Controlling What We Can Control: Limiting Embryonic/ Fetal Losses

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Abstract

While there is sometimes a feeling of hopelessness when we are asked to solve a herd problem involving "occult" loss of pregnancies, there are a few things we as veterinarians can do to reduce the risk of embryonic and fetal losses. For many infectious causes of pregnancy attrition, there are vaccines of various safety and efficacy. Some of them are effective because elegant research on host-pathogen interactions has pointed the way to exquisitely specific and effective antigens and adjuvants; some are effective in spite of a lack of such information; and a few are not particularly effective. But not all pregnancy losses are the direct result of fetal infections. This paper will attempt to address the increasingly long list of causes of pregnancy disruption in cattle, and to point out some preventive measures reported to be effective, or in the case of some as yet unproven interventions, at least make pathophysiologic sense. Topics will include measures to diminish the impact of non-infectious causes of pregnancy disruption (trauma, heat stress, twinning, plant and other ingested toxins); a few infectious causes where preventive intervention is likely to pay off (e.g. infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD) venereal campylobacteriosis, leptospirosis); some "iffy"areas where the payoff is not as certain; and a discussion of the expectations of a vaccine against any reproductive pathogen. A brief mention will be made of some current thinking on efforts to reduce early pregnancy loss through nutritional management of dairy cows.

Introduction

The list of factors that directly cause or contribute to pregnancy loss in cattle is a long one. But the list of those factors that we can do something about is considerably shorter, and will be the subject of this presentation. The list is not exhaustive, but attempts to address factors that all of us confront at some time or another. Many, if not most of those in the audience are already doing much of what can be practically done. The presentation will discuss major categories of insults to the conceptus, and an example or two of either a well-tested approach to correcting or preventing the consequences of these insults, or an approach that has at least some basis in science.

Definitions

Early embryonic death (EED) is taken to mean death of the conceptus before maternal recognition of pregnancy (MRP), i.e., death prior to day 16-19 of gestation. Such an event theoretically has no effect on estrous cycle length, and accounts for as much as 70-80% of total pregnancy wastage (which is about 40-60% of all fertilizations). [47,86,88] Death of the conceptus after MRP, but before organogenesis is complete (~day 42) will be labeled as "late embryonic death", and accounts for an additional 10+/- 5% loss.[86] And death of the conceptus after day 42 will be termed fetal death, which can roughly be divided into early, middle, and late fetal deaths that correspond to the three trimesters of gestation. These are abortions of diagnosed pregnancies, and occur in 5-12% of pregnant dairy cattle.[28,97] Different studies use different delineations, which makes comparison of mortality data confusing. Some studies refer to "late ED" as death that occurs between day 28 and day 84.[86] Most studies agree that only about half of this "late embryonic death" occurs between day 28 and 42, i.e., while the conceptus is still technically an embryo.[44,86]

Major Factors Affecting Embryonic Survival

Genetic factors

At the grossest level of genetic defects, chromosomal abnormalities (e.g., aneuploidy, polyploidy,) account for a very large proportion of embryonic losses in many mammalian species, including cattle.[6] That so many early embryos fail (as many as 25-50% in humans [39]) because of chromosomal problems indicates just how difficult it is to properly match up maternal and paternal chromosomes at fertilization. Other wayward chromosomes occur either by duplication or deficiency, in which case the total amount of DNA is also changed. A rearrangement that does not change the DNA amount is a translocation, in which two chromosomes break and

reunite such that all coding material remains attached to one centromere while the other centromere is lost. Robertsonian translocations may still allow formation of gametes at meiosis, but typically there is an increased proportion of non-fertilizing or non-fertilizable gametes. If fertilization by a bull with a 1/29 Robertsonian translocation does occur, the resulting zygote is at increased risk of embryonic death.[6] In a Darwinian world, this bull would be selected against, but artificial insemination allows the sowing of his seed across a swath of the population, especially if he has a high PTA/reliability for important production traits.

At the level of the gene, there are well-known heritable defects associated with embryonic death, a single example of which is the deficiency of uridine monophosphatase synthase (DUMPS). This gene defect is well characterized, and represents a single point mutation (from a C to a T), transmitted as a Mendelian recessive.[84] Polymerase chain reaction (PCR) tests are available to identify heterozygous (carrier) animals and homozygous embryonic cells.[83]

"Fertile" bulls whose offspring die very young

Bulls have well-documented differences in conception rates, but there is also a difference in conceptus survival between bulls. For example, Lopez-Gatius *et al* showed in a single herd longitudinal study that one of six bulls used had similar conception rates to the other five bulls, but a significantly higher proportion of early fetal deaths[44] in the absence of known infectious disease influence.

What we can do today about genetic factors

Some constitutive chromosomal abnormalities can be diagnosed in the sire or dam, e.g., the Robertsonian translocations (1:29 or 16:20, e.g.),[78,103] and the stud industry is generally good about listing such defects in their sire catalogs. It's important to note that failure to find such defects in a pedigree analysis does not rule out Robertsonian translocations, which can occur *de novo*.[78] Regarding molecular-level genetic defects, again, when the gene for the defect is characterized, most bull studs list the sire's genotype for this trait right alongside that bull's PTA or other production parameters. Where diagnostic tests (e.g., PCR) exist, a sample of citrated blood is often all that is needed for diagnostic karyotyping. In such cases, although we can't do much about the defect once it is manifest, we can prevent it by selective mating. Since such defects are typically identified as Mendelian recessives, it is not necessarily contraindicated to breed a carrier bull to females, so long as the genotype of the female is known. All offspring of a carrier sire x homozygous normal dam should be normal, but half of them will be carriers, and these should probably not be bred to carrier bulls. So a little old fashioned "Aa x AA" genetic counseling can be helpful here.

To identify bulls with low conceptus survival rates, you'll probably need software that can trace cows back to their artifical insemination (AI) mates of record for a lost pregnancy. Depending on timing of the embryonic deaths and pregnancy diagnosis, some of these bulls could show favorable conception rates, so it would be important to look beyond simple conception rates and examine lost pregnancies by AI bull. Dairy software that allows you free access to the database will be necessary. Before condemning that bull, it would be informative to have a laboratory culture a few representative straws (include *Mycoplasma* cultures), to be sure that the problem is the bull's, and not the processor's. While bacterial contamination of frozen semen will typically reduce its fertilizing ability (and therefore not affect cycle lengths), slow-growing organisms may not act until the embryo has developed for awhile.

Possible developments

Those genes that are expressed early in the embryo's life, and that confer faster development of the pre-implantation embryo, are being identified in various laboratories around the world. For example, the protein product of the *Ped* gene in mice promotes faster cleavage, better survival to birth, birth weight, etc.[27] The hunt is on for a similar gene in cattle, with some interesting early results so far. Whether this line of research will lead to practical on-farm applications isn't known at this time. Possibilities include everything from development of a protein product that could be added to semen extenders, to gene insertion in a bovine zygote to make a cow transgenic for this gene, to genetic selection for cows that produce the product naturally.

Heat Stress

We all know that it's hard to get and keep a cow pregnant when environmental temperatures/humidity are consistently above the "comfort index", i.e. above a temperature-humidity index of 72°F (22°C).[4,22] The early conceptus is vulnerable to heat shock, especially in the first week. Although heat stress later in gestation influences placental function and can contribute to modestly altered gestation periods, smaller placentae and fetuses, as well as increased PGE_2 (from the conceptus) and $PGF_{2\alpha}$ (from endometrium) in a ruminant model,[3,5,74,75] it generally does not contribute to the overall proportion of cows that abort a diagnosed pregnancy, i.e., after about day 42. This relative resistance to heat-induced, mid-to-late-term abortion may be due to an adaptive mechanism, whereby during prolonged heat loads, conceptus growth is down-regulated. But cows with sustained exposure to temperature/humidity conditions above the comfort index any time during the first week

following estrus and ovulation suffer significant losses, with many embryos failing to survive to the period of maternal recognition. Most have found that fertilization itself is not blocked, but that heat-stressed embryos created by that fertilization develop at a slower pace, such that by day 7, when most "normal" embryos are at the late morula-early blastocyst stage, heat-stressed embryos will have developed more slowly, and suffered significant intracellular damage. This damage occurs whether the oocytes were fertilized and heat stressed *in vivo*[71,72,74] or *in vitro.*[73,91] Moreover, within this one-week window, the earlier the embryo is heat-stressed, the less likely it is to survive to MRP. In addition, the uterine environment of the heat-stressed cow is compromised, with altered blood flow, and probably alteration of uterine secretions ("uterine milk") that are critical for the pre-implantation embryo's survival.[24]

The retarded development of such heat-stressed embryos results in insufficient interferon tau (IFNτ) production by embryonic trophectoderm, and a lost pregnancy before we (or she) even knew she was pregnant (early embryonic death-EED). In general, as development proceeds, the conceptus becomes more resistant to heat stress such that by day 42, near the end of organogenesis, it is relatively tolerant of an increased heat load. [24] Hence, the finding that heat stress contributes little to the risk of aborting a *diagnosed* pregnancy. [45]

Magnitude of the problem

In the United States, from Florida to Fresno, and Minnesota to the Mexican border, conception rates (diagnosed pregnancies per insemination) fall dramatically in the summer for lactating cows. Summer conception rates of 12% are not unusual in the famously hot states.[55,72] An interesting paper by Dr. Lopez- Gatius in northeast Spain examined fertility parameters in large dairy operations over a 10-year period, and found that – to no one's surprise - fertility is declining, if measured in terms of cyclicity rates, pregnancy rates, inactive ovaries, etc., but only in cows examined or inseminated in the warm season. Cows examined or inseminated in the cool season have maintained the same fertility indices for the past decade, during which time these Spanish Holsteins' milk production has risen by 31%.[45] Similarly, Sartori *et al*[80] found that fertility (in this discussion, taken to mean early embryo survival and quality) of virgin heifers or non-lactating cows inseminated in summer was higher than for summer-bred lactating cows, while during the winter, there was little difference in embryo survival between lactating and non-lactating animals. This and other evidence suggest that falling embryo survival rates are not a direct consequence of increased milk production per se, but increased milk production plus heat stress pushes many cows beyond their ability to cope.

What we can do about embryo losses due to heat stress

"Everyday solutions" include use of fans and evaporative cooling. A whole body of literature on evaporative and convectional cooling exists, and I'd refer you to that literature.[4,22] Essentially, strategic cooling in the following areas has been shown to be beneficial, in terms of milk production and reproductive performance: solid shade for all adult cows (milking and dry); holding pen cooling (beyond udder-cleaning – this can lower core body temperature as much as 3.5° F (-15.7°C); exit lane cooling; corral shade cooling; and feed line cooling.

More involved solutions

Embryo transfer, using fresh embryos created *in vitro* (IVF) under controlled conditions, has been shown to increase pregnancy rates when transfers are made to synchronized heat-stressed recipients by appointment, or so-called timed embryo transfer (TET).[1] However, when the freezing method was vitrification, pregnancy rates were lower than when fresh IVF embryos were used in a TET program,[1] suggesting that the art of vitrification needs improvement. Alternatively, some have successfully collected *in vivo*-fertilized embryos during the cool season, frozen them, and transferred them to heat-stressed recipients.[25,34] Frozen, cool-season embryos, unlike their hot-season counterparts, reached the blastocyst stage unmolested by heat shock. This does not guarantee them successful gestation to term, but gives them more of a chance to survive until MRP, and since the effects of heat stress diminish with advancing embryo development, this procedure buys some valuable time.

Cross breeding?

Within *Bos taurus*, there are differences in the ability to tolerate heat loads, at least as measured by increases in rectal temperatures[64,69] or in respiratory rate,[56] with corresponding increases in environmental temperatures. These studies generally rank dairy breeds' resistance to heat shock as Jersey>Brown Swiss> Holsteins. Cross-breeding with *Bos indicus* gains a considerable amount of heat tolerance,[15] but compromises production.[53] Recently a major (single?) gene that influences hair length and heat tolerance has been described in *Bos taurus* cattle.[65] Perhaps, through transgenic technology, this gene could be introduced into the major domestic milking breeds.

Hormonal Deficiencies

This is a catch-all term that serves as a useful "backdoor" diagnosis when we can't determine what's causing embryo/fetal death. Many of us use the term without knowing which hormones might be involved, what the timing of the "deficiency" is, or what we can

do about it. The hormone that gets the most attention is progesterone (P_4). Certainly pregnancy won't proceed without it, but P_4's deficiency is likely an effect rather than a fundamental inability of the CL to make progesterone. There is a relationship between circulating plasma levels (or milk levels) of P_4 and progesterone production by the corpus luteum of early pregnancy, and several factors influence that relationship. The effective concentration of P_4 at the endometrium is a function of production rate, distribution, and catabolic rate. Production rate undoubtedly has some genetic influences, manifest via gonadotropin and other hormone receptors, and numbers of activated copies of genes for steroidogenic synthetic enzymes. Distribution can be influenced by environmental temperature, as already mentioned. Under prolonged heat stress, uterine blood flow can be altered such that distribution of progesterone to the pregnant uterus can be compromised. Additionally, progesterone metabolism, which occurs mostly in the liver, can be influenced by the rate at which P_4 is delivered to that organ, i.e., by hepatic blood flow.[79] Several studies have noted that in cows with high dry matter intakes (and especially high intakes of protein – i.e., in high-producing dairy cows), there is increased hepatic blood flow, with resultant increased metabolism of progesterone. The latter phenomenon is sometimes blamed for the apparent association between milk production and fertility, but closer scrutiny (see Dr. Thurmond's paper in these *Proceedings*) suggests that a direct relationship between milk production and abortion of a diagnosed pregnancy has yet to be established. It *is* likely that such a metabolic explanation for "progesterone failure" is valid at the time of establishment of pregnancy (MRP), well before we can make a definitive pregnant/open diagnosis. Evidence for this failure comes from two types of studies: (1) those in which naturally circulating P_4 levels at various post-insemination/pre MRP days were directly correlated with pregnancy rates,[12,33,48] and (2) those in which increases in pregnancy rates were associated with (a) direct boosting of P_4 levels by administration of exogenous progesterone or (b) indirect boosting of endogenous P_4 via exogenous gonadotropin treatment before MRP.[8,82,92,94]

What we can do about "hormonal deficiencies"

As mentioned above, supplemental progesterone has been advocated to get the early embryo past the period of maternal recognition. But direct supplementation, e.g., via intravaginal progesterone devices, has had mixed results. Professor Bill Thatcher has advocated boosting endogenous P_4 by injecting an LH-like product, namely human chorionic gonadotropin (hCG) at day 5 after insemination. In studies conducted at multiple sites in high-producing dairy cows, his group showed that >85% of cows given hCG (3300 IU, intramuscularly) on day 5 developed accessory CLs, had approximately 3 to 6 ng/ml higher plasma progesterone concentrations than controls in cows and heifers, respectively, and had significantly higher conception rates as determined by examination at days 28, 45, and 90. How does hCG accomplish this? The increased P_4 output probably doesn't hurt. Some studies have shown increased early embryonic growth when P_4 levels increase. An additional possibility in heifers is that hCG will change the follicular wave pattern in most individuals to a "three-wave" pattern, such that the dominant follicle of the final wave isn't secreting much estradiol until almost day 20, which means that prostaglandin synthesis and release is likely to be delayed as well, a condition that favors maternal recognition of pregnancy.[94]

A similar approach to raising endogenous progesterone is to inject a GnRH analog, or better, to implant a sustained-release GnRH agonist. Such a scheme would take advantage of the analog's ability to cause immediate gonadotropin release from the pituitary, which in turn will produce accessory CLs, as well as its ability to subsequently down-regulate pituitary gonadotrophs, thus inhibiting further follicular development while existing luteal function is enhanced.[93] This GnRH agonist implant approach is currently experimental, but watch for developments in this area.

A third approach to nurturing the conceptus through the period of pregnancy recognition involves the use of non-steroidal anti-inflammatory drugs to suppress $PGF_{2\alpha}$ synthesis from arachidonic acid precursors, and thus sustain the CL through the critical 15-17 day period. Only a few studies are available on the use of compounds like flunixin meglumine for this purpose. In one such study,[63] pelleted flunixin was fed multiple times/day to cycling heifers, beginning at day 14. Luteolysis was delayed in a dose-dependent manner, such that animals treated four times per day delayed luteolysis until after treatment ceased. Clearly, more practical means of delivery, not to mention studies on the abomasal effects of prolonged flunixin intake, are necessary before this practice can be recommended.

Bovine somatotropin (BST), given at the time of the second GnRH injection in an OvSynch protocol, can increase the proportion of females that conceive in lactating, but not in non-lactating cows. BST acts synergistically with IFNτ, to regulate prostaglandin secretion, at least *in vitro*.[93] BST probably reduces the endometrial estrogen receptor α (involved in stimulation of $PGF_{2\alpha}$), while IFNτ probably down-regulates the oxytocin-induced secretion of $PGF_{2\alpha}$. So this is not a P_4-raising strategy, but rather a P_4-saving strategy, designed to sustain the CL of pregnancy through the MRP period.

Dietary and Metabolic Influences on Embryo Survival/Death

Diet (General)

Negative energy balance following calving is a well-described risk factor for anestrus and extended calving-conception intervals. But it also contributes to poor survival of those embryos that *are* conceived. One proposed mechanism of this embryonic death is a relative progesterone deficiency, predicted by P_4 levels manifest in the cycles preceding insemination. Cows with greater negative energy balance in the first month after calving are more likely to show these low P_4 cycles.[14,44,45] The reason for the apparent "memory" by which negative energy balance is manifest as low P_4 may be that follicles that are in their early growth phase at the time of this metabolic stress (early post partum) will be compromised by decreased levels of growth factors, including IGF1, and when recruited to ovulate 40-50 days later, will make less competent CLs (i.e., bad follicles make bad CLs).[102] In addition, increased metabolism of P_4 by the liver of cows fed high energy/high protein diets[79] would exacerbate the low progesterone environment created by such bad CLs.

Protein metabolism

Blood/Milk Urea Nitrogen - There is now a fairly large body of literature that collectively points a finger at elevated blood urea nitrogen BUN) as an agent of fertility disruption, although its precise role in embryonic and early fetal death is not well defined.[54] The high BUN is usually traced to excessive rumen degradable protein is the ration. Rumen microbes convert excess amino groups to urea and ammonia. There is disagreement among studies on effect of elevated BUN/MUN levels on embryo survival. Some beef cow studies show little effect of elevated urea on embryo quality or viability, while several dairy cow and *in vitro* studies strongly suggest a negative effect. Among other effects, elevated plasma urea levels are inversely related to uterine luminal pH, with BUNs in the 20 mg/dl range corresponding to uterine pHs of 6.0 or less.[13] Normal luminal pH is dynamic, but is generally above 7.1.[36,49] Early embryos tolerate small changes in pH for very short periods (minutes to a few hours), but increasing the hydrogen ion concentration by a factor of 10 (1 pH unit decrease) is likely to be very hard on embryo viability. Moreover, function of the dam's uterine epithelial cells is altered, such that the normal pH gradient across polarized cells is changed, and more $PGF_{2\alpha}$ is secreted.[13]

Dietary dysfunction – Carbohydrates

Excessive carbohydrate fermentation, leading to rumen acidosis and the resulting tissue insult suffered by the rumen epithelium, allows otherwise harmless microbes access to the circulation, and thus to all organ systems, including the uterus. This is likely to be the route of infection for some (most?) of the non-specific bacterial abortions that occur sporadically. Bacteria can disrupt pregnancy directly, by transplacental migration and infection of the fetus, or indirectly via endotoxin production (see below, under "Indirect infectious processes").

Negative metabolic influences on embryo survival: What we can do

As a practicing non-nutritionist, I will keep my remarks at a very general level here: densify the ration; that is, since dry matter intake is compromised in the early postpartum period, get more calories per pound or kilogram of feed. The feeding of fats has become routine in today's high-producing dairies. Several studies suggest that feeding supplemental fat not only has a positive influence on P_4 production (perhaps by providing cholesterol as a progesterone precursor), but also reduces clearance of P_4.[50,51] Recent work suggests that the poly-unsaturated fats, in particular the so-called omega-3 fatty acids, may have a role in maintaining early pregnancy, at least through MRP.[70,89] Briefly, the omega-3 fatty acids (so-called because the first double bond occurs in the third position) include linolenic acid (LNA), an 18-carbon essential fatty acid that constitutes a major component of cell membranes. It is metabolized by $\Delta 6$ desaturases, elongases, and $\Delta 5$ desaturases to eicosapentaenoic acid (EPA), a 20-carbon molecule that is a precursor for the synthesis of "series 3" prostanoids. Additionally, linoleic acid (LA) is an 18-carbon omega-6 essential fatty acid that is metabolized by the same desaturases and elongases to arachidonic acid, which is the 20-carbon precursor for the "series 2" prostanoids. Arachidonic acid is processed by the prostaglandin H synthase complex (PGHS) to produce prostaglandins and thromboxane, or by lipooxygenase to produce leukotrienes. So what does all this alphabet soup have to do with embryonic survival? There is some evidence of competition between EPA and arachidonic acid for the PGHS complex, a situation that may slow the production of $PGF_{2\alpha}$.[12a,52] Several studies have suggested that feeding high levels of omega-3 and omega-6 fatty acids has a "sparing" effect on the corpus luteum. For example, dairy cows fed supplemental fish meal (high in EPA and another omega-3 fatty acid metabolite, docosahexaenoic acid, or DHA) at 5.4% of the dry matter, had a lower endometrial release of $PGF_{2\alpha}$ in response to estradiol and oxytocin given on day 15. This was measured as a reduction in $PGF_{2\alpha}$ metabolites in the peripheral plasma.[51,52] Furthermore, *in vivo* cow studies suggest that the sensitivity of the CL to injected $PGF_{2\alpha}$ is reduced in animals fed fish meal, a rich source of omega-3 fatty acids.[12a] Keep in mind that PGI_3, the

prostanoid produced by metabolism of omega-3 fatty acids, has significant vasodilatory effects that could counter the vasconstrictive effect of $PGF_{2\alpha}$, and thus perhaps help spare the CL from "starvation." The total picture is still not in focus, and some studies have come to different conclusions, i.e., that LA and/or LNA cause a reduction in early luteal phase P_4.[77] But a case is developing for feeding of polyunsaturated fats, especially omega-3 fatty acids, as a way to reduce endometrial $PGF_{2\alpha}$ production, and to desensitize the existing CL to the luteolytic action of the $PGF_{2\alpha}$ that is produced. In theory, it should be desirable to include omega-3 fatty acids in the ration for at least a month after insemination.

What we can do about nutritional effects on embryonic death

Nurture the CL and the early, elongating embryo. The practical means of doing this are to (a) establish a nutrition program that minimizes body condition losses in the first month of lactation; (b) evaluate protein utilization by monitoring urea levels as BUN or MUN; (c) minimize rumen acidosis; (d) consider supplementing with polyunsaturated fats, including omega-3 fatty acid sources like linolenic acid. Obviously, work closely with a qualified nutritionist in all of these plans.

Nitrates

Nitrate-induced abortion is something we used to understand. The concept was simple: Nitrates accumulated in nitrogen-fertilized or heat-stressed forages/hays. Nitrites, either pre-formed by the plant material, or converted from nitrates by rumen microbial action, were quickly absorbed from the victim's GI tract, and rapidly caused a met-hemoglobinemia by oxidizing hemoglobin Fe^{++} to Fe^{+++}.[16,100] Methemoglobin doesn't carry O_2 well at all. And if that weren't enough, the nitrite also acts directly as a vasodilator, thus reducing arterial pressure significantly, with the effect of reducing peripheral perfusion, including the uterine vasculature. Given that the fetus's pO_2 is dependent on the dam's pO_2 and the placental perfusion rate, this "one-two punch" would seem to be sufficient to either kill the fetus outright, or in a late-term exposure, stress the fetus to the point where its adrenal glands would begin the cortisol cascade that will prematurely initiate parturition. But the literature has many contradictions. Several papers report a failure to cause abortion in the absence of overt signs of nitrate/nitrite poisoning (muscle tremors, weakness, dyspnea, falling, cyanosis) in the adult herd. In those cases where abortion did occur following acute exposure, it was delayed by about a week after the first deaths occurred among adults. In one study, increases in nitrate/nitrite intake of > 0.5% of dry matter were associated with an increase in parturient paresis and puerperal endometritis, but a decrease in abortion.[46] Some authors discount altogether the occurrence of nitrate-induced abortion in cattle that have not shown symptoms. Others see an all-or-none presentation, i.e., in some herds, abortion-only, without overt illness is seen; in others, only acutely poisoned cows are seen, with little or no abortion among unaffected cows.[105] These differences in presentation are probably manifestations of the dose/intake rate of the offending nitrites, since prospective studies have shown acute toxicity leading to death of adults only when methemoglobinemia reaches about 70% (i.e., 70% of Fe^{++} Hb is converted to Fe^{+++} Hb). Overt signs in cannulated late-term fetuses were seen when fetal methemoglobinemia was only 30-40%. Lesser amounts failed to elicit overt signs in dams, but in some cases were able to induce an abortion about one week after nitrite exposure.[98] One study from Mexico fed increasing amounts of $Na\text{-}NO_3$ to pregnant cows every three-to-four days. Only two cows showed any effects: one had symptoms of nitrite poisoning without aborting, and one cow aborted later without showing signs. The abortion was attributed to IBR.[30]

What we can do about nitrate-induced pregnancy losses

Typically, we don't see this one coming. But oat, grass, sudan, green-chop corn, and other forages from nitrogen-fertilized fields and harvested after a drought, or put up damp in hot weather, should be tested for nitrate/nitrite levels before feeding. If levels are elevated (>1% dry matter [DM] DM), they can still be fed, but need to be diluted in low-nitrate roughages. If symptoms are already evident in cows, methylene blue is still the treatment of choice; up to 50 ml of a 5% solution (25 grams of methylene blue powder dissolved in 500 ml of sterile saline) is given by rapid intravenous injection.[100] Even if you save the cows, expect some abortions in the next week. While there may be some scientific logic behind attempting to save the pregnancies of affected cows by repeated doses of antiprostaglandins (e.g. flunixin), there is no data to support or refute such a treatment. If cows are in late gestation, and the fetus survives its dam's acute methemoglobinemia attack, it probably will have already begun the cortisol cascade to parturition, which is difficult to stop.

Mycotoxins

Mycotoxins, including zearalenone, ergot alkaloids, and aflatoxins, rarely cause abortion without overt illness. Their prevalence varies regionally, such that warmer, humid climates are associated with a higher risk. Cattle are quite resistant to the abortifacient properties of zearalenone. Aflatoxins, however, have been

associated with acute toxicity and abortions, but always accompanied by significant morbidity and mortality in the victims. See Panter and Stegelmeier for a practical review.[66]

Indirect Infectious Processes - Endotoxin

Even if a live gram-negative bacterium does not reach the uterine lumen, it may still trigger abortion. Several studies, using bolus or "trickle" infusions of endotoxin, have shown a sharp rise in $PGF_{2\_}$ within hours of endotoxin delivery, with resultant luteolysis and abortion.[31,32] Most of these studies indicate that endotoxin-induced abortion is more effective in disrupting early pregnancies (late embryonic/early fetal period). It is important to note that the dose-rate of endotoxin infused (in mg/hr) in some of these studies induced only very mild signs of endotoxemia in the cow (indeed, they could have been easily missed in a large herd), and yet resulted in luteolysis and abortion.[31] The same dose-rate of endotoxin that induced abortion in 42-day pregnancies was unable to cause abortion in 90-day pregnancies. It is tempting to speculate that a larger, more developed corpus luteum at 90 days may be able to better withstand a mild, transient $PGF_{2\_}$ "attack" than the CL of a 42-day pregnancy,[81] but since the endotoxin immune status of the cows in these studies was imprecisely known, we have to be careful- circumspect, even- in interpreting these results. In nature, the source of endotoxin, at least in theory, could be almost any gram-negative infection in the body, including rumenitis, mastitis and pneumonia. Carlos Risco *et al* showed an association between clinical mastitis and abortion in dairy cows.[76] Similarly, work by Dale Moore and colleagues at UCD's VMTRC in Tulare (in press) is showing that cows with clinical mastitis and those with subclinical mastitis (linear somatic cell count [SCC] scores > 4.5) were more likely to lose a pregnancy.

What we can do about endotoxin–induced conceptus losses

What follows is speculation, based on a very limited number of published studies. Vaccination with coliform core antigen, e.g., J-5 strain of *Escherischia coli*, or Endovac Bovi's *Salmonella* strain, would seem to make sense. While these vaccines generally do not prevent infection (at least not of the mammary gland, which they were designed to protect), they do reduce the severity of inflammation, possibly by neutralizing endotoxin before it can work its mischief, or by increasing opsonic activity of serum and secretions.[35] The same vaccine strategies that reduce the severity of signs of coliform mastitis should reduce the incidence of endotoxin-induced abortion by ablating the $PGF_{2\alpha}$ surge normally associated with endotoxemia.[104] Because of conservation of core antigens among gram negative bacteria, these *E. coli*- or *Salmonella*-derived vaccines offer cross protection against many other bacteria and/or their endotoxins. As an example, J-5 immunization of humans generated antibodies that cross-reacted with *Neisseria gonorrhoeae*.[21] Early attempts at similar cross protection against the cattle pathogen, *Haemophilus somnus*, an organism that can be transmitted sexually, were only partially successful in mouse models, i.e., J-5-immunized mice had only slightly higher fetal survival after challenge with *H. somnus* than control (unvaccinated) mice.[38] This is another area to watch for advances. It may be that we protect against sexually transmitted diseases by vaccinating with something other than the etiologic agent or even its parts.

Whether antiprostaglandins, such as the nonsteroidal anti-inflammatory drug flunixin, are useful against endotoxin-induced abortion is academic. They may help quell the initial surge of $PGF_{2\_}$, but only if they're on board at the time of or before the endotoxemic event.[32] In cases of inadvertent exposure to endotoxin, e.g., when a contaminated medication or vaccine has been injected, anti-prostaglandins make sense. If flunixin injectable is used, it should be given immediately, intravenously, at 1.1 – 2.0 mg/kg, and followed by the same dose intramuscularly every eight for up to seven total doses.[2]

Trauma

Two specific types of trauma are much discussed with respect to their effect on pregnancy. The first, already addressed in this symposium, is the inadvertent damage to the conceptus that may occur during palpation of the early-pregnant uterus *per rectum.* Several studies, some of which created quite a stir, seemed to implicate palpation as a significant risk factor for conceptus death. Some implicated specific techniques used in early pregnancy diagnosis (e.g., chorio-allantoic membrane slip), while others seemed to establish a risk to the timing of palpation, with increased risk of fetal death among cows palpated at specific post-insemination intervals. This discussion will not revisit the works mentioned, but instead will attempt to get two points across concerning palpation and pregnancy loss: 1) palpation of early pregnancies does carry a risk, which is almost certainly different for each palpator. But in well-trained individuals palpating outside of the "higher risk" intervals (~36-42 days after insemination), that risk is often quite small and, when compared with the other risk factors for pregnancy loss described in this presentation, is probably negligible.[97] And 2) even when the palpator does kill the conceptus during palpation of a 40-50-day pregnancy, for example by rupturing the amniotic vesicle, there is typically a considerable lag between this

event and either expulsion of an identifiable abortus or a return to estrus. In a study done years ago, we intentionally crushed the amniotic vesicle in 42-49-day pregnancies, and monitored progesterone, prostaglandin metabolites, and uterine palpation findings for a month. The earliest return to estrus was about two weeks, with others going as long as 28 days. This was not because estrous behavior was missed, but because a luteolytic burst of $PGF_{2\alpha}$ occurred well after the amnion was ruptured. In fact, the chorio-allantoic membranes continued to grow for a few days after the amnion had been destroyed, so that if only a membrane slip technique had been employed, these cows might have been declared pregnant.[40] These observations are presented for the benefit of those of us who have been confronted by a herdsman or owner who notices an aborting early pregnancy the afternoon after a morning herd check. In our experience, fetuses killed at 42-49 days by palpation take longer (1-4 weeks) to be expelled, so the conceptus your client is concerned about probably died some time ago.

The second type of "trauma" is the $PGF_{2\alpha}$ treatment or intrauterine insemination of a cow that is already pregnant. This can be the result of Type II errors in heat detection (calling a cow in heat when she's not), or from some of the intensive estrus and ovulation control programs used on dairies, where 'non-pregnant cows' are identified as early as possible as candidates for resynchronization and re-insemination. Estimates of the magnitude of embryo losses due to inseminating pregnant cows vary, with reports of >19% Type II errors, and 17% to >60% of cows so inseminated losing the existing pregnancy.[90]

What we can do about trauma-induced conceptus loss

As a former instructor once put it, "Be kind to the tissues". Use palpation methods that do not pinch, i.e., keep your fingers and thumb flatly opposed, vs fingertip-to-thumbtip, during exploration and slipping of the uterine horns/membranes. If you are assessing the amniotic vesicle, cradle it gently, and do not hold the entire weight of the retracted uterus by the vesicle. As for inseminating pregnant cows – don't! If uncertain about the pregnancy status, the old advice about placing the semen just in the cervical os is still good advice. If the dairy has estrus detection problems, use plasma or milk progesterone results of samples taken at the time of breeding to demonstrate to a skeptical employee that the cows he/she is breeding can't be in estrus. If you want quantitative results, use a diagnostic laboratory that can run progesterone ELISAs or radio-immunoassays. Small animal lab services may offer P_4 assays, and progesterone is progesterone, across all mammals, so the "dog assay" will recognize the cow progesterone. Alternatively, the cow-side progesterone test kits can be useful in these situations, even though their results are only semi-quantitative at best. If milk or serum taken on the day of insemination shows "high" progesterone, that cow is not only NOT in estrus, she's not even close to estrus. (Recall that in a cycling cow, there are 5-8 days out of every 21-day cycle in which her P_4 levels will be low, below the detection range of these cow-side kits.) So if her P_4 is high on the day she is artificially inseminated, she is likely to be well into the luteal phase, or pregnant. In dairies practicing timed AI following Ov Synch or similar protocols, the use of GnRH at day 21 following insemination would seem to allow an early start to resynchronization (i.e. before a firm diagnosis of pregnant/not pregnant is possible) without doing any harm.[17] Definitive (ultrasound) diagnosis at day 28 occurs just before the cow would have been injected with $PGF_{2\alpha}$ if she were diagnosed not pregnant. The cost-benefit of such programs needs to be evaluated for each client.

Infectious Agents

These are discussed last, to ensure that we consider the non-infectious influences discussed above. Basically, the approach to minimizing the effects of abortifacient organisms is the same as for any infectious disease, which means reducing the probability of introducing the organism to the herd (biosecurity), minimizing intra-herd transmission of endemic or introduced agents (environmental management) and reducing the consequences of infection for exposed animals (herd immunity.) For intensive operations such as today's modern dairy enterprises, the density of the population presents significant challenges. Respiratory and gastrointestinal pathogens don't have to travel far to find the next potential victim. And in the 60+% of dairies that use natural service for some part of their overall breeding program, obligate venereal agents are easily passed from one cow to another via the bull.

For this discussion, the major specific reproductive pathogens are taken to be:

- Bovine Herpes I (IBR) virus
- Bovine viral diarrhea (BVD) virus, genotypes I and II
- *Leptospira borgpetersenii* serovar *hardjo (*type *hardjo-bovis)*
- Neospora caninum
- Campylobacter fetus venerealis
- Tritrichomonas foetus

There are lots more, but these are the agents that operate in many of our practice areas, and that can cause significant herd-scale problems.

What we can do to blunt the reproductive wastage cause by these reproductive pathogens

Bovine Herpes I (IBR) virus is as close to a "death ray" for a bovine conceptus as we're likely to see. In the unprotected pregnant female, this virus rapidly crosses the placenta and establishes a fetal viremia, with peracute necrosis in many organs, and death within 24-48 h. The fetus is apparently susceptible throughout gestation. Prevention of fetal infection is based on preventing maternal viremia through immunization of the dam. Even rather low serum virus neutralizing titers (1:8, for example – check with your lab for their corresponding ELISA titer[23,42]) are generally sufficient to prevent maternal viremia. The basic choices we have to make as clinicians are: 1) What should I vaccinate with - live virus, chemically altered virus, or killed virus? And (2) When should I vaccinate? Because of the widespread prevalence of IBR and the lack of a regulatory eradication program, the fundamental question, "Should I vaccinate?" has already been answered. For years, the dogma has been to avoid vaccinating any pregnant animal, ever, because live vaccine virus was perfectly capable of destroying the fetus. (The live-virus vaccines are attenuated, but still potent enough to do in the fetus if they are not checked before reaching the placenta.) Now, it appears that what we suspected all along is true – that if the dam has been properly immunized with almost any form of IBR vaccine virus between six months of age and her first pregnancy, her immune "readiness" should be sufficient to prevent viremia when inoculated with live virus during pregnancy. This makes it a bit easier to sift through the technical data that the pharmaceutical and biologics companies present. Essentially, live virus is safe for use in a pregnant cow if she is already immune at the time you boost her with the live virus product. The immune status could be conferred by vaccinating before breeding with either live or chemically modified virus products.[26,87] A commonly recommended protocol for protection against IBR's reproductive effects in dairy cows is to wait until 5-6 months old to vaccinate calves with live virus, in order to avoid neutralization of vaccine virus by colostral antibodies; boost with live virus again just before heifers go into breeding groups. After this time, it shouldn't matter which form of vaccine she's given, since she satisfies the "already immune" prerequisite for live virus use. Boosting her immunity annually, or at the time of postpartum exam, should spare her from the reproductive consequences of IBR infection, even though it may not absolutely prevent infection nor clear a persistent (e.g., trigeminal nerve) infection. At least one vaccine manufacturer has obtained FDA approval for labeling its live-virus IBR as safe for previously immunized pregnant females,[26] and another has similar claims for its chemically modified IBR vaccine.[87]

The situation with BVD is only a little more complicated, although the literature is bewildering. Most field BVD strains are of the non-cytopathic (NCP) biotype, meaning only that they replicate in cell cultures without killing the cells. They are quite capable of causing significant problems in the pregnant cow, however. The NCP types are those responsible for establishing persistent infection (PI) in an unprotected fetus exposed before its immune system is competent (before ~ 125 days). Subsequent exposure of the PI individual to an immunologically identical but cytopathic (CP) biotype will cause fatal mucosal disease. It appears that the "immunological identity" of the CP virus is not a coincidence, but rather arises directly from the NCP virus as the result of a single mutation. Theoretically, protection of the fetus is as for IBR, i.e., circulating antibody and tissue-resident cytotoxic T cells, generated in response to vaccine, apparently neutralize wild-type virus before it can colonize the placenta.[68] The problem has been that most vaccines could protect the dam, but could not completely prevent fetal infection, with the result that PI calves are born, ensuring that the herd will continue to be exposed.[29] Calves born to PI dams are themselves PI, so the description of PI calves as the Trojan Horse of BVD infection is pretty apt.

In unprotected pregnancies, NCP biotypes of either of two distinct genotypes, BVD I and BVD II, can cause significant losses. Vaccination with modified live virus (MLV) Type I products has been shown to provide protection against fetal infection by Type I strains, and to cross-protect against type II strains. This is generally less evident with killed products, i.e., there is less complete cross-genotype protection with existing killed BVD products, although some have been shown to reduce morbidity of the complementary genotype. Combined genotypes (I and II) are available in MLV or killed formats; two doses of the killed product at a 3-4 wk interval, or a single dose of the MLV product, confers initial immunity against Type I and Type II BVD, with annual boosting commonly recommended. As with IBR, live BVD virus can be given as colostral anti- BVD neutralizing titers wane to $\leq$ 1:16 (~five months[59]). Vaccinating a PI calf with MLV may kill her, but probably won't, unless it's a CP type that is an immunological match for the NCP strain she's already carrying, in which case she'll probably develop mucosal disease. Failing that, she is likely to continue to quietly shed NCP virus, in spite of the antibody titers she develops to an immunologically different vaccine virus. Therefore, if the goal is to work toward elimination of BVD from the herd, simply vaccinating won't do it. All PI animals need to be identified and eliminated. The reader is referred to cited literature on the subject[57,58] and urged to consider screening groups of animals by pooling samples (e.g. whole blood samples), which can be assayed for BVD virus by PCR. All individuals in the pool are tested only if the pooled sample is PCR-positive.[57,58]

Suggested BVD vaccination protocol: assuming a low prevalence of PI animals, use of an MLV product at ~ 5+ months of age seems well-justified. A second MLV dose prior to first breeding (~12-13 months) should provide more than adequate protection to the conceptus after she conceives. In the past, opinions have differed on BVD vaccination protocols after the birth of her first calf. Booster immunizations at the time of post-partum checks (~30 days), using MLV, have been widely used, on the theory that the cow is not pregnant, and therefore not at risk for fetal disease caused by the MLV booster injection. Others argued that the continued use of MLV put a large amount of virus on the premises, and increased the risk of vaccine virus shedding from non-pregnant vaccinated cows to pregnant cows whose immune status was unknown. Recent claims by some vaccine manufacturers state that newer formulations of MLV immunogens for BVD provide better fetal protection (i.e., are less likely to allow a PI calf to occur), and are safe for use in pregnant cows. If true, these two attributes represent a significant advance in what appears to be an eternal battle against BVD. In any case, once initial immunity is established, annual boosting may use either live or killed, but should provide protection against Type I and Type II BVD viruses.

Leptospirosis

Leptospira-induced pregnancy wastage has had us all befuddled over the years. Nomenclature alone was difficult to stay abreast of, and the efficacy of vaccinating was frequently called into question. The difficulties in obtaining a definitive diagnosis only added to our befuddlement. But several advances have made a rational approach to lepto more achievable. For one, diagnostic labs have developed an impressive array of tools beyond microscopic agglutination titers, including IFA, ELISA, immunohistochemistry, and PCR. So today, we are getting a more accurate look at the incidence of lepto infertility and abortion, which is the good news. The bad news is that this "better look" has resulted in more nomenclature for us to remember. It seems we may have been immunizing for the wrong "lepto" for years, or in some cases not immunizing at all, in spite of sticking needles in cows.[9,11,60] The organism that some of us may remember as *Leptospira interrogans*, serovar *hardjo* type *hardjo-prajitno,* which is the *hardjo* component of nearly all of our tri- or pentavalent lepto vaccines, is apparently not the *hardjo* that is most prevalent in USA. Our domestic Lepto is another species altogether, namely *Leptospira borgpetersenii*, serovar *hardjo* (type *hardjobovis*). The confusion in nomenclature is partly because of the hybrid means by which these two leptos are named: They are genetically quite different, hence the assignment to different species; but their surface antigens, as detected by serum antibodies, are identical, making them serologically indistinguishable; hence, the identical "type" designation.[11,61] The *L. borgpetersenii* organism apparently causes the most trouble in the United States, and yet the only available vaccines for many years contained the *L. interrogans* organisms, which might explain some of our profession's frustration with the subject of lepto abortion. But nothing is ever that simple. Ultimately, Dr. Carol Bolin and her colleagues showed that a monovalent *L. borgpetersenii* serovar *hardjo* bacterin (Spirovac™) and a Schering Plough monovalent *L. interrogans hardjo* bacterin (Leptavoid™) achieved the remarkable goal of preventing infection and tissue colonization.[9,10,60,61] Moreover, working with Dr. Cindy Baldwin's group at the University of Massachusetts (Amherst), it was shown that the Spirovac™ and Leptavoid™ bacterins induced a Th1 type of immune response, which is unusual for an extracellular parasite like lepto.[10,60,61] In addition, higher humoral antibody titers were achieved with either of the monovalents than with a representative pentavalent. So why did the monovalents work better? Was it the cell-mediated immunity (CMI) they generated? Was it something about the way the organisms were grown before harvesting? It probably wasn't because of the particular species used for their hardjo antigen, since the monovalent bacterin using *L. interrogans hardjo* worked nearly as well as the *L. borgpetersenii hardjo*. The significance of these findings may require a brief detour for a review of the essential immunology involved.

Two-minute Immunology drill

T helper lymphocytes are the lymphocytes that help determine which elements of the immune system will be brought to bear on a pathogen, and generally invoke those elements by secreting cytokines. Depending on the nature of the pathogen, the T cell response will be either Th1 or Th2, or a combination of the two. Th1 cytokines include interferon gamma (IFNγ), which in turn has many effects on the immune system. The IFNγ and other cytokines released by Th1 cells essentially direct the immune response towards CMI, and towards a limited antibody response that is dominated by the IgG_2 isotype In contrast, in a Th2 helper cell response, the resulting immune response tends to be directed towards a humoral (antibody) response, dominated by IgA, IgE, and IgG_1. Most extracellular pathogens are dealt with by a predominantly humoral response, where antibody binds to the organism's surface (opsonization), after which the opsonized organism is either phagocytized by a macrophage or neutrophil, or killed outright by complement-mediated lysis or antibody-dependent cell cytotoxicity (ADCC). Intracellular pathogens, including most viruses, many bacteria and some protozoa, are handled by the CMI wing of the

immune system, whereby epitopes (small pieces of antigen) of the now intracellular pathogen are displayed on the host's cell surface. A cytotoxic lymphocyte that can recognize these epitopes then binds to and kills the infected host cell. Hence, the statement above that the new lepto vaccine is unusual, in that it spurs a CMI response to what is essentially an extracellular parasite. The CMI response may arm lymphocytes in the kidney where leptospires would presumably have to be intracellular for at least a brief time, on their way to the urine from which they are often identified. Regarding protection against lepto *hardjo*'s reproductive effects, it is likely that humoral responses are important, too, especially since pregnancy has been shown in some mammals to direct immune resources toward a Th2 (antibody-dominated) response.[41] In any case, whether the apparent improvement in efficacy over previous products is due to the CMI response, or to a more appropriately prepared antigen, is not fully known.

Using the "new" lepto vaccines

Spirovac™ label instructions call for the usual two doses at four-week intervals, followed by annual boosters. However, the pre-existing carrier animal is a problem: The *hardjo* serovars set up shop in the kidney and are not necessarily eliminated by vaccination. So it's preferable that the animal be lepto-free before vaccinating. ("lepto-free" is inferred from serological testing and urine culture/IFA/PCR.) According to literature provided by Spirovac's manufacturer (see http://www.spirovac.com/country.asp?lang=EN&drug=SV&country=US&species=OO), in non-lactating animals, a single dose of LA-200 will clear most infected animals. In lactating animals, Excenel can be used (off-label). In either case, the first vaccine and antibiotic can be given at the same time. It will be interesting to see if the duration of immunity is as long as controlled trials suggest (at least four months,[60] and perhaps seven months.[61] Previous lepto vaccines have been notorious for the short-lived immunity they imparted, but now we realize that they may not have been imparting immunity of any duration, at least not for *L. borgpetersenii*, serovar *hardjo* (type *hardjo bovis*).

In the trials conducted at University of Massachusetts-Amherst, there was also cross-protection with *L. kirschneri* serovar *grippotyphosa* (more nomenclature!).[11] So, the current pentavalent lepto vaccines will apparently protect against *L. interrogans* serovars *pimona, canicola, icterohemorrhagiae*, and possibly against *L. kirschneri* serovar *grippotyphosa,* but not against *L. hardjo* serovars of either the *borgpetersenii* or *interrogans* species.[11] This means that the monovalent *hardjo* vaccines need to be used in addition to a pentavalent lepto vaccine if any serovars other than *hardjo* are operating on the premises. At Spirovac's current price, the initial two-injection series and annual booster vaccinations, either at postpartum check or as the cow finishes her voluntary waiting period, are probably all that dairymen will want to pay for. If and when the cost comes down, it may be advisable to vaccinate at least once during pregnancy, to boost immunity during the period when the cow needs it most. The Spirovac™ product is specifically labeled as safe for use in pregnant cows.

Neospora caninum

Essentially everyone agrees that *Neospora* can be a significant cause of conceptus losses in dairy cattle. And that's about where the agreement ends. Because, like BVD, congenitally infected calves grow up to have congenitally infected calves of their own, we know that transplacental transmission of *N. caninum* is common, and represents the chief means by which the disease agent remains in the herd. Horizontal transmission (ingestion of oocysts passed in dog feces, or ingestion of tachyzoites perhaps from licking infected placentae) also occurs, but at much lower frequency. The only commercially available vaccine, a killed, whole-cell preparation of *N. caninum*, has limited efficacy in preventing congenital infection, but in company-sponsored trials has shown some benefit in decreasing the proportion of infected cows that abort.[18] However, we now know that a great many dams that are infected do not abort, although their infection status may be associated with production losses other than abortion.[95,96] So, in order to make the decision about whether to vaccinate, it would be very helpful to calculate the proportion of abortions in the herd that can be attributed to *N. caninum*. (See Dr. Thurmond's discussion of "herd-based approaches to abortion" in these *Proceedings* for details of making that calculation.) Vaccinating will render all immunized cows seropositive, making it impossible to use serology as a tool for cleaning up a herd. Look for new developments in more sophisticated vaccines for *Neospora,*[37,62] but they will take awhile. If one elects not to vaccinate, then the only means of control is to identify and remove infected (seropositive) animals. Given the prevalence in many California dairies of seropositive animals, the culling rate of such a strategy would be unacceptable. More involved measures include using ET to remove pre-implantation embryos from seropositive (presumed infected) cows, and transfer them into tested negative recipients. Most diagnostic labs can now run the appropriate serological test (ELISA, competitive ELISA, or IFA) to determine the recipients, *Neospora* status. Because vertical transmission is presumed to occur hematogenously, (i.e. tachyzooites are delivered by the dam's circulatory system to the endometrium, from which they colonize the placenta), infection of the conceptus is unlikely to occur before placentation is well

under way (after day 30). So pre-implantation embryos are considered safe with respect to *Neospora*, even if they come from an infected (seropositive) donor.

Trichomonosis and Campylobacteriosis

These two diseases have essentially identical epidemiology. Both are obligate venereal pathogens, both cause relatively early demise of the conceptus in the absence of clinical signs in the dam or sire, and in both diseases the infection of the female is temporary while males, especially older males, are chronically infected, perhaps for life. However, our ability to deal with them differs significantly, because there is no legal, efficacious treatment for "trich," and because of a difference in the efficacy of vaccines. An oil-adjuvanted product was shown to be effective not only for prevention of early abortion in females,[20] but also for prevention of permanent infection in bulls.[19] There are even reports of clearing *Campylobacter. fetus venerealis* infection from cows, and remarkably, bulls, by vaccinating in the face of infection.[85,99] The humble *C. fetus venerealis* bacterin is one of the triumphs of veterinary biologics, and represents the first successful vaccine for a purely sexually transmitted disease in any species. But we don't really have a clue how it works It's difficult for any immunologist to explain how a systemically injected vaccine for an extracellular pathogen can generate a response that is effective on the surface (skin) of the penis and prepuce, both of which are covered by a stratified squamous epithelium. In any case, in dairies that use bulls for any part of the breeding program, we generally recommend they be immunized, using a double dose of the Vibrin™ product (marketed by Pfizer) given twice, at a four-week interval with annual booster, if the bull stays around that long. (Caution: inject on the side of the neck; there will be post-vaccination welts, usually mild.) In beef cattle, where the bull's access to females is less controlled than on a dairy, (i.e., the neighbor's bull may come a-calling, or shared grazing may be practiced), we recommend vaccinating males and females. Initial immunization requires two injections at 4-6 wk intervals, with the second injection given two weeks before turn-in, and annual boosters thereafter. This timetable makes immunological sense, i.e., immune responses peak at about the time of potential exposure, but the reality of range cattle management is that it's not easy to get one's hands on the cattle in perfect agreement with an immunologist's schedule. Compromises include vaccinating at weaning and again just before turn-in. although this may provide a weak anamnestic response. Even well vaccinated females can still be vaginally infected, although they will be resistant to uterine infection, and clear the organism from the vagina more quickly than controls. Interestingly, this endpoint, i.e. personal protection but still able to infect others, has been a sticking point in development of human STDs; but as our MD colleagues become more aware of the concept of "herd immunity," some of these objections are softening.

Tritrichomonas foetus

We have seen quite a bit of trichomonosis in both dairy and beef operations in California and the West. Like campylobacteriosis, females are infected at coitus, and clear the infection 1-4 months later, while bulls become chronic carriers. Interestingly, conception occurs normally, even though the parasite and the sperm arrive in the reproductive tract at the same time, a phenomenon that has been shown both *in vivo* [67] and *in vitro.*[7] Most fetal death from trich occurs in the 50-70 day range. The goal of vaccination of females is to provide sufficient immunity so that a cow exposed to an infected bull can clear the organism from the tract before this 50-70 day deadline. There is a commercial vaccine available with partial efficacy for beef females shown in rigorous challenge studies.[43] The vaccine is a killed, whole-cell product. As with campy, vaccination did not prevent infection in most (80%) cases, but it did allow more rapid clearance of the organism from the female reproductive tract, and was associated with a significantly higher calving rate, although that rate was still lower than what is commercially acceptable. The vaccine is not universally used, probably because of questions about its efficacy (no efficacy has been demonstrated in bulls), and cost (~$2.75/dose). In dairies, where bulls cohabitate year-round with females, most control programs focus on testing and removal of infected bulls. This involves culture of preputial smegma on a suitable medium, e.g., InPouch (Biomed Diagnostics, White City, OR), with PCR of positive cultures to confirm the diagnosis. All this sounds fine, until you walk onto a 1000+ cow dairy, and realize that, in many instances, there is no place to safely collect the preputial smegma. The lockups over the feeders aren't built to hold bulls, and even if they can, a quick side-step by the bull can be dangerous. If a foot-trimming chute or tilt table is available, and strong enough, use one of these to restrain the bull. If one bull has a positive diagnosis, he should be culled, and the remaining bulls tested twice more each, at one week intervals. It's important that the bulls be removed from the cows during this week, to give the pathogen time to build up its numbers before sampling.

When there is a positive diagnosis of trich in a bull

In the dairy situation, this is a great opportunity to tout the advantages of an all-artificial insemination program. Expect less than a jubilant response. (There is probably a reason they were using bulls.) If the client

won't go for a complete, permanent switch to AI, some of our more creative practitioners have persuaded clients to try AI for six months - enough time for infected cows to clear the organism - and instituted ovulation control/timed AI programs. If that is similarly unacceptable, then we'd recommend the following: 1) declare a one-month breeding holiday (stand by to purchase springers later, to make up for the "sag" in total milk 10 months from now); 2) sell all bulls, and replace them with virgin bulls; 3) vaccinate all females twice during the holiday. This will reduce the number of trichomonads in the female herd (most will completely clear in this time), and reduce the opportunity for transmission of trichomonads from cow to cow, via the bull. Trichomonads (and Campy, too) like to live in the crypts created by epithelial folding of the preputial/penile skin. Because young bulls have very shallow epithelial crypts compared to bullsover three years old, using virgin bulls exclusively denies these venereal organisms a place to thrive.

In a seasonally breeding beef herd, annual testing of bulls is the most cost-effective course of action, although when other risk factors are present (older bulls, a neighbor who has/had trich in his herd, shared grazing.), vaccination becomes more cost-effective.[101]

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