



Managing Reproductive Disorders in Dairy Cows

Dairy Updates

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Introduction

Reproductive efficiency is a critical component of a successful dairy operation, whereas reproductive inefficiency is one of the most costly problems facing the dairy industry today. Reproductive disorders occur frequently in lactating dairy cows and can dramatically affect reproductive efficiency in a dairy herd. Some of the most common disorders include ovarian cysts, twinning, early embryonic loss, and retained placenta. These are diverse disorders that are similar in that they all can result in impaired reproductive function. Deciding whether to breed, treat, or cull dairy cows exhibiting one or more of these reproductive disorders is a challenge for both veterinarians and dairy producers. In addition, there is considerable controversy among dairy scientists and bovine practitioners regarding the economic impact of these disorders in a dairy operation and the most effective management or therapeutic intervention for treating these disorders. Because of this controversy, dairy managers should focus on prevention and control of risk factors associated with each disorder rather than on prescriptive therapeutic interventions. Dairy producers should work

closely with their herd veterinarian to develop such management strategies and discuss appropriate interventions when necessary.

Normal ovarian function in cattle

In postpubertal cattle, two ovarian structures, follicles and corpora lutea (CL), exist in various stages of growth or regression during normal reproductive cycles. Unlike males, which continually produce gametes throughout their life span, females recruit their gametes from a finite population of oocytes established early during embryonic development. Folliculogenesis is the process of forming mature follicles capable of ovulation from the pool of non-growing, primordial follicles in the ovary. As follicles grow, they progress through several stages of development (Figure 1). In cattle, less than 1% of the 100,000 follicles present at puberty will develop to maturity and ovulate.

The primary functions of ovarian follicles are to:

- 1) Support and nurture a developing oocyte that is capable of being fertilized after ovulation,
- 2) Secrete steroid hormones which regulate the morphology and function of the reproductive organs, as well

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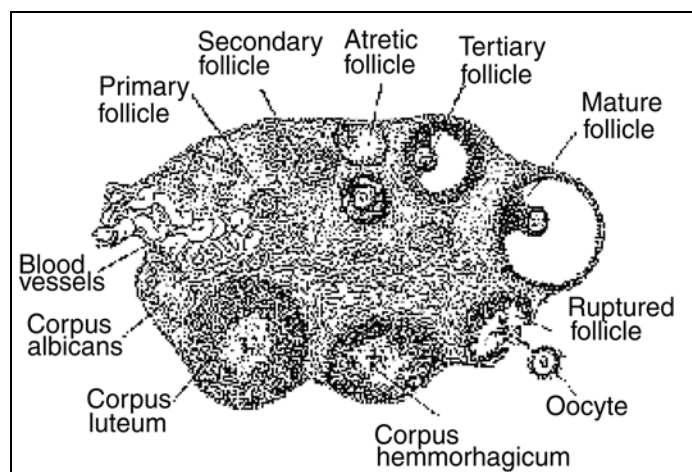


Figure 1: Schematic diagram of an ovary showing the sequence of events in the origin, growth, and ovulation of follicles and formation and regression of the corpus luteum. Follow clockwise around the ovary starting with the primary follicle.

Adapted from Patten and Carlson. 1974. Foundations of Embryology (3rd ed) McGraw-Hill. Reproduced with permission.

as reproductive behavior during estrus, and

- 3) Provide the precursor cells that will luteinize and form the *corpus luteum* (CL) after ovulation (Iranni and Hodgen, 1992).

Corpora lutea are transient endocrine glands that form after ovulation from the tissues that previously constituted an ovarian follicle. Luteal formation begins when increasing peripheral concentrations of estradiol secreted by a developing follicle indirectly trigger a surge of luteinizing hormone (LH) to be released from the anterior pituitary. This LH surge initiates ovulation and luteinization of follicular granulosa and thecal cells, which shifts steroid biosynthesis from estrogens to progestins. Progesterone is the primary steroid product of the CL, and it is required for normal implantation and maintenance of pregnancy in cattle. If pregnancy does not occur or fails to be established, the CL regresses in response to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) secreted by the uterus (Brunner *et al.*, 1969; Boding, 1974). In nonpregnant cows, luteal regression normally occurs around Day 16 to 18 after ovulation. Administration of $PGF_{2\alpha}$ or one of its analogues from Day 6 to 16 after ovulation induces

luteolysis in nearly all animals, whereas the CL is refractory to $PGF_{2\alpha}$ -induced luteolysis from Day 1 to 5 after ovulation.

◆ Follicular waves

Scientific studies using transrectal ultrasonography have led to clarification of the nature of antral follicular development in cattle (Pierson and Ginther, 1984). The first studies using ultrasound revealed that follicular growth occurs in waves, each wave culminating with formation of a large follicle (Figure 2).

A follicular wave begins with emergence of a group or cohort of small antral follicles just before the day of ovulation. During the next several days, one of the follicles in this cohort continues to grow and becomes dominant, thereby suppressing emergence of a new follicular wave. As the dominant follicle continues to grow, growth of the remaining follicles in the cohort ceases or slows, and these subordinate follicles eventually undergo atresia. A second wave of growth emerges on approximately Day 10 after ovulation and, for three-wave cycles, an additional wave emerges at Day 16 after ovulation. For both two- and three-wave cycles, the ovulatory follicle arises from the final wave (Ginther *et al.*, 1996).

Under most circumstances, follicular waves ensure that only one follicle capable of undergoing ovulation is present at any given time during the estrous cycle. In general, primiparous and multiparous lactating dairy cows exhibit more two-wave cycles, whereas nulliparous dairy heifers tend to exhibit more three-wave cycles. Some factors that may influence the number of waves per estrous cycle in dairy cattle include dietary intake (Murphy *et al.*, 1991), age, parity, and lactational status (Lucy *et al.*, 1992).

Ovarian cysts

For the purpose of this review, ovarian cysts are defined as anovulatory fluid-filled structures ≥ 25 mm in diameter that persist on the ovaries for more than 10 days (Archibald and Thatcher, 1992). Ovarian cysts in dairy cows have been

reported to be a major cause of economic loss and reproductive dysfunction in dairy operations (Garverick, 1997), and cows diagnosed with cysts often exhibit extended calving intervals (Bartlett *et al.*, 1986). The reported incidence of ovarian cysts in dairy cows varies from 10 to 13% (Erb and White, 1973; Bartlett *et al.*, 1986), and problem herds may have a much greater incidence (30 to 40%) for brief periods (Archibald and Thatcher, 1992). Based on these incidence rates, ovarian cysts probably affect at least one million dairy cows in the United States each year (Garverick, 1997).

◆ Classification of ovarian cysts

Ovarian cysts can be classified as either follicular or luteal (Table 1). Follicular cysts are thin-walled, fluid-filled, ovarian structures ≥ 25 mm in diameter. Many cows exhibit more than one cystic structure on one or both ovaries at any given time. Early studies reported that cows with cysts exhibited intense and prolonged estrual behavior termed nymphomania (Kessler and Garverick, 1982) resulting from low progesterone due to the absence of a functional CL and increased estradiol from the cystic follicle. Normally, estradiol from a preovulatory follicle initiates a cascade of endocrine events that induce ovulation. In the case of cystic follicles, this endocrine cascade is uncoupled, and normal progression of the estrous cycle is disrupted, causing infertility. The etiology of follicular cysts is also difficult to study because of the unpredictability of the onset of cyst formation within an individual cow (Garverick, 1997). The precise mechanism responsible for this endocrine uncoupling is poorly understood.

Luteal cysts are thick-walled, fluid-filled structures ≥ 25 mm in diameter that secrete normal to above normal amounts of progesterone. Most luteal cysts probably form

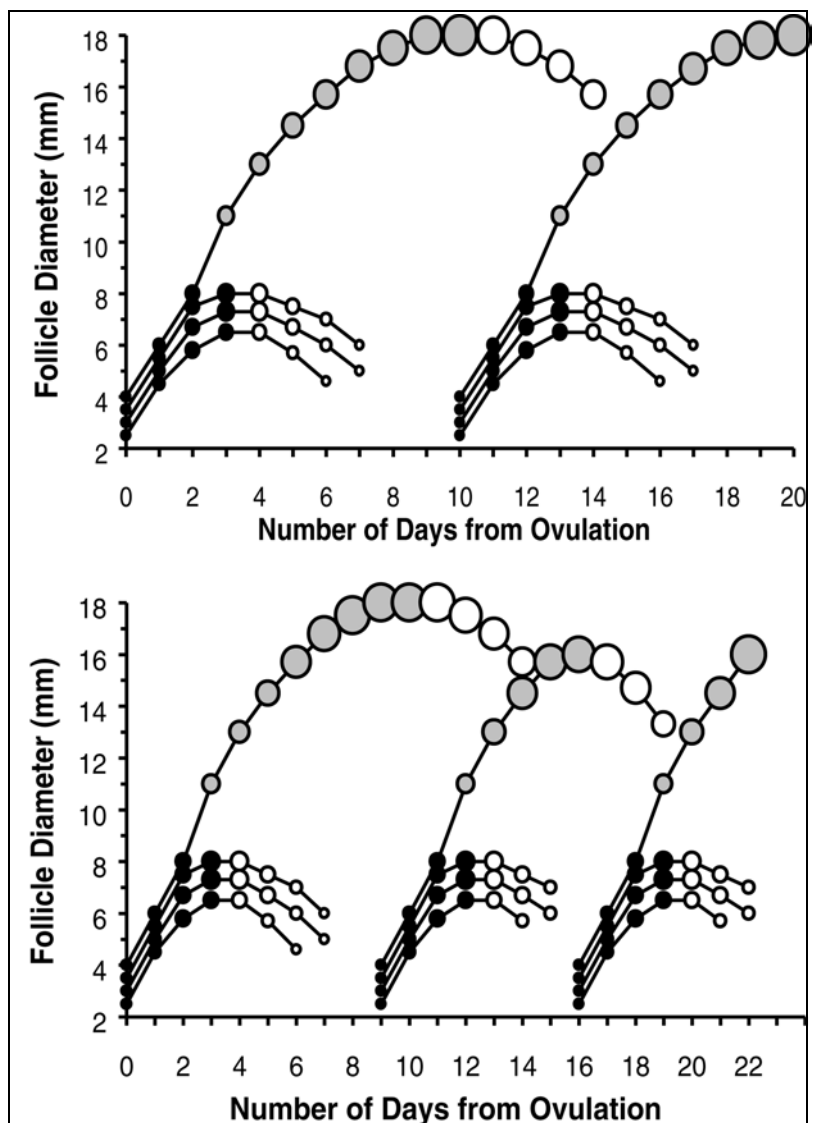


Figure 2: Schematic diagram depicting two-wave (top panel) and three-wave (bottom panel) patterns of follicular growth during the bovine estrous cycle. Growing follicles before selection of the dominant follicle are depicted as black circles, the dominant follicles of each wave are depicted as gray circles, and atretic follicles are depicted as open circles.

through luteinization of a follicular cyst (Garverick, 1997), and can cause infertility if they persist and maintain systemic progesterone at concentrations that inhibit the LH surge and ovulation. The thick wall of luteal cysts is composed of luteal tissue and, in contrast to cystic follicles, the fluid-filled cavities of cystic CL often contain numerous intertwining trabecula that can easily be resolved using ultrasonography. Luteal cysts should not be confused with normal CL containing a fluid-

filled cavity. Based on ultrasonographic examinations in heifers, 79% of otherwise normal CL contain cavities ranging from less than two to greater than 10 mm in diameter at some time during the estrous cycle and early pregnancy (Kastelic *et al.*, 1990).

Based on recent field research using ultrasound to monitor follicular development in lactating dairy cows (Fricke *et al.*, unpublished), a new classification of follicular cysts may predominate in lactating cows (Table 1). These cysts appear similar to follicular cysts using ultrasound, but do not inhibit normal progression of follicular waves and ovulation of normal dominant follicles. Therefore, cows exhibiting this type of cystic structure do not normally exhibit nymphomania. Because these cysts do not impair normal reproductive function, we have termed them benign follicular cysts. The presence of benign follicular cysts may complicate both diagnosis and treatment of follicular cysts in lactating dairy cows.

◆ Risk factors and nutritional factors affecting ovarian cysts

The physiology and etiology of ovarian cysts is poorly understood, and there is much conjecture regarding risk factors for ovarian cysts. Heredity has been implicated; however, heritability estimates are low (Casida *et al.*, 1951; Ashmaway *et al.*, 1990) and selection against cysts is probably not a profitable management strategy for dairy producers (Garverick, 1997). Other factors include increased milk production (Johnson *et al.*, 1966), estrogen content of forages (Barga, 1987), and uterine infections (Bosu and Peter, 1987; Peter *et al.*, 1989). Garverick (1997) has also suggested that compounds with estrogenic activity in feedstuffs may play a role in cystic ovarian disease. Zearalenone is a mycoestrogen produced by the fungi *Fusarium spp.* that may be present in moldy feed (Diekman and Green, 1992). Zearalenone ad-

versely affects fertility in swine and, although cattle are not as sensitive to its effects, it should be limited to less than 500 ppb in the total diet DM (Whitlow and Hagler, 1993).

Cows with excessive body condition at dry off are 2.5 times more likely to develop cystic ovaries (Gearhart *et al.*, 1990), and the incidence of cysts for cows that were normal and over-conditioned during the dry period were 12% and 29%, respectively (Butler and Smith, 1989). However, in some studies the over-conditioning of cows at calving has not been associated with development of ovarian cysts (Gearhart *et al.*, 1990; Ruegg *et al.*, 1992). Risk of ovarian cysts also increases in primiparous cows with elevated milk ketone concentrations (Odds Ratio = 8.7; Andersson *et al.*, 1991). Harrison *et al.* (1984) reported cystic ovary incidence rates for cows fed a selenium-deficient diet during the dry period of 50% for control cows versus 19%, 44%, and 19%, respectively, for cows supplemented with selenium, vitamin E, or selenium/vitamin E. Feeding 300 mg β -carotene daily per cow from Days 3-98 postpartum did not influence the incidence of cystic ovaries or reproductive performance (Wang *et al.*, 1988). Control and treated cows were supplemented with 75,000 IU vitamin A per cow per day. There was no reproductive benefit to feeding 1,000,000 IU versus 100,000 IU vitamin A per cow per day (Tharnish and Larson, 1992). The current practice on commercial dairies is to supplement dry cows and lactating cows with vitamin A at about 100,000 IU and 150,000 IU per cow per day, respectively (Weiss, 1998).

◆ Diagnosis and treatment of ovarian cysts

Diagnosis of cysts in dairy cattle most often occurs during routine postpartum rectal

Table 1: Classification, functional status, and response to GnRH or PGF_{2 α} of ovarian cysts in dairy cows

Classification	Steroid secreted	Response to GnRH	Response to PGF _{2α}
Follicular	Estradiol	Luteinization	None
Luteal	Progesterone	None	Regression
Benign follicular	None	None	None

examinations conducted by a bovine practitioner. Palpation per rectum of a large, fluid-filled structure is commonly used as a clinical indication of a follicular cyst. Unfortunately, differentiation between follicular and luteal cysts via rectal palpation is difficult, even for experienced practitioners (Dawson, 1975; Farin *et al.*, 1992). Accuracy of diagnosis increases when using transrectal ultrasonography, with correct identification of greater than 90% of luteal and nearly 75% of follicular cysts (Farin *et al.*, 1990, 1992). Follicular and luteal cysts also can be classified based on serum progesterone concentrations (Farin *et al.*, 1990). Diagnosis of a cyst in conjunction with low serum progesterone is indicative of a follicular cyst, whereas a cyst in conjunction with high serum progesterone is indicative of a luteal cyst. Using these criteria, benign follicular cysts would fall into either category depending on the stage of the estrous cycle when they were detected.

Treatment for ovarian cysts depends on the classification of the cyst (Table 1). Follicular cysts are most commonly treated by administration of synthetic GnRH analogs approved for use in lactating dairy cows (Bierschwal *et al.*, 1975; Seguin *et al.*, 1976; Whitmore *et al.*, 1979). Manual rupture of cysts via rectal palpation is not recommended because of the reduced efficacy compared with GnRH (Ijaz *et al.*, 1987) and because adverse side effects including adhesions around the ovary and adnexa may impair fertility (Archibald and Thatcher, 1992). Interestingly, approximately 20% of untreated cows with follicular cysts recover spontaneously (Bierschwal *et al.*, 1975), supporting the notion that many of these cysts may indeed be benign. Treatment with GnRH induces luteinization rather than ovulation of the follicular cyst, and ultimately results in formation of a luteal cyst (Garverick, 1997; Fricke *et al.*, unpublished observation). Once formed, regression of a luteal cyst can be induced by administration of PGF2 α (Nanda *et al.*, 1988). Administration of GnRH to cows with benign follicular cysts often induces ovulation of a normally growing dominant

follicle rather than the cyst itself (Fricke *et al.*, Table 2), and other researchers have reported similar observations (Archibald and Thatcher, 1992; Garverick, 1997).

The ideal treatment for ovarian cysts would be one that is effective for all three classifications of ovarian cysts. Ovsynch, a protocol for synchronizing ovulation in lactating dairy cows, uses injections of both GnRH and PGF2 α (Pursley *et al.*, 1995, 1997), and may be an effective treatment for ovarian cysts (Table 2). A recent field trial using Ovsynch and ultrasonographic monitoring of ovarian structures (Fricke *et al.*, unpublished) revealed that 11% of lactating cows exhibited a large ovarian structure that would have been diagnosed as a cyst using rectal palpation. Treatment with Ovsynch induced ovulation of a follicle other than the cyst that was present at the time of the second GnRH injection in 73% of cows. Nearly 37% of these synchronized cystic cows conceived after a timed AI. These preliminary data support use of Ovsynch as a treatment for cows exhibiting all classifications of ovarian cysts, and may be the treatment of choice when rectal palpation is used to diagnose cysts.

Twinning

Twinning is an unavoidable outcome of reproduction in dairy cattle and is undesirable in a dairy operation because it reduces overall profitability and reproductive efficiency (Eddy

Table 2: Effect of ovarian cysts on synchronization rate and conception rate in lactating dairy cows after synchronization of ovulation using Ovsynch.

Item	Ovarian cyst ^a		Overall
	Yes	No	
Incidence	11.0%	89.0%	
Synchronization rate ^b	73.1%	85.3%	84.0%
Conception rate ^c	36.8%	48.8%	47.6%

^aA fluid-filled ovarian cyst ≥ 25 mm in diameter present at the time of the second GnRH injection of the Ovsynch protocol.

^bOvulation of a normal dominant follicle after the second GnRH injection of the Ovsynch protocol.

^cUltrasonographic determination conducted at 28 d post AI.

et al., 1991; Beerepoot *et al.*, 1992). One study estimated that every twin birth incurs a \$108 economic loss compared with a singleton birth (Beerepoot *et al.*, 1992). Twinning also reduces reproductive performance by increasing average days open and services per conception of the dam during the subsequent lactation (Nielen *et al.*, 1989). In addition, the etiologies of many periparturient diseases in cattle are associated with twin births. Cows calving twins have higher risks for stillbirth, retained placenta, metritis, displaced abomasum, ketosis, and acidurea (Pfau *et al.*, 1948; Markusfeld, 1987; Nielen *et al.*, 1989). Incidences of abortion (29.3% vs. 12.0%), neonatal calf mortality (15.7% vs. 3.2%), reduced birth weight (43.5 vs. 30.6 kg), and retained placenta (34% vs. 7%) also are greater among twins compared with singleton calves, probably due to the reduced gestation length and increased incidence of dystocia among cows calving twins (Pfau *et al.*, 1948; Erb and Morrison, 1959; Neilen *et al.*, 1989; Day *et al.*, 1995). Culling rates are also greater for cows calving twins (Eddy *et al.*, 1991). One impact of twinning is a reported reduction in the number of fertile heifers available for use as replacements in the dairy herd (Table 3). This decrease arises from increased neonatal calf mortality of twins and a skewed gender ratio resulting in more homozygous male pairs.

◆ Mechanisms of twinning

Cattle are a monotocous species meaning that, under most circumstances, a successful pregnancy results in the birth of a single calf. The physiologic mechanism responsible for regulating the number of follicles that become dominant within each follicular wave usually results in selection of a single dominant follicle

capable of ovulation (see Figure 2). Initiation of an induced or naturally occurring ovulatory stimulus causes release of a single oocyte from the dominant follicle at ovulation. If the subsequent events from fertilization to parturition occur normally, the pregnancy will result in birth of one calf. On occasion, however, two follicles are selected to continue growth from among the group of growing follicles in a follicular wave resulting in a phenomenon termed codominance. If the appropriate stimulus for ovulation occurs naturally or is induced when codominant follicles are present, two oocytes, one from each follicle, will be released. If the subsequent events from fertilization to parturition occur normally for both oocytes, twins will result. The single cell that is formed after an oocyte is fertilized by a sperm is called a zygote. Thus, twins that occur as a result of ovulation and fertilization of two oocytes are called dizygous twins.

Most twins in cattle are of the dizygous type (Erb and Morrison, 1959; Johansson *et al.*, 1974; Ryan and Boland, 1991). Dizygous twins can be the same or opposite in sex and are no more alike phenotypically or genetically than siblings with the same parents born during different gestations. Ovulation of a single dominant follicle also can, on rare occasions, result in twins. Twins resulting from ovulation and fertilization of a single oocyte are called monozygous twins. Monozygous twins are genetically and phenotypically identical and, therefore, are always of the same sex. The mechanism by which monozygous twinning occurs is not clearly understood, but monozygous twinning can be considered a natural cloning of the original zygote in vivo.

The rate of monozygous twinning in cattle is low, with estimates ranging from 7.4% (Erb and Morrison, 1959) to 13.6% (Ryan and Boland, 1991) of all twin births or less than 0.3% of all births.

Table 3: Effect of twinning on the number of replacement heifers per pregnancy.

Reference	Replacement heifers per pregnancy	
	Single births	Twin births
Erb & Morrison, 1959	0.43	0.34
Nielen <i>et al.</i> , 1989	0.48	0.42
Day <i>et al.</i> , 1995	0.42	0.29

◆ Twinning rates in dairy cattle

Risk factors for twinning in cattle include effects of breed type and parity (Nielen *et al.*, 1989; Ryan and Boland, 1991). The percentage of twins born also varies with the season of the year, with a trend toward more twin births during the summer months. This seasonal effect on twinning has been attributed to an increased plane of nutrition during the fall when cows calving during the summer would have conceived, a decreasing light period, and a decrease in the viability of early stage embryos conceived during summer months compared with those conceived during cooler fall months (Cady and Van Vleck, 1978; Nielen *et al.*, 1989). High cumulative milk production and previous twinning are additional factors that increase the risk of twinning (Nielen *et al.*, 1989; Kinsel *et al.*, 1998). In general, the twinning rate for most beef breeds of cattle is less than 1% (Rutledge, 1975). The reported incidence of twinning in dairy cattle ranges from 2.5 to 5.8% and is dramatically affected by parity, ranging from 1% for first parity to nearly 10% during later parities (Table 4).

The effect of parity on twinning rate is not clearly understood but may be explained by an increased ability of older cows to support twins throughout gestation, an increase in the rate of double ovulation, or an interaction of both of these factors. Increased uterine capacity of cows calving twins has been reported (Ryan and

Boland, 1991). Furthermore, the incidence of double ovulation in lactating dairy cows is around 14% (Kidder *et al.*, 1952; Fricke *et al.*, 1998), and, as with the incidence of twinning, increases with parity (Labhsetwar *et al.*, 1963). Kinsel *et al.* (1998) reported an increased rate of twinning over a 10-year period. The single largest contributor to this increase was the increase in peak milk production that occurred over that period. They also suggested that feeding higher energy diets to high-producing cows may be increasing the incidence of double ovulations, and hence the rate of twinning. This nutritional effect is similar to the practice of "flushing" in ewes (Dunn and Moss, 1992), but further research is required in dairy cattle.

Increasing levels of dietary bypass protein can increase ovulation rate and incidence of twinning in ewes (Nottle *et al.*, 1988). Therefore, the high levels of bypass protein fed to lactating cows may partially account for the increased twinning rate. The overall twinning rates reported for dairy cows in recent studies are greater than those reported in many earlier reports (Day *et al.*, 1995), indicating that twinning rate may be increasing over time in the dairy cattle population as a whole. If twinning is related to nutrition and/or milk production (Nielen *et al.*, 1989), this increase in twinning would not be unexpected considering recent trends in feeding practices and yearly increases in milk production per cow.

Table 4: Effect of parity on twinning rate (%) or relative risk (R)^a of twinning in dairy cattle.

Ref ^b	No. of calvings	Parity						All parities
		1	2	3	4	5	6	
1	937	0.7	5.0	4.2	5.0	7.0	6.7 ^c	4.2
2	7,387	1.3	4.4	5.3	4.6	5.8	6.0	4.6
3	11,951	0.8	2.7	4.1	4.5	4.9	4.8 ^c	3.2
4	19,755	0.9	2.1	3.5	3.4	3.7	3.2	2.5
5	24,843	1.0	7.0	7.5	7.9	9.1 ^c	-	4.2
6	19,497	1.3	6.0	9.4 ^c	-	-	-	-
7	52,362	1.0	2.9	3.2	3.9	3.3	4.1 ^c	2.4
8	8,521	R=0.2	R=1.5	R=1.6	R=1.6	R=2.0 ^c	-	5.8

^aRelative risk (R) of twinning for each parity group is calculated against all others pooled together.

^bReference: 1=Pfau *et al.*, 1948; 2=Erb & Morrison, 1959; 3=Nielen *et al.*, 1989; 4=Eddy *et al.*, 1991; 5=Ryan & Boland, 1991; 6=Berry *et al.*, 1994; 7=Kinsel *et al.*, 1998; 8=Markusfeld, 1987.

^cIncludes all cows $\geq$ the parity listed.

◆ Freemartinism

Freemartinism in heifers results from twinning when embryonic membranes of a male and female conceptus fuse during gestation resulting in exchange of blood between the male and female fetuses. Endocrine factors or cells from the male calf cause abnormal development of the reproductive organs of the female calf resulting in infertility. Freemartinism occurs in about 92% of heifers born as a result of heterosexual twin pregnancies (Buoen *et al.*, 1992). Thus, about 8% of heifers from heterosexual twin pregnancies will be fertile, presumably because the fetal membranes fail to fuse or because membrane fusion occurs after the critical period of reproductive organ differentiation (Buoen *et al.*, 1992).

The earliest developmental abnormalities of the female reproductive tract resulting in freemartinism occur between 49 to 52 days post fertilization (Jost *et al.*, 1972). Interestingly, freemartinism has been documented in singleton female calves, which probably results due to loss of a male twin after fusion of the embryonic membranes but before parturition (Wijeratne *et al.*, 1977). In addition, many bulls born twin to a heifer exhibit various degrees of impaired reproductive function including inability to produce semen, reduced sperm production, or increased incidence of abnormal spermatozoa (Dunn *et al.*, 1979). From a practical perspective, bulls born twin to a heifer can be used for breeding purposes after passing a breeding soundness and semen quality examination (Long, 1979).

◆ Management of twins

Management of cows carrying twins depends on accurate identification of the presence of twins during early gestation. Cows carrying twin pregnancies can be accurately identified at 40 to 55 days post AI using transrectal ultrasonography (Echternkamp and Gregory, 1991; Davis and Haibel, 1993; Dobson *et al.*, 1993). Palpation per rectum between 50 to 70 days post AI also results in an acceptable degree of accuracy among experienced bovine practitioners (Day *et al.*, 1995).

Several management scenarios could be considered upon diagnosis of a twin pregnancy in a dairy cow. Continued management of the cow carrying twins could be avoided either by culling the cow or by aborting the twin pregnancy, usually through administration of an ecboic agent such as PGF_{2α}. Several factors would argue against aborting a twin pregnancy with the intent of rebreeding the cow. First, the estimated average lactation length of cows subjected to induced abortion and rebreeding would approach 500 days (~18.5 month calving interval) based on average reproductive performance and management indices for lactating cows (Table 5). Second, the risk for a twin pregnancy during the subsequent gestation is increased because cows calving twins are at greater risk for subsequent twinning (Nielen *et al.*, 1989). Based on these considerations and depending on the value of the dam and calf, culling may be a better alternative to aborting the pregnancy.

If a cow carrying twins is to be maintained in the herd until parturition, several management

Table 5: Estimated intervals and cumulative days in milk associated with events after a management decision to terminate the pregnancy of a cow carrying twins.

Mean interval from:	Interval (Days)	Cumulative Days in Milk
Calving to twin pregnancy diagnosis and induction of abortion	184 ^a	184
Induction of abortion to second conception	84 ^b	268
Second conception to dry off	232 ^c	500

^aAverage days open (144 days; voluntary waiting period = 60 days, conception rate = 40%, and service rate = 40%)+ day of gestation at diagnosis of twins (40 days).

^bMedian days to second conception using AI breeding (84 days; conception rate = 40%, and service rate = 40%).

^cAverage gestation length (282 days) - average dry period (50 days).

practices should be considered. First, based on research in beef cows (Wheeler *et al.*, 1979; Koong *et al.*, 1982), dairy cows carrying twins should be fed a higher plane of nutrition, especially during the last trimester of gestation (Nielen *et al.*, 1989; Day *et al.*, 1995). Second, because the gestation length of cows calving twins is reduced by seven to 10 days (Pfau *et al.*, 1948; Nielen *et al.*, 1989; Ryan and Boland, 1991), most cows calving twins would miss the transition diet feeding period beginning about two to three weeks before the estimated calving date. Earlier dry off and feeding of a transition diet may reduce the incidence of postpartum metabolic problems associated with cows calving twins (Ryan and Boland, 1991). Finally, assistance at calving for cows carrying twins may reduce complications associated with dystocia and may reduce economic losses by reducing the rate of neonatal calf mortality.

Early embryonic loss

Pregnancy loss contributes to reproductive inefficiency in lactating dairy cows because fertility assessed at any point during pregnancy is a function of both conception rate and pregnancy loss. Conception rates at 28 to 32 days post-AI in lactating dairy cows range from 40 to 47% (Pursley *et al.*, 1997; Fricke *et al.*, 1998), whereas conception rates in dairy heifers are nearly 75% (Pursley *et al.*, 1997). Similarly, pregnancy loss in lactating dairy cows is greater than that in dairy heifers (20% vs. 5%; Smith and Stevenson, 1995). Although the specific factors responsible for early embryonic loss in dairy cows are not known, they may be similar to those factors responsible for reduced conception rates.

◆ Timing of early embryonic loss

Early embryonic loss in cattle is difficult to study because no sensitive test similar to that used for women and mares exists. The fertilization rate after AI in beef cows is 90%, whereas embryonic survival rate is 93% by Day 8 and only 56% by Day 12 post AI (Diskin

and Sreenan, 1980). In dairy cattle, only 48% of embryos were classified as normal on Day 7 after AI (Weibold, 1988). Thus, substantial pregnancy loss probably occurs within two weeks post AI.

Rectal palpation from 40 to 60 days post AI is the most common method of pregnancy diagnosis in dairy cattle. Several studies have used pregnancy diagnosis based on rectal examination to establish a conception rate from which pregnancy loss can be determined as gestation ensues. Using this technique, pregnancy loss is about 10%, with greater losses in lactating cows compared with heifers (Thurmond *et al.*, 1990; Markusfel-Nir, 1997). Furthermore, the risk of pregnancy loss was more than four times greater during the first trimester compared with the second and third trimesters of gestation (Markusfel-Nir, 1997).

Recently, transrectal ultrasonography was used to determine the timing of pregnancy loss from 28 days post AI to calving in lactating dairy cows (Vasconcelos *et al.*, 1997). Pregnancy diagnosis was conducted at 28, 42, 56, 70, and 98 days post AI for 1,600 dairy cows in three herds with a rolling herd average >23,000 pounds. The conception rate of cows at 28 days post AI was 32%, and overall pregnancy loss from Day 28 to calving was nearly 25%, with most losses occurring during the first 60 days of gestation (Figure 3).

◆ Factors affecting early embryonic loss

Because fertility assessed at any point during pregnancy is a function of both conception rate and pregnancy loss, factors associated with pregnancy loss may be similar to those responsible for low fertility. Nutrition can have a major impact on dairy cow fertility. A recent review (Ferguson, 1996) indicated that nutritional causes of low fertility are first due to energy management, second to excessive protein feeding, and third to trace element and vitamin deficiencies. In addition, greater body condition score losses from calving to breeding result in reduced fertility (Ferguson, 1996).

Specific physiologic mechanisms responsible for pregnancy loss in lactating dairy cows are unknown, but may include:

- 1) Lactational stress associated with increased milk production (Oltenacu *et al.*, 1980; Nebel and McGilliard, 1993),
- 2) Negative energy balance (Butler and Smith, 1989),
- 3) Toxic effects of urea and nitrogen (Butler *et al.*, 1995) or
- 4) Reduced ability to respond to increased environmental temperature (Stevenson *et al.*, 1984; Hansen *et al.*, 1992).

Beef cows losing weight have a higher incidence of early embryonic loss than those gaining weight (Dunn and Moss, 1992). This suggests that negative energy balance may be involved when a high incidence of early embryonic loss is observed in dairy cows. Recommendations for minimizing the severity of negative energy balance in high-producing dairy cows include:

- 1) Maximizing dry matter intake in early lactation and
- 2) Feeding diets containing 0.78 Mcal NEI per pound and 5%-7% total fat (DM basis).

High circulating urea and ammonia from the feeding of diets high in degraded intake protein (DIP) may adversely affect early embryonic development (Butler, 1998). Further, the feeding of excess DIP may exacerbate negative energy balance and related reproductive problems. Elrod and Butler (1993) reported increased early embryonic loss for heifers fed an energy-restricted diet containing high levels of DIP. Ferguson (1996) indicated that cows fed high amounts of DIP showed more irregular intervals between first and second service. Dietary DIP should be restricted to 10%-12% (NRC, 1989; DM basis). Also, nitrate concentrations in water

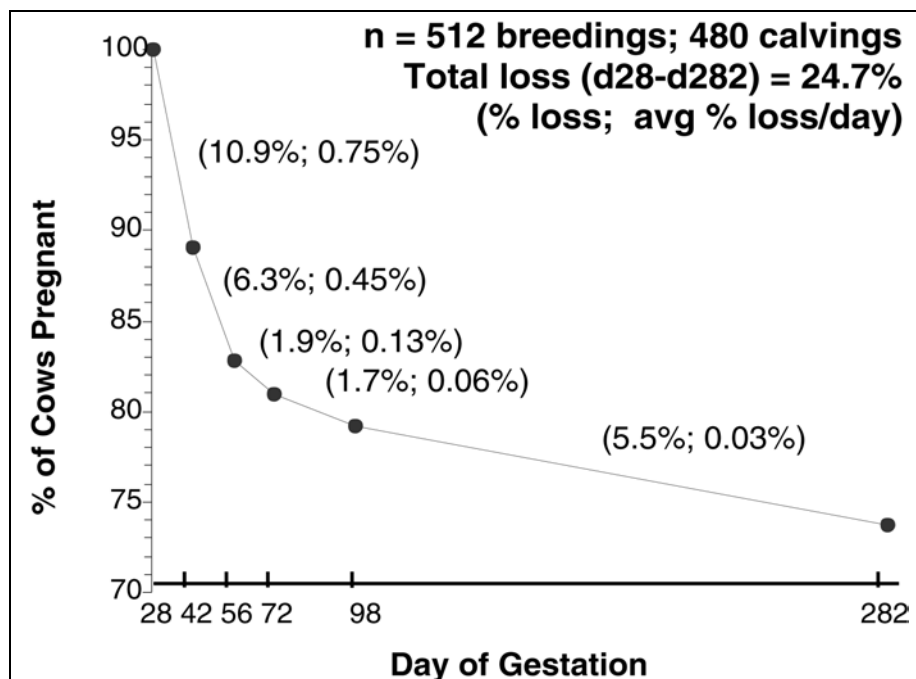


Figure 3: Pregnancy losses from 28 days post AI to calving in lactating dairy cows. Pregnancy status was diagnosed using ultrasound at 28, 42, 56, 70, and 98 days post AI, and calving data were recorded at parturition. The conception rate at 28 days was 32%. Data adapted from Vasconcelos *et al.*, 1997.

and forages should be evaluated when herds are experiencing a high incidence of abortions or early embryonic loss (Davison *et al.*, 1965).

◆ Reproductive management of early embryonic loss

At present, there is no practical way to reduce early embryonic loss in lactating dairy cows. However, recognizing the occurrence and magnitude of early embryonic loss may actually present management opportunities by taking advantage of new reproductive technologies that increase AI service rate in a dairy herd. One such technology is the use of transrectal ultrasonography for early pregnancy diagnosis. If used routinely, transrectal ultrasonography has the potential to improve reproductive efficiency within a herd by reducing the period from AI to pregnancy diagnosis to 26 to 28 days with a high degree of diagnostic accuracy (Pierson and Ginther, 1984). Furthermore, use of ultrasound could minimize embryonic loss that may occur after manipulation of the reproductive tract and conceptus during

pregnancy diagnosis using rectal palpation (Paisley *et al.*, 1978; Vaillancourt *et al.*, 1979).

There are several reasons that transrectal ultrasound is not widely used among bovine practitioners for pregnancy diagnosis at present. First, ultrasound machines are relatively expensive, costing between \$15,000 to \$20,000. Second, most ultrasound machines are large and require an external power source, thereby making them cumbersome to use under field conditions. Because of these factors, use of ultrasound has been restricted to research or specialized procedures, such as fetal sexing, transvaginal oocyte recovery, or embryo transfer. Fortunately, several companies are currently marketing newer generations of ultrasound machines that are cheaper, smaller, and battery operated. Continuation of this trend will foster future use of this technology by bovine practitioners.

There are two main caveats to using ultrasound for routine early pregnancy diagnosis in a dairy herd. First, emphasis must be given to identifying nonpregnant rather than pregnant cows. Of cows diagnosed pregnant at 28 days post AI, 14 to 16% experience early embryonic loss by 56 days post AI (Vasconcelos *et al.*, 1997; Fricke *et al.*, 1998). Therefore, cows diagnosed pregnant at 28 days post AI using ultrasound should be scheduled for reexamination around 56 days post AI, when the rate of embryonic loss per day begins to decline (Vasconcelos *et al.*, 1997; Figure 3). Second, a management strategy must be developed to return the nonpregnant cows to service as quickly as possible after pregnancy diagnosis. Such strategies include administration of PGF_{2α} to cows with a responsive CL, use of estrus detection aids, or a combination of both methods. Unfortunately, the service rate was only 58% when using a system combining PGF_{2α} and Kamar heat mount detectors (Britt and Gaska, 1998), probably due to the inherent inefficiencies of estrus expression and detection in lactating dairy cows.

◆ One strategy for managing reproduction

An attractive strategy for managing reproduction in a dairy herd would combine use of synchronization of ovulation and timed AI (Ovsynch), an estrus detection aid, and early pregnancy diagnosis using ultrasound. Every two weeks, groups of cows past the voluntary waiting period would receive their first postpartum insemination after synchronization of ovulation using Ovsynch. This would dramatically reduce median days to first AI by eliminating estrus detection for the first postpartum breeding. At the time of AI, an estrus detection aid such as a Kamar device or estrus detection tail paint would be applied to the cow. This would aid in detection of cows that return to estrus between 18 to 28 days post AI due to failure of conception or early embryonic loss. Cows detected in estrus during this period could then be inseminated based on the detected estrus. At 28 days post AI, a veterinarian using ultrasound would identify any nonpregnant cows, which would be scheduled for resynchronization using Ovsynch along with the next group of cows. This would eliminate reliance on estrus detection for the next breeding, thereby reducing the interval from pregnancy diagnosis to rebreeding. All cows diagnosed pregnant at 28 days post AI would be scheduled for a second ultrasound examination at 56 days post AI to determine if pregnancy loss had occurred. This is an aggressive reproductive management system that would improve reproductive efficiency by maximizing AI service rate in the herd. This is accomplished through use of early pregnancy diagnosis using ultrasound. Although estrus detection would not be completely eliminated using this system, it would be minimized through the use of Ovsynch and timed AI.

Retained placenta

The time shortly before and after calving, also known as the periparturient period, is an extremely important time for dairy cows in

terms of milk production and reproduction during the ensuing lactation. The endocrine events that occur during this time are responsible for coordinating placental separation from the uterus, uterine involution, resumption of reproductive cyclicity, and lactation.

During the later stages of gestation, the conceptus comprises two parts: the fetus and the membranes surrounding the fetus, or the placenta. The placenta serves as the functional unit of exchange between the maternal and fetal systems. Classifications of placentas vary widely among eutherian or placenta-bearing mammals. Dairy cattle exhibit a cotyledonary placenta in which exchange between the maternal and fetal systems occurs at specialized regions called placentomes. Each placentome comprises a portion of the maternal endometrium called a caruncle and a portion of the fetal membranes called a cotyledon. In dairy cattle, there are between 70 and 120 placentomes attaching the fetal membranes to the endometrium, each of which may attain a diameter of up to 10 cm during the later stages of pregnancy. Shedding of the placenta after parturition depends upon separation of the caruncular and cotyledonary portion of each of the 70 to 120 placentomes attaching the fetal membranes to the endometrium. Most cows expel the placenta within eight hours after delivery of their calf. Retention of fetal membranes for eight to 12 hours or more after parturition is indicative of the abnormal condition termed retained placenta. Interestingly, cows and water buffalo are the only domestic ruminants in which retained placenta routinely occurs (Laven and Peters, 1996).

◆ Mechanisms responsible for retained placenta

Retained placenta is costly to a dairy operation because it results in economic loss. Milk from cows treated with antibiotics must be discarded. In addition, a temporary reduction in appetite resulting in decreased milk production occurs in 55% to 65% of affected cows (Sandals *et al.*, 1979).

The exact mechanisms responsible for placental separation after parturition are not known and remain poorly understood (Laven and Peters, 1986). Eight factors that may interfere with normal release of the placenta include uterine atony, necrosis, edema of the chorionic villi, advanced involution of placentomes, hyperemia, placentitis and cotyledonitis, acute postpartum metritis, and immature placentomes (Paisley *et al.*, 1986). It is interesting to note that most pharmacological therapies for retained placenta aim to accelerate removal of the placenta by increasing uterine contractility; however, uterine atony is one of only eight causes of clinical cases of retained placenta.

Joosten and Hensen (1992) have proposed a novel hypothesis for the underlying mechanism causing retained placenta. They speculate that products of the major histocompatibility complex (MHC) genes may be involved in the etiology of retained placenta. Briefly, specific MHC gene compatibility between dam and calf would lead to impaired production of the appropriate lymphokines (immune factors), which in turn could inhibit the maturation of the placenta that is necessary for placental shedding (Joosten and Hensen, 1992). In contrast, MHC gene incompatibility between dam and calf would result in an appropriate immune response resulting in timely shedding of the placenta after parturition. Joosten and Hensen (1992) add that most risk factors correlated with retained placenta, such as twinning, abortion, reduced gestation length, and low calf birth weight are all in some way associated with fetal and/or placental immaturity, which can be associated with defective fetal-maternal signaling brought on by insufficient triggering of an alloresponse. Further research in this area may lead to new strategies for preventing or treating retained placenta in cattle.

◆ Risk factors and nutritional factors affecting retained placenta

Reported incidence rates of retained placenta vary widely depending on the clinical definition

of retained placenta and country of origin (Laven and Peters, 1996). Based on placenta retention for >24 hours, incidence ranges from 3.8% in the United Kingdom (Esslemont and Peeler, 1993), 7.7% in the United States (Muller and Owens, 1974), and 2.0% in New Zealand (Moller *et al.*, 1967).

Many factors are associated with retained placenta including:

- 1) Dystocia,
- 2) Abortion,
- 3) Milk fever,
- 4) Low protein diets prepartum, and
- 5) Deficiencies on selenium, vitamin E, and/or vitamin A (Roberts, 1986).
- 6) Others include breed, year, season, herd, gestation length, induction of parturition, twinning, age, and fatty liver (Laven and Peters, 1986).

Cows with milk fever at calving are more likely to retain their placenta (Curtis *et al.*, 1985; Odds Ratio = 4.0). Prepartum feeding strategies for controlling hypocalcemia, such as restricting intake of potassium and sodium,

reducing dietary cation/anion difference through supplementation of anionic salts, and regulating intake of calcium and phosphorus should aid in the prevention of retained placenta.

Julien *et al.* (1976a) reported that cows fed an 8.5% CP diet during the dry period had a higher incidence of retained placenta than cows fed a 15% CP diet (50% vs 20%). The NRC (1989) protein requirement for dry cows is 12% CP (DM basis). Lead feeding extra protein during the three-week steam-up period prior to calving may reduce the likelihood of retained placenta (Curtis *et al.*, 1985; Odds Ratios = 0.4 and 0.7), but controlled feeding trials are lacking.

Data on the effect of selenium and vitamin E supplementation during the dry period on the incidence of retained placenta are presented in Table 6. Feeding selenium-deficient diets during the dry period (<0.10 ppm on a DM basis) resulted in a high incidence of retained placenta. Supplementation with selenium (Julien *et al.*, 1976a), selenium and vitamin E (Harrison *et al.*, 1984; Julien *et al.*, 1976a; Julien *et al.*, 1976b; Eger *et al.*, 1985), or vitamin E (Erskine *et al.*,

Table 6: Effect of selenium (Se) and Vitamin E (E) on incidence of retained placenta in dairy cows

Ref ^a	Basal Se-E	Supp. Se	Supp. E	Timing	RP C ^b	RP T ^b
1	NA ^c	-	3,000 mg IM ^d	8-14 d PreP ^e	12.5%	6.4%
2	0.05 ppm Se 320 mg E/d	50 mg IM	740 IU/d oral	21 d PreP - Se Dry Period - E	16.0%	17.0% - Se 30.0% - E 0.0% - Se/E
3	0.02-0.06 ppm Se E was NA	50 mg IM 12.5 mg/wk oral	680 IU IM -	21 d Prep - Se/E Dry Period - Se	38.0%	0.0% - Se/ E 0.0% Se
4	0.02-0.04 ppm Se E was NA	50 mg IM	680 IU IM	40 & 20 d PreP 20 d PreP	51.0%	9.0% 10.0%
5	0.10-0.20 ppm Se E was NA	45 mg IM 6 g IR ^f	2,000 IU IM -	10 - 21 d PreP - Se/E 60 d PreP - Se	21.0%	25.0% 26.0%
6	0.04-0.10 ppm Se E was NA	4.6 - 23.0 mg IM	140 - 700 IU IM	21 d PreP - Se/E	23.6%	12.6%

^aReference: 1=Erskine *et al.*, 1997; 2=Harrison *et al.*, 1984; 3=Julien *et al.*, 1976a; 4=Julien *et al.*, 1976b; 5=Hidiroglou *et al.*, 1987; 6=Eger *et al.*, 1985.

^bIncidence of retained placenta in control (C) and treatment (T) groups.

^cNA = Not available.

^dIM = Intramuscular injection.

^ePreP = Prepartum.

^fIR = Intraruminal bolus.

1997) reduced the incidence rate for retained placenta, but effects of either selenium or vitamin E supplemented alone were not consistent (Harrison *et al.*, 1984). In the trial of Hidiroglou *et al.* (1987) with dietary selenium at 0.10 to 0.20 ppm (DM basis) during the dry period, the selenium and vitamin E treatment regimen did not reduce the incidence of retained placenta.

◆ Treatment of retained placenta

Manual removal of the placenta is one of the oldest and most widely practiced therapies for retained placenta and is advocated by some authors (Arthur, 1979). However, most research indicates that manual removal results in impaired rather than improved fertility (Paisley *et al.*, 1986; Bolinder *et al.*, 1988). This is because manual removal induces trauma, hemorrhage, hematomas, and vascular thrombi within the uterus. Furthermore, even when removal of the placenta appears complete, portions of fetal cotyledons remain attached to the maternal caruncles (Paisley *et al.*, 1986).

Drugs that increase uterine motility including PGF_{2α} and its analogs, oxytocin, and estrogens have shown limited benefit for treating retained placenta (Paisley *et al.*, 1986). Use of PGF_{2α} to treat retained placenta is widespread; however, many reports show no beneficial effects after spontaneous or induced retained placenta (Garcia *et al.*, 1992). In fact, PGF_{2α} metabolites (Bosu *et al.*, 1984) and uterine contractility (Burton *et al.*, 1987) are greater in cows with retained placenta, suggesting that placental detachment, rather than uterine atony, is the cause of retention. These results indicate that the efficacy of PGF_{2α} for treating retained placenta is questionable. In contrast, oxytocin may slightly decrease the incidence of retained placenta and subsequent metritis (Mollo *et al.*, 1997). Use of estrogenic compounds are thought to act by increasing uterine tone through increased uterine sensitivity to oxytocin (Roberts, 1986). However, one side effect of using estrogens to treat retained placenta is an increased incidence of ovarian cysts (Roberts, 1986). Considering the diverse causative factors

for retained placenta, it is understandable that most pharmacological treatments for retained placenta have little chance for success unless the cause of retained placenta is uterine atony.

◆ Metritis

Cows with retained placenta have an increased risk for metritis (Correa *et al.*, 1993; Emanuelson *et al.*, 1993), and metritis is thought to be the main factor by which retained placenta affects fertility (Lavan and Peters, 1986). In addition, cows with veterinarian-assisted dystocia, retained placenta, and displaced abomasum are at increased risk of contracting metritis (Odds Ratios = 4.9, 5.7, and 3.6, respectively; Curtis *et al.*, 1985). Gearhart *et al.* (1990) reported that cows that were over-conditioned at dry off and lost more condition during the dry period were more prone to veterinary-assisted dystocia. The incidence of metritis for cows that were normal and over-conditioned during the dry period was 14% and 31%, respectively (Butler and Smith, 1989). Dry cow and transition cow feeding programs that control over-conditioning and calving-related metabolic and reproductive disorders should aid in prevention of metritis.

Injection of 3,000 mg vitamin E IM once at eight to 14 days prepartum reduced the incidence of metritis from 8.8% to 3.9% (Erskine *et al.*, 1997). Harrison *et al.* (1984) reported metritis incidence rates for cows fed a selenium deficient diet during the dry period of 83% for control cows versus the following for cows supplemented with:

- 1) 65% selenium (50 mg injected IM once at 21 days prepartum),
- 2) 84% vitamin E (740 mg per day orally during the dry period), and
- 3) 57% selenium/vitamin E (combination of the two treatments).

◆ Management of retained placenta

Until the specific causes of and an effective treatment for retained placenta are discovered, prevention of the incidence of retained placenta

is the best strategy for dealing with this problem. Critical factors include nutritional management of transition and fresh cows, and

cleanliness of the calving area. Treatment for specific cases of retained placenta should be based on veterinary recommendations.

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