

Highlighting factors contributing to pregnancy loss in beef cattle*

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Abstract

Pregnancy loss in beef cattle remains a costly problem for producers, leading to diminished calf crop uniformity and reduced percentages of cows with a calf at the end of calving season. Although several tools exist to ascertain pregnancy status, the first 30 days of pregnancy encompasses the period with the greatest proportion of pregnancy losses and these losses often occur before traditional methods permit pregnancy determination. The ability to accurately predict pregnancy failure remains a major limitation. Blood-based assays detecting chemical changes in maternal circulation have provided insight into embryonic and fetal monitoring and are used to make predictions for pregnancy loss. Although there are certain unknown aspects to the etiology of pregnancy loss, there is growing body of work to identify physiological biomarkers within the maternal, paternal, and embryonic systems to clarify risk factors for pregnancy failure. This review highlights a few of the factors contributing to pregnancy loss and the rapidly evolving methods utilized to predict pregnancy failure. Further, this review highlights a few of the changes to parental physiology after exposure to various environmental factors, the consequences on the physiology of pregnancy and the likelihood of pregnancy success.

Keywords: Cattle, pregnancy, placentation, maternal, sire, environmental

Introduction

Embryonic and fetal mortality remain as major issues in domestic livestock production. Although embryonic mortality and pregnancy loss research have garnered industry-wide attention, a major limitation within is the inability to accurately predict pregnancy failure and determine early embryonic and fetal viability. Additionally, predicting pregnancy loss in beef cattle is difficult in enterprises that are less intensely managed than dairy operations that collect data to compile reproductive outcomes. Early pregnancy contains several critical developmental milestones and pregnancy losses can occur throughout this period. There are continued discussions within reproductive physiology to determine the period of most substantial embryonic loss. Fertilization rates in beef cattle are estimated quite high (~90%) but attrition occurring throughout pregnancy indicated that the underlying issue of pregnancy loss extends beyond the period of successful conception.¹

Indeed, embryonic mortality (0-45 days after insemination) is estimated to affect 54% of beef cattle after a single insemination.¹ Within this period, roughly 16% of this embryonic loss

occurs between days 16-32 and ~6% of these losses occurs between days 29-45.¹ Some suggest that embryonic mortality is most substantial between embryo hatching and elongation although others suggest that it is most substantial during and after maternal recognition of pregnancy and early placentation.^{2,3} These periods of embryonic development encompass the processes of maternal recognition of pregnancy and early placentation, possibly indicating that pregnancy loss may be attributed to either failed embryonic signaling or improper placentation events. Therefore, the issue of pregnancy loss is likely multifactorial.

Although the timing of embryonic mortality and pregnancy loss in cattle can vary substantially based on production status, parity, genetic composition, breeding method, and management conditions there are apparent embryonic, maternal, paternal, and environmental factors known to contribute to pregnancy failure (Figure 1). Aim of this review is to highlight some of the current known and hypothesized contributions to pregnancy loss experienced in beef cattle and the evolving methods on how pregnancy loss is predicted.

Measuring reproductive efficiency

Before discussing various components of pregnancy loss, it is important to first understand how to measure and manage

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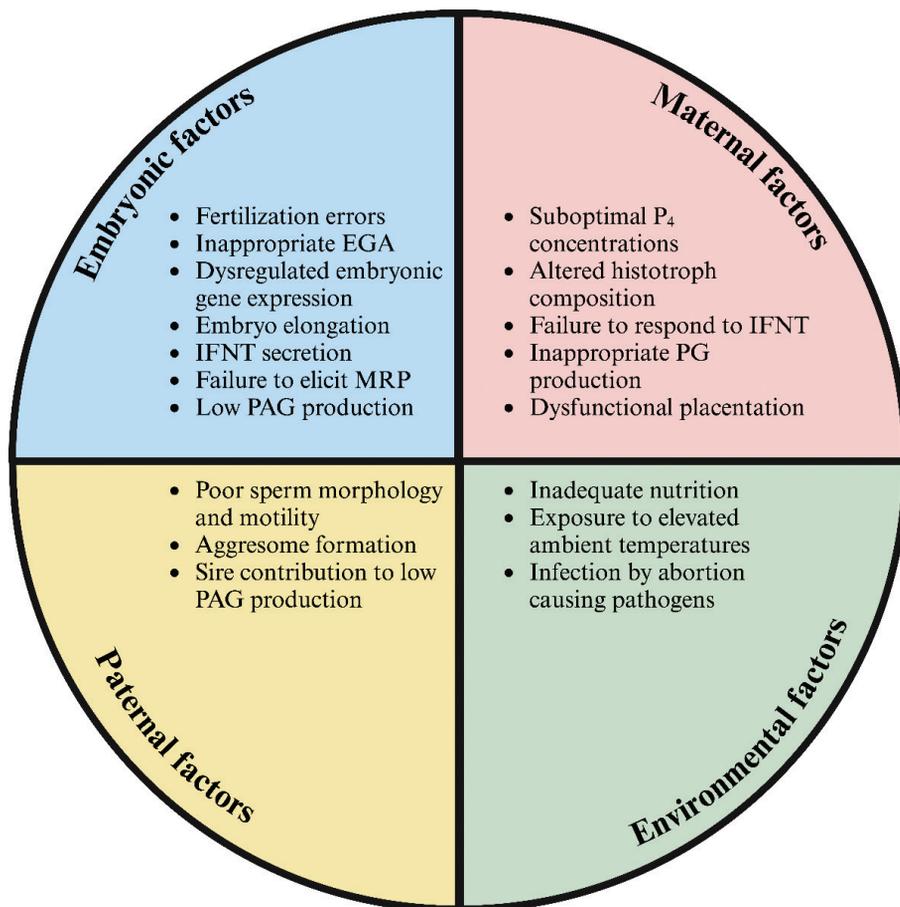


Figure 1. Proposed factors contributing to pregnancy loss in beef cattle; pregnancy loss is a multifactorial issue and is likely a reflection of inadequacy at the embryonic, maternal, paternal and environmental levels (EGA: Embryonic genome activation, MRP: maternal recognition of pregnancy, PAG: pregnancy associated glycoproteins, IFNT: interferon-tau, P₄: progesterone PG: prostaglandins).

these losses. Pregnancy determination is a vital tool to measure reproductive efficiency; the generated data are used to directly monitor farm reproductive success or to indirectly study trends in national fertility. It has been estimated that pregnancy loss in beef cattle in the USA results in a gross loss of \$3.7 billion in profit per year.⁴ Despite this, only 20% of USA beef operations utilize pregnancy diagnosis. Thus, there remains a clear disconnect between the financial benefits and the utilization of pregnancy data. The lack of adoption of pregnancy diagnosis likely stems from a lack of trained technicians, increased input costs associated with labor and service fees, the need for adequate handling facilities, and/or the effects of handling stress on cattle.

Fortunately for producers, there are 3 primary methods to consider for pregnancy diagnosis; each method has advantages and disadvantages over the others. The most traditional method is transrectal palpation of uterine contents. Transrectal palpation is often completed by certified personnel at least 40 days after breeding and offers an affordable means to detect conceptus and associated membranes without the use of costly equipment. Despite the cost effectiveness of transrectal palpation, the accuracy of pregnancy determination remains dependent on the skill of the technician. The precise determination of aging via transrectal palpation before day 40 is dependent solely on the detection of the cardinal signs of pregnancy (displacement of

the chorio-allantoic membrane, palpation of amniotic vesicle, palpation of fetus or palpation of placentomes).⁵ Although transrectal palpation of uterine contents are used to estimate age of pregnancy, this method is limited in its ability to accurately estimate embryonic viability as assessed by the heartbeat.⁶

Ultrasonography is becoming quickly adopted amongst beef producers. Unlike transrectal palpation of uterine contents, ultrasonography can determine pregnancy as early as 28 days after breeding and is a more accurate method to determine fetal age through the measurement of crown rump length.^{7,8} Ultrasonography also provides producers an earlier opportunity to make a management decision compared to transrectal palpation.^{6,7} Another benefit of ultrasonography is the ability to determine fetal sex from days 60-90 of pregnancy. Knowing fetal sex is an advantage for seedstock producers who can plan what sex of breeding animals they will market in upcoming sales. Although ultrasonography is becoming more economical and more accurate as technology advances, it remains a costly tool for practitioners that imparts costs to producers. Likewise, although ultrasonography can detect pregnancy earlier than transrectal palpation of uterine contents, it does not prevent the possibility of loss after pregnancy diagnosis.

Lastly, one of the newest technologies utilized for pregnancy determination are blood-based pregnancy tests. Similar to

ultrasonography, blood or milk-based tests are performed as early as 28 days after breeding and rely on the detection of a family of proteins called pregnancy associated glycoprotein (PAG) in maternal circulation. Detectable concentrations of PAG are only present in maternal circulation when the animal is pregnant or just recently calved or aborted. One advantage of blood or milk tests is that producers do not require substantial training or, in the case of rapid tests, expensive equipment to perform. Additionally, blood or milk-based tests also offer pregnancy status determination for operations that are located far away from a technician capable of performing transrectal palpation or ultrasonography; furthermore, travel expenses for technicians to get to an operation are eliminated. Blood or milk tests are performed either chute side or shipped to laboratories across the country for PAG quantification. Chute-side results are generated within 20 minutes, whereas blood shipped to the laboratory generally have results within a week. Therefore, the ability of producers to quickly make management decisions pertaining to pregnancy status relies on the type of test utilized. Commercially available blood-based pregnancy tests have true-positive rates of 93-98% and false-positive rates of 1-7%.^{9,10} Thus, one clear limitation of blood or milk-based pregnancy determination is the risk of false-positives for animals experiencing embryonic mortality occurring after collection of blood or milk used for initial pregnancy status determination and the period of secondary confirmation via blood or milk test. Cows that recently experienced embryonic mortality or immediately after parturition maintained elevated concentrations of PAGs.^{11,12} Collectively, each of these methods of pregnancy determination are suited for various operations but each of them ultimately results in the same outcome, increased reproductive efficiency by determining nonpregnant status earlier. Ability to accurately determine pregnancy status allows producers to make management decisions, ultimately improving farm profitability and sustainability of beef production.

Embryonic contributions to pregnancy loss

First week of pregnancy encompasses the greatest proportion of pregnancy loss in beef and dairy cattle.^{1,2} During this period there are major developmental milestones that are critical for pregnancy success such as fertilization, embryonic genome activation, blastocyst formation, and hatching from the zona pellucida. A meta-analysis compiling data from 12 studies investigating pregnancy loss in beef cattle through day 7 of pregnancy was 28.4% and within these studies, before day 4, pregnancy loss was 23%.¹ In dairy cattle, it has been estimated that only ~ 50% of ovulated oocytes will generate viable embryos between days 6 and 8 after insemination.² Given the large proportion of attrition occurring during the first week of pregnancy, discussing specific embryonic factors associated with pregnancy failure is warranted.

Estimated rates of fertilization in beef and dairy cattle are predicted to be ~ 90%, indicating that substantial losses occur after gametic syngamy.¹³⁻¹⁵ Although the consensus is that ovulated oocytes do not differ in the ability to undergo fertilization, given compliance with accurate artificial insemination (AI) techniques, there are factors prior to ovulation that can impact oocyte developmental competence. Indeed, manipulating the periovulatory hormonal environment can influence fertility outcomes. One such example is in cows that ovulated smaller follicles at estrus had fewer pregnancies per AI compared to cows that ovulated larger follicles.¹⁶ Evidence from dairy cattle indicated that follicular growth is altered when progesterone concentrations are lower, hindering oocyte maturation and ability to establish pregnancy.¹⁷ Although it

should be acknowledged that many determinants of embryonic competence are influenced by preovulatory factors, the activation of the embryonic genome also presents a critical period of embryonic mortality. Embryonic genome activation, also called the maternal-to-embryonic transition, is a transitory period occurring around the 8-16 cell stage of embryonic development whereby the early embryo degrades maternal RNAs and proteins and begins transcription and translation of the newly formed genomic products.^{18,19} The molecular mechanisms regulating embryonic genome activation have yet to be fully elucidated; however, failure of the embryo to complete this transition results in the inability of the embryo to continue. Collectively, identifying optimal periovulatory physiology and regulators of the maternal-embryonic transition present opportunities to improve embryonic survival and mitigate pregnancy losses.

The high incidence of pregnancy loss during the first weeks of pregnancy has driven concerted efforts to try and predict embryonic competence to sustain pregnancy to term.²⁰ These efforts have been focused on identifying biomarkers indicative of pregnancy success during the preimplantation and postattachment periods of development. Early statistical models utilizing calving data from more than 4,500 embryo transfers attempted to predict embryo survivability to term and revealed that only 50-70% of embryos and recipients are competent to result in a calf.²¹ These authors concluded that factors external to the embryo (e.g. recipient) are critical regulators to embryonic survival or loss.²¹ As technology has advanced, the tools utilized to predict embryonic competence have also expanded. Indeed, the use of machine learning to incorporate highly detailed models with developmental outcomes has been explored to identify embryonic genes predictive of competence. One study that combined transcriptomic data from blastocysts of known developmental competence with transcriptomic data of long and short conceptuses identified differentially expressed genes amongst the populations and integrated these genes into pathways predictive of embryonic competence and conceptus elongation.²⁰ The 341 differentially expressed genes associated with embryonic competence were annotated to pathways relating to metabolic processes, glycolysis/gluconeogenesis, and glycerolipid metabolism.²⁰ Similarly, 669 genes associated with embryonic incompetence were annotated to the spliceosome, RNA processing, and cell cycle regulation.²⁰ Together, these authors suggest that specific transcriptional patterns within the first weeks of pregnancy are identified as predictors of embryonic success, but also, the pathways identified may reveal novel targets for reducing pregnancy failure. The authors further utilized machine learning to discriminate differentially expressed genes into genes predictive of pregnancy success or failure and identified 8 genes (*CHSY1*, *GSTO1*, *TPI1*, *CCNA2*, *CDK7*, *EIF4A3*, *LSM4*, and *YWHAG*); the first 3 are predicted to be expressed within competent blastocysts and last 5 expressed in incompetent blastocysts.²⁰ Collectively, this work has provided insights into the molecular signature of embryos predicted to lead to pregnancy success. Further works remain to test the validity of these candidate biomarker genes and to interrogate the pathways regulating embryonic competency; however, the increased utilization of next generation sequencing and machine learning will permit further understanding of the processes leading to pregnancy success.

Failure to elicit maternal recognition of pregnancy

Around day 16 of the normal estrous cycle, follicular estradiol concentrations begin to increase that stimulate the actions of estrogen receptor alpha leading to increased

expression of oxytocin receptors in the endometrium, ultimately perpetuating pulsatile secretion of prostaglandin $F_{2\alpha}$ (PGF_{2 α}).²² These PGF_{2 α} pulses act as the primary luteolytic signaling molecule causing regression of the corpus luteum, thus beginning a new estrous cycle. When a conceptus is present within the uterine lumen, however, interferon tau (IFNT) secreted by the trophoblast cells in the placenta work to block the luteolytic cascade and maintain the corpus luteum. This process, deemed maternal recognition of pregnancy, is also heavily reliant on embryonic secreted factors. Although IFNT is primarily attributed to its role in cycle extension, IFNT also elicits robust changes in the local endometrial landscape and to peripheral physiology. For example, IFNT secreted by the conceptus will exit from the uterine vein and increase the expression of interferon stimulated genes (ISG) such as *ISG15*, *MX1*, *MX2*, and *OAS1* in extrauterine tissues such as circulating blood cells and the corpus luteum compared to nonpregnant controls.²³⁻²⁵ The dynamic changes to extrauterine expression in ISGs have been utilized as a predictor of pregnancy loss. Indeed, recent works have demonstrated an association between elevated concentrations of ISGs and pregnancy maintenance in dairy cattle.²⁶ Currently, the physiological implication of IFNT-induced changes to maternal immune cells in the periphery are not clear. Transcriptomic analyses of peripheral leukocytes collected 21 days after embryo transfer have been conducted to compare between cows that maintained or lost pregnancy.²⁷ Sequencing revealed that the top upregulated pathways were related to inflammatory chemokine activity and immune defense response, suggesting that IFNT has a role in modulating immune tolerance.²⁷ The roles of conceptus-derived IFNT promoting immune tolerance and recognition of pregnancy in extrauterine tissues require further study, and understanding the mechanisms of immune tolerance in cattle presents a unique opportunity to mitigate pregnancy failure by identifying key regulatory pathways.

Another consideration when discussing IFNT is the abundance or dose of IFNT secreted by the trophoblast cells. It is known that endometrium responds differently to shorter and longer conceptuses.²⁸ This finding is interesting given recent *in vitro* data indicating that endometrial explants had dose- and period-dependent changes to gene expression in response to IFNT.²⁹ Taken together, these data implied that inadequate or inappropriately timed secretion of IFNT by the conceptus can fail to elicit a response within the endometrium and ultimately fail to rescue corpus luteum. Thus, failure of the conceptus to secrete sufficient amounts of IFNT could contribute to pregnancy loss. Given that secretion of IFNT is dependent on the embryo, future studies should investigate whether the conceptus must secrete baseline concentrations of IFNT to elicit a maternal response. Lastly, although IFNT is known to induce changes to the endometrial transcriptome, endometrium also responds to other secreted factors independent of IFNT.³⁰ A novel experiment cultured endometrial explants with either a day 15 conceptus derived from *in vitro* fertilization, day 15 conceptus derived from AI, 100 ng/ml of recombinant IFNT, or medium alone revealed 240 differentially expressed genes in endometrial explants cultured in the presence of conceptus, regardless of origin.³⁰ Indeed, infusion of conceptus-derived prostaglandins into the uterine lumen of cyclic ewes induced changes in the expression of classical Type I interferon-stimulated genes *ISG15* and *RSAD2*.³¹ Collectively, roles of prostaglandins during the periattachment period warrant further study. In particular, roles of prostaglandins secreted by the conceptus and the endometrium as they pertain to attachment need further exploration.

Attachment failure at the embryonic-maternal interface

Although evidence strongly indicates that the proportion of pregnancy loss decreases as pregnancy progresses, the incidence of pregnancy loss during the late embryonic and early fetal period is limited in the characterization.¹ This period of embryonic development occurs after maternal recognition of pregnancy ~ days 21-28 of pregnancy, encompassing the earliest phases of placental apposition, adhesion, and attachment.^{32,33} During this period of pregnancy, the placental trophoblast cells begin to differentiate into 2 morphologically recognizable populations delineated by the number of nuclei (mononucleated or binucleated).³² Binucleated trophoblast cells comprise 15-20% of trophoblast cells and will migrate through microvillar junctions of the uterine luminal epithelium, ultimately forming a fetal-maternal syncytium.³⁴ Trophoblast binucleate cells upon final maturation begin to rapidly express and secrete PAG.³⁵ The PAG family of proteins represent a group of aspartic proteinases that are highly expressed products of the cetartiodactyla placenta.³⁵ At the placental-epithelial interface, there is an abundance of PAG-positive cells beginning around day 21.³³ Functional roles of PAG are still explored; a study treated endometrial explants isolated from pregnant and nonpregnant animals 18 days after estrus with an equal mixture of PAG 4, 6, and 9 resulted in transcriptional changes for proteins associated with matrix remodeling, chemokine production and prostaglandin release.³⁶ Thus, changes elicited in response to PAG locally in the endometrium and peripherally need to be investigated. In particular, the immunomodulatory roles of PAG during the periattachment period warrant further study.

As aforementioned, PAG concentrations within maternal circulation are an accurate marker to predict embryonic mortality.³⁷ Pregnant cows with higher peripheral concentrations of PAG experienced increased embryonic survival compared to those with lower circulating PAG concentrations (Figure 2).^{11,38} Additionally, it has been documented that cows undergoing late embryonic mortality had differing circulating PAG patterns compared to those that maintained pregnancy (Figure 2).¹¹ Given that PAG concentrations in maternal circulation are associated with embryonic competence or mortality, roles of PAG in the physiology of pregnancy competence are explored. Recent evidence has indicated that cows with higher placentome blood perfusion have elevated concentrations of circulating PAG compared to cows with lower placentome blood perfusion.³⁹ In lactating dairy cattle, delayed increases in PAG was indicative of inappropriately timed or insufficient embryonic attachment.²⁶ Collectively, the PAG-mediated physiological changes at the placental-maternal interface are still early in the characterization. The literature has established that PAGs, an embryonic product, are a strong measure of fetal monitoring and are incorporated in parametric analyses to predict pregnancy failure. However, the specific molecular and cellular functions of PAG have not been completely elucidated and therefore researchers are interested in understanding the roles of PAG in quintessential aspects of placentation, such as tissue remodeling.

In summary, the embryo represents a major contributing factor to the success or failure of pregnancy. Therefore, there are many pivotal risk periods for determining embryonic success for pregnancy. Current and future works should investigate and test more precise predictors of embryonic competence. Furthermore, understanding the molecular and cellular physiology regulating positive pregnancy outcomes could be used

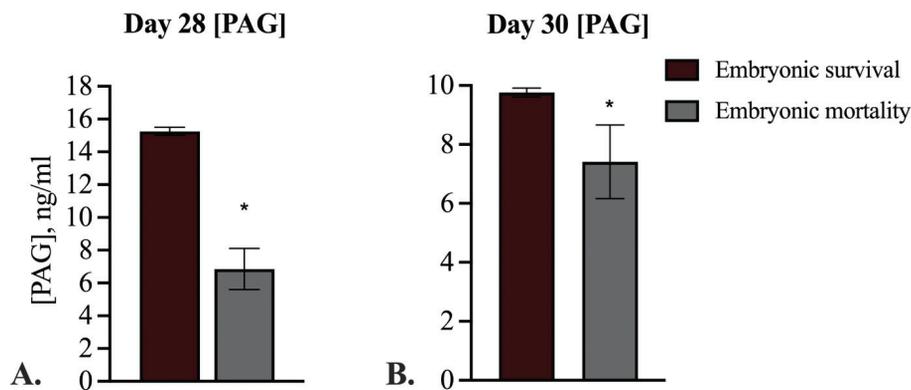


Figure 2. Relationship between peripheral PAG and embryonic survival: A. Cows with lower concentrations of plasma PAG on day 28 of pregnancy experience higher pregnancy loss compared to cows that maintained pregnancy (adapted¹¹); B. Cows experiencing embryonic mortality have lower concentrations of plasma PAG on day 30 of pregnancy compared to cows with embryonic survival (adapted³⁸).

to study the supplementation of factors that may be lacking in embryos progressing towards failure.

Contributions of the maternal uterine environment to pregnancy loss

Maternal-embryonic communication is critical in achieving reproductive success, and the uterus responds dynamically to the embryo throughout pregnancy. Secretion of specific proteins, hormones, and growth factors by the embryo and endometrium within the lumen are essential for the establishment and maintenance of pregnancy. Therefore, understanding this dialogue is necessary for elucidating the underlying reasons for pregnancy loss. The fundamental role of the endometrium prior to the development of a functioning cotyledonary placenta is to support rapid embryonic development and conceptus growth.⁴⁰ Thus, the uterine environment is a critical regulator in pregnancy success or failure. Prior to conceptus attachment to the luminal epithelium, the conceptus relies on endometrial secretions, also called histotroph, to support cell proliferation, migration and the morphological changes that occur during elongation.⁴¹ Perturbing the native uterine histotroph composition by flushing the uterine lumen with saline on days 4 or 7 after estrus reduced pregnancy per embryo transfer by 33.1 and 30.6%, respectively.⁴² This evidence is supported by ovine models of uterine gland ablation, whereby histotroph composition is dramatically altered, and hatched blastocysts are unable to undergo elongation.⁴³ Furthermore, machine learning has been utilized to integrate the endometrial transcriptome 7 days after estrus with pregnancy outcomes to predict genes linked to uterine receptivity.⁴⁴ In this discriminant analysis, 50 genes were identified and could predict uterine receptivity with an overall accuracy of 96.1%, regardless of the breed of the animal.⁴⁴ Therefore, inadequate transcription or secretion of necessary factors within the uterus can contribute to pregnancy loss. Coincidentally, identifying secreted factors within the uterine lumen that are critical for embryonic development presents an opportunity to improve pregnancy outcomes and mitigate pregnancy losses.

Failure to respond to conceptus-derived signals and maintain the corpus luteum

As previously mentioned, the bovine conceptus begins to secrete IFNT around day 16, and this type 1 interferon elicits

robust responses in the endometrium, ultimately extending the life of the corpus luteum. The signaling actions of IFNT are perpetuated in a paracrine manner are reviewed.⁴⁵ Evidence from sheep has demonstrated that IFNT signals via the interferon alpha and beta receptors on luminal endometrial epithelial cells to inhibit expression of *ESR1* and *OXR*, ultimately diminishing the pulsatile release of endometrial $PGF_{2\alpha}$.^{45,46} Thus, the ability of the endometrium to respond to IFNT is critical to successful maternal recognition of pregnancy and maintenance of the corpus luteum. Recent work in dairy heifers has revealed that the endometrial responsiveness to IFNT is variable among individuals, and this variability is associated with subsequent fertility.⁴⁷ After intrauterine infusion of recombinant IFNT, the endometrial transcriptome had differences in expression between heifers classified as highly fertile and subfertile and these genes were associated with cell signaling, metabolism, attachment, migration, and extracellular matrix proteins.⁴⁷ Further, subfertile heifers had lower concentrations of glycerol and oxylipins derived from arachidonic acid within uterine luminal fluid after IFNT infusion.⁴⁷ Together, this work indicated that individual animals may have differential responsiveness to IFNT signaling. In the context of pregnancy loss, identifying regulators of IFNT sensitivity present an opportunity to improve fertility outcomes, and although the secretion of IFNT is dependent on the embryo, it is worthy of considering if the endometrium has a threshold of sensitivity to various doses of IFNT that is sufficient to elicit a signaling response.

Roles of aberrant maternal prostaglandins in pregnancy loss

Prostaglandins are lipid-based signaling molecules that have essential roles in regulating bodily processes. Prostaglandins are synthesized from arachidonic acid, and the synthesis of various prostaglandins is mediated by the cyclooxygenase (COX) family of proteins. In cattle, prostaglandins ($F_{2\alpha}$ and E_2) begin a definitive increase between days 31-35 of pregnancy and increases in the concentrations of these hormones have been positively correlated with pregnancy.^{48,49} The expression of *PTGS2* (COX2) is upregulated during ovine implantation and is directly related to the degree of invasion of the trophoblast cells.⁵⁰ Furthermore, *PTGS2* in the mouse model had upregulated during placental development and is therefore involved in decidualization and angiogenesis, promoting placental development.⁵¹ As mentioned above, estrus cyclicity in

cattle is highly dependent on appropriate secretion and regulation of prostaglandins from the endometrium. Indeed, during the maternal recognition period, the ablation of $\text{PGF}_{2\alpha}$ pulses is necessary for the maintenance and prolonged lifespan of the corpus luteum; however, during normal cyclic scenarios in the absence of a conceptus, prostaglandin will reach the corpus luteum and initiate luteal regression. Although $\text{PGF}_{2\alpha}$ is of critical importance to maintain normal cyclicity, the exact roles of $\text{PGF}_{2\alpha}$ during placentation are unknown.

The conceptus begins to synthesize and secrete prostaglandins around day 13 of pregnancy, eliciting changes to endometrial gene expression.³¹ Further, circulating $\text{PGF}_{2\alpha}$ concentrations increased during days 31-35 in beef cows that maintained pregnancy compared to cows that underwent embryonic mortality.⁴⁸ This period of pregnancy corresponds to the period of active placentation, suggesting that $\text{PGF}_{2\alpha}$ may be necessary in facilitating proper attachment of the bovine embryo. Indeed, placental cells are under strict hormonal control; therefore, these hormones tightly regulate the abundance and activity of proteins involved in extracellular matrix remodeling at the placental-endometrial interface.⁵² Treating caruncular epithelial cells isolated from pregnant cows with $\text{PGF}_{2\alpha}$ increased cell viability and adhesion.⁵² Therefore it is likely that appropriate secretion of prostaglandins from either the conceptus and/or endometrium are required to facilitate early placentation events. Further, bovine endometrial cells treated in vitro with indomethacin and aspirin (prostaglandin inhibitors), experienced decreases in cell proliferation and cell viability.⁵³ Collectively, it is becoming more apparent that prostaglandins are having a critical role in facilitating dialogue between the conceptus and endometrium, having a role in placentation events such as extracellular matrix remodeling and adhesion, and therefore may be targets for improving pregnancy loss.

There are still many unknowns regarding the maternal contribution to pregnancy loss in cattle. Likewise, there are consistently novel findings published relating to the structure and

establishment of the bovine placenta, the dynamic endocrine milieu of pregnancy, and the delicate cell-cell interactions at the placental-endometrial interface that will aid in the understanding of the mechanisms leading to late embryonic/fetal loss in bovine. A proposed model of key maternal factors contributing to pregnancy failure is depicted in Figure 3.

Paternal contributions to pregnancy loss

Although the sire is traditionally speculated to only contribute half of the genetic material to the oocyte during fertilization, this line of thinking is rapidly challenged as more information pertaining to paternal contributions to pregnancy are revealed. Further, although research regarding pregnancy loss has mainly focused on maternal and embryonic contributions, there is considerable variation in pregnancy loss among sires.⁵⁴⁻⁵⁷ Consequently, variation in genetic indices to measure sire fertility have been developed and rapidly utilized in dairy bulls; however, genomic-based tools have been less utilized in beef herds because of the lack of data and validation of quantitative trait loci.^{58,59} Consequently, sire phenotypic data have been utilized to make associations with fertility. Indeed, associations among bull libido, scrotal circumference, backfat thickness, testis weight, semen quality parameters and sire residual feed intake have all been linked with variation in reproductive function.^{60,61} Given that genetic selection parameters for beef bull fertility are limiting, other avenues have been explored to evaluate sire fertility and predict sires that will support embryonic development.

Although poor sperm morphometric parameters are useful for chute-side breeding soundness exams to quickly rule out unacceptable bulls, other more quantitative measurements are utilized to improve sire fertility. Computer assisted sperm analysis (CASA) monitors sperm movement and applies algorithms to the behavior of sperm cells to estimate sperm cell morphokinetic defects in real time⁶²; CASA is used to predict beef bull fertility in timed AI systems.⁶³ Compared to other

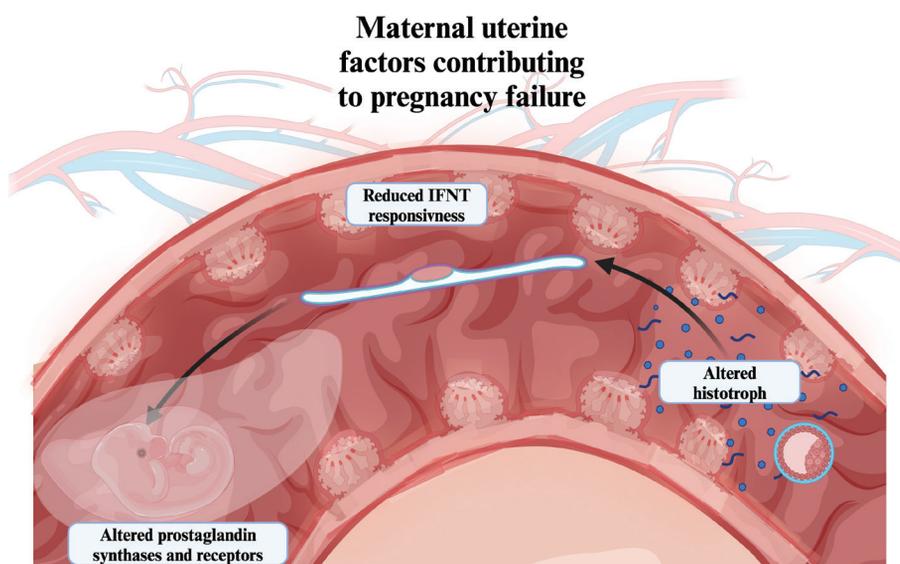


Figure 3. Conceptual model of maternal factors contributing to pregnancy failure. Perturbations in luminal histotroph composition, variance in the endometrial response to interferon-tau, and altered prostaglandin metabolism may all contribute to pregnancy failure at various stages of pregnancy. Identifying key regulators of embryonic survival during milestones of pregnancy will permit a deeper understanding of pregnancy loss.

various qualitative measurements assessed in the study, CASA had the greatest coefficient of determination for fertility.⁶³ This study also generated a composite model by incorporating CASA data with image-based flow cytometric analyses, and together these 2 metrics were successful in predicting bull fertility.⁶³ One recent study classified 2 groups of bulls as high or low fertility sires and attempted to objectively measure sperm quality.⁶⁴ One promising method was the detection of aggresomes located in the head of the sperm. Aggresomes are a buildup of unwanted proteins that occur after a failure in protein modification and are associated with cell death.⁶⁴ Interestingly, low fertility sires had a significantly higher amount of aggresomes located in the head of the sperm cell (via fluorescent microscopy) prior to a gradient purification commonly used for in vitro fertilization, but this difference between fertility groups disappeared after purification.⁶⁴ Further study of aggresome defects identified that the low fertility sires had a much higher percentage of aggresome defects via image-based flow cytometry.⁶⁴ Collectively, new molecular insights are developed to quantitatively evaluate sperm cell dynamics and integrate these data into predictive indices independent of paternal genomics. Advancing sperm cell diagnostic and predictive abilities will work in part to reduce pregnancy failure by minimizing errors attributed to inherent sperm defects.

As mentioned above, indices such as sire conception rate (SCR) (defined as the probability of a single straw of semen to yield a pregnancy as compared to the means of other bulls in the population) have been developed and are heavily utilized as proxies to estimate sire fertility.⁶⁵ Despite these indices, the relationship between estimated sire fertility and the paternal contributions to pregnancy failure has remained elusive. One study utilized 10 sires classified as either high or low SCR for in vitro and in vivo embryo production; low SCR sires produced fewer blastocysts compared to high SCR.⁶⁶ After superovulation, low SCR sires produced a higher percentage of unfertilized oocytes and degenerated embryos than high SCR sires.⁶⁶ Together, these data indicated that low SCR sires may have altered ability to fertilize oocytes and support early embryonic development, ultimately reducing pregnancy establishment.

Although the relationship between SCR and the ability of the sire to support embryonic development during early pregnancy is interesting, a further point should be highlighted. In these studies, there are presumed no differences in sperm cell morphology and motility such that the sperm utilized between high and low fertility bulls would pass routine screening examinations, indicating that differences at the molecular level may be facilitating the reduced ability to support embryonic development. Research into the molecular contributions and alterations of sperm to embryonic development will permit more robust fertility screening types and minimize the proportion of early embryonic failure as a result of improper paternal factors.

Sire contributions to placentation

One of the proposed reasons why individual sires have variable incidence of pregnancy loss is the possibility of variation in the paternal contributions to placentation during the late embryonic period of development. In this hypothesis, it is proposed that differences in PAG expression or transmission of PAG to maternal circulation (via placental vasculature) may be a major component behind the variation in pregnancy

Influence of sire fertility on [PAG]

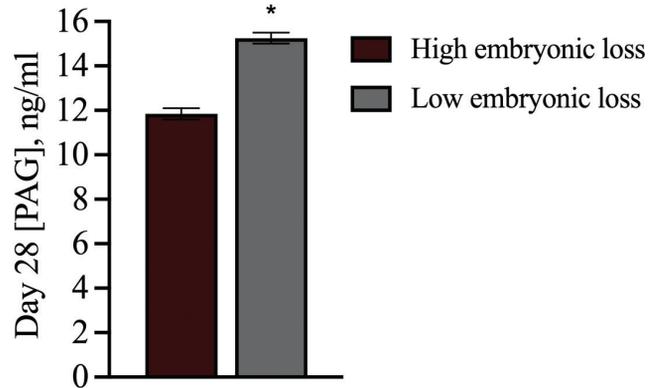


Figure 4. Relationship between sire and PAG concentrations in maternal circulation; sires classified as low embryonic loss have higher PAG concentrations compared to sires classified as high embryonic loss (adapted³⁷).

losses amongst sires. Indeed, pregnancies sired by some sires consistently yielded higher peripheral concentrations of PAG and experienced lower percentages of pregnancy loss compared to pregnancies generated by sires yielding lower concentrations of PAG (Figure 4).^{11,37,56,67}

Given that complete and successful placentation is required for the continuation of pregnancy to term, one of the tools utilized to further understand the contributions of the sire in successful pregnancy and placentation is the use of uniparental embryos. Parthenogenic (PA) embryos are embryos that contain only the maternal genome and are therefore lacking the entire paternal genome.⁶⁸ Seminal experiments conducted in mice demonstrated that zygotes generated using 2 male pronuclei yielded poorly developed embryos with normally developing trophoblast, whereas zygotes produced with 2 female pronuclei generate relatively normal embryos, but poor extraembryonic tissue.^{69,70} Previous studies in cattle have suggested that the sire may be one of the primary contributing factors to a fully developed and functioning placenta.^{56,71} Some evidence indicated that although PA embryos are capable of survival past the period of maternal recognition of pregnancy, the transfer of a single PA embryo on day 8 after estrus failed to increase *ISG15* expression in peripheral granulocytes compared to an in vivo-produced embryo.⁷² Further, the transfer of multiple PA was sufficient to increase uterine IFNT protein concentrations, indicating that PA embryos are capable of transcribing and translating the signal required to extend the estrous cycle.⁷² Despite the ability of PA embryos to extend the estrous cycle, the role of the sire in specific aspects of placentation requires further research. Collectively these data strongly suggest that sires have critical roles in pregnancy that expand beyond fertilization but also that unknown sire factors are required for complete and successful attachment, implantation, and overall placentation.

Environmental contributions to pregnancy loss

There is a close relationship between environmental exposures and reproductive success in cattle. Some of the factors that have garnered the most attention in beef cattle reproductive physiology are the interactions of pregnancy and nutritional status, exposure to elevated environmental temperatures,

and disease. These factors have drawn attention because of the negative relationship with fertility.

Although many of the studies investigating the relationship between nutritional status and fertility have been conducted in dairy cattle, beef cattle also face drastic challenges relating to nutritional availability. Adequate nutrition is critical not only during the periconceptual and postpartum period, but proper management of nutrition for prepubertal heifers can impact lifetime reproductive performance.^{73,74} Indeed, nutritional requirements are influenced by cow's breed, season, and parity, but are also dynamic depending on the physiological status of the cow. For example, nutrient requirements increase as pregnancy progresses.⁷⁵ The importance of available nutrients is evident through experiments in cows with a lower body condition score (BCS) had lower pregnancy rates after AI.⁷⁶ This relationship is due to the imbalance between the hypothalamic-gonadal axis, whereby cows with lower BCS did not return to appropriate cyclicity.⁷³ Cows supplemented with 100 or 150% of their energy and protein requirement during the third trimester of pregnancy had faster ovarian follicular growth and more ovulatory follicles 21 days postpartum compared to cows maintained on pasture.⁷⁷ This change in ovulatory capacity directly related to increased pregnancy rates in cows fed 150% of their energy and protein requirement, ultimately increasing offspring sale value.⁷⁷ Although fertility increases in supplemented cows, it must be noted that over supplementation of nutrients can also yield negative effects on fertility. For example, cows with elevated BCS (obese) had lower pregnancy rates after embryo transfer, and lower blastocyst rates after superstimulation compared to

cows with a moderate BCS.^{24,25} These data provide strong evidence that metabolic status and nutrient supply are 2 factors that require close management for optimal reproductive efficiency.

Approximately 70% of the world's cattle population are in locations considered tropical or subtropical. Reproductive processes in cattle are sensitive to hyperthermic condition.⁷⁸ Indeed, heat stress is attributed to reduced estrous behavior, impaired follicular development, reduced oocyte competence, and inhibited embryonic development.⁷⁸ Therefore, cattle that are well adapted to these environments are necessary to remain efficient and sustainable. For example, oocytes collected from Nelore (*Bos indicus*) cows were more tolerant of artificial heat stress compared to Jersey and Angus (*Bos taurus*) cows.⁷⁹ This was evident by Nelore-derived embryos having higher blastocyst production, higher expression of cell division markers, and lower expression of apoptotic markers when compared to *Bos taurus*-derived embryos⁷⁹; furthermore, there was a tendency for Angus-derived embryos to yield fewer pregnancies compared to Nelore embryos.⁷⁹

Another source of environmental influence of pregnancy loss is the role of infectious diseases in populations of cattle. Although infectious agents can hinder embryonic development, the most pronounced and costly period of abortion occurs between 42 and 260 days. Recent reviews identified the most common infectious agents associated with abortion in cattle and revealed that *Neospora caninum*, *Trueperella pyogenes*, bovine viral diarrhoea virus, infectious bovine rhinotracheitis, *Leptospira* species and fungal infections are the most diagnosed abortive agents

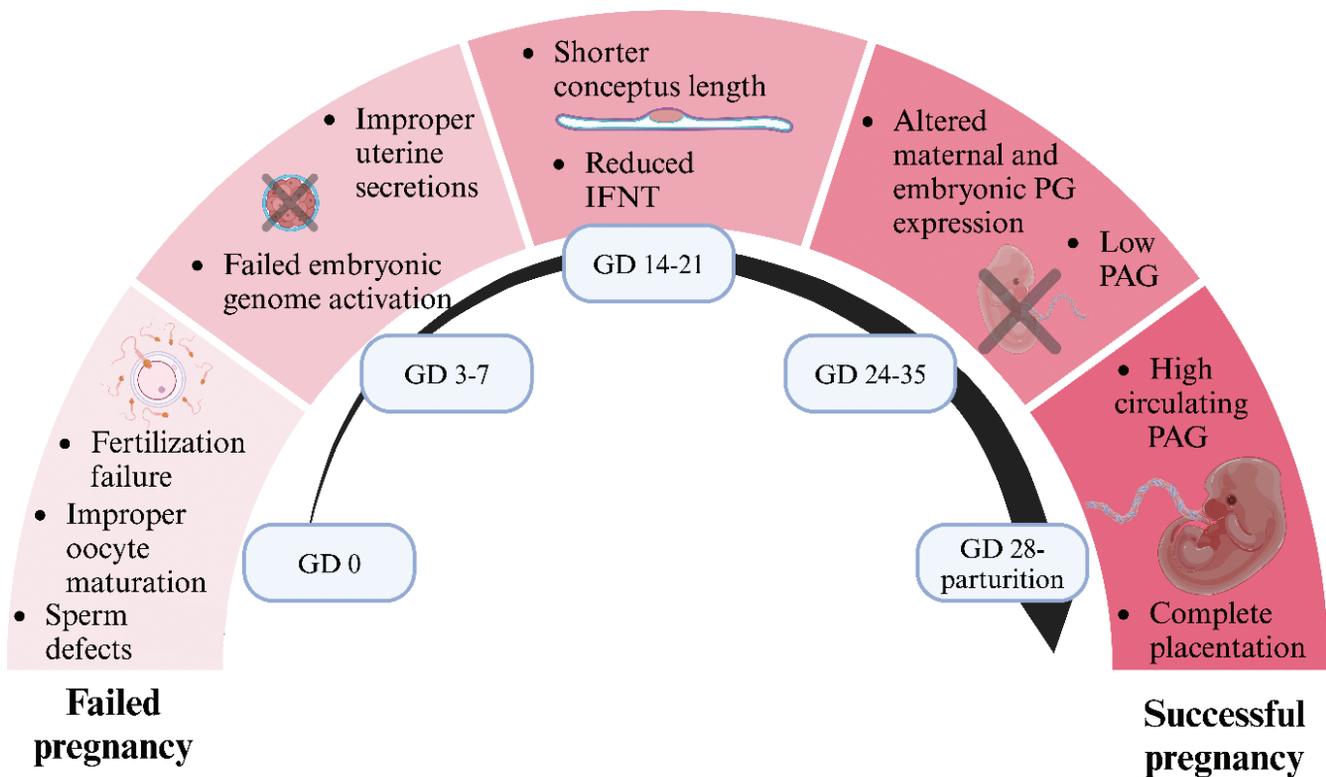


Figure 5. Schematic of critical factors contributing to pregnancy success and failure. Pregnancy loss is multifactorial, and the incidence of pregnancy failure changes depending on the timepoint of pregnancy. There are specific temporal failures that contribute collectively to pregnancy loss, and reducing these losses at each point will contribute to higher reproductive efficiency and sustainability of beef production.

identified in beef cattle.^{80,81} The best management practices to mitigate the impacts of disease-induced abortion are to prevent transmission via rapid diagnostics, maintaining strong biosecurity practices, and appropriate management of positive cattle. Monitoring and reducing the incidence of infectious diseases in cattle populations will aid in minimizing pregnancy loss.

Conclusion

Pregnancy success is highly dependent on numerous conditions, and therefore, the etiology of pregnancy loss is multifactorial. A schematic of critical factors contributing to pregnancy success and failure is depicted in Figure 5. The maternal contribution to pregnancy begins well before the period of fertilization, whereby complete maturation and final development of the oocyte must be completed. From here on, the maternal endocrine milieu must be conducive for ovulation and rapid reprogramming of ovarian function to prepare for pregnancy. Indeed, the changes to the uterine luminal environment are likely driven by changes to circulating ovarian steroids, and these changes are paramount for supporting the earliest phases of embryonic development. Shortly afterwards, a symbiosis must occur where the embryonic and maternal systems must work together in concert through cell signaling and proliferation to allow for complete attachment and placentation. Although the maternal and embryonic physiological systems are typically center stage in the discussion of pregnancy loss, the roles of the paternal contribution to pregnancy loss are rapidly discovered. Furthermore, managing the impacts of environmental factors can improve embryonic, maternal, and paternal competency for pregnancy success. In conclusion, identifying the specific factors contributing to pregnancy failure will aid in improving the efficiency, sustainability, and profitability of beef cattle systems.

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