Factors Affecting Preimplantation Embryonic Development in Dairy Cows

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Take Home Messages

- Pregnancy losses during the preimplantation period (up to day 20 of development) are substantial in lactating dairy cows.
- Genetic and non-genetic factors influence the likelihood of preimplantation conceptus development and survival.
- Genetic factors refer to genetic variations in genomes of cows, sires, and embryos that are linked to the success of preimplantation conceptus development.
- Recessive lethal alleles are good examples of genetic variations in the embryo genome that directly affect conceptus survival.
- The non-genetic factors known to impair preimplantation conceptus development include extensive loss of body condition postpartum, inflammatory diseases postpartum, low concentration of progesterone during the ovulatory follicle development, and hyperthermia of cows caused by heat stress before or shortly after breeding.
- Strategies to improve preimplantation conceptus development and survival include 1) genetic selection for reproductive traits; 2) elimination of recessive lethal alleles from the population; and 3) reduction of the prevalence of non-genetic predisposition factors for pregnancy failures.
- Development of pharmaceutical and nutraceutical strategies to improve preimplantation conceptus survival is ongoing.

Introduction

Reproduction is a major component in dairy sustainability because it impacts the overall milk yield in a herd and its production efficiency. Establishment of pregnancy in an optimal time postpartum, and maintenance of the pregnancy until term have important economic consequences (DeVries, 2006; Ribeiro et al. 2012). Although reproductive management in dairy farms has evolved in the last 15 years, reproductive efficiency in dairy cattle is still not optimal. For instance, the average 21-day cycle pregnancy rate in lactating dairy cows in Canada is only 17% (Denis-Robichaud et al., 2016), and increments beyond 17% are economically attractive (Ribeiro et al. 2012).

Low pregnancy rate is a result of low insemination rate and/or low pregnancy per artificial insemination (P/AI). The development of strategies and technology to improve estrous detection, and the development of ovulation synchronization programs for insemination of cows not detected in estrus have optimized insemination rate and time of first breeding postpartum in lactating cows (Bisinotto et al., 2014). On the other hand, low P/AI remains a major contributor to suboptimal reproduction in lactating dairy cows and, although considerable progress has been made on the understanding of early pregnancy biology in cattle, only modest progress has been made on the understanding of developmental failures.

Pregnancy losses are substantial in dairy cows. Fertilization of single ovulated oocytes (eggs; n = 419) was 83% in commercial North American dairy herds in which the survival of the potential zygotes (fertilized eggs) at the end of first and fourth week of development averaged 67 and 41%, and the proportion of pregnancies resulting in a calving averaged 33% (Ribeiro et al. 2016a). These numbers indicate that approximately 19% of the potential zygotes fail to survive the first week of development; 39% of the live morulas (6-day embryos) fail to survive until the end of the fourth week of development; and 19.5% of the fourth week pregnancies do not result in a calving. Thus, overall, 60% of the fertilized oocytes are lost during uterine development (Ribeiro et al. 2016a).

The success of pregnancy establishment and maintenance until calving is influenced by a multitude of factors. Suboptimal uterine conditions and less competent embryos are the major reasons for pregnancy failures, and these two traits are affected complexly by genetic and non-genetic (environmental) factors. In this article, success of pregnancy establishment and survival will be discussed as a phenotypic trait, whose variability would be explained mostly by a genetic component, an environmental component, and their interaction. Genetic and environmental components will be discussed below after a brief review of early conceptus development in cattle for appreciation of crucial events in developmental biology and their complexity.

▪ Preimplantation Conceptus Development in Cattle

This section is a summary of a more extensive review on the same subject (Ribeiro et al., 2015) in which an extensive list of references containing the following information can be found.

Early embryonic development up to the blastocyst (day 7) stage is very similar among eutherian species and can be reproduced in vitro, which is a valuable source of information for better understanding of developmental biology during these early stages of development. After fertilization of the oocyte in the oviduct, maternal and paternal pronuclei are formed and merged to form the zygote. Embryonic cells derive from cleavages of the zygote and stay enclosed in the zona pellucida, forming a morula by day 4 of development. These early events are highly dependent on oocyte inherited molecules and on lactate, pyruvate, glucose, amino acids, growth factors, cytokines, vitamins, lipids, oxygen and other metabolites secreted by the oviduct.

The morula undergoes compaction and enters the uterus by day 5 of development, where the embryonic cells undergo the first round of cell differentiation. Cells differentiate into either inner cell mass or trophectoderm cells, forming the blastocyst around day 6 of development. The inner cell mass gathers in one pole of the embryo and will originate the embryonic tissues. The trophectoderm cells create the outside layer of cells and will originate the extra-embryonic membranes. After additional rounds of cell differentiation, the spherical blastocyst expands and hatches from the zona pellucida by day 9 of development.

Embryonic development after hatching from the zona pellucida in ruminants is distinct from other eutherian species and difficult to reproduce in vitro, which limits our understanding of the processes required for maintenance of pregnancy. Instead of starting implantation soon after hatching from the zona pellucida as it occurs in rodents and humans, trophectoderm cells of the spherical blastocyst have to proliferate and elongate along the uterine lumen before the initiation of implantation. In a first moment, the spherical embryo stays free-floating into the uterine lumen and cell proliferation leads to formation of an ovoid conceptus (embryo and associated extra-embryonic membranes) by day 13. Up to this point, endometrial physiology is coordinated mainly by progesterone and there is no major distinction between the endometrium of a pregnant and a nonpregnant female.

Around day 14, however, the 1-mm ovoid conceptus starts to elongate by intensive proliferation of trophoblast cells and becomes a 20-cm filamentous structure by day 17. This process of conceptus elongation is dependent on histotroph secretion by the endometrium. The uterine histotroph is a complex combination of molecules including glucose, amino acids, proteins, ions, growth factors, and cytokines, among others that are fundamental for the early embryo development in all mammalian species, but especially important for ruminants whose implantation is shallow and late, starting only at day 20 of development.

Concomitant with conceptus elongation, the highly active trophoblast cells secrete bioactive products that affect endometrial physiology, establishing a complex crosstalk between the two tissues that coordinate critical events for pregnancy establishment, formation of a functional placenta, and pregnancy survival to term. Among these critical events are: 1) maternal recognition of pregnancy associated with corpus luteum (CL) maintenance; 2) establishment of a servomechanism of conceptus nourishment; 3) differentiation of binucleated trophoblast cells; and 4) immunomodulation in the endometrium. Completion of implantation resulting in a fully functional synepitheliochorial placenta will occur only around day 60 of development.

In addition to the changes in the trophoblast and endometrial physiology, important changes also occur on and around the embryoblast. Cells continue to proliferate, and differentiate in the many tissues of the body. Functional structures of main embryonic organs are formed by day 42 of development, and the embryo is then called fetus.

All the aforementioned events highlight the importance, complexity, and potential reasons for developmental failures during early pregnancy, which impairs reproductive efficiency in dairy cattle. Reduced oocyte quality, impaired competence of embryonic development, altered oviduct environment, unbalanced histotroph composition, and impaired receptivity of endometrial cells are just a few examples of things that can go wrong during early conceptus development in cattle. Nonetheless, little progress has been made on the holistic understanding of reproductive failures and on the development of strategies to reduce embryonic mortality.

▪ Predisposition Factors for Embryonic Mortality

Pregnancy failures do not occur randomly in dairy herds, but have predisposition factors, which can be of genetic or non-genetic nature. The genetic factors refer to allelic variations in the genetics of the cow, in the genetics of the breeding sire, or in the resulting genetics of the embryo that influence the likelihood of pregnancy establishment and survival to term. Chromosomal abnormality is another genetic cause of embryonic mortality. The non-genetic factors refer to environmental factors that impact reproductive biology of dairy cows with direct consequences for pregnancy success, and include: nutritional status, health, anovulation or low progesterone during ovulatory follicle development, and heat stress.

Genetics

A small but important portion of the variation in pregnancy success among dairy cows within and across herds is explained by the genetics of the cow, the genetics of the breeding sire, and the resulting genetics of the embryo. In general, the genetic heritability of reproductive traits is relatively low, generally less than 10%, and suggests that reproductive success is affected mainly by non-genetic factors. Nonetheless, fertility traits should not be neglected in the genetic selection program of a dairy farm because they affect reproductive performance of the herds in the long-term.

Incorporation of fertility traits in the genetic evaluations of dairy sires started in 2003 in the U.S. and in 2004 in Canada. Predicted transmitting ability (PTA) for traits such as daughter pregnancy rate (DPR) started to be evaluated and be provided in sire proofs (VanRaden et al., 2004). Fertility traits were also included in composite indexes such as the Lifetime Profit Index and Pro$ (Canada) and Net Merit (USA). Despite estimated low heritability, implementation of these new reproductive traits in the genetic evaluation likely contributed for improvements observed in the reproductive performance of Holstein and Jersey breeds in recent years, and represented an important change in breeding strategies of dairy herds. More recently, new fertility traits such as sire conception rate, cow conception rate and heifer conception rate were developed and are available in some sire proofs. Selection for reproduction traits currently available does not need to be performed at the expense of productive traits, considering that a significant proportion of the active AI sires have predicted genetic gains for both production and reproduction traits (Santos et al., 2010).

The development of next generation sequencing methods, sequencing of the bovine genome, and development of bovine chip arrays for identification of single nucleotide polymorphisms (SNPs) resulted in the advent of the genomics era in dairy cattle breeding. Genomic predictions of genetic merit for several traits can now be determined at affordable prices. Samples of DNA of individual animals are placed on a chip that provides information on genotypes of thousands of SNPs distributed across the 30 bovine chromosomes. The genomic information can then be incorporated into the traditional genetic evaluations for estimation of genomic PTA (GPTA). Compared to proven sires, the reliability of genomic prediction by itself is good (60-80%) for most traits evaluated, and the inclusion of genomic information into genetic predictions of young animals by parent average increases the reliability results expressively. Not surprisingly, genomics has cause significant changes in genetic selection of dairy cattle. Today, every young bull acquired by AI companies is genotyped, and more than 60% of all AIs performed in North America use semen from young sires (with no traditional proofs), reducing the average generation interval and speeding the genetic gains.

In addition to genomic information of AI bulls, the number of females genotyped in commercial herds has also increased significantly in the last decade. In fact, the total number of genotyped dairy cattle in North America has exceeded 1 million (over 100,000 in Canada). Several of these genotyped females have phenotype data on fertility traits and, therefore, this information is also used to estimate genetic contributions in fertility traits of dairy cows.

This new information has also increased our understanding on developmental failures and improved the selection methods for fertility in dairy cattle.

One important contribution of genomic information in dairy cattle was the identification of recessive disorders, including the identification of lethal recessive alleles that cause embryonic mortality (Cole et al., 2016). In general, this information is obtained by identifying haplotypes that are common in the population but never as homozygous. The genome of the embryo is a composite of the cow's genome (inherited from the maternal pronucleus) and the bull's genome (inherited from the paternal pronucleus). When lethal allelic variations in specific genes are inherited in haplotypes from both mother and father resulting in a homozygous embryo for the specific gene, the embryo fails to develop and to survive in the uterus, pregnancy is lost, and homozygous individuals are never born. On the other hand, heterozygous embryos are capable of developing and surviving in the uterus, generating live individuals that are carriers of the recessive lethal alleles. The frequency of lethal alleles in the population can be increased by increasing inbreeding, which has been reported in dairy breeds. Several lethal alleles have been identified and the genomic information of individual animals can now be used in genetic selection to avoid homozygotes or to eliminate carriers from the population (VanRaden et al., 2011).

Among the lethal recessive alleles/haplotypes that have been identified are: deficiency of uridine monophosphate synthase (DUMPS), complex vertebral malformation (CVM), brachyspina, HH1, HH2, HH3, HH4, and HH5 (VanRaden et al. 2011). The causative mutation has been identified in some cases, such as in the HH1 recessive haplotype, in which a nonsense mutation was found in the gene called apoptotic protease activating factor 1 (*APAF1)* and predictably result in a truncated and nonfunctional protein (Adams et al., 2016). This mutation was traced back to the bull Pawnee Farm Arlinda Chief that was born in 1962. This sire and several of his sons were used extensively in the dairy industry worldwide and were important for the evolution of the breed. Their recessive lethal mutation, however, was estimated to have caused more than 500,000 abortions over 30 years (Adams et al., 2016).

In addition to recessive lethal alleles, the allelic variation of other genes (not lethal) might influence the developmental competence of the embryo in more subtle differences, increasing or decreasing the likelihood of survival. Similarly, allelic variation in a cow's genome could also be associated with oocyte quality and its developmental competence, as well as with the uterine receptivity to pregnancy, influencing the success of pregnancy establishment and maintenance. Finally, allelic variation in the sire's genome could be associated with the sperm potential to fertilize oocytes and to activate the zygote's genome for development. Several research groups worldwide are working to identify allelic variations associated with these traits, and new platforms for identification of genetic markers of fertility will likely be developed in the near future. As examples, Ortega et al. (2016) reported that allelic variation in the gene coenzyme Q9 (*COQ9*) explained 3.2% of the genetic variation for DPR; and Han and Peñagaricano (2016) found 8 regions in bulls' genomes significantly associated with sire conception rate.

Nutritional Status

Transition from the dry period (nonlactating pregnant state) to lactation (nonpregnant lactating state) requires the dairy cow to drastically adjust her metabolism so that nutrients can be partitioned to support milk synthesis by homeorhesis. With the onset of lactation, a sharp increase in nutrient requirements occurs. Feed intake, however, is usually depressed around parturition, and consequently, the caloric and nutrient requirements of the cow postpartum are only partially met by feed consumption, which causes extensive mobilization of nutrients from body tissues. Adipose tissue is particularly affected by reduced circulating concentrations of glucose and insulin that up-regulate lipolytic signals for hydrolysis of stored triglycerides and increase availability of nonesterified fatty acids (NEFA) to be used as an energy source. The imbalance in energy, however, is extended to nutrients such as amino acids, minerals and vitamins, which need to be mobilized from muscle and bones. Consequently, lactating dairy cows usually lose significant amounts of body mass postpartum. The amount of body weight loss, however, varies according to the extent of the negative energy balance which, in turn, is mainly determined by energy intake.

Body condition scoring reflects the amount of subcutaneous body fat of cows. In commercial herds, cows are often scored during the dry and early lactation periods to monitor overall nutrition management, which is linked with subsequent lactation performance. Santos et al. (2009) evaluated the body condition score (BCS) in cows at parturition and at the time of first breeding postpartum after synchronized ovulation. The authors observed that cows with extensive reductions in BCS between parturition and AI (1 unit or more in a 1 to 5 scale) are likely to have an extended anovulatory period, decreased P/AI, and increased risk of pregnancy loss compared with cows that had moderate loss of BCS (< 1 unit) or no loss in BCS. Ribeiro et al. (2016a) reported similar results for cows with low BCS (BCS < 3.0) at the moment of AI compared to those with moderate BCS (BCS ≥ 3). These studies suggest the existence of a negative effect of extensive loss of BCS postpartum and resulting low BCS at the time of AI in the success of pregnancy establishment and maintenance. The biological mechanisms involved, however, are still not completely elucidated.

Lactating cows are usually around the peak of milk production by the time of the first breeding postpartum. Similar to high-performance athletes, modern high-producing dairy cows have remarkable nutrient requirements, with total requirements averaging 4 times the maintenance requirements. Accordingly, feeding nutritionally balanced diets undoubtedly plays a central role in any dairy operation. Meeting all nutrient requirements is crucial not only for cows to demonstrate their full genetic potential to produce milk, but also to demonstrate their full genetic potential to support an early developing embryo and generate a healthy pregnancy. Several micronutrients such as glucose, arginine, trace minerals and fatty acids, and growth factors affected by nutritional status such as IGF-1 impact conceptus development and survival; therefore, nutritional deficiencies during preimplantation conceptus development might also lead to pregnancy losses (Ribeiro et al., 2015).

Health

The metabolic and physiological scenario of dairy cows postpartum explained above does not favor function of immune cells. Studies have drawn attention to the negative impact of periparturient metabolic profile on immune cell competence to fight infections. Increased concentrations of NEFA and ß-hydroxybutyrate (BHBA), and reduced concentrations of glucose, insulin, calcium, and vitamins A and E have all been associated with impaired immune cell function and increased susceptibility to infection. In fact, capability of cells to migrate into tissues and kill pathogens is, in general, compromised during the peripartum period. As a consequence of the impaired immune competence during early lactation, the incidence of infectious diseases is substantial. Epidemiological studies indicate that approximately 40% of lactating cows present at least one case of clinical inflammatory disease in the first 60 days postpartum (Santos et al., 2010; Ribeiro et al. 2016a).

Although 78% of these diseases occur in the first 3 weeks postpartum and breeding occurs usually after day 50 postpartum, inflammatory diseases occurring before breeding have a carryover effect, and reduce fertilization of oocytes and development to morula, and impair early conceptus elongation and secretion of interferon-tau in the uterine lumen. These changes in conceptus development are concurrent with inflammation-like changes in the transcriptome of conceptus cells, increased pregnancy loss, and reduced pregnancy and calving per breeding (Ribeiro et al. 2016a). The negative impacts of disease on reproduction are observed independently of estrous cyclicity status and the BCS of cows at the onset of the synchronization program, both of which are known to influence fertility of dairy cows as discussed above. Moreover, the 3 factors (disease postpartum, low BCS, and anovulation) have negative additive effects on fertility of cows bred after synchronized ovulation (Ribeiro et al., 2016a). Furthermore, disease at the preantral or at antral stages of ovulatory follicle development has similar negative impact on pregnancy per AI and pregnancy losses.

The fact that diseases postpartum also increase late pregnancy losses (after day 90 of development) suggests that the carryover consequences of diseases on developmental biology are longer than four months. Interestingly, the compromised maintenance of pregnancy caused by inflammatory diseases postpartum is also observed in cows receiving a random viable blastocyst on day 7 of the estrous cycle. Thus, not only reduced oocyte competence is a likely reason for the low fertility of this cohort of cows, but impaired uterine environment is also involved (Ribeiro et al., 2016a).

Anovulation

Anovulation is a normal and temporary physiological condition of cows during pregnancy and early postpartum. It is characterized by lack of regular estrous cycles and ovulation, although follicle growth remains. Time for first ovulation postpartum and resumption of estrous cyclicity is variable among cows and directly associated with the energy balance postpartum. Low concentrations of glucose, insulin and IGF-1, and high concentrations of NEFA and BHBA in blood, and consequently in follicular fluid, are all resulting scenarios from severe negative energy balance postpartum that restrict follicular growth and synthesis of estradiol, and delay resumption of postpartum ovulation.

Anovulation becomes a problem for reproductive management because on average 18 to 43% of dairy cows are still anovular at the end of the voluntary waiting period (Santos et al., 2009). If the reproductive management relies on breeding after detection of estrus, insemination of these cows will be delayed and, consequently, time to pregnancy will be extended causing reproductive inefficiency and economic loss. Adoption of timed AI programs maximizes submission rates to AI and lessens the problem of anovular cows in reproductive efficiency. Nevertheless, pregnancy per AI of anovular cows after synchronized ovulation is reduced compared with estrous cyclic herdmates (Santos et al., 2009; Ribeiro et al., 2016b) and, therefore, anovulation still impairs pregnancy rates and reproductive efficiency even when a timed AI program is used. Because there is no evidence that synchronization of ovulation in anovular cows is less efficient, it has been hypothesized that their reduced fertility results from impaired capability to establish and/or maintain pregnancy (Bisinotto et al., 2010).

Low concentration of progesterone during the ovulatory follicle development seems to be the major cause of alterations in developmental biology in anovular cows. Estrous cyclic cows that are induced to ovulate a follicle that grew under low concentrations of progesterone, mimicking the endocrine scenario observed in anovular cows, have similar reduction in pregnancy per AI as that observed in anovular cows (Bisinotto et al., 2010). Moreover, sufficient progesterone supplementation during ovulatory follicle development is able to rescue pregnancy per AI in anovular or low progesterone cows (Bisinotto et al., 2013). Nonetheless, timing and biology of the events leading to impaired embryonic development by anovulation before the synchronization of the estrous cycle are not completely understood.

One of the consequences of low concentrations of progesterone during the development of the ovulatory follicle is overexposure of the oocyte to luteinizing hormone, that in turn could impair the kinetics of oocyte meiotic resumption, the maturation process, and its developmental competence (Santos et al., 2016). Additionally, low concentration of progesterone during development of the ovulatory follicle alters the composition of the follicular fluid and uterine physiology in the subsequent estrous cycle (Santos et al., 2016). Interestingly, day 15 conceptuses from anovular cows were longer and secreted greater amounts of IFN-τ than conceptuses from estrous cyclic cows, likely a consequence of differences in the progesterone concentrations before and after breeding (Ribeiro et al., 2016b). Anovular cows, as expected, had lower concentrations of progesterone during ovulatory follicle development. This difference allowed accelerated growth of the dominant follicle and ovulation of a larger follicle that, in turn, resulted in the formation of a larger CL in the following cycle and greater concentrations of progesterone that likely anticipated conceptus elongation. Nonetheless, anovular cows had reduced concentrations of IGF-1 in plasma, and their conceptuses presented remarkable differences in the transcriptome. Some of the altered transcripts indicate that conceptus cells from anovular cows were under greater cellular stress and present increased rates of apoptosis and autophagy, which could lead to increased conceptus mortality after day 15 of development (Ribeiro et al., 2016b).

Heat Stress

Dairy cows undergo hyperthermia during the summer months in most of the world (including Canada), which causes a dramatic reduction in establishment and maintenance of pregnancy. Hyperthermia has numerous effects on cellular metabolism and function that help explain reductions in fertility. Some of these effects directly affect reproductive biology of dairy cows, resulting in altered periods of follicle dominance, reduced steroidogenic capacity of follicular and luteal cells, altered endometrial activity, and impaired oocyte quality (Hansen, 2009). Other effects are caused indirectly by reduced nutrient intake and impaired immune system that lead to high incidence of diseases postpartum as discussed above, and consequently, reduced reproductive efficiency. Elevated temperature and humidity during the hot months also alter the environment, and might increase the pathogen challenge and facilitate infection. Although data on the associations between season and risk of uterine diseases in dairy cattle are scarce, recent epidemiological studies indicate that incidence of retained placenta and metritis increases during the hot season. This multitude of direct and indirect effects causes reduced fertilization and impairs early embryo development, reducing establishment and maintenance of pregnancy, and compromising reproductive efficiency during the summer months (Schüller et al., 2014).

▪ Strategies to Improve Preimplantation Conceptus Survival

The best strategy to improve preimplantation conceptus development and survival in dairy cows is to minimize the prevalence of predisposition factors for developmental failures. Genetic selection of bulls for AI and replacement heifers should always consider fertility and health traits. Moreover, if genomic information is available, recessive lethal alleles should be avoided and eliminated from the herd if possible. Culling decisions of lactating dairy cows should also consider genetic potential for reproductive success and health traits. Adequate nutritional management during the transition period is critical to minimize the incidence of extensive loss of BCS, metabolic problems, clinical diseases, and anovulation at the end of the voluntary waiting period. Monitoring the prevalence of all non-genetic predisposition factors (disease, anovulation, low BCS) is important, and the information should be used to take decisions. Training of farm personnel for fast identification of sick cows, and administration of adequate treatment in early stages of the health problem is critical. In addition, heat abatement system in barns and parlor is recommended to avoid losses in reproduction related to heat stress during the summer months.

In addition to genetic selection for fertility traits and minimization of non-genomic predisposition factors for early pregnancy loss, we have also worked to develop pharmaceutical and nutraceutical treatments to improve preimplantation conceptus development and consequently minimize early pregnancy losses. For example, our group tested a translational approach by supplementing low doses of growth hormone (GH) to lactating dairy cows during the pre and peri-implantation periods, between days 0 and 28 relative to AI, with the objectives to increase circulating concentrations of IGF-1 and improve embryo development and survival. Both objectives were obtained with success. Supplementation of low doses of GH during the preimplantation period resulted in increased concentrations of IGF-1 in plasma, improved pregnancy per AI, and reduced pregnancy losses, resulting in a 28% increase in calving per AI (Ribeiro et al., 2014). Signaling of IGF-1 plays an important role in different stages of preimplantation conceptus development and might be deficient in some cows at time of breeding. Additional measurements obtained in this study demonstrated that the treatment was effective on improving preimplantation conceptus development and survival.

Conclusions

Pregnancy per AI in dairy cows in North America has not changed over the last 15 years, and advances in reproductive efficiency observed in the same period were obtained mainly by improving strategies of reproductive management and genetic selection. Losses of pregnancy during the preimplantation period are still significant and cause important economic losses. Further improvements in reproductive efficiency will require reductions in early embryonic mortality, which in turn will require a better elucidation of critical events in developmental biology and commitment of producers to reduce the prevalence of predisposition factors for pregnancy losses and to include fertility traits in the genetic selection and culling decisions.

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