there is marked difference in the response to LH by large and small luteal cells (O'shea, 1987)

Harrison *et al.* (1987) reported that small luteal cells possess higher number of LH receptors than large luteal cells. Niswender *et al.* (1985) found that 20% of the progesterone (P_4) in the ovarian vein in mid-cycle is secreted by small luteal cells while nearly 80% of P_4 is secreted by large luteal cells which have only few functional receptors for LH. O'shea (1987) indicated that on per cell basis large luteal cells produce more progesterone than small cells.

Luteal function during early pregnancy: Plasma and milk progesterone concentration rise similarly in early luteal phase in pregnant and non-pregnant animals but the higher concentrations are maintained in pregnant cows for duration of pregnancy which are essential for maintenance of pregnancy. Anti-luteolytic substances secreted by embryo around day 13 are probably responsible for differences in progesterone patterns between pregnant and nonpregnant animals.

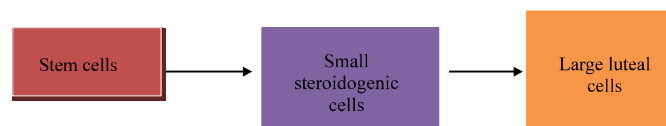


Fig. 1: Conversion of luteal cells

Lamming *et al.* (1989) found that milk progesterone concentrations in pregnant and nonpregnant cows rise indifferently until day 9, which later on diverged and the concentrations in pregnant cows remained higher. They also reported a significant dip in progesterone concentrations in pregnant animals on day 11, followed by a rise which reflects a rescue effect of the corpus luteum by the embryo.

Shelton *et al.* (1990) reported that the rate of rise in progesterone concentration was lower in the postovulatory period in cows identified for sub-fertility than in pregnant and nonpregnant heifers.

Role of oxytocin, oxytocin receptors (OTR) in luteal function: Wathes and Lamming (1995) have reported that during luteal regression, pulses of oxytocin stimulate synthesis and pulsatile release of $\text{PGF}_2\alpha$ following an increase in endometrial oxytocin receptors (OTR). Oxytocin receptor synthesis and $\text{PGF}_2\alpha$ release are inhibited by interferon production by the conceptus during early pregnancy. They also reported that OTRs are present during anoestrus, oestrus and late luteal phase and during most of pregnancy while the plasma oxytocin concentration causes parallel changes in plasma progesterone. These concentrations are basal at oestrus and rise from about day 2 of the cycle peaking around day 9 and falling from about day 12-13 before the onset of luteolysis (Wathes *et al.*, 1993). The pattern is similar in the pregnant animal and plasma concentrations fall from 12-13 days after inseminations.

Plasma concentrations of $\text{PGF}_2\alpha$ are generally low for most of the cycle but pulsatile release starts at day 13 and the pulse frequency increases until luteolysis (day 17). In nonpregnant ewes most oxytocin pulses occur in association with $\text{PGF}_2\alpha$ whereas, in pregnancy most pulses are not associated with $\text{PGF}_2\alpha$ (Hooper *et al.*, 1986). This pattern might be similar in cattle.

Progesterone blocks the increase in OTR for about the first 10-12 days of the luteal phase, but the mechanism of its prolonged effect, followed by upregulation, is still unclear. McCracken *et al.* (1984) found that increasing progesterone concentration in the luteal phase down regulates the progesterone receptor for about 10 days. But there was no evidence whether the progesterone receptors increase prior to the OTR increase or the treatment with progesterone reduces its own receptor (Wathes and Lamming, 1995).

Interferon, oxytocin receptors interaction and luteal function: During early pregnancy the rise in OTR concentration is inhibited probably by the action of embryo derived IFN. The mechanism of action of IFN may involve suppression of both oestradiol and oxytocin receptors probably at the transcriptional level (Bazer *et al.*, 1994). Bovine Interferon-alpha ($\text{bIFN}\alpha$) has been shown to stimulate progesterone production by luteal cells *in vitro*, without affecting oxytocin output (Luck *et al.*, 1992). Imakawa *et al.* (1993) reported that maternal granulocyte macrophage colony stimulating factor might be involved in stimulating embryo IFN production.

Types of abnormal luteal function: Abnormal luteal function is associated with reduced pregnancy rates (Hommeidaa *et al.*, 2004). Two distinct type of abnormal luteal function have been reported (Troxel and Kesler, 1984):

Type-I (short luteal phase): It is observed after a period of sexual rest and when breeding is initiated for the first time. In this condition short life span of corpus luteum (6-12 days) is observed.

Type-II (inadequate luteal phase): It is observed at any stage during the reproductive life. Life span of corpus luteum is of more than 14 days but with depressed plasma progesterone.

Mechanisms contributing to reduced luteal function: Mechanism contributing to reduced luteal function can be classified into three main categories:

- Deficiencies in the maturational process within the preovulatory follicle and/or inadequacies of ovulatory stimulus
- Shortcomings in the support of the CL once they have formed
- Premature activation of the luteolytic process

Follicular deficiencies may reflect in the form of subnormal progesterone concentration and short lived CL may be the consequence of premature activation of the luteolytic process.

WHY IS THE FIRST POSTPARTUM OVULATIONS RESULTS IN SHORT CYCLES?

During early postpartum period when the reproductive system is recovering to its normal anatomical and functional status, especially ovaries (ovarian rebound), presence of dormant follicles in ovaries and adequate pattern of LH secretion leads to first postpartum ovulation after few weeks (Perea and Inskoop, 2008) but the fertility in the first postpartum oestrus is poor as compared with subsequent cycles. During normal luteal phase of estrous cycle progesterone (P_4) causes increase in estrogen concentration to promote synthesis of progesterone (P_4) receptors (Zollers *et al.*, 1993) and to inhibit synthesis of uterine receptors for oxytocin (OTR) through the mid-luteal phase (Kieborz-Loos *et al.*, 2003). But lack of priming of uterus for progesterone followed by estrogen prior to first ovulation causes decrease in progesterone receptors and up regulation of oxytocin receptors which results in premature $PGF_{2\alpha}$ secretion (Cooper *et al.*, 1991). During the transition period from morula to blastocyst, i.e., during first 2 weeks of insemination (Inskoop, 2002) maternal recognition of pregnancy is prevented by premature release of $PGF_{2\alpha}$ from uterus and luteal origin resulting in early demise of CL (Cooper *et al.*, 1991; Inskoop, 2004). Since, the $PGF_{2\alpha}$ is secreted prematurely the exogenous supplementation of progesterone is also ineffective in prevention of embryonic death.

Theoretically, CL could be influenced by two tropic stimuli:

- Increasing the CLs secretory activity
- Prolonging CLs life span

Deficiencies prior to ovulation: Proper maturation of preovulatory follicle is essential as follicle after maturation results in CL formation (Garverick *et al.*, 1988). Gonadotropin treatment prior to the induction of ovulation results in improved functioning of resulting CL (Sheffel *et al.*, 1982). Changes within maturing follicle may influence functional CL in the form of decreased cell numbers, cell sizes or proportions of large to small luteal cells (O'shea *et al.*, 1984) or deficiency of receptors to luteotropin could result in the CL failing to recognize or respond luteotropin (Rutter and Randel, 1984). Increased concentrations of preovulatory LH could promote secretion of P_4 via cells of granulosa or thecal origin (Fitz *et al.*, 1982), with the evidence favouring an effect of LH on the small luteal cells.

Why are the mature follicles scarce at the time of sexual rest?: During the period of anoestrus or sexual rest no. of mature follicles is less due to low plasma LH concentration (Nett, 1987). This was partly the result of low pituitary reserves of LH during the early postpartum period consequently, the deficiencies were considered to include inadequate release of LH, either for several days prior to ovulation, or in short coming (duration and/or peak concentration) of preovulatory surge of LH.

Hormonal mechanisms involved in follicular maturation: The final stages of follicular maturation are associated with marked increase in the frequency of LH episodes (Karsch *et al.*, 1983). The frequency of LH increases from one per 3 to 12 h from luteal regression to 1 to 2 immediately per LH peak (McLeod *et al.*, 1982). During ovarian acyclicity these LH frequency were low (Savio *et al.*, 1990). Thus, during the early postpartum period in dairy cows, the pulsatile release of LH was insufficient to promote the final maturation of follicles (Hunter and Southee, 1987). To support follicular maturation a pulsatile delivery of LH to the ovary may be preferable to constant exposure (McNatty *et al.*, 1981). However, widely differing patterns of LH stimulation during the pre-ovulatory period have resulted in normal follicular maturation and some CL function (Keisler *et al.*, 1985). PGF₂ α may also be involved in follicular development in early postpartum cows (Guilbault *et al.*, 1987). Accordingly, either the quantity or pattern of PGF₂ α release is inappropriate prior to ovulation resulting in subnormal CL function.

Why LH pulses infrequent during early postpartum?: Lowered pituitary concentration of LH is one of the limitations to the re-establishment of oestrous cycles rather than alterations in the sensitivity of the organ to GnRH (Moss *et al.*, 1985). Nett (1987) suggested that deficiencies of the hypothalamic hypophysial axis could result in a reduced secretion of LH during the early part of the postpartum period.

Nett (1987) concluded that during the postpartum period the hypothalamus although, contained sufficient stores of GnRH to stimulate the anterior pituitary. Once the pituitary stores of LH had been replenished, then pulses in the secretion of LH could increase in frequency to culminate in the first postpartum oestrus (Humphrey *et al.*, 1983). Ovarian acyclicity thus appeared to be the result of a failure of follicle development, possibly due to an inadequate frequency of LH pulses reflecting inadequate pulsatile release of GnRH (Wise *et al.*, 1989).

Feedback response to estrogen: Peters (1984) has proposed that although, the positive feedback mechanism of E₂ in triggering the preovulatory LH release may be functional, recovery of maximal activity may continue over an extended period. Therefore, the hypothalamic-pituitary axis would need to recover responsiveness to increasing plasma concentrations of E₂ before the first ovulation can occur.

Parfet *et al.* (1986) stated that the absence of oestrous cycles in suckled cows near 30 days postpartum was not due to deficiencies in:

- Ovarian follicular development
- Anterior pituitary concentration of LH and FSH or *in vitro* releasibility of LH or
- Pituitary receptors for GnRH

In the properly fed cow, at about one month after calving, the hypothalamic-pituitary axis is fully able to support resumption of ovarian cycles, but the suckling stimulus inhibits the release of LH (Williams, 1989).

Is FSH deficit during early postpartum period?: FSH plays a permissive role in the onset of ovarian cycles postpartum. Deficiency of FSH during the late luteal to early follicular phase could inhibit the development and function of the preovulatory follicle (Fortune and Quikre, 1988).

FSH concentrations are lower over the last four days before the first preovulatory LH surge induced by weaning in cows. FSH concentrations were also lower compared to the same period prior to second preovulatory LH surge (Remirez-Godinez *et al.*, 1982). The former preceded short lived CL, while the later preceded normal luteal function.

Lack of follicular maturation in the postpartum cow: Nett (1987) used a model to explain the inadequate maturation of follicles in the postpartum, anoestrous cows. For low concentrations of LH during early lactation he suggested that during pregnancy the high concentrations of P_4 and E_2 resulted in a prolonged negative feedback on the hypothalamic-hypophyseal pituitary axis. Accordingly, the synthesis of LH was inhibited and pituitary stores become depleted so that basal release of LH was reduced. After parturition, a two phase recovery of the hypothalamic-pituitary gonadal axis occurred with the first phase (lasting 2-5 weeks) characterized by infrequent release of GnRH (one pulse/4-8 h). Once pituitary stores of LH had been replenished then the amplitude of the LH pulses was sufficient to stimulate follicular growth. This denoted the start of second phase of the recovery process, during which the increased circulating concentration stimulated growth of ovarian follicles, which in turn produced E_2 . At this point of time, the frequency of release of GnRH also increased with a consequent increase in the frequency of LH pulses. The final stages of follicular development ensued and culminated in the first ovulation.

Intrafollicular receptors for LH and the role of estrogen (E_2): Even if luteotropin secretion is sufficient to support normal CL function, then short lived or inadequate CL may not recognize LH. This might be due to insufficient LH receptors possibly within the maturing follicle and in the subsequent CL. Both E_2 and FSH are necessary for production of LH receptors before luteinization of granulosa cells (Richards, 1980).

According to Fortune and Quirke (1988), estrogen (E_2) also acts within the follicle to regulate its development and function. They proposed that as the preovulatory follicle mature the initial action of E_2 of positive feedback is on its own production, via increased androgen synthesis.

During preovulatory growth, follicles become more responsive to LH and acquire an increased ability to synthesize E_2 . This increased responsiveness to basal levels of LH was due to increased LH receptors in the granulosa cells.

Estrogen (E_2) and luteal lifespan: Larson (1987) reported that administration of hCG (to induce ovulation) leads to CL with a normal lifespan in cows with high plasma E_2 than in cows with low E_2 . Inskeep *et al.* (1988) reported largest preovulatory follicle possessed decreased numbers of receptors for LH in granulosa and theca cells in cows which were predicted to have short luteal phases than in Norgestomet pretreated cows which were expected to show normal luteal lifespans.

Garcia-Winder *et al.* (1987) and Inskeep *et al.* (1988) found a higher concentration of E_2 in largest follicle and CL with normal life span in cows implanted with synthetic progestogen

(Norgestomet). Evidences from number of sources support that estradiol within the preovulatory follicle is associated with function of resulting CL.

Deficiencies at the time of ovulation/postovulation

Could abnormalities of preovulatory LH surge result in a short lived CL or defective CL?:

Troxel and Kesler (1984) stated that the magnitude and duration of GnRH induced LH surge appeared to be associated with enhanced CL function and lifespan. Cruz and Kesler (1988) reported that cows with normal luteal function had a greater GnRH induced LH release than cows with short luteal phases.

Shirar *et al.* (1989) emphasized the importance of duration of the preovulatory LH release. They observed that progestogen treatment, prior to GnRH, changed the pattern of LH release and possibly thereby improved luteal function.

Can the reduced lifespan or lowered secretory activity of the first CL be attributed to suboptimal luteotropic support?:

Manns *et al.* (1984) reported the presence of FSH receptors in luteal cells of cows. Walters and Schallenberger (1984) suggested that since 97% of separate FSH pulses during the mid-luteal phase in cow were associated with P₄ pulses, it is possible that FSH could be the principal hormone that stimulates P₄ secretion. They maintained that this did not exclude a luteotropic action of LH in addition to a stimulatory action of FSH on release of P₄ in the cow. Pekala *et al.* (1983) proposed that there might be an interaction between oxytocin (from the CL) and LH in regulating P₄ synthesis, with oxytocin increasing the response to LH.

Mallory *et al.* (1986) reported premature luteal regression or reduced CL function if the luteotropic support from pituitary is disrupted during the formation of CL (\pm day 1 of the cycle).

Deficiencies in luteotropic support: Deficiency in luteotropic support might be due to:

- Short lived CL
- Inadequate CL

Short lived CL: Several studies have been carried out on insufficient luteotropic stimulus to maintain lifespan of subsequent CL during early postpartum period. Injection of microencapsulated GnRH promote normal luteal function by elevating LH concentration (Roberts *et al.*, 1989).

Inadequate CL: If it is accepted that luteotropin may not be sufficient to realize normal luteal function, then the question arises that when should the endogenous supplies need to be augmented.

Pearson and Lishman (1989) demonstrated that a luteotropic stimulus provided early in the induced cycle (day 3-5) improved luteal function to a greater extent than when PMSG was provided after day 5.

Progesterone concentration on day 10 after AI were higher in pregnant buffaloes than in buffaloes that showed embryonic mortality not associated with infectious agent. Similarly higher progesterone concentrations on day 20 of AI were found in pregnant buffaloes as compared to non-pregnant ones and buffaloes that showed embryonic mortality (Campanile *et al.*, 2005).

LH receptors within the CL and luteal function: Increased numbers of receptors for LH, on the granulosa cells of the bovine CL, could be a major factor in regulation of secretion of P_4 by the ensuing CL (McNeilly *et al.*, 1981). They also believed that there may not be a simple relationship between the binding of LH to luteal cells and the secretion of P_4 . Consequently, the failure of the short lived CL to recognize LH was not a factor that caused early luteal regression (Rutter *et al.*, 1985).

LH receptors are not deficient in the inadequate CL (Braden *et al.*, 1989). Hunter *et al.* (1988) concluded that lack of gonadotropin receptors was probably not a fundamental cause of premature regression of the short lived CL.

The role of the uterus and $PGF_2\alpha$ in subnormal luteal function

Presence of $PGF_2\alpha$ during early lactation: Thatcher *et al.* (1980) have demonstrated increased jugular plasma concentrations of prostaglandin metabolite (PGFM) during the early postpartum interval in milked dairy cows. Odde *et al.* (1980) suggested that premature regression of CL, which appeared to function normally for approximately 7 days after induced ovulation, was due to the luteolytic effect of $PGF_2\alpha$ needs consideration.

Oxytocin and the release of $PGF_2\alpha$: Luteolysis occur due to positive mechanism between luteal oxytocin and uterine $PGF_2\alpha$ which occurs at specific time of the oestrus cycle. Progesterone suppresses $PGF_2\alpha$ secretion when the concentration of progesterone receptors is high by inhibiting oxytocin in early part of oestrus cycle but as the cycle progresses these progesterone receptors decreases resulting in secretion of estrogen and oxytocin receptors. This result in uterine responsiveness to oxytocin which further facilitates positive mechanism between oxytocin and endometrial $PGF_2\alpha$. Various research studies conducted on normal oestrus cycles suggests that progesterone regulates the $PGF_2\alpha$ release by timing the initial peaks of secretion early in the oestrus cycle and later on by modulating the $PGF_2\alpha$ secretion until the corpus luteum regresses completely.

Increase in oxytocin concentrations in association with increase concentration of PGFM in cows with short luteal phases has also been reported by Peter *et al.* (1989). Zollers *et al.* (1989) compared short and normal luteal phases in beef cattle and found oxytocin induced premature release of $PGF_2\alpha$ on day 5 in short cycle which was not seen in normal cycles but the oxytocin induced $PGF_2\alpha$ release was similar to that observed at luteolysis on day 16 of normal cycle. Lesser No. of progesterone receptors as compared to more No. of endometrial oxytocin receptors were reported on Day 5 after estrus in postpartum beef cows expected to have short luteal phase than in cows with normal luteal phase (Zollers *et al.*, 1993). Evidences indicate involvement of oxytocin in release of $PGF_{2\alpha}$ in cows with short luteal cycles.

Research studies on anoestrus cows have suggested that progesterone treatment results in development of larger preovulatory follicles containing more estradiol 17- β than in control or short cycled cows and greater concentration of LH receptors in granulosa and theca layer in largest preovulatory follicle in postpartum cows pretreated with norgestomet than controls (Inskeep *et al.*, 1988). Increase in concentration of estradiol is associated with induction of endometrial progesterone receptors (Ing and Tornesi 1997). Cooper *et al.* (1991) and Kieborz-Loos *et al.* (2003) reported lower concentration of estradiol secretion by preovulatory follicle in short cycled cows will result in less no. of progesterone receptors in uterine endometrium and the first rise in progesterone and early $PGF_2\alpha$ secretion with short luteal phases.

Oxytocin administration during the early part of the cycle has short luteal lifespan in cycling cows and it was suggested that oxytocin functioned via elevated levels of uterine $\text{PGF}_2\alpha$ (Milvae and Hansel, 1980) in a manner similar to that of an IUD inserted early in the cycle in the ewes. Suckling has same effect via the pituitary release of oxytocin (Troxel and Kesler, 1984). When the CL releases oxytocin, it may cause $\text{PGF}_2\alpha$ release which in turn regresses the CL (Schams *et al.*, 1985). An imbalance in luteal levels of oxytocin was proposed as a cause of the eventual demise of the CL. Peter *et al.* (1989) were able to detect parallelism between concentration of PGFM and oxytocin in early postpartum dairy cows. Inskeep (1995) reported oxytocin to be an important factor that plays an important role in embryonic death of cows with short luteal phases. While McCracken *et al.* (1984) had earlier reported that luteal oxytocin and uterine prostaglandin facilitate luteolysis through reciprocal positive feedback mechanism. Zollers *et al.* (1993) reported that concentration of endometrial oxytocin receptors are greater at day 5 after estrus in cows with short lifespan of CL than in cows receiving progesterone and are expected to have normal lifespan.

Niswender *et al.* (2007) concluded that luteal $\text{PGF}_2\alpha$ is involved in luteolysis in sheep. According to him uterine $\text{PGF}_2\alpha$ initiates increased secretion of $\text{PGF}_2\alpha$ by corpus luteum and it also initiates mobilization of Ca^{++} in large luteal cells and secretion of oxytocin in large luteal cells. Oxytocin secreted by large luteal cells acts on small luteal cells and triggers mobilization of Ca^{++} in small luteal cells. Increase in intracellular levels of calcium leads to apoptotic death of the large and small luteal cells.

Negating the effect of $\text{PGF}_2\alpha$: Involvement of $\text{PGF}_2\alpha$ in premature regression of the CL is supported by the observation that treatment with substances which block the action of $\text{PGF}_2\alpha$ increased the functional lifespan of CL destined to be short lived in dairy cows (Dobson *et al.*, 1987).

Removing the source of $\text{PGF}_2\alpha$ (experimentally): Strong evidence that a premature release of $\text{PGF}_2\alpha$ from the uterus could be responsible for the early demise of the first induced CL in cows has been provided by Copelin *et al.* (1987). They demonstrated that the CL formed after early weaning of calves did not possess an inherently short lifespan because removal of the uterus did not result in premature luteolysis. Wright *et al.* (1988) showed that the previously gravid uterus had to be present to induce premature regression of the first CL, subsequent to induced abortion in heifers.

Demonstrating the release of $\text{PGF}_2\alpha$: Peter *et al.* (1989) showed increased concentrations of metabolite of $\text{PGF}_2\alpha$ (15 keto-13, 14 dihydro [PGFM] $\text{PGF}_2\alpha$) were detected early in the cycle when, the CL was short lived. Schrick *et al.* (1993) found greater concentrations of $\text{PGF}_2\alpha$ with lower embryo quality in uterine flushing of cows with short lived CL than normal luteal phases on day 6 and concluded that detrimental uterine environment was major cause of lowered fertility in cows with short luteal phases. Buford *et al.* (1996) concluded that inhibition of prostaglandin synthetase and lutectomy prevents detrimental effects of endogenous $\text{PGF}_2\alpha$ on the early embryonic stage in postpartum cows whereas lutectomy alone was effective in doing so in non-lactating cycling cows. Therefore, the lower survivability of embryos in postpartum cows might be due to the combined effect of uterine $\text{PGF}_2\alpha$ secreted on Days 4 through 9 (Cooper *et al.*, 1991) and luteal secretion of $\text{PGF}_2\alpha$ in response to the uterine signal (Buford *et al.*, 1996). Hockett *et al.* (2004) reported that $\text{PGF}_2\alpha$ might interfere with process of compaction of morula stage of embryo resulting in lower quality and viability of embryos. This might occur as alterations in gap junctions and cell adhesion

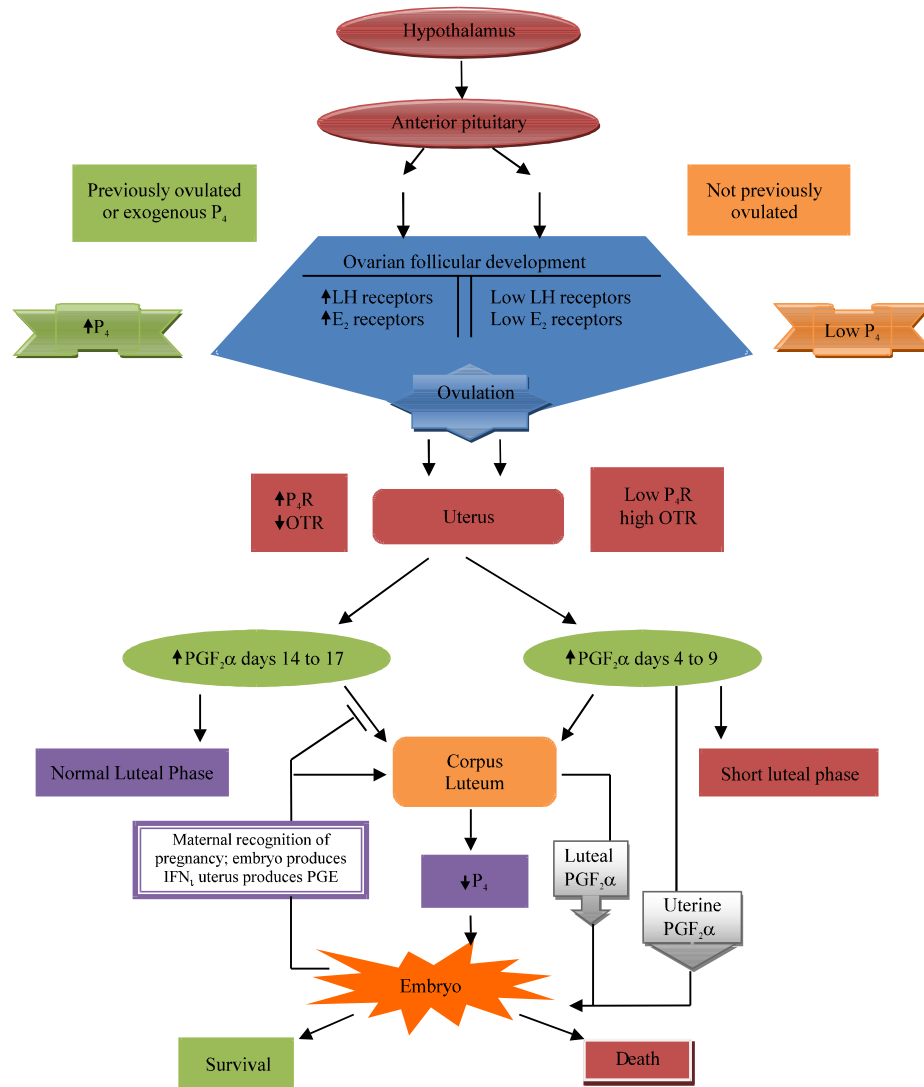


Fig. 2: Sequence of endocrinal events conducive to embryonic death or survival in short and normal estrous cycles (Perea and Inskeep, 2008)

molecules, induction of apoptosis mechanisms or alterations of gene transcription during embryonic development in compaction morula stage of embryo (Scenna *et al.* 2004).

Perea and Inskeep (2008) described the sequential endocrine events conducive to embryonic death or survival in short and normal estrous cycles (Fig. 2). According to them after parturition, the hypothalamic-pituitary axis stimulates progressive ovarian follicular development, estradiol production and expression of LH receptors in follicle. Without a previous cycle or exposure to progesterone (P₄)/progesterone priming followed by estrogen (E₂), uterine progesterone receptors (P₄R) are low and increase in progesterone after ovulation initiates immediate secretion of uterine PGF₂α which causes luteolysis and, if the animal has been mated, it is embryotoxic. Exposure of the uterus to progesterone followed by estrogen prior to first postpartum ovulation up regulates uterine progesterone receptors and contributes to establishment of progesterone dominance of the uterus. As the estrus cycle progresses progesterone binding to its receptors delays uterine secretion of

PGF₂α. If the animal is not mated/pregnant luteolysis is initiated by uterine secretion of PGF₂α that starts around day 14 to 17 and decreases progesterone production. If the animal is pregnant, the embryo produces Interferon-tau (IFN) while the uterus increases PGE and the corpus luteum is maintained allowing the embryo to survive. The whole process is known as maternal recognition of pregnancy.

DIAGNOSIS

Early embryonic deaths before regression of CL are indistinguishable from fertilization failure in that both cow and ewe return to estrus at the normal time. Death of one embryo in twin ovulating ewes may be undetected as pregnancy will continue. Several methods are used to determine embryonic mortality in cattle. The main being:

Examining embryos: Examining embryos collected by *in vivo* flushing of reproductive tract at different days after breeding.

Determining progesterone in blood, milk and saliva: Determination of progesterone in blood and milk, 21 days after oestrus is the most common method used in the pregnancy diagnosis of ruminants until the early nineties (Zoli *et al.*, 1992; Karen *et al.*, 2003). Prvanovic *et al.* (2009) in his study on monitoring of early pregnancy and embryonic mortality using blood progesterone concluded that it is impossible to determine embryonic mortality alone on the basis of progesterone profile while pregnant and non-pregnant cows can be easily distinguished 21 day post AI. They also concluded that it is very easy and accurate to distinguish non-pregnant cows from cows that have suffered early embryonic mortality.

CL dysfunction in human reproduction is identified by either of two criteria namely estimation of plasma or salivary P₄ levels or endometrial biopsy. Two or three plasma samples could be processed for the assay. Progesterone levels should not be less than 10 ng/ml during the luteal and mid luteal phase.

Pregnancy associated glycoprotein (PAG) test: The main advantage of Pregnancy Specific Proteins (PSP) for pregnancy diagnosis in cattle is their ability to prove the existence of placentation and the presence of live, vital embryos, while progesterone only proves the existence of corpus luteum. The most commonly used pregnancy specific protein for pregnancy diagnosis in cows is PAG (pregnancy-associated glycoprotein). Pregnancy-associated glycoprotein has been found in the serum of pregnant cattle and used as a pregnancy marker (Perenyi, 2002). As pregnancy failure occurs, PAG concentrations drop and disappear from maternal blood. The Pregnancy-Associated Glycoproteins (PAG) are synthesized by the monothand binucleate cells of the ruminant's trophoderm. Apart of it is released into maternal blood circulation which can be assayed by RIA and ELISA. RIA methods are very precise for measuring PAG concentrations in the maternal blood and milk of the ruminants. The sensitivity and specificity of this method are very high. The results are encouraging and use of milk and in blood for PAG test is helpful in detection of embryonic mortality in the ruminants (Sousa *et al.*, 2008). Prvanovic *et al.* (2009) in his study on monitoring of early pregnancy and embryonic mortality using PAG test concluded that embryonic mortality between 18-24 days after AI was evident from drastic decrease in PAG seven and half to nine days later and using PAG for pregnancy diagnosis enables us to prove the existence of live, vital embryos *in utero* 24 days after conception.

Ultrasound examination: Transrectal ultrasonography has been used to detect early pregnancy and to determine embryo/fetal death in recent years (Kahn, 1992; Romano and Magee, 2001). It is advantageous as it is a safe technique with no effects on embryo/fetus viability (Kahn, 1992; Ball and Longue, 1994; Baxter and Ward, 1997). It is advantageous over palpation per rectum pregnancy diagnosis in earlier diagnosis of pregnancy/non-pregnancy, determination of embryo/fetus viability, determination of number of embryos, reduction of misdiagnosis (false negatives and false positives) and reduction of potential iatrogenic embryo/fetus attrition (Romano and Magee, 2001). In research studies maximum sensitivity and negative predictive values were obtained from day 29 on in cows and from day 26 on in heifers.

TREATMENT TO IMPROVE PREGNANCY RATES

Various methods have been tried using different preparations for improving pregnancy rates by reducing embryonic mortality. They are as under:

Supplementing progesterone/progestogen: Research studies have reported that low concentration of progesterone can result in the development of a stronger luteolytic signal and hence it might be concluded that cows with lower plasma concentrations are apparently more prone to embryo loss. Macmillan and Peterson (1993) found that the conception rates to first insemination were increased when the CIDR device was inserted 6-8 days after insemination. Broadbent *et al.* (1992) recorded a significant increase in conception rate among cattle when Crestar ear implants (Norgestomet) were given on day 7.

Peters and Ball (1995) stated that progesterone supplementation might also cause suppression of endogenous luteotropic support due to increased negative feedback. While, Mann and Lamming (1999) demonstrated that supplemental progesterone was beneficial to fertility increasing conception rates when administered prior to day 6 after AI in lactating dairy cows.

Use of human chorionic gonadotropin (hCG): An alternative approach to increase the progesterone levels is by use of Human Chorionic Gonadotropin (hCG) to enhance the production of progesterone by the animals own corpus luteum. Administration of human chorionic gonadotropin (hCG) induces ovulation with the subsequent formation of a functional accessory CL which in turn increases progesterone and may enhance embryo survival. Studies have supported that conception rate is better in cows with three follicular waves after insemination as compared to cows with two follicular waves and hCG induction of three-wave cycles may also contribute to higher pregnancy rates. Thatcher and Collier (1986) and associates have indicated that the injection of hCG (2000 IU) i.m.; (1000 IU) i.v. 5 days after oestrus induces ovulation of the first wave dominant follicle and formation of accessory corpus luteum and increases plasma progesterone levels during the luteal phase. Santos *et al.* (2001) demonstrated that injecting 3300 IU of hCG in lactating cows 5 days after AI resulted in increased number of CL and higher plasma progesterone concentrations. Conception rates on days 28, 42 and 90 were improved by hCG treatment. The findings of Santos *et al.* (2001) were supported by findings of Nishigai *et al.* (2002), they administered hCG on day 6 and the pregnancy rates were increased (67.5%) with formation of accessory corpora lutea as compared to control cows (45.0%). or cows receiving hCG on day 1 (42.5%) after AI Lopez-Gatius *et al.* (2002) reported that cows having an additional spontaneous CL were eight times less prone to fetal loss than those with a single CL.

PMSG (pregnant mare serum gonadotropin) administration: Hirako *et al.* (1995) in their study on luteotropic effect of PMSG in cattle and reported significant increase in progesterone concentration on administration of 500 IU of PMSG on day 7 after estrus. They concluded that PMSG treatment increases progesterone secretion and luteal function without excessive follicular development.

Gonadotropin releasing hormone (GnRH) treatment: Administration of GnRH (250 µg or greater) at the time of insemination increases pregnancy rates by 12.5% and effect was more pronounced in repeat breeder cows (22.5%) (Morgan and Lean, 1993). Administration of GnRH at oestrus increases serum progesterone levels and the proportion of large luteal cells in the corpus luteum (Mee *et al.*, 1995).

MacMillan *et al.* (1986) reported an enhancement of the conception rate in dairy cows when GnRH (10 µg Buserelin) was injected on day 11 after breeding by AI. Injecting Buserelin at 3 days interval from day 12 (luteal phase) increases progesterone concentrations and maintains at luteal levels till the injections are continued, i.e., until day 48 after the preceding oestrus (Thatcher *et al.*, 1989). This indicates that buserelin exerts a continued luteotropic or antiluteolytic effect under these circumstances.

Administration of hCG during luteal phase of the cycle induces ovulation of the dominant follicle (Price and Webb, 1989). Administration of GnRH on day 6 of the cycle resulted in ovulation in 75% animals with the formation of accessory corpora lutea (Webb *et al.*, 1992). This not only induces additional progesterone secretion, but also downregulates oestradiol production.

Mann *et al.* (1995) reported that administration of buserelin on day 12 after insemination results in reduced plasma oestradiol concentrations and suppression of pulses of the PGF₂α metabolite (13, 14-dihydro-15-keto-PGF₂α (PGFM) from about day 13 onwards, confirming its antiluteolytic effect. Reduction in oestradiol concentrations at this time might inhibit the luteolytic mechanism and hence, pregnancy is maintained. They also concluded that GnRH treatment weakens rather than delays the luteolytic signal. The timing of buserelin/GnRH treatment appears critical since treatment at other times after insemination did not have any effect (MacMillan *et al.*, 1986; Drew and Peters, 1994).

Interferons: Intrauterine administration of recombinant ovine/bovine IFN_t resulted in extension of the oestrous cycle and abolished the oxytocin induced PGF₂α secretion on day 17 (Meyer *et al.*, 1995). IFN_t is more effective than IFN_α in preventing embryonic mortality as it has no side effects.

Omega-3 and reproductive performance: Research studies by workers (Mattos *et al.*, 2000, 2002; Thatcher *et al.*, 2001) have indicated that omega-3 fatty acids decreases secretion of PGF₂α. Trials by research workers (Bonnette *et al.*, 2001; Mattos *et al.*, 2002; Petit and Twagiramungu, 2002; Ambrose and Kastelic, 2003) with natural sources of omega-3 fatty acids such as Eicosapentaenoic Acid (EPA), Dehydroascorbic Acid (DHA) and α-linolenic acids have indicated that these fatty acids are capable of decreasing the secretion of PGF₂α and compliment the antiluteolytic action of IFN_t thereby improve pregnancy rates. EPA and DHA also have anti-inflammatory and immunosuppressive effects that compliment the normal immunosuppressive and anti-inflammatory effects of progesterone and IFN_t in early pregnancy.

CONCLUSIONS

EED is the major cause of reproductive and economic loss in cattle. Though many factors have been incriminated but luteal function plays an important role. Embryonic death occurs the time of maternal recognition of pregnancy, probably related to a failure of the IFN γ secretory mechanism. Recent research, both in terms of physiological mechanisms and pharmacological treatments has mostly focused on the period of maternal recognition of pregnancy or the anti-luteolytic effect. Ovarian examination, blood/milk progesterone levels, PAG test and ultrasound appear to be the only practical tool presently available. hCG/GnRH/P₄ supplementation have shown positive results. Supplementation of interferon as anti-luteolytic agent and supplementing Omega-3 has shown encouraging results.

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