PREGNANCY LOSSES: PREVALENCE, TIMING AND ASSOCIATED CAUSES

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ABSTRACT

It is clear that high-producing lactating dairy cows experience substantial losses of pregnancy from fertilization to delivery of a live newborn. On most farms, producers and veterinarians only recognize fetal losses that occur after day 40 or 50 of gestation. In most cases, causative agents cannot be determined. However, the majority of losses of pregnancy occur during the embryonic stages of development. Among the several factors that increase the risk for pregnancy loss are oocytes of low quality, due to stresses such as high body temperature or prolonged follicular dominance; diseases; the metabolic status of the cow and consequent changes in BCS, which result in delays in resumption of ovarian cyclicity; and embryo toxicants such as gossypol.

Key words: Pregnancy loss, embryonic death, body condition

PÉRDIDAS EMBRIONARIAS: PREVALENCIA, CAUSAS ASOCIADAS Y ESTRATEGIAS PARA INCREMENTAR LA SUPERVIVENCIA EMBRIONARIA

RESUMEN

La eficiencia reproductiva en la industria lechera, es sin duda alguna, directamente proporcional con la rentabilidad. A través de los años se ha estudiado el impacto negativo que tienen las perdidas gestacionales, en la productividad de las fincas. Adicionalmente se ha creído que, aunque sin causa etiológica determinada, la mayoría de perdidas gestacionales ocurren después del día 40-50 de la gestación durante el periodo fetal. El presente artículo tiene como objetivo mostrar el impacto otros factores de perdida gestacional haciendo énfasis en otros factores que incrementan el riesgo de pérdida gestacional tales como el la calidad del oocito asociado a cambios medio ambientales que inducen altas temperaturas corporales, o que pueden generar folículos persistentes, enfermedades, así como los cambios en la condición corporal y el estado metabólico de las vacas el cual puede retardar la reactivación ovárica, y finalmente algunos factores tóxicos al embrión como el gosipol.

Palabras clave: perdida gestacional, muerte embrionaria, condición corporal

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INTRODUCTION

Revenue from the dairy industry is directly dependent on reproductive efficiency. Conception rate, estrous detection rate and pregnancy loss are factors that determine the reproductive efficiency in dairy herds. However, pregnancy loss alone can have devastating effects on economic success of dairy herds. It has been estimated that for every pregnancy lost, there is an average loss of US$640,00 (1).

Reproductive failure in inseminated dairy cattle results from poor fertilization and embryo survival. Recent studies with lactating dairy cows indicate that fertilization rate averaged 76,2%, and ranged from 55,3 to 87,8% (2). Once the egg is fertilized, the fate of the pregnancy is then determined by the survival of the embryo and fetus. The incidence of pregnancy losses in dairy cattle vary with time when pregnancy is diagnosed. Unfortunately, little is know about the risk factors for pregnancy loss in the first 25 to 28 d of gestation, as no accurate, noninvasive method for pregnancy diagnosis is available at this point for detection prior to day 25.

Losses of pregnancy can be characterized as early embryonic death, which occurs prior to maternal recognition of pregnancy; late embryonic death, which occurs from day 24 to 42 of gestation; and fetal death, which occurs after day 42. Frequency of losses is presumed to be greater during the early embryonic period, which can be demonstrated by the use of embryo transfer. When 7 day old embryos were transferred to recipient cows and then recovered by uterine flushing 9 days later, only 55 to 60% of the concep were recovered (3). In lactating dairy cows, transfer of fresh or frozen/thawed embryos results in pregnancy rates that are rarely greater than 50%; thereby indicating that early embryonic loss can affect more than 50% of the fertilized oocytes. For AI, late embryonic losses are more prevalent than fetal losses. When taken together, embryonic and fetal losses can account for more than 60% of the gestations.

Several factors affect pregnancy losses in cattle, some of which were discussed in other presentations in this proceedings. However, common risk factors associated with reduced fertility in cattle include: abnormalities of the oocytes associated with impaired quality due to stresses or prolonged dominance, impaired embryonic development and compromised pregnancy recognition signal, prolonged postpartum anestrous, uterine and other periparturient diseases, infectious diseases, excessive loss of body condition score early postpartum, heat stress, increased parity, and dietary ingredients.

CHARACTERIZATION OF PREGNANCY LOSSES

Pregnancy losses can be characterized by death of an embryo or fetus. Most producers recognize losses of pregnancy when the fetus is expelled from the uteri of pregnant cows. However, it is estimated that most losses of pregnancy occur in the first 50 d of gestation, during the embryonic period. Humblot (4) suggested that luteolysis and return to estrus prior to day 24 might be linked with early embryonic death; but, if the corpus luteum (**CL**) is maintained and return to estrus is delayed beyond day 24, it could point to embryonic loss occurring after day 16 of gestation. Therefore, losses of pregnancy prior to day 24 after AI indicate early embryonic losses, and those between days 24 and 50, indicate late embryonic losses. Pregnancy losses detected after day 50 characterize fetal losses.

PREVALENCE OF PREGNANCY LOSSES

Sartori et ál. (5) collected embryos on day 6 after ovulation from lactating dairy cows and observed that only 33,3 and 52,8% of them were considered viable when cows were exposed to either heat stress or thermoneutrality, respectively. Based on their results, when heat stress is not present, more than 45 % of the pregnancies have been lost by day 7 of gestation.

McDougall et ál. (6) studied the prevalence and risk factors associated with pregnancy losses in lactating dairy cattle in pasture-fed dairy cattle in New Zealand. A total of 2,004 pregnant cows were enrolled in the study and 128 animals (6,4%) lost their pregnancy. The pregnancy loss incidence rate was greater between weeks 6 to 10 of gestation than to weeks 10 to 14. Vasconcelos et ál. (7) evaluated pregnancy at various intervals after AI. Of the initial pregnancies on day 28 after AI, 89,5%, 83,8%, 82,4%, and 80,9% remained pregnant on days 42, 56, 70, and 98 of gestation, respectively. Santos et ál. (2) summarized data from several experiments and observed that the risk for pregnancy loss was much greater early than later in gestation. Between gestation days 30 and 45, approximately 12,8% of the pregnancies were lost; whereas after day 45, when the interval between pregnancy diagnoses were much longer than 15 d less than 11% of the pregnancies were lost. Figure 1 depicts survival curves for late embryonic and fetal losses in lactating dairy cattle (2). It is clear that for cows in the US, the rate of loss is greater during the early than the later stages of gestation.

In most dairy farms in the US, approximately 10 to 15% of the pregnancies are lost between 40 d of gestation and calving. Therefore, early embryonic losses are more prevalent than late embryonic losses, which in turn are more prevalent than fetal losses.

EARLY PREGNANCY LOSS

Estimations of fertilization rate in lactating dairy cows range from 55,3% during the summer to almost 87,8 % during periods of thermoneutrality (5). However, conception rates 27 to 31 d after AI are usually lower than 50% in lactating dairy cows.

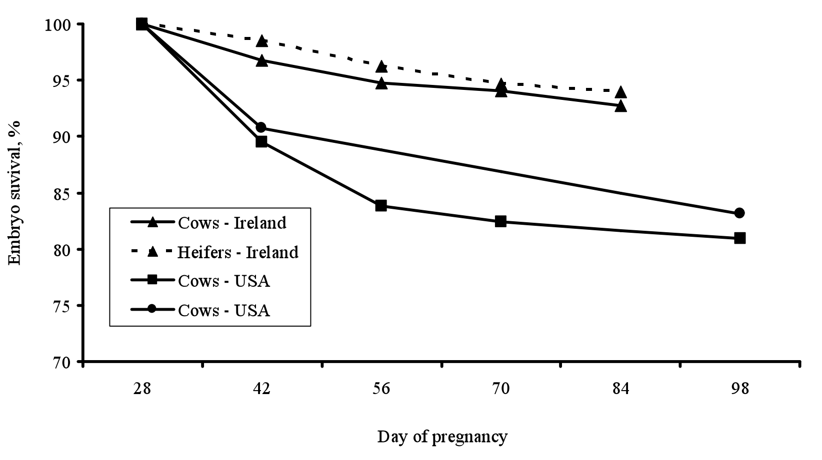

Figure 1. Curves for embryo survival after day 28 of gestation in lactating dairy cows and primigravid dairy heifers in Ireland and the USA. Adapted from Santos et ál. (2).

Furthermore, when 6 to 7 d old embryos are transferred to lactating dairy cows, only 35 to 45% of the cows remain pregnant 22 days later. Therefore, a tremendous loss of pregnancy occurs early after insemination, and this loss can represent up to 40% of the fertilized oocytes.

OOCYTE QUALITY

The female and male gamete quality is a major factor involved in fertilization rate and development of the newly formed zygote. Different studies have shown deleterious effects of poor quality oocytes on fertilization rate, embryo development, and conception rate. Embryonic survival is reduced if embryos are derived from oocytes of cows with follicles of prolonged dominance, diets containing gossypol or that result in increased concentrations of urea and ammonia in blood, or exposure to heat stress. Because it might take 40 to 50 d for a follicle to develop to the ovulatory stage (8), events taking place months before ovulation can influence fertilization and early embryonic survival.

PERSISTENT FOLLICLE

The ovulation of aged follicles results in oocytes of lower quality. Revah and Butler (9) compared the quality of the cumulus oophorus complex of persistent follicles recovered on day 13 of the estrous cycle (d 0 = ovulation) and follicles in the growing phase recovered on day 7. The cumulus oophorus complex of 13 day old follicles had degenerative characteristics, while cumulus oophorus complex of 7 day old follicles were intact. The long exposure of the cumulus oophorus complex to increasing concentration of LH causes premature maturation of the oocyte, resulting in compromised viability (10) and in reduced conception rates (11). Early embryonic survival was compromised when inseminated cows ovulated a persistent follicle compared to cows ovulating a growing follicle (12). Similarly, as the period of dominance was prolonged, embryo quality on day 6 was compromised (13). These results indicate that factors resulting in prolonged dominance of the ovulatory follicle can compromise early embryonic development, likely resulting in greater embryonic loss.

HEAT STRESS

Lactating dairy cows are very sensitive to heat stress. The high milk yield is related to increased feed intake and metabolic rate, compromising the cow's thermoregulatory mechanisms. Zeron et ál. (14) evaluated the developmental competence and the composition of the membrane of oocytes exposed to heat stress. The exposure to high environmental temperatures resulted in decreased production of estradiol and inhibin by the follicles and decreased rate of cleavage and development to the blastocyst stage. Oocytes collected during the summer had worsened morphology, which could have been due to changes in the fatty acid profile of the oocyte membrane. Although heat stress influences fertilization, early and late embryonic and fetal survival (15, 16, 5), it is during the early stages of gestation, from fertilization to the initial cell divisions, that high temperatures have the most deleterious effects.

A recent study by Sartori et ál. (5) demonstrated that lactating cows under heat stress have lower fertilization rates than nulliparous animals and lactating cows exposed to thermoneutral temperatures. This was thought to be related to oocyte quality, because the number of accessory spermatozoa was similar between embryos and unfertilized oocytes. Moreover, embryos produced by lactating cows in hot climates had lower quality than embryos produced by lactating cows under thermoneutral temperatures (5). Drost et ál. (17) demonstrated transfer of *in vivo* produced embryos from cows exposed to thermoneutral temperatures increased pregnancy rates in heat stressed cows compared to AI. This demonstrates that negative effects of heat stress can compromise oocyte quality, fertilization, and early embryo development; thus reducing establishment of pregnancy. The results of Drost et ál. (17) also indicate it is possible to overcome the negative effects on oocyte quality and fertilization of high ambient temperature and the consequent high body temperature (5).

PREGNANCY RECOGNITION

At day 15 to 17 of the estrous cycle the elongated embryo undergoes a critical period of its development as it is faced with the eminent possibility of luteal regression and pregnancy termination due to secretion of prostaglandin (PG) $F_{2\alpha}$ by the endometrial cells. The mononuclear cells of the trophectoderm in early stages of development (15 to 17 days) are responsible for the production and secretion of interferon-tau (IFN-τ) (18). The antiluteolytic effect of IFN-τ results from the inhibition of endometrial expression of oxytocin receptors and possibly the transduction mechanism once oxytocin binds to its receptor on the endometrial cells; thereby inhibiting the episodic release of $PGF_{2\alpha}$ that would regress the CL and terminate the pregnancy (19). Compromised development of the embryo and underdevelopment of the trophectoderm are; therefore, responsible for early pregnancy loss. This is thought to be mediated by the inability of the embryo to suppress the luteolytic mechanism during the period of CL maintenance, further compromising the implantation of the embryo (18).

DISEASES

Many diseases have been reported to reduce conception rate, an indication of reduced fertilization or increased early and late embryonic losses. Although diseases reduce fertility in cattle, it is unknown the stage of gestation when pregnancy is most affected by previous or concurrent illnesses. Cerri et ál. (20) reported that cows with subclinical endometritis, diagnosed by increased neutrophil influx into the uterus, tended to have reduced fertilization rates. It is clear that clinical (21, 22) and subclinical (22, 23) endometritis reduce conception rates in cattle and this is likely the result of reduced fertilization and embryonic survival.

LATE PREGNANCY LOSS

In primigravid cows, embryonic and fetal losses in dairy cattle are usually low, and average 4,2% (2). However, in high-producing lactating dairy cows, pregnancy losses are prevalent. Survival curves for pregnancy maintenance in three studies with lactating dairy cattle clearly indicate that the rate of pregnancy loss is more pronounced in the first 42 days of gestation in the US; however, results from Ireland indicate a similar rate of pregnancy loss throughout the first 80 to 90 days of gestation (Figure 1).

CYCLING STATUS

Rhodes et ál. (24) indicated that between 11 and 38% of the cows in year-round calving production systems are still anovulatory by 50 to 60 days postpartum; whereas 13 to 43% of the cows in pasture-based systems are anovulatory prior to the beginning of the breeding season. Rutigliano and Santos (25) evaluated cyclic status in 5,767 lactating dairy cows in the first 65 d postpartum and observed that multiparous cows were 2,1 times more likely to be cyclic than primiparous cows (81,9 vs. 69,5%; P< 0,001). In that population of lactating Holstein cows from five dairy herds, 22,5% of the cows remained anovular by day 60 postpartum.

It is interesting to note that even when cows ovulate following a period of anovulation or anestrous, fertility is low. These cows usually have poor insemination rates after the voluntary waiting period when subjected to estrous detection systems (26). When subjected to a timed AI program utilizing GnRH, the majority of anovular cows experience a synchronized ovulation, but conception rates remain low (26). The first postpartum luteal phase can be of short duration (< 12 days), which is usually associated with lack of previous exposure to progesterone (27) or adequate estradiol during proestrus (28). Lower plasma concentrations of progesterone in the preceding estrous cycle resulted in premature release of $PGF_{2\alpha}$ in the subsequent cycle (29). Therefore, anovulation poses a risk to establishment and maintenance of pregnancy in cattle.

It is known that anovulation and anestrous is a major risk factor for conception rates (6, 24, 30), but less characterized is the risk for late embryonic and fetal losses. Rutigliano and Santos (25) evaluated the risk for late embryonic loss in lactating dairy cows classified as cyclic or anovular at 65 d postpartum and subjected to synchronization of estrus or ovulation for first postpartum AI. They observed that anovular cows were 1,3 times more likely to lose a pregnancy than cyclic cows (Table 1).

Santos et ál. (2) reviewed several studies with high-producing dairy cows and observed that 15,7 and 26,3% of the pregnancies were lost in cyclic and anovular cows, respectively. Anovular cows were 2,01 times more likely to experience pregnancy loss than cyclic cows (OR = 2,01; 95% confidence interval 1,41, 2,88; P < 0,001). Similarly, in New Zealand, working with dairy cows in pasture-based system, those cows classified as anestrous at the beginning of the breeding season had lower pregnancy rate and increased risk for pregnancy loss. In fact, the risk for pregnancy loss increased 1,6 times in anestrous compared with cyclic cows (Figure 2). Therefore, reducing the prevalence of anovulatory cows prior to first postpartum AI is expected to increase conception rates and minimize pregnancy losses in cattle.

Table 1. Risk for pregnancy loss between 30 and 58 d of gestation in cows classified as cyclic or anovular by 65 d postpartum (25).

Cyclic status	Pregnancy loss	Adjusted OR	P value
Cyclic	14,5 (249/1716)	Referent	0,09
Anovular	18,6 (63/339)	1,30 (0,95, 1,79)	

[1] OR = odds ratio.

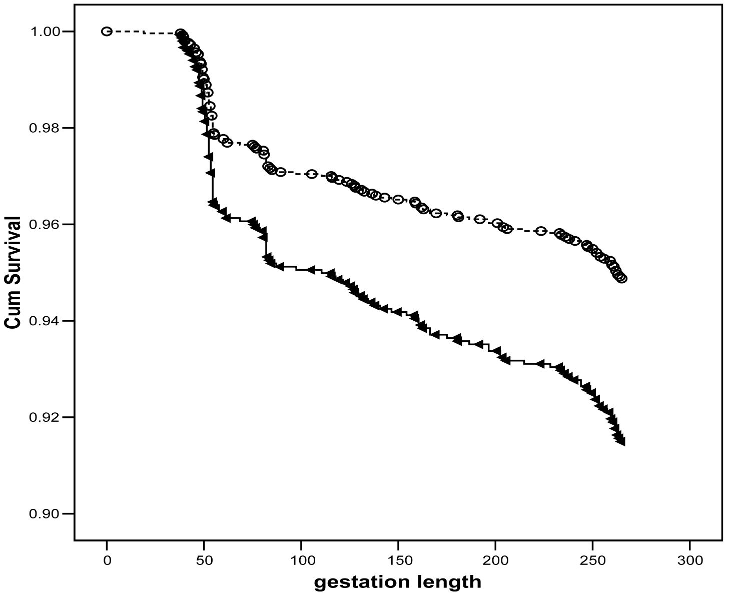

Figure 2. The survival of cows considered cyclic (open circle) or anestrous (closed triangle) by 7 days prior to the beginning of the breeding season (6).

MILK YIELD

Reproductive performance of dairy cattle has decreased in North America, Europe, and Israel (31, 32, 33, 14), which has partially been attributed to the emphasis on high milk yield per cow (31). Increased milk yield is accompanied by increases in feed intake and overall metabolic rate in dairy cows. Increased feed intake related to increased milk yield has been associated with increased blood flow through the liver and increased clearance of progesterone and estradiol. Sangsritavong et ál. (34) demonstrated that feed intake corresponded with acute increases in hepatic blood flow and reduction in peripheral concentrations of progesterone and estradiol. Sub-optimal concentrations of estradiol (28) and of progesterone (35) can have impacts on the reproductive physiology of the cow that adversely affects fertility. Increased milk yield also results in increased catabolic state during the first few weeks of lactation. A decline in energy balance early postpartum can delay resumption of cyclicity and negatively influence fertility. Snijders et ál. (36) observed that cleavage rate and the number of oocytes developing into blastocysts were reduced when they were derived from high versus medium genetic merit cows. However, the same study indicated that 120-day milk production was not associated with cleavage rate and blastocyst development in *in vitro* cultured oocytes.

We have recently summarized nine studies in which milk yield in the first 90 d postpartum and embryo survival were evaluated (25). Cows were categorized into quartiles for milk yield according to parity (primiparous and multiparous) and late embryonic loss was evaluated from days 30 to 58 of gestation. Pregnancy loss did not differ according to level of milk production (Table 2).

Table 2. Association between milk yield in the first 90 d postpartum and pregnancy loss between 30 and 58 d of gestation (25).

Milk yield quartiles	Pregnancy loss	Adjusted odds ratio and 95 % CI
Q1, 34,5 kg/d	14,9 (73/491)	Referent
Q2, 41,4 kg/d	13,7 (73/533)	0,91 (0,64, 1,30)
Q3, 45,7 kg/d	14,4 (79/550)	0,94 (0,66, 1,35)
Q4, 51,6 kg/d	18,1 (87/481)	1,20 (0,84, 1,72)

Table 3. Association between changes in body condition score (BCS, 1 to 5 scale) in the first 70 d postpartum and pregnancy loss between 30 and 58 d of gestation (25).

BCS change	Pregnancy loss	Adjusted odds ratio and 95% CI	P value
Lost 1 unit or more	22,5 (29/129)	Referent	0,01
Lost < 1 unit	16,8 (176/1047)	0,66 (0,41, 1,04)	
No change	12,2 (107/879)	0,50 (0,30, 0,81)	

BODY CONDITION SCORE

López-Gatius et ál. (37) indicated that a 1 unit drop in body condition score using the 1-5 scale from calving to 30 days postpartum increased risk for pregnancy loss by 2,41 fold. Similarly, Silke et ál. (38) observed that cows losing 1 unit in BCS from day 28 to 56 of gestation had a 3,2 fold increase in risk of pregnancy loss in the same period. We observed similar results with high-producing cow in the US (25). Cows losing more than 1 unit of BCS in the first 70 d postpartum were more likely to experience late embryonic loss than those losing less than 1 unit (Table 3).

These data indicate that the metabolic status of the cow, as evidenced by changes in BCS, affects embryonic and fetal survival. Therefore, nutrition and health programs during late gestation and early lactation that minimize tissue mobilization are expected to improve maintenance of pregnancy in lactating dairy cows.

MASTITIS

Bacterial mastitis can be caused by either gram-negative or gram-positive organisms. The former releases endotoxins from its lipopolysaccharides-containing cell wall that can induce endogenous release of $PGF_{2\alpha}$. Similar to gram-negative, gram-positive bacteria can cause inflammatory responses, pyrexia, and septic shock. It is known that mastitis, either clinical or subclinical, is associated with reduced conception rates in dairy cattle (39). Several epidemiological studies have indicated a strong relationship between mastitis and risk for pregnancy loss in lactating dairy cows.

Risco et ál. (40) evaluated the risk for fetal loss in 2087 cows diagnosed pregnant. The authors observed during the study period 127 abortions and 60 cases of clinical mastitis. After evaluating the risk for abortion, they concluded that cows diagnosed with clinical mastitis during the first 45 d of gestation were at 2,7 (95% confidence interval = 1,3 to 5,6) times greater risk of abortion within the next 90 d of gestation than herdmates without mastitis.

Santos et ál. (41) demonstrated that animals developing mastitis prior to AI, from AI to pregnancy diagnosis, and after pregnancy diagnosis had greater incidence of abortion than cows not developing mastitis throughout the lactation. Similarly, Chebel

Table 4. Risk for pregnancy loss between 30 and 58 d of gestation in cows inseminated following timed AI or synchronized estrus (25).

Method of AI	Pregnancy loss	Adjusted OR[1]	P value
Timed AI	15,2 (227/1493)	Referent	0,99
Estrus	15,1 (85/562)	1,00 (0,75, 1,33)	

[1] OR = odds ratio

et ál. (16) observed that clinical mastitis between pregnancy diagnosis and reconfirmation was associated with increased late embryonic loss (P = 0,02). Cows experiencing clinical mastitis were 2,80 (95% confidence interval: 1,16, 6,78) times more likely to lose their pregnancy than those not experiencing mastitis. In pasture-based systems, clinical mastitis was also associated with risk for pregnancy loss (6). Therefore, it is clear that clinical mastitis is associated with increased risk for pregnancy loss, although it is less clear whether this is a direct causal relationship or that the underlying mechanisms leading to mastitis also influence maintenance of pregnancy in cattle.

SYNCHRONIZATION PROGRAMS

The current low estrous detection rate achieved by commercial dairies has encouraged the wide use of synchronization of ovulation/estrus protocols. These programs are based on combinations of hormones such as GnRH and $PGF_{2\alpha}$ in the case of the Ovsynch and CoSynch protocols, which synchronize the emergence of a new follicular wave, cause luteal regression, and synchronize the final development and ovulation of the dominant follicle. Other programs for estrous or ovulation synchronization might also incorporate the use of intravaginal inserts containing progesterone.

It has been suggested that pregnancy losses might have increased with the use of ovulation synchronization programs (32). Several studies have compared late embryonic losses in lactating dairy cattle when inseminated following a synchronized estrus or ovulation. Lactating cows inseminated following a timed AI program (Ovsynch) had similar pregnancy losses from day 31 to 45 post-AI compared with cows inseminated after spontaneous estrus (10,4 vs. 13,2 %) (16). When Santos et ál. (2) summarized six studies, only one observed a tendency to increase pregnancy loss for cows inseminated following timed AI compared to synchronized estrus (15). Recently, Rutigliano and Santos (25) evaluated the risk of late embryonic loss in lactating dairy cows inseminated either at detection of a synchronized estrus or at timed AI. The authors observed similar pregnancy loss between the two methods of insemination (Table 4).

It is interesting to note that when cows were inseminated following secondary signs of estrus using mounting detectors or following timed AI after a single treatment with $PGF_{2\alpha}$, the risk for abortion increased by 1,7 times compared with cows inseminated after being observed in estrus based on visual observation of mounting activity (40). Therefore, when properly implemented, it is unlikely that synchronization protocols increase the risk for pregnancy losses in dairy cattle.

GOSSYPOL

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 (42, 43, 44).

When lactating dairy cows were fed diets differing in free gossypol content, those receiving the greater gossypol diet had reduced conception rates and experienced greater fetal losses (45). Transfer of embryos into lactating dairy cows from gossypol-fed donor heifers reduced pregnancy rates compared with embryos from heifers not fed gossypol (46). Therefore, diets that increase plasma gossypol pose a risk to establishment and maintenance of pregnancy in dairy cattle.

CONCLUSIONS

It is clear that high-producing lactating dairy cows experience substantial losses of pregnancy from fertilization to delivery of a live newborn. On most farms, producers and veterinarians only recognize fetal losses that occur after day 40 or 50 of gestation. In most cases, causative agents can not be determined. However, the majority of losses of pregnancy occur during the embryonic stages of development. Among the several factors that increase the risk for pregnancy loss are oocytes of low quality, due to stresses such as high body temperature or prolonged follicular dominance; diseases; the metabolic status of the cow and consequent changes in BCS which result in delays in resumption of ovarian cyclicity; and embryo toxicants such as gossypol.

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