

## **Epidemiology of Pregnancy Losses and Practical Strategies for Prevention**

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### **Abstract**

Pregnancy losses from fertilization to term are extensive in lactating dairy cattle. In many cases, they are underestimated because of the inability to determine conception early after insemination. For most producers, pregnancy losses are only quantified after the initial diagnosis is performed at approximately 30 to 45 d of gestation. Recent studies that provided reliable assessments of fertilization have led to the conclusion that more than 60% of all pregnancies are lost before term in lactating dairy cows. The critical period is from fertilization until the end of the differentiation phase (embryonic period) in which approximately 85% of those losses occur. Therefore, strategies to prevent embryonic losses should result in the greatest improvement in reproductive performance. Nevertheless, although fetal losses are minor (approximately 15%) compared with embryonic losses, those are more costly than embryonic losses; therefore they cannot be overlooked. Known risk factors for pregnancy loss include prolonged periods of follicular dominance, low progesterone concentration during ovulatory follicle development, heat stress, postpartum diseases and disorders such as dystocia, metritis, endometritis, mastitis, fever, ketosis, lameness, and digestive problems, negative energy balance, excessive body weight loss, toxins found in feedstuffs such as gossypol, mycotoxins, and ergot alkaloids, and infectious agents such as *Neospora caninum*, *Leptospira spp.*, *Campilobacter spp.*, *BVD virus*, and *IBR virus* among others. Strategies that prevent or control known risk factors should help decrease pregnancy loss and improve reproductive performance.

### **Introduction**

Pregnancy losses exert a major detrimental impact on the reproductive efficiency and

profitability of dairy herds. Average cost of a pregnancy loss was estimated at \$555 but varied depending on days in milk at conception and stage of gestation when the pregnancy is lost (De Vries, 2006; Table 1). The Committee on Bovine Reproductive Nomenclature (1972) established that the embryonic period of gestation extends from conception to the end of the differentiation stage, at approximately 42 days of gestation, and that the fetal period extends from gestation day 42 to the delivery of the calf. Losses of pregnancy before day 24 indicate early embryonic losses (usually associated with failure of maternal recognition of pregnancy), and those between days 24 and 42 days, indicate late embryonic losses. Pregnancy losses detected between 42 and 260 days of gestation characterize fetal losses or abortion, whereas a calf that is born dead between 260 days and full term (approximately 280 days) is defined as a stillbirth (Peter, 2000; Santos et al., 2004a).

Two sources of pregnancy failure exist after breeding, fertilization failure and pregnancy loss. In dairy cattle, fertilization rates are similar between lactating and nonlactating cows and they averaged 76.2% (ranging from 55.3 to 87.8%) and 78.1% (ranging from 58.0 to 98.0%), respectively (Santos et al., 2004a). Fertilization rate was recorded at 100% in heifers (Sartori et al., 2002). Nonetheless, in lactating cows, more than 60% of the fertilized oocytes are lost until term (Figure 1). The critical period is from fertilization until the end of the differentiation phase (embryonic period) in which approximately 85% (54%/63%) of those losses occur. Therefore, strategies to prevent embryonic losses should result in the greatest improvement in reproductive performance. Nevertheless, although fetal losses are minor (approximately 15%) compared with embryonic losses, those are

more costly than embryonic losses (De Vries, 2006; Table 1); therefore they cannot be overlooked. In this review we will focus on the known risk factors for pregnancy loss and strategies to reduce them.

#### **Risk Factors for Pregnancy Loss** ***Prolonged Periods of Follicular Dominance and Oocyte Quality***

Oocyte quality is critical for the control of fertilization and subsequent development of the newly formed zygote. One major factor determining oocyte competence is the duration of follicular dominance. Cumulus-oophorus complexes recovered from persistent follicles (13 days after ovulation) presented degenerative characteristics that were absent in complexes recovered from follicles during the growing phase (7 days after ovulation; Revah and Butler, 1996). It has been suggested that such morphological changes are linked with long-term exposure to slightly elevated LH pulse frequency, advancing oocyte maturation, germinal vesicle breakdown, and decreased oocyte quality (Mihm et al., 1999). Negative effects of prolonged follicle dominance on fertility of dairy cows have been associated with impaired embryo quality detected as early as 6 days after AI, and not with fertilization failure (Ahmad et al., 1995; Cerri et al., 2009b). Abnormal persistency of ovarian follicles is not required to reduce fertility, because embryo quality was diminished following a 2-day extension of follicle dominance (Cerri et al., 2009b). This is an important observation because dominant follicles from lactating dairy cows must grow larger in order to induce estrus compared with dairy heifers because of the increase in steroid clearance in the liver in lactating cows (Sangsrivong et al., 2002; Wiltbank et al., 2006). Such reduction in embryo quality caused by prolonged follicle dominance has been implicated with embryonic mortality and reduced pregnancy per AI (P/AI) between days 30 and 34 after AI (Austin et al., 1999). An approach that has been shown to improve conception rates in lactating dairy cows is to reduce the interval between the first GnRH injection of the Ovsynch program

and the PGF<sub>2α</sub> injection from 7 to 5 days to reduce the period of follicle dominance (Santos et al., 2010). This approach increased pregnancy per AI (P/AI) from 30.9 to 37.9% when all cows were evaluated and from 33.9 to 39.3% when only cows that ovulated were evaluated (Santos et al., 2010). The increase in P/AI probably resulted from increased embryonic survival because fertilization rate was not found to be affected in persistent follicles (Ahmad et al., 1995; Cerri et al., 2009b). The only downside of this program is the need for a second PGF<sub>2α</sub> 24 hours after the first one to ensure luteolysis occurs.

#### ***Cycling Status and Progesterone Concentration during Follicle Growth***

One in every 4 U.S. high-producing dairy cows fails to resume ovulation by the end of the voluntary waiting period, which has been associated with reduced fertility to the first postpartum AI (Santos et al., 2009). A decrease in P/AI for anovular cows compared with their cyclic herd mates is very consistent, and P/AI in anovular cows is approximately 40% lower than cyclic cows (Santos et al., 2009; Galvão et al., 2010; Bisinotto et al., 2010). In the study by Santos et al. (2009) P/AI was 24.5 and 35.8% for anovular and cyclic cows at 58 days after AI. Reduced P/AI is probably a consequence of early embryonic loss because most anovular cows ovulate synchronously but are found open at pregnancy diagnosis between 30 and 40 days after AI (Gümen et al., 2003). Increased risk of late embryonic death and fetal loss in previously defined anovular cows, however, has been described in some but not all studies (Table 2). Failure to detect a significant increase in pregnancy loss for anovular cows could be explained by the lack of power of individual studies, because the risk is consistently greater among anovular cows. In fact, when the results from 7 studies were combined, anovular cows were 2 times more likely to lose their pregnancies than cyclic cows (Santos et al., 2004a).

Reduced or basal concentrations of progesterone during growth of the preovulatory fol-

llicle have been found to affect uterine endometrial morphology (Shaham-Albalancy et al., 1997), which is thought to affect endometrial secretory function during early gestation and also to increase the synthesis of PGF<sub>2α</sub> in response to oxytocin (Shaham-Albalancy et al., 2001), which may affect embryo development and lead to premature luteolysis (Garverick et al., 1992; Cerri et al., 2011a), respectively. Furthermore, reduced concentrations of progesterone during growth of the preovulatory follicle have been associated with greater LH concentrations (increased LH pulse frequency), faster growth of the ovulatory follicle, reduced intra-follicular IGF-1 concentrations, and compromised embryo quality (Cerri et al., 2011a, Cerri et al., 2011b; Rivera et al., 2011).

Recently, it was observed that ovulation of a follicle from the first wave of the estrous cycle that develops under subluteal concentrations of progesterone is associated with a reduction in fertility similar to anovular cows (Bisinotto et al., 2010; Denicol et al., 2012). In the U.S., a progesterone insert containing 1.38 g of progesterone (Eazi-Breed CIDR Cattle Insert, Pfizer Animal Health, Madison, NJ) is approved for resynchronization of return to estrus (Chenault et al., 2003). Use of 1 CIDR insert to supplement progesterone in anovular cows has produced inconsistent results (Stevenson et al., 2006; Chebel et al., 2010) probably because progesterone concentrations are only increased by approximately 0.7 to 0.8 ng/mL (Cerri et al., 2009c). Recent studies have been able to reestablish progesterone to concentrations consistent with that of mid diestrus cows by using 2 CIDR inserts, and have been able to restore embryo quality (Rivera et al., 2011) and P/AI (Denicol et al., 2012; Bisinotto et al., 2013). In the work by Bisinotto et al. (2013), P/AI at 62 days after AI was 28.6, 43.7, and 47.3% for cows without a CL at initiation of Ovsynch. Cows without a CL at initiation of Ovsynch that received 2 CIDRs and cows with a CL at initiation of Ovsynch, respectively.

Therefore, given a 25% prevalence of anovulation and the large impact on early embryon-

ic loss and also on late embryonic and fetal loss, management practices that improve cyclicity are expected to decrease pregnancy losses. Recently, we observed that cyclicity by 21 days in milk was negatively associated with parity (less in first-lactation cows), metritis, metabolic or digestive problems, loss of > 28 kg of body weight and a dry period > 76 days (Vercouteren et al., 2014). Therefore, strategies that prevent extended dry period duration and loss of body weight, together with reductions in the incidence of metritis, metabolic problems, and digestive problems should improve postpartum cyclicity and decrease pregnancy losses.

### **Heat Stress**

High-producing dairy cows are particularly sensitive to heat stress because of increased feed intake and metabolic rate, which challenge thermoregulation (Wolfenson, 2000). At air temperatures approaching 27°C (80°F) in humid climates, the body temperature of lactating cows rises above normothermic values, and severe hyperthermia develops as air temperatures rises above 30°C (86°F) (Wolfenson et al., 2000). Air temperatures above 30°C (86°F) are seen in most states in the U.S. during summer, and as temperatures may rise because of global warming, heat stress is expected to become a bigger problem. Negative effects of heat stress on fertility have been recorded since the 1970's (Gwazdauskas et al., 1973; 1975). Badinga et al. (1985) reported that P/AI in lactating dairy cows decreased from approximately 48% in February and March to approximately 20% in July August and September as maximum air temperature increased from approximately 21 to 32°C.

Heat stress affects fertilization and particularly early embryo development. Sartori et al. (2002) observed reduced fertilization and fewer grade 1 to 3 embryos recovered on day 6 of gestation when lactating dairy cows were inseminated during summer compared with AI performed during winter. Similar results were observed by Vieira et al. (2014). The embryo at the very early stages of its devel-

opment, during days 1 to 3 of pregnancy, is highly susceptible to hyperthermia. Later during days 5 to 8 of pregnancy, embryos become more resistant to high temperatures because of their increased production of heat-shock proteins, particularly heat-shock protein 70 after day 2 of pregnancy (Ealy et al., 1993; Edwards and Hansen, 1997; Hansen and Aréchiga, 1999). Exposure to high environmental temperatures resulted in decreased number of small follicles per ovary, altered fatty acid composition of phospholipids from follicular fluid, granulosa cells and oocytes, decreased embryo cleavage, and decreased development to the blastocyst stage (Zeron et al., 2001). Such findings were linked to changes in oocyte morphology and in the fatty acid profile of the oocyte membrane. As a consequence of poor oocyte quality, *in vitro* developmental competence of embryos generated from oocytes obtained from heat-stressed cows was largely reduced. Similar responses were observed following *in vivo* production of embryos. Therefore, most of the negative effects of heat stress on fertility affect processes involved with oocyte maturation, fertilization, and early embryo development to the blastocyst stage (Wolfenson, 2000).

The best strategy to minimize the negative effects of heat stress is to provide adequate cooling (Collier et al., 2006). Collier et al. (2006) states that successful cooling strategies for lactating dairy cows are based on maximizing available routes of heat exchange, convection, conduction, radiation, and evaporation, and lists the following strategies for heat abatement: air movement (fans), wetting (soaking) the cow's body surface, high pressure mist (evaporation) to cool the air in the cow's environment, and facilities designed to minimize the transfer of solar radiation. A study in Israel (Flamenbaum and Ezra, 2003) compared farms that employed intensive cooling (cows cooled in both the holding and feeding areas for a total of 10 cooling periods and 7.5 cumulative hours per day), moderate cooling (cows cooled in the holding area only for a total of 6 cooling periods and 4.5 cumu-

lative hours per day), and farms that provided no cooling in summer (July to September). They observed that P/AI for multiparous cows were 46.6, 45.8, and 43.5% during winter (December to February) and 33.8%, 34.5% and 16.7% during summer for farms using intensive, moderate, or no cooling regimens, respectively. In primiparous cows, P/AI were 55.8, 53.5, and 53.9% during winter, and 40.4, 34.0 and 14.6% during summer for farms using intensive, moderate, and no cooling regimens, respectively. This and other studies with similar findings were summarized by Flamenbaum and Galon (2010). These results show that although the negative effects of heat stress cannot be eliminated, they can be largely alleviated with effective cooling methods.

Another strategy that has been suggested, and is being implemented in some herds is the transfer of embryos collected during winter or the transfer of *in vitro*-produced embryos. Drost et al. (1999) demonstrated that the transfer of *in vivo*-produced embryos from cows exposed to thermo-neutral temperatures increased pregnancy rates in heat-stressed cows compared with pregnancy rates of cows after AI. Similarly, transferring fresh embryos produced *in vitro* resulted in greater P/AI than AI during summer (Block et al., 2010). Conversely, Demetrio et al. (2007) showed that the probability of pregnancy at 28 days of gestation was similar between AI and ET (fresh *in vivo*-produced embryos) when cows were under heat stress (body temperature > 40°C), whereas the probability was greater for ET when body temperature was < 39.5°C. Furthermore, pregnancy loss between 28 and 42 days of gestation was greater for ET compared with AI (21.5 vs 10.8%;  $P = 0.06$ ), and the differences were greater when cows were under heat stress, which would make ET in the summer less appealing (Demetrio et al., 2007).

### ***Diseases of the Reproductive Tract***

Fertility is strongly associated with the health status of the cow, especially regarding calving-related disorders and diseases that affect

the reproductive tract. The stage of gestation at which such disorders affect embryonic survival, however, is mostly unknown. Dairy cows that presented dystocia, metritis, or clinical endometritis were 50 to 63% less likely to resume ovarian cyclicity by the end of the voluntary waiting period (Santos et al., 2011), which as mentioned before has major implications for embryo development. In addition, cows that presented at least 1 of the previously cited disorders was 25 to 38% less likely to become pregnant following the first postpartum AI compared with healthy cows (Table 3), and were 55 to 67% more likely to lose their pregnancy during the first 60 days of gestation (Table 4). The exception was the lack of effect of metritis, although a negative effect on pregnancy loss has been observed in other studies (Ribeiro et al., 2013; Bittar et al., 2014). Although dystocia, metritis, and clinical endometritis are less prevalent in grazing-based cows, their impact in reproductive performance is similar to that observed in confined cows (Ribeiro et al., 2013). Those that presented at least 1 of the previous disorders was less likely to be cyclic by 49 days in milk, less likely to become pregnant to the first postpartum AI, and more likely to lose the pregnancy during 30 to 65 days of gestation.

It has been suggested that such reduction in fertility is related to both fertilization failure and embryonic mortality. It is clear that both clinical (McDougall et al., 2007; Galvão et al., 2009) and subclinical (Gilbert et al., 2005; Galvão et al., 2010; Vieira-Neto et al., 2014) endometritis reduce P/AI in cattle and this is likely the result of reduced fertilization and embryonic survival (Lima et al., 2013). Cows with subclinical endometritis, diagnosed by increased neutrophil influx into the uterus, had reduced proportion of fertilized structures (Cerri et al., 2009c). Using an in-vitro model to elucidate the effects of intrauterine inflammation on embryonic survival, Hill and Gilbert (2008) cultured bovine embryos in media conditioned by either inflamed or non-inflamed endometrium. Embryos cultured in the presence of the inflamed-conditioned media had fewer total and trophectoderm

cells, although no major morphological changes were described. Peripartum disorders of the reproductive tract have long-term carry over effects on fetal viability. High-producing dairy cows that experienced dystocia and those diagnosed with clinical endometritis were 67 and 55% more likely to lose their pregnancies between the first and second month of gestation compared with healthy cows, respectively (Santos et al., 2011; Table 4). Similarly, grazing dairy cows presenting dystocia, metritis, or clinical endometritis were 2 to 4 fold more likely to lose their pregnancies during 30 to 60 days of gestation (Ribeiro et al., 2013). Lima et al. (2013) observed that the greatest effect on pregnancy loss was when cows had both clinical and subclinical endometritis; those cows had 3.3 times the risk of losing their pregnancy compared with healthy cows. The inflammatory process associated with uterine diseases leads to occlusion of the endometrial glands, dilation of underlying glands with deposit of connective tissue and formation of scar tissue in the uterus (McEntee, 1990), which may affect placentation and maintenance of pregnancy. Furthermore, activation of the inflammatory cascade stimulates the release of PGF<sub>2α</sub> from the endometrium and luteal cells, hence inducing luteolysis (Skarzynski et al., 2005).

Cows with uterine diseases have been found to have decreased dry matter intake pre- and postpartum (Hammon et al., 2006; Huzzey et al., 2007) and decreased immune function postpartum. Therefore, strategies that maximize dry matter intake pre- and postpartum are expected to have a positive impact on immune function, decrease the postpartum incidence of uterine diseases, and decrease pregnancy losses.

Another very important factor affecting the incidence of uterine disease is hypocalcemia (Martinez et al., 2012; Martinez et al., 2014). In a recent study at University of Florida, it was observed that inducing subclinical hypocalcemia compromised leukocyte function (Martinez et al., 2014), and spontaneous sub-

clinical hypocalcemia resulted in an increased incidence of metritis (Martinez et al., 2012). In fact, the probability of metritis markedly increased as serum calcium concentrations decreased during the first 3 days postpartum (Figure 2; Martinez et al., 2012). A 1 mg/dL decline in serum calcium between calving and the lowest value during the first 3 days postpartum increased the risk of metritis by 28% (adjusted risk ratio = 1.28; 95% CI = 1.10 to 1.49). Therefore, strategies to prevent clinical and subclinical hypocalcemia such as the use of acidogenic prepartum diets are expected to have a positive impact on immune function, decrease incidence of uterine diseases postpartum, and decrease pregnancy losses.

### ***Fever and Mastitis***

Other diseases not directly associated with the reproductive tract also have been implicated with impaired embryo survival. Cows with fevers ( $> 39.5^{\circ}\text{C}$ ) during the first 2 weeks postpartum were 40% less likely to conceive following the first postpartum AI and twice as likely to lose their pregnancies between the first and second months of gestation (Santos et al., 2011; Tables 3 and 4). Bacterial mastitis can be caused by either gram-negative or gram-positive organisms. The former releases endotoxins from its lipopolysaccharide-containing cell wall that can induce endogenous release of  $\text{PGF}_{2\alpha}$ . Similar to gram-negative, gram-positive bacteria can cause inflammatory responses, pyrexia, and septic shock. Several studies have demonstrated the negative effects of mastitis on maintenance of gestation. Both clinical and subclinical mastitis are associated with reduced conception in dairy cattle (Schrack et al., 2001). Furthermore, cows that developed clinical mastitis during the first 45 days of gestation were 2.7 times more likely (95% CI = 1.3 to 5.6) to abort during the next 90 days of gestation than healthy herd mates (Risco et al., 1999). These results were later confirmed by Santos et al. (2011; Table 4). Santos et al. (2004b) demonstrated that cows developing mastitis before AI, from AI to pregnancy diagnosis, and after first pregnancy diagnosis had greater incidence of pregnancy losses

than cows that did not develop mastitis throughout the entire lactation. Finally, clinical mastitis was also associated with reduced P/AI and greater risk for pregnancy loss in pasture-based systems (McDougall et al., 2005; Ribeiro et al., unpublished results). Furthermore, Hernandez et al. (2011) reported recently that occurrence of mastitis increased the risk of pregnancy loss in cows with reduced body condition score ( $\text{BCS} \leq 2.75$ ) at the end of the voluntary waiting period, but not in those with a  $\text{BCS} \geq 3.00$ . Therefore, additive effects of diseases and poor body fat reserves might exacerbate their impacts on fertility of dairy cows by increasing losses of pregnancy.

Mastitis is a complicated and costly problem for the dairy farmer. No simple solutions are available for its prevention. Mastitis control programs involve: (1) maintaining clean free stalls and bedding; (2) using proper milking procedures; (3) regular maintenance of milking equipment; (4) dipping teats before and after milking; (5) treating cows at dry-off; (6) early identification and adequate treatment of clinical cases; and (7) culling from the herd chronic, non-responsive cows, and those with contagious pathogens such as *Staphylococcus aureus* and *Mycoplasma spp.*

### ***Changes in Body Condition Score***

Metabolic status of the periparturient high-producing dairy cow is closely related to fertility. Cows should be managed to minimize the negative nutrient balance observed during late gestation and early lactation. López-Gatius et al. (2002) indicated that a 1-unit drop in BCS from calving to 30 days postpartum increased risk for pregnancy loss by 2.4 times. Similarly, Silke et al. (2002) observed that cows that lost 1 unit in BCS from days 28 to 56 of gestation had a 3.2-fold increase in risk of pregnancy loss during the same period. In U.S. dairy herds, cows with reduced BCS either at calving or at the time of the first postpartum AI are at a greater risk of losing their pregnancies from day 30 to 58 of gestation (Santos et al., 2009). The detrimental effect of negative nutrient balance on embryon-

ic or fetal mortality can be depicted through changes in BCS from calving to the time of AI. Cows that maintained their body condition during the first 70 days postpartum and those that lost less than 1 unit of BCS were 62 and 48 % less likely to lose their pregnancies between the first and second month of gestation. The negative effect of BCS loss is probably at least in part related to its association with anovulation. Cows that lose > 1 BCS during the first 60 days in milk (Santos et al., 2004a) or lose > 28 kg in the first 14 days in milk (Vercouteren et al., 2014) are less likely to be cyclic. Strategies that improve early postpartum dry matter intake should help prevent excessive BCS or body weight loss and their negative effect on fertility.

### **Toxins Found in Feedstuffs**

Cottonseed is extensively used in diets of lactating dairy cows as a source of protein, fat, and fiber. Cottonseed contains gossypol, a polyphenolic compound produced by the pigment glands of the cotton plant that can be toxic to mammalian cell. Several studies have demonstrated that both *in vivo* and *in vitro* gossypol influences embryo quality and development (Hernández Cerón et al., 2005; Villaseñor et al., 2008). When lactating dairy cows were fed diets differing in free gossypol content, those receiving the greater gossypol diet had reduced P/AI and greater fetal losses (Santos et al., 2003). Transfer of embryos into lactating dairy cows from gossypol-fed donor heifers reduced P/AI compared with embryos from heifers not fed gossypol (Galvão et al., 2006). Diets that increase plasma gossypol pose a risk to establishment and maintenance of pregnancy in dairy cattle; therefore, should be avoided.

Some experimental studies have shown that mycotoxins such as zearalenone in very high concentrations can cause abortions in cattle, although these concentrations are not normally found in "naturally contaminated" feedstuffs. Likewise, the only reports of abortions associated with aflatoxin seem to be situations in which the health of the cow also was severely compromised by the toxin.

Ergot alkaloids are toxins produced by the *Claviceps fungus*, which grows in the seeds of various grasses and small grains such as fescue, brome grass, wheat, oats, and rye. These toxins have been associated with abortions in dairy cattle as well as causing other health problems.

### **Infectious Agents**

As mentioned previously, abortions (loss between 42 and 260 days of gestation) represent only 15% of all pregnancy losses, but they are the most costly. In addition, these are the losses that are easier to prevent or to stop in the case of an outbreak (abortion storm); therefore, they can be the most rewarding to resolve. Most (55 to 77%) of the abortion cases submitted to diagnostic laboratories in the U.S. go undiagnosed (Anderson, 2007). The best laboratories such as the California Animal Health and Food Safety Laboratory System (University of California-Davis), diagnose approximately 45% of the abortion cases submitted. Of those with a diagnosis, infectious agents are the most important causes (Table 5). The causative agent with the largest prevalence (Neosporosis), is also the most difficult to control because no commercial vaccine is available in the U.S. and the parasite (*Neospora caninum*) can be vertically transmitted very effectively (95% efficacy) from dam to fetus (Dubey et al., 2007). Sero-prevalence in the U.S. ranges from 10.3% in Texas to 60.6% in one study in California (Dubey et al., 2007). Because a vaccine is not available and vertical transmission is very efficient, prevention strategies should focus on identifying and segregating or culling seropositive animals and preventing horizontal transmission by avoiding contamination of feed and water with feces from definitive hosts (dog and coyote). The second most commonly diagnosed cause of abortion is sporadic bacteria. A diverse group of bacterial species is associated with opportunistic infections of the placenta and fetus resulting in abortion. These bacteria are not contagious pathogens but are commonly found in the environment or on mucosal surfaces. A ma-

ternal bacteremia is the presumed means by which they reach the gravid uterus and subsequently infect the placenta. Bacteremia is a common occurrence in cows with metritis, mastitis, and ruminal acidosis, but other diseases such as pneumonia also may lead to bacteremia. Common bacteria in this group include *Trueperella* (*Arcanobacterium*) *pyogenes*, *Bacillus* spp., *Escherichia coli*, *Haemophilus somnus*, *Pasteurella* spp., *Pseudomonas* spp., *Serratia marcescens*, *Staphylococcus* spp., and *Streptococcus* spp. (Anderson, 2007). Because this group of bacteria cause sporadic abortions, good herd health practice and disease prevention should decrease its impact on pregnancy loss. Epizootic bovine abortion is mainly found in cattle grazing foothill rangelands in California, Nevada, and Oregon. The infection is transmitted to susceptible pregnant cattle by an argasid tick (*Ornithodoros coriaceus*), that feeds on deer and cattle. Following an abortion affected cattle are resistant to repeat abortion (Anderson, 2007). Because no vaccine for epizootic bovine abortion is available, preventing exposure of naïve cattle to the argasid tick is so far the best strategy to prevent abortions. Leptospirosis is a very important cause of pregnancy loss because it cannot only cause abortion but also embryonic loss. For years, the major bovine pathogenic leptospira was presumed to be *L. interrogans* serovar *hardjo*. This serovar was considered the most important of the antigens in the pentavalent vaccines that also contained serovars *pomona*, *canicola*, *ictero-hemorrhagiae*, and *grippotyphosa*. In contrast, recent research using genetic sequencing found that *L. interrogans* serovar *hardjo* was only found in the U.K. and the main *Leptospira* species found in the U.S. is *Leptospira borgpetersenii*, serovar *hardjo*, type *hardjobovis*. Serovar *hardjo* is host adapted to cattle and can establish lifelong infections in their kidneys and reproductive tracts. Convincing data have shown that *L. hardjobovis* is responsible for not only abortions of established pregnancies, but also for reduced P/AI (increased embryonic loss) associated with carrier cows and bulls (Bondurant, 2007). Currently, vaccines exist that contain *L.*

*hardjobovis* separately or in combination with other important *Leptospira* serovars mentioned previously and with the main respiratory viruses (BVD, IBR, BRSV, and PI3). Prevention strategies should include: (1) vaccination with a 5-way bacterin that includes *L. hardjobovis* every 6 months; and (2) elimination of infection in carrier animals by administration of antibiotics before first breeding or during the dry period. The vaccine prevents infection, but does not cure existing infections. The following treatments have been found to eliminate the renal carrier state: a single injection of oxytetracycline (20 mg/kg i.m.); a single injection of tilmicosin (10 mg/kg s.c.); ceftiofur (5 mg/kg i.m. once daily for 5 days or 20 mg/kg i.m. once daily for 3 days); or amoxicillin (15 mg/kg i.m., 2 injections 48 hours apart); (3) elimination of sources of infection such as feed or water contaminated by dogs, rats, or wildlife (Bondurant, 2007). Bovine viral diarrhea virus (BVD) remains an important cause of abortion and its main means of prevention is to eliminate persistently infected animals from the herd combined with vaccination against BVD every 6 months. The BVD virus is immunosuppressive and is found in many fetuses infected by other agents. Outbreaks of abortions by organisms that normally cause sporadic abortion should raise suspicion of possible concurrent BVD virus infection (Anderson, 2007). As mentioned before, current vaccines available in the market are multivalent (BVD, IBR, BRSV, and PI3); therefore, vaccination also should protect against IBR, another important cause of abortion and early embryonic loss in cattle (Anderson, 2007). Mycotic abortion is mainly caused by *Aspergillus fumigatus*, and is probably a result of feeding moldy feed. Infection with *Listeria monocytogenes* also has been associated with feeding spoiled silage, so rigorous control of quality of feed being fed to cows should prevent these types of abortions. Campylobacteriosis is a venereal disease caused mainly by *Campylobacter fetus* subspecies *venerealis*, although *C. fetus* serovars *fetus*, and *C. jejuni* also have been involved. Use of AI or vaccination (cows and bulls) should prevent abortions



(Bondurant, 2007). *Salmonella* species cause sporadic abortions. Most bovine abortions are associated with *Salmonella dublin*, but other serotypes can be involved (Anderson, 2007). Clinical salmonellosis and abortions are observed mostly when other stressors are present; therefore, good herd health practices should prevent abortions.

### Conclusions

Recent studies that provided reliable assessments of fertilization have led to the conclusion that more than 60% of all pregnancies are lost before term in lactating dairy cows. The critical period is from fertilization until the end of the differentiation phase (embryonic period) in which approximately 85% of those losses occur. Therefore, strategies to prevent embryonic losses should result in the greatest improvement in reproductive performance. Nonetheless, although fetal losses are minor (approximately 15%) compared with embryonic losses, those are more costly than embryonic losses; therefore they cannot be overlooked. Known risk factors for pregnancy loss include prolonged periods of follicular dominance, reduced or basal progesterone concentration during ovulatory follicle development, heat stress, postpartum diseases and disorders such as dystocia, metritis, endometritis, mastitis, fever, ketosis, lameness, and digestive problems, negative energy balance and excessive body weight loss, toxins found in feedstuff such as gossypol, mycotoxins, and ergot alkaloids, and infectious agents such as *Neospora caninum*, *Leptospira spp.*, *Campilobacter spp.*, *BVD virus*, and *IBR virus* among others. Strategies that prevent or control the known risk factors should help decrease pregnancy loss and improve reproductive performance.

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**Table 1.** Cost of pregnancy loss after 1, 4, or 7 months of gestation by lactation number and days in milk at conception<sup>1</sup>

Lactation	61 days in milk at conception			243 days in milk at conception		
	Month of gestation			Month of gestation		
	1	4	7	1	4	7
1	110	279	578	489	739	962
2	285	525	756	310	517	933
3	336	562	726	121	298	711

<sup>1</sup> Adapted from De Vries, 2006.

**Table 2.** Risk for pregnancy loss between the first and second months of gestation in dairy cows according to cyclic status

Reference	Pregnancy loss, % (no./no.)		P value
	Cyclic	Anovular	
Santos et al., 2004	15.7 (195/1,245)	26.3 (56/213)	< 0.001
Bisinotto et al., 2010	13.2 (235/1,780)	15.0 (56/374)	0.17
Chebel et al., 2010	8.7 (18/207)	13.3 (8/60)	0.30
Stevenson et al., 2006	16.0 (33/208)	31.0 (25/81)	< 0.05
McDougall et al., 2005	5 (73/1,470)	9 (48/534)	0.007
Overall	11.2 (550/4,910)	15.4 (85/552)	0.004
Risk ratio	-	1.4 (1.1 – 1.7)	

**Table 3.** Impact of health problems during the first 60 days postpartum on pregnancy at first postpartum AI of dairy cows<sup>1</sup>

Health status	Pregnant, %	Adjusted OR (95% CI) <sup>2</sup>	P value
Health problem	51.4	1.0	
Healthy	43.3	0.8 (0.7 – 0.9)	0.001
1 case of disease	34.7	0.6 (0.5 – 0.7)	< 0.001
> 1 case of disease			
Type of health problem <sup>3</sup>			
Calving problem	40.3	0.8 (0.6 – 0.9)	< 0.001
Metritis	37.8	0.7 (0.6 – 0.8)	< 0.001
Clinical endometritis	38.7	0.6 (0.5 – 0.7)	< 0.001
Fever postpartum	39.8	0.6 (0.5 – 0.7)	< 0.001
Mastitis	39.4	0.8 (0.6 – 1.1)	0.20
Lameness	33.3	0.6 (0.4 – 0.8)	< 0.001
Pneumonia	32.4	0.6 (0.3 – 1.3)	0.20
Digestive problems	36.7	0.8 (0.46 – 1.3)	0.38

<sup>1</sup> 5,719 postpartum dairy cows evaluated daily for health disorders on 7 dairy farms in the U.S. Adapted from Santos et al. (2011).

<sup>2</sup> OR = odds ratio; CI = confidence interval.

<sup>3</sup> Calving problem = includes dystocia, twin birth, stillbirth, and retained placenta, which was characterized by presence of fetal membranes 24 hours after calving; Metritis = watery fetid uterine discharge during the first 14 days postpartum; Clinical endometritis = vaginal mucus score > 2 (> 10% pus in the mucus); Fever = rectal temperature > 39.5°C during the first 14 days postpartum; Mastitis = presence of abnormal milk in at least 1 mammary gland; Clinical ketosis = lack of appetite and presence of ketonuria using test strips; Pneumonia = increased lung sounds and respiratory frequency concurrent with fever; Digestive problem = indigestion caused by displacement of abomasum, bloat or diarrhea.

**Table 4.** Impact of health problems during the first 60 days postpartum on risk of pregnancy loss during the first 60 days of gestation in dairy cows<sup>1</sup>

Health status	Pregnancy loss, %	Adjusted OR (95% CI) <sup>2</sup>	P value
Health problem	8.9	1.0	
Healthy	13.9	1.7 (1.3 – 2.4)	< 0.001
1 case of disease	15.8	2.1 (1.4 – 3.2)	< 0.001
> 1 case of disease			
Type of health problem <sup>3</sup>			
Calving problem	15.9	1.7 (1.2 – 2.4)	< 0.01
Metritis	11.3	1.0 (0.7 – 1.6)	0.76
Clinical endometritis	15.1	1.6 (1.0 – 2.3)	0.03
Fever postpartum	18.0	2.0 (1.2 – 3.1)	< 0.01
Mastitis	19.8	2.6 (1.5 – 4.6)	< 0.001
Clinical ketosis	14.6	1.6 (0.8 – 3.6)	0.22
Lameness	26.4	2.7 (1.4 – 5.1)	< 0.01
Pneumonia	16.7	1.9 (0.4 – 8.7)	0.42
Digestive problems	15.8	1.8 (0.5 – 6.3)	0.35

<sup>1</sup> 5,719 postpartum dairy cows evaluated daily for health disorders on 7 dairy farms in the U.S. Adapted from Santos et al. (2011).

<sup>2</sup> OR = odds ratio; CI = confidence interval.

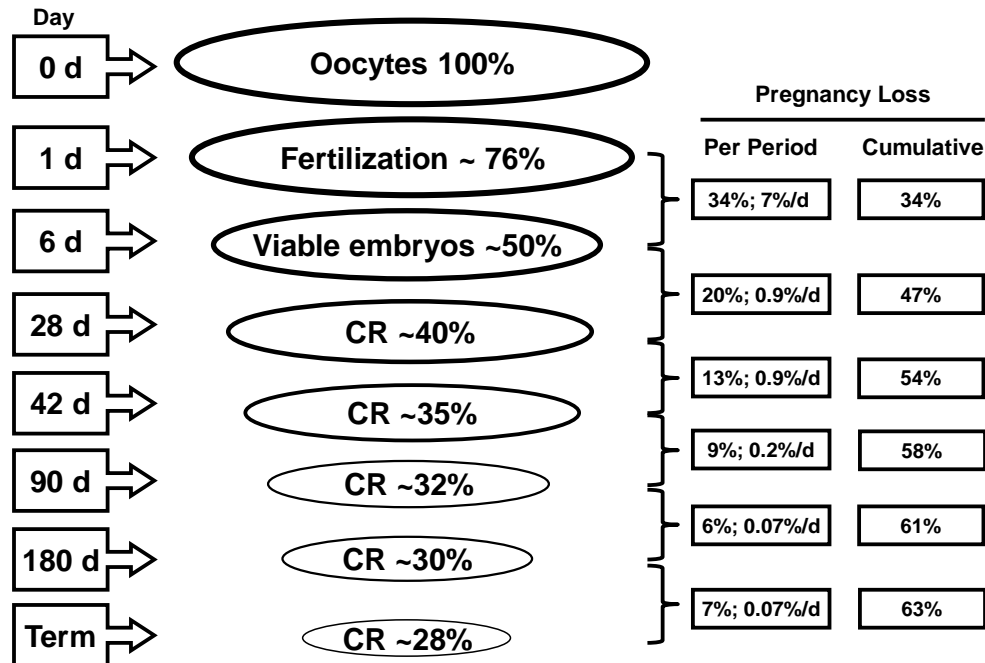
<sup>3</sup> Calving problem = includes dystocia, twin birth, stillbirth, and retained placenta, which was characterized by presence of fetal membranes 24 hours after calving; Metritis = watery fetid uterine discharge during the first 14 days postpartum; Clinical endometritis = vaginal mucus score > 2 (> 10% pus in the mucus); Fever = rectal temperature > 39.5°C during the first 14 days postpartum; Mastitis = presence of abnormal milk in at least 1 mammary gland; Clinical ketosis = lack of appetite and presence of ketonuria using test strips; Pneumonia = increased lung sounds and respiratory frequency concurrent with fever; Digestive problem = indigestion caused by displacement of abomasum, bloat or diarrhea.

**Table 5.** Diagnosis of bovine abortion from 2,296 cases presented to the California Animal Health and Food Safety Laboratory System, University of California-Davis, Davis, CA )1998-2003)<sup>1</sup>

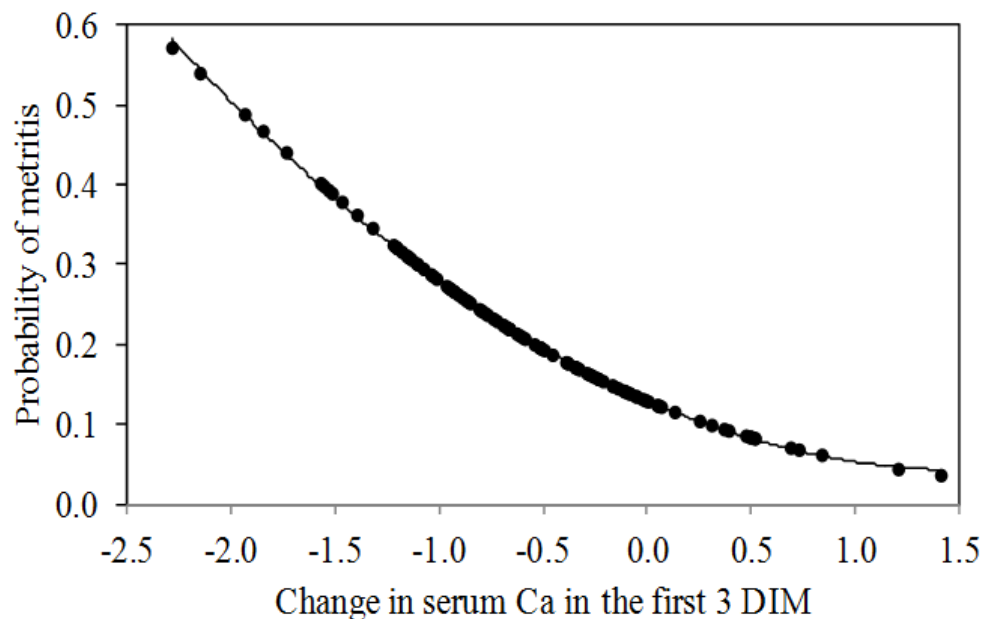
Total etiologic diagnosis	44.4% (1,019/2,296)
Categories of etiologic diagnoses with percentage of diagnosed abortions	
Sporadic bacteria	24.7%
Brucellosis	0.0
Listeriosis	< 1%
Salmonellosis	1.6%
Leptospirosis	5.9%
Capilobacteriosis	3.7%
Epizootic bovine abortion	6.5%
Mycotic	3.0%
Infectious bovine rhinotracheitis virus	3.0%
Bovine viral diarrhea virus	4.0%
Neosporosis	50.9%

<sup>1</sup> Adapted from Anderson et al. (2007).





**Figure 1.** Timing and extent of pregnancy losses in the high-producing lactating dairy cow. Day = day after breeding; CR = conception rate. Adapted from Santos et al. (2004a).



**Figure 2.** Probability of metritis relative to the change in serum calcium (Ca) concentrations between the day of calving and the lowest serum Ca concentration during the first 3 days in milk (DIM). Adapted from Martinez et al. (2012).