Epidemiologic Approaches for Measuring and Understanding Abortion in Dairy Cows

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Abstract

This paper offers methods for measuring abortion risk and undertaking abortion diagnostic investigations. In addition, some of the associations found between demographic and reproductive features of the dam and subsequent abortion are described. The cohort life table method is used to compute risk of fetal loss (abortion) for pre-defined time intervals (eg. days or weeks) during the gestation period, as well as overall proportion of cows that abort. In contrast, the abortion density method calculates abortion risk for a defined calendar time (eg. per month) as the number of abortions per cow-days-at risk. Both methods are standardized to account for the changing number of cows at risk of abortion, as a result of culling, death, abortion, and calving. Thus, both methods will produce higher and more realistic estimates for abortion risk than are typically calculated. These measures and statistical modeling have been used to estimate the expected magnitude of abortion for dairies (ranging from 8% to 19%) and to identify associations between demographic or reproductive features of the dam and subsequent risk of abortion. Some factors discussed are effects of a previous abortion, dam gravidity and age, days open at time of conception, and season. Analytic methods also are presented for herd-based diagnosis of abortion related to infectious agents or other exposures. These methods permit estimates of the risk, or proportion, of abortions attributable to exposure to an infectious agent or to other putative abortifacient agents.

Introduction

Abortion in dairy cows continues to represent a significant loss of production efficiency. Risks of abortion for large, intensively managed dairies typically are believed to range from 8 to 19% of cows confirmed pregnant.[2,4,16] Unfortunately, standardized measures have not been applied to estimating most rates of abortion or to investigating abortion problems, which has impaired our ability to make comparisons within and among herds and to begin to understand the vagaries and multicausal nature of abortion in dairy cows. This review illustrates some methodology for estimating abortion risk in dairy herds and for undertaking abortion investigations. In addition, a brief review is provided for demographic features of the dam and environmental exposures that may contribute to abortion risk.

Measuring abortion rates and risk

Two approaches to measuring abortion risk are presented. One uses a cohort life table that estimates the cumulative proportion of cows that abort (or fetuses that die) and the risk of a cow aborting at any specified time in gestation.[2,4,6,7] The other approach estimates abortion risk for a specified calendar time period (eg. month of December or the year 2003), using methods to calculate abortion density.[4] Both methods provide a standardized approach to measuring abortion risk because each approach adjusts for the changing number of cows at risk of aborting over the course of gestation or some other period of time.

Cohort life table method

The cohort life table method will be illustrated using the fetal life table below (Table 1). The life table utilizes current herd data to project the overall fetal survivorship (inverse of the proportion aborting) and provide estimates of high abortion risk periods during gestation. Data needed for a fetal survival life table are obtained for all cows known to be pregnant at some specified time or period. For example, the table might be used to measure fetal survival of all cows diagnosed pregnant in the past year, or of all cows that were pregnant during the last year. The following information on each cow is required to be compiled for the life table: a) days carried calf (DCC) at time of first pregnancy diagnosis; b) DCC at the time fetal death was expected to have occurred; c) DCC when the cow died, was culled, or was lost-to-follow-up for any reason; and d) DCC when the cow calved. Although the approach may be referred to as a fetal life table, generally the method measures loss of pregnancy between pregnancy diagnosis and calv-

Table 1. A fetal survival or abortion life table measuring abortion , fetal survival, and risk of abortion for a hypothetical group of 100 cows. The overall abortion risk is indicated as 1-**P**, or 1-0.807 = 0.193. Calculations are carried out to 3 decimals for the purpose of illustration; two significant digits normally suffice.

Interval (DCC) (i)	No. pregnant (n)	No. aborting (a)	No. dying, culled, not followed (c)	No. at risk of aborting (r)	Proportion aborting (q)	Proportion not aborting (p)	Risk of abortion (h)	Cumulative proportion not aborting (**P**)
31-65	100	1	2	99	1/99=0.01	1-1/99=0.989	1/98.5= *0.010*	**1.0**
66-100	97	4	1	96.5	4/96.5=0.041	1-4/96.5=0.959	4/94.5= *0.042*	(1 x 0.989) = **0.989**
101-135	92	3	3	90.5	3/90.5= 0.033	1-3/90.5=0.967	3/89= *0.034*	(0.989 x 0.959) = **0.948**
136-170	86	5	2	85	5/85=0.059	1-5/85=0.941	5/82.5= *0.061*	(0.948 x 0.967) = **0.917**
171-205	79	3	2	78	3/78=0.038	1-3/78=0.962	3/76.5= *0.039*	(0.917 x 0.941) = **0.863**
206-240	74	2	3	72.5	2/72.5=0.028	1-2/72.5=0.972	2/71.5= *0.028*	(0.863 x 0.962) = **0.830**
241-275	69	0	1	68.5	0/68.5=0	1-0= 1.00	0	(0.830 x 0.972) = **0.807**
Overall		18						**0.807**

ing, which can include embryonic loss (<45DCC), fetal loss (45-260 DCC),[1] and premature calving (>260 DCC).

Attempts should be made to include all pregnancy loss, including directly observed abortion as well as losses evident by an open cow with a previously diagnosed pregnancy and by a pregnant cow with a fetus younger than estimated from a previously diagnosed pregnancy and estimated conception date. For most abortions, detection is made one-to-three months after the abortion actually occurred and, therefore, it is often not possible to obtain the precise date of fetal death. It is important, however, that the date of fetal death be estimated as closely as possible, and that the date of abortion diagnosis not be used in calculating DCC at abortion (unless abortion was actually observed). The goal is for abortion estimates to refer to a specific gestation period , and not reflect the one-to-three month lag in diagnosing abortion. In addition, it is important that the DHIA 152-day rule for abortion dating not be considered in estimating the true time of abortion; otherwise, estimated abortion risk will be biased. Some guidelines (based on personal experience) for assessing when a fetus died include a) following an abortion, a new conception from a bull breeding typically takes place, on average, 1.5 heat cycles after abortion, thus an approximate abortion date can be obtained if the conception date of the second pregnancy is estimated and b) uterine involution to a 'normal' non-pregnant status typically occurs within three-to-four weeks following abortion. Practitioners should develop their specific guidelines for estimating the date of abortion.

The data on DCC indicated above for each cow are compiled in a life table based on pre-specified gestation intervals. These intervals can be quite wide (ie 30-40 days) or narrow (1-2) days), depending on the number of cows included in the table. For small herds with relatively few cows, wider intervals should be used; otherwise, a general assessment of gestational risk groups will not be possible because of the paucity of data in most intervals. Smaller intervals can be used for herds with a large number of cows. Generally, however, the interval used within a practice should be consistent so that abortion risk and patterns can be compared among herds. A description of each column in the life table follows.

i: The interval number; i=1 is the first interval, i=4 is the fourthth interval, etc. Number of days in the interval was set here at 35, in part for convenience of illustration.

n: The number of pregnant cows beginning each interval. Cows should enter the interval encompassing the time pregnancy was first diagnosed. Thus, if the pregnancy of a cow was diagnosed at 70 days, her data would be included beginning with the 66-100 day interval, not before then. For simplicity, however, the pregnancies of all cows in this example were assumed to have been diagnosed in the first interval.

a: The number of cows that aborted (or the number of fetuses that died) in the interval. The designation here assumes a reasonable estimate of the actual death date.

c: The number of pregnant cows that were censored during the interval. This represents the cows that died, were lost, culled or had not advanced in gestation beyond this interval. The example given here is simplified to exclude pregnant cows that had not advanced beyond a given gestation period. It is assumed that, on average, cows censored during the interval are at risk of aborting only for half of the interval. Or, stated in terms of the number of cows, only half of the censored cows are at risk of aborting during the entire interval.

r: The number at risk of aborting during the interval. This number represents an adjustment to n that considers that censored cows only spend about half their time in the interval. It is calculated as $r = n - \frac{1}{2} c$

q: The proportion of cows at risk of aborting in the interval that actually abort during the interval. It is calculated as $q = a/r$.

p: The proportion of cows at risk of aborting that do not abort in the interval. It is calculated as $p = 1- q$.

h: The incidence or risk (sometimes referred to as hazard) of abortion during the interval. It assumes that cows that abort do so on average halfway through the interval, or that aborted cows are at risk of aborting only for half of the interval. Thus, $h = a/(r - \frac{1}{2}a)$.

S: The cumulative proportion of cows that remained pregnant to begin the interval (or that remained pregnant at the end of the previous interval). This value represents a measure of the overall survival of the fetuses, or of the overall cumulative proportion of cows that had not yet aborted at a given time. The value of **S** for the first interval is, by definition, 1.0. $\mathbf{S}_i$ is calculated as $\mathbf{S}_{i-1} \times p_{i-1}$, indicating that the proportion surviving up to the beginning of an interval is the proportion that survived up to the beginning of the previous interval times the proportion of those that survived during the previous interval.

The life table above presents historical data for cows that aborted, died, were culled, or successfully completed a pregnancy. The beginning interval values assume rectal palpation is used to diagnose pregnancy. Intervals would begin sooner if ultrasound was used to diagnose pregnancy, and thus overall abortion risk would be higher than if rectal palpation was used to diagnose pregnancy. A variation of this table would be a 'real-time' table that includes all currently pregnant cows, including those for which pregnancy has not yet been terminated, either by abortion or by calving. In such a case, the cows still pregnant would be removed from column (c), indicating the DCC for today's date for each cow. Thus, in such a case, the number of cows censored would increase with each gestation interval.

Overall gestation-specific abortion risk is calculated as 1-**P** for the last interval. In this illustration, the abortion risk is 1-0.807, or 19.3%. Note that if one calculates an abortion risk considering simply the 18 of 100 cows aborting, the risk would be 18%. Thus, if an adjustment is not made for the cows censored over the course of gestation, as well as for cows that are no longer at risk because they aborted, an artificially deflated risk of abortion is obtained. In this case, abortion would have been underestimated by 6.7% ({0.193-0.180}/ 0.193), compared with the life table approach. Estimates of the risk of abortion for each period (h) can be plotted to assess when fetuses experience the highest risk of death (Figure 1). Such information can help guide diagnostic investigations and sampling. As shown in Table 1 and Figure 1, risk of fetal loss in this hypothetical illustration is greatest for the mid-gestation period, around five months.

Abortion density method

The abortion density method calculates the risk of abortion per pregnant-cow-days-at-risk (or cow-months or cow-years at risk) during some defined time period, such as a month or year. Using the changing number of cows at risk as the denominator, the method accounts for cows that are no longer at risk of aborting because they died, were culled, aborted or calved out. As an example, suppose there were five abortions in one month and the sum of all pregnant cow-days during the month

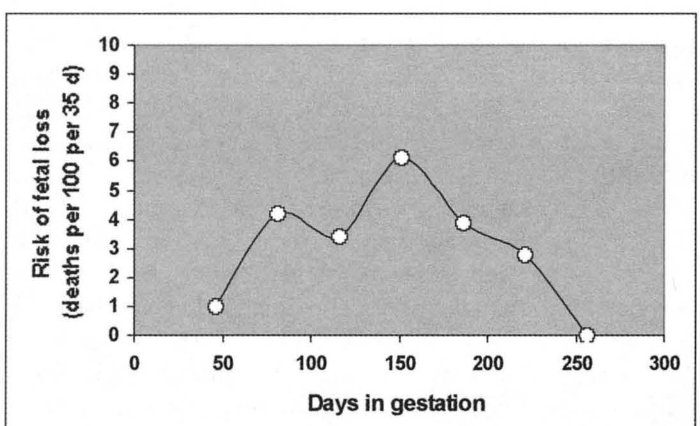

Figure 1. Risk of fetal death over the course of gestation for a hypothetical group of pregnant cows, as calculated using a fetal survival life table (see Table 1).

was 12,000. The abortion density would be 5/12,000= 0.000417 abortions per one pregnant cow-day at risk, or 0.0417 abortions per 100 cow-days at risk (eg. 4.17%). Table 2 provides hypothetical data for monthly abortion density calculations for a 1000-cow dairy. The abortion density method offers an overall standardized method to calculate abortion risk for any specified time period. These risks can be specified further for designated stages of gestation. For example, the abortion density could be calculated for cows pregnant at least three months, or pregnant between three and five months.

Herd-based diagnostic approach to abortion

The causes of most abortions remain unknown, in part because herd-based diagnostic designs have not generally been used.[6,10,13,14] In this section a herd-based diagnostic approach is illustrated which addresses a commonly asked question as to whether a herd, or a multiple-herd, abortion problem is related to some exposure, such as an infectious disease agent. A case-control diagnostic design is used in collecting serum samples from cows that have recently aborted (cases) and from at least as many pregnant cows in the same environment that have not aborted (controls).[13] If the herds are small and a large number of aborted cows cannot be identified at one time, then cows can be sampled over some period of time (perhaps even a year or more) to obtain a large and representative sample from those that have aborted and from those that have not aborted. The samples are tested, preferably at the same time, and the data organized in a 2 x 2 table, as shown below. The first question is whether there is evidence of a relationship between abortion and the exposure; that is, are abortion and exposure independent of each other. Independence is examined using a chi-square test, or Fisher's Exact test. If the P-value is <0.10, one generally has some confidence that the relationship observed between abortion and exposure did not simply occur by chance alone. The interpretation depends on the sample size, so that for small samples (<30) one might prefer to use $P<0.10$, whereas for large samples (>50), one might use $P<0.05$. If a large P-value is obtained, say $P>0.15$ or greater, then one would conclude with reasonable confidence that the data collected show no association between abortion and the exposure. At this point, one either can stop because there is no reason to proceed with the other calculations, or one can collect more data to improve the power of finding an association if one truly exists.

If there is convincing evidence that an association exists between abortion and the exposure, then the next step is to estimate the strength of the association. Strength of the association is measured by the odds ratio (OR), which in turn is used to estimate the attributable proportion (AP). The OR indicates how much greater the risk of abortion is for exposed compared with unexposed cows. For example, an OR=3 would indicate the abortion risk in exposed cows would be three times that of unexposed cows. The AP is calculated as (OR-1)/OR and is a measure of the proportion of abortion in aborted, exposed cows that was actually attributable to the exposure. The AP is a key herd diagnostic parameter because it provides an estimate of the importance of the exposure in the context of the herd. If OR=3, then AP = 66%, or 66% of the abortions in the aborted and exposed cows is attributable to the exposure. An example of an assessment for an association between *Neospora caninum* serologic status and a herd abortion

Table 2. An illustration of monthly abortion density calculations for a hypothetical 1000-cow dairy. Pregnant cow-months at risk was calculated by dividing the total number of pregnant cow-days in a given month by the number of days in the month. For example, 14,779 cow-days were tallied for January, which represents 476.7 cow-months (14,779/31).

	Month											
	J	F	M	A	M	J	J	A	S	O	N	D
No. abortions	9	11	7	9	12	10	13	11	8	9	7	9
No. pregnant cow-months at risk	476.7	540.8	508.5	536.9	522.6	524.2	503.2	491.4	492.0	460.3	472.0	472.6
Abortion density (%) (per 100 pregnant cows per month)	1.9	2.0	1.4	1.7	2.3	1.9	2.6	2.2	1.6	2.0	1.5	1.9

problem is given in Table 3. Here, the P-value is significant (<0.05), indicating the *N. caninum* serologic status and abortion are not independent (there is probably a link between the two). The OR of 4.6 suggests that the risk of abortion among seropositive cows in the herd could be expected to be about four times greater than among seronegative cows and the AP of 78% suggests that about 78% of the abortions among aborted, seropositive cows is attributable to *N. caninum*. It should be noted, as an aside, that it is common to find no evidence of an association between abortion and *N. caninum* in some herds, even though the herds may have a high prevalence of *N. caninum* infection.[13] This lack of association suggests that other factors, which vary from herd to herd, may predispose or help trigger fetal infection or death in cows infected with *N. caninum*.

Demographic and environmental factors associated with abortion:

Milk production

No clear evidence has emerged for a link between abortion and milk or fat production,[5] in part because of the methodologic difficulties in following a large number of cows to identify whether or not milk or fat production changes precede fetal death, and if production and abortion are confounded by disease that affects both production and fetal viability.

Age and gravidity

In women, advancing age is believed to carry with it an increased risk of spontaneous abortion, mainly associated with aged ova and development of trisomy. No studies have been reported, however, that specifically examine trisomy in cattle and related abortion risk. Nevertheless, age may play a role in reducing a cow's general ability to maintain a pregnancy through diminished immune function or other declining physiologic functions associated with senescence. One of the problems in assessing whether increasing age imposes an increased risk of abortion in dairy cows relates to the culling of aborted cows such that the older cows remaining in a herd have been preferentially retained, in part, because they experienced little if any previous abortion. Thus, an age-related risk of abortion could be seen to decline, rather than to increase, with age because each successive age group remaining in the herd represents a cohort that has experienced less abortion.

In addition, the possible relation between age and abortion is confounded by a cow's gravidity, which represents the number of her total lifetime pregnancies. It is believed by some that the greater the number of pregnancies (higher gravidity) the greater the reproductive 'wear and tear' experienced by the cow, and thus the greater risk of pregnancy loss in cows with high gravidity. Because gravidity and age increase together and are thus highly correlated, it becomes difficult to separate effects of increasing age and increasing gravidity on abortion risk. Using newly developed statistical methods that were able to tease out the effects of correlated variables,[3] a large study of dairy cows found that the risk of abortion did increase as cows aged.[16] Although the age-effect was significant statistically, the effect was quite small, and probably by itself would not contribute noticeably to overall herd abortion rates for most herds. The age factor could contribute to a trend toward higher herd abortion risks, however, for purebred herds that retain a high proportion of registered old cows and for herds expanding in size and that may not be culling many older cows.

In contrast to some prevailing dogma, however, the study found that after adjusting for the effect of age, the risk of abortion was lower for cows with high gravidities than for similarly aged cows with fewer lifetime pregnancies. Some possible explanations for why cows with many successful pregnancies might experience a lower risk of abortion could include a genetic predisposition to greater fertility and fecundity (number of offspring),

Table 3. An illustration of a herd-based, case-control diagnostic approach for assessing causes of abortion on dairies, using a hypothetical exposure to *Neospora caninum* as an example.

Neospora serostatus	No. aborted	No. not aborted	Odds of abortion
Positive	10	8	10/8
Negative	6	22	6/22
Total	16	30	46

Chi square = $[\{(10)(22)-(8)(6)\}^2 (46)] / \{(10+8)(10+6)(8+22)(6+22)\}$ = 5.6, P-value = 0.023
Odds ratio (OR) = (10)(22)/(6)(8) = 4.6
Attributable proportion (AP) = (4.6-1)/4.6 = 0.78

where there is a common mechanism operating both to maintain fertility and pregnancy after 42 days. Another explanation could be that higher gravidity cows remaining in a herd are those that withstood the culling pressure, in part by not aborting. Thus, low-risk cows (those with no history of abortion) would tend to be over-represented in high gravidity groups of retained cows.

Days open at conception

Cows conceiving early in lactation (30-60 days) can be expected to experience a higher risk of aborting the pregnancy, compared with cows conceiving later than 60 days or so.[3,16] Even though an increased risk of abortion for cows conceiving early has been found to be statistically significant, the effect per se of early breeding on overall herd abortion risk is likely to be small. Thus, for most dairies, efforts to breed cows later would probably not result in a perceptible decline in overall herd abortion.

Previous abortion

A cow's previous abortion history is probably the single greatest predictor of whether she aborts in the future.[3,16] Cows that abort tend to experience twice the risk of subsequent abortion, compared with cows that have not aborted. Further, cows that aborted their previous pregnancy after 75 days experienced a much higher risk of aborting the next pregnancy, compared either with those aborting less than 75 days, or with those not aborting the previous pregnancy at all.[16] Cows that had aborted and that were infected with *N. caninum* experienced an additionally higher risk of subsequent abortion, compared with aborted cows that were not infected with *N. caninum.*[12] There is one possible notable exception to the 'rule' that previous abortion increases risk of subsequent abortion. Cows that aborted their previous pregnancy early in gestation (before 75 days) experienced a lower risk of aborting the next pregnancy, even compared with cows that did not abort the previous pregnancy.[16] This sparing effect of early pregnancy loss may be related to the higher risk of abortion for cows conceiving early in lactation, as discussed above.

Summer heat

A common perception is that abortion risk increases during the summer, presumably because of increased environmental temperatures. Efforts to find an association between high ambient temperatures and increased abortion risk (proportion of cows aborting) in the Central Valley of California, however, have been unsuccessful so far.[8] A likely explanation for the observed increase in number of abortions during the summer is that the seasonal variation in conception puts the number of high-risk fetuses (those 3-5 months of age) at their highest during the summer months.[11] Typical seasonal variation in the number of fetuses between three and eight months' gestation for dairies in the Central Valley is shown in Figure 3. For most herds, the number of fetuses in the high-abortion-risk period of 3-5 months in gestation tends to peak during the hot summer months, even though the total number of fetuses at risk (or pregnant cow-days at risk) might be declining for those months (Figure 2). Thus, the perceived increase in abortions may be a reflection of the increased numbers of fetuses at a high risk of being aborted during those months; overall herd abortion risk generally has not been observed to increase during the summer.

Other factors

Very few formal studies have investigated the effects of environmental exposures on abortion risk. Evidence has been found for a seasonal influence on aborted fetuses that were infected with *N. caninum*[11] and a seasonality in the proportion of mummified fetuses was observed for month of conception (Figure 4).[4] The latter finding illustrates how various measures of abor-

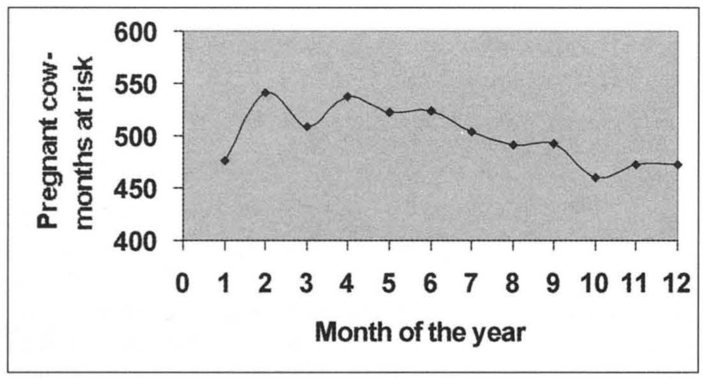

Figure 2. Seasonal variation expected in number pregnant cow-days at risk for a hypothetical 1000-cow dairy (see Table 2).

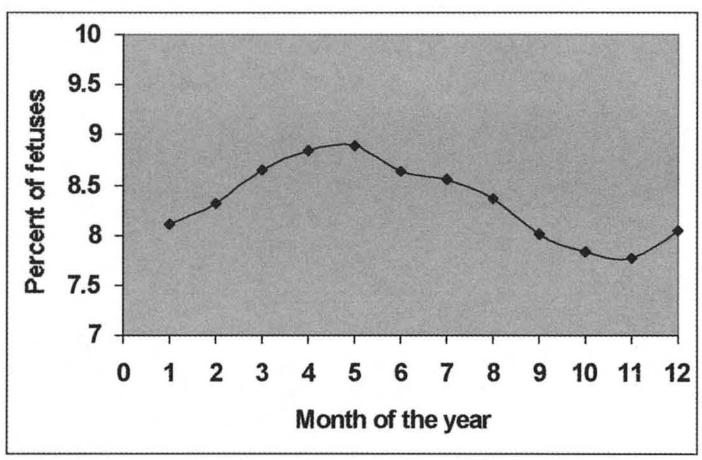

Figure 3. Seasonal variation expected in percent of fetuses 3-8 months of age. Graphed from data presented elsewhere.[11]

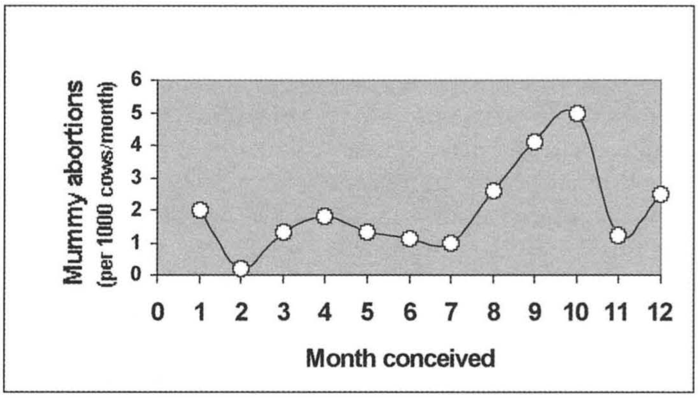

Figure 4. Variation in estimated risk of mummified fetuses based on month of conception. Graphed from data presented elsewhere.[4]

tion can be used to identify possible diagnostic hypotheses. For example, the elevated risk of mummies for cows conceiving in the early fall in California could suggest early embryonic exposure to agents prevalent at that time of the year (ie bluetongue virus, feeding of seasonal feedstuffs). Palpation per rectum has not been found to be associated with abortion, at least for veterinarians experienced in palpation.[9]

Summary

Methods are presented that permit a standardized measure of abortion risk for dairy herds, and a standard approach to investigating a herd abortion problem. The methods permit abortion risk assessment for a given month and over the course of gestation. Standardized risk measures and methods can be used to evaluate changes in herd abortion risk, identify possible management factors contributing to an abortion problem, and to develop diagnostic strategies. Some of the demographic features of the dam that can increase or predict her risk of abortion include early conception, advancing age, poor reproductive performance in general, and a previous abortion.

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