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PREGNANCY DIAGNOSIS

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Introduction

Since the beginning of civilization animal owners have been interested in determining whether or not conception has taken place, and various clinical signs and superstitions have been used for millennia to diagnose pregnancy (Bayton, 1939). Over time, a number of more accurate methods for detection of pregnancy in cows and other female domestic animals have been developed including observation, physical examination, chemical tests and electronic instruments.

Indications

The purpose for examining cows for pregnancy is not to detect those that are pregnant, but to detect those that are not pregnant so that they can be inseminated again or culled from the herd. For profitable production, cows should calve for the first time at approximately 24 months of age and deliver a calf annually thereafter. The annual cost of maintaining a beef cow varies by geographic region and from year to year, but estimates range from approximately $350.00 to $450.00 (Guthmiller, 2002). Feed costs represent approximately 70% of the annual expenditure (Eller, 1996). Thus, in most management systems, non-pregnant cows are culled from the herd to save the cost of maintaining non-productive animals, but in herds that have both spring and fall calving seasons, cows are sometimes moved to the other group and given a second opportunity, although this decision may be difficult to justify economically. Beef cows are usually examined for pregnancy when their calves are weaned at 5 to 7 months of age, although in intensively managed herds, individual cows may be examined earlier. Although the cost to maintain a non-pregnant cow is high, recent surveys indicate that only 17.7% of herd owners examine cows and 15.9% examine heifers for pregnancy (National Animal Health Monitoring System, 1994).

Management Methods for Pregnancy Diagnosis

Exposure to a bull or artificial insemination
A history of cohabitation with a bull, the observation of mating, or artificial insemination is used by some to suggest that a cow is pregnant. While fertilization rates are high, only about 50% of inseminations result in a detectable pregnancy. Conversely, unobserved, unplanned, or unrecorded matings are not uncommon. Thus, history is not a reliable indicator of pregnancy status and may sometimes be deceptive.

Cessation of the estrous cycle
Bovine embryos signal their presence around day 15 to 17 after ovulation, the corpus luteum is maintained, and the maternal estrous cycle is suspended. Thus, failure of a cow to return to estrus 18 to 24 days after breeding suggests that conception has occurred (Zemjanis, et al., 1969).

In beef herds that use natural service, perceptive managers may observe that a greater than expected number of cows return to estrus after mating. This situation suggests an infertile bull, the presence of a venereal disease, or some other cause of infertility and there is an opportunity to take corrective action before the breeding season ends. Conversely, undernutrition is a common cause of anestrus (failure to cycle) in lactating beef cows and the observation that few cows return to estrus after the first few weeks of the breeding season may mislead managers to believe cows have become pregnant when they are, in fact, not cycling.

A few pregnant cows show mild to conspicuous signs of estrus and may be mistakenly thought to be non-pregnant. Artificial insemination of pregnant cows may result in abortion if the insemination instrument is passed completely through the cervical canal and the fetal membranes are disrupted.

Metestrus hemorrhage
A bloody vaginal discharge is common in cows 24 to 48 hours after estrus and is the result of hemorrhage from capillaries in the lining of the uterus due to rapid decline in estrogen that follows ovulation. If metestrus bleeding is observed in a cow that was not seen in estrus a few days previously, it is implieid that estrus was unobserved and the animal is not pregnant.

Palpation per Rectum

Palpation of the reproductive tract through the rectal wall (rectal palpation) has been the customary method for pregnancy diagnosis since early in the last century (Cowie, 1948; Benesch and Wright, 1951; Zemjanis 1970; Roberts 1986; Jephcott and Norman, 2004). Depending upon the skill of the examiner and the age and size of the dam, rectal palpation is useful to diagnose pregnancy as early as approximately day 30 and can be utilized thereafter until term. Although a number of changes in the size, texture, location, and content of the uterus occur during pregnancy, there are only four *positive* signs of pregnancy that are detectable by rectal palpation, and the examiner must find detect at least one of these four signs before declaring the cow pregnant. The four positive signs of pregnancy in cows are:

Palpation of the amniotic vesicle
Palpation of the fetal membrane slip
Palpation of placentomes (cotyledons and caruncles)
Palpation of the fetus

Amniotic vesicle

The amnion is a portion of the placenta that contains the developing conceptus and the amniotic fluid is palpable as early as 28 days after conception in heifers and by 32 to 35 days in older cows. The vesicle is recognized as nearly spherical, turgid, fluid-filled structure that is approximately 1 cm in diameter at 28 days and increases in size as pregnancy advances. In a bovine conceptus, the heart is external until approximately day 42; therefore caution must be exercised when attempting to detect early pregnancies and undue pressure must not be applied to the amniotic vesicle. Intentional rupture of the amniotic vesicle has been used in the past as a method to intentionally provoke abortion in cattle (Ball and Carroll, 1963).

Fetal membrane slip

The examiner can detect the chorioallantois (developing placenta) within the lumen of the pregnant uterus by compressing the uterine horn between the thumb and forefinger, lifting the uterus, and then allowing the horn to slowly "slip" from the grasp. If the cow is pregnant, the chorioallantois can be felt to slip through the fingers just prior the uterine wall. The membranes can be slipped in the pregnant horn as early as 30 days and can be reliably detected by day 35. During early pregnancy, the fetal membranes are thin, and a delicate touch and some experience are required to recognize this sign of pregnancy.

Placentomes

In ruminants, cotyledons of the fetal placenta fuse with the maternal caruncles to form placentomes. Seventy five to 120 maternal caruncles arranged in two dorsal (upper) and two ventral (lower) rows are present in the uterus of cows. Placentomes begin to form early in gestation and are of sufficient size to be palpable by 75 to 80 days. The size of placentomes varies with the stage of gestation and their location in the uterus. They are most consistent in size just in front of the cervix and are palpated at that location to estimate the stage of gestation.

Fetus

The fetus becomes palpable at approximately 65 days when the amniotic vesicle softens and remains palpable for the balance of gestation. In the early stages, the fetus can be grasped directly. Later, the fetus is detected by ballottement; the examiner sets the fetal fluids in motion by rocking the hand against the uterine wall and recognizes the fetus as it rebounds against the hand. The fetus is identified as a free-floating firm object within the fluid-filled uterus during the first 4 months of gestation. As pregnancy advances, increased weight of the fetus and fluid pulls the uterus downward and forward until the fetus comes to rest on the abdominal floor during the fifth and sixth months. Continued growth of the fetus positions it closer to the maternal pelvis during the last trimester and palpation of the fetus is facilitated.

Estimation of the Stage of Gestation

The stage of gestation can be estimated on the basis of palpable characteristics of the uterus and fetus. Estimation of the stage of pregnancy is most accurate during the first half of pregnancy. In early pregnancies, stage of gestation can be estimated on the basis of the size of the pregnant horn and size of the amniotic vesicle. In more advanced pregnancies, age of the fetus is estimated based on determination of the size of the

placentomes at the base of the pregnant horn, the size of the fetus, fetal crown-to-nose length, and position of the uterus (Zemjanis, 1970; Ball, 1980; Roberts, 1986).

Safety of Rectal Palpation

Fetal death—It is difficult to separate fetal attrition that might be caused by rectal palpation from spontaneous fetal death that would occur in non-palpated animals. Therefore, in light of the information currently available, it seems reasonable to conclude that if rectal palpation is a cause of fetal death, the incidence is probably low and the value of the information gained is greater than the risk of fetal loss (Abbitt et al., 1978; Vaillancourt et al., 1979; Paisley et al., 1987; Alexander et al., 1995). However, clinicians must be aware of the possibility of negative effects of rectal palpation on early pregnancies and conduct examinations meticulously, cautiously, and with dispatch.

Fetal damage—While there are suggestions that pregnancy diagnosis by rectal palpation contributed to abnormal development of the digestive tract (atresia coli; Ness et al., 1982; Ducharme et al., 1990), more extensive investigations have found no association between anatomical defects and rectal palpation (Constable et al., 1989; Syed and Shanks, 1993) .

Disease transmission—Bovine leucosis virus has been experimentally transmitted by infusion of relatively large amounts of blood from viremic animals (Henry et al., 1987). Others report that leucosis virus transmission by rectal palpation of cows either does not occur or is uncommon (Lassauzet et al., 1989). In herds in which other measures to control transmission of the virus are practiced, it may be prudent to use a separate clean obstetric sleeve for palpation of each cow. The role of common obstetric sleeves in transmission of other infectious diseases is unknown.

Electronic Methods of Pregnancy Diagnosis

Real-time ultrasonography

The use of transrectal real-time ultrasonography (also called B-mode or brightness-mode) for detection of pregnancy in cows has been extensively described (Ginther, 1995; Ginther, 1998). While there is some variation among operators, image quality of the instrument, and animals, an accurate diagnosis of pregnancy can be made by approximately 26 to 28 days after ovulation when a 5 MHz transducer and a high quality scanner are used (Pierson and Ginther, 1984). There are reports that pregnancy can be accurately detected earlier with a 7.5 MHz transducer (Boyd et al., 1998). Formulae for estimation of fetal age with ultrasonography have been published. The fetal heartbeat can be first detected at approximately day 21 and is the “gold standard” for proof of presence of a viable conceptus.

Embryonic loss confounds pregnancy diagnosis by ultrasound as well as other methods of early pregnancy determination. Recent reports indicate that 10 to 16% of cows diagnosed pregnant early after insemination by ultrasound will undergo embryonic loss. Therefore, a second examination at approximately 60 days after insemination to confirm pregnancy is required (Mee et al., 1994; Vasconcelos et al., 1997; Fricke et al., 1998; Thatcher et al., 2002; Lopez-Gatius et al., 2002).

Other ultrasonic instruments

Ultrasound scanners less expensive than the widely-used real-time scanners have been advertised from time to time but critical evaluations have shown them not sufficiently accurate for reliable detection of pregnancy (Ducker et al., 1985; Cameron and Malmo, 1993).

Chemical Methods of Pregnancy Diagnosis

Early pregnancy factor/early conception factor

Early pregnancy factor (EPF) is a protein that was first detected in the serum of pregnant mice within 4 to 6 hours after mating. EPF is made of two components (EPF-A and EPF-B). EPF-A is secreted by the uterine tube (oviduct) and EPF-B by the ovary. Production of EPF-B requires a signal from the fertilized egg (ovum factor). Ovum factor is released in the presence of prolactin after sperm penetration. EPF is an attractive marker for pregnancy in that it appears within hours after conception and disappears rapidly after death or removal of the embryo (Koch et al., 1983; Sakanju et al., 1993; Fan and Zhen, 1997).

Initially, EPF was detected by the rosette inhibition test; a sensitive but time-consuming assay that is not suitable for routine use (Yoshioka et al., 1995). More recently, a lateral flow dipstick has been developed for detection of EPF as a "cow-side" method to detect pregnancy. While an initial report indicated that the method was reliable to correctly diagnose non-pregnancy in 94.6% of cows at 24 to 48 hours after insemination (Threlfall and Bilderbeck, 1998), more recent reports indicate that the cow-side test is not sufficiently accurate to be used as a management tool for dairy cattle (Adams and Jardon, 1999; Whisnant et al., 2000; Grandy et al., 2001; Cordoba et al., 2001). The manufacturer (EDP Biotech Corporation; http://www.edpbiotech.com) of the EDP/ECF™ test recommends that the test be used to identify non-pregnant cows. It is recommended that milk or serum samples be tested at 7 days after insemination. According to information supplied by the manufacturer, ECF becomes non-detectable in milk and serum by 20 days after conception.

Pregnancy-associated glycoproteins

The process by which the dam recognizes the presence of an embryo varies among species (Thatcher et al., 1995; Roberts et al., 1996). Numerous signals are exchanged between dam and embryo to prevent luteal regression and maintain receptivity of the uterus to the presence of an embryo and its membranes. Detection of one (or more) of these signals could be a useful method to detect pregnancy. because: 1) the protein(s) is a specific marker for pregnancy, and 2) the protein appears very early and failure to conceive could, in theory, be detected prior to the next anticipated ovulation.

In cattle and sheep, the embryo begins its efforts to prevent regression of the corpus luteum prior to attachment to the endometrium. Large quantities of interferon-tau are released by the mononuclear cells of the placenta as the developing embryo begins to elongate on days 14 to 16 in cattle. Interferon-tau would seem to be an excellent indicator of pregnancy since it is specifically associated with pregnancy and it is present prior to the next anticipated ovulation. Unfortunately, interferon-tau remains within the

uterine lumen and does not appear in measurable quantities in maternal blood or other body fluids (Baxer et al., 1996; Roberts et al., 1999; Demmers et al., 2001).

After maternal recognition of pregnancy in ruminants, attachment of the embryo to the uterus begins. Invasiveness of the trophoblast is limited. In cattle, areas of attachment are first observed at 20 days. Fetal binucleate (two nuclei) cells migrate out of the trophectoderm and fuse with maternal epithelial cells forming fetomaternal hybrid tissue. The binucleate cells are responsible for successful implantation and subsequent growth of the placentomes and produce and deliver protein and steroid hormones to the maternal circulation. Hormones are synthesized in binucleate cells and stored in granules and released into the maternal tissue (Wooding, 1992).

Two pregnancy-specific proteins were isolated from the bovine placenta (PSP-A and PSP-B; Butler et al., 1982). PSP-A is not limited to pregnant animals but PSP-B was shown to be specific to the placenta and can be detected by 24 days after conception. Pregnancy diagnosis by assay of PSP-B is available commercially (BioPRYN^{TM}; BioTracking, LLC; http://www.biotracking.com). Serum samples are taken after 28 days post-insemination from heifers and after 30 days from lactating cows. The actual test procedure requires 27 hours after the samples have been received by the laboratory. Samples can be sent to the central laboratory in Idaho or to one of several cooperating laboratories that have been licensed to use the test. Measurement of PSP-B can also be used for detection of pregnancy in other ruminants including sheep, goats, bison, deer, elk, and moose. Detection of PSP-B cannot be used to diagnose pregnancy in llamas, however. PSP-B has a long half-life and disappears slowly from the maternal circulation after parturition. The slow disappearance of PSP-B after calving may interfere with use of the test for diagnosis of pregnancy if blood samples are taken too soon after calving (less than 90 days; Sasser et al., 1986).

A bovine pregnancy-associated glycoprotein (bPAG-1) was isolated from fetal cotyledons and subsequently, an assay was developed for detection of pregnancy. b-PAG-1 was detected in maternal serum at day 22 of pregnancy in some cows and by day 30 in all pregnant animals. Peak concentrations were found 1 to 5 days prior to calving and became undetectable by 100 days after parturition (Zoli et al., 1991; Zoli et al., 1992).

Currently, the function of PGG's is unknown. It has been estimated that there are more than 100 bPAG's, many of which are expressed in the placenta (Xie et al., 1997). These proteins appear, some for only a few days, at various times throughout gestation. Detection of these products of the placenta presents a unique opportunity for early and accurate detection of pregnancy. PAG's are not limited to ruminants and other members of the PAG family have been found in pigs, horses, and other species (Green et al., 1994; Szafranska et al., 1995; Gan et al., 1997).

Most of the research on the clinical application of detection of PAG's for diagnosis of pregnancy in cattle and other species has utilized radioimmunoassay systems to detect the proteins. This type of assay is not suitable for field use and must be conducted under

controlled conditions where equipment and personnel suitable for utilization of radioactive material are available. However, there is currently considerable interest in commercial development of "cow-side" assay systems that can be used for rapid and accurate identification of cows that are not pregnant. One such assay system that was under development when this manuscript was being prepared is SurBred™ (AspenBio Pharma; http://www.aspenbioinc.com). Information provided by the manufacturer indicates that the test will detect pregnancy by approximately 18 days after insemination. They claim to be in the final stages of development but details regarding availability of the test are unknown at this time (spring 2006).

As noted above, early embryonic death is common during the first few weeks of pregnancy. Thus, cows could accurately be diagnosed as pregnant shortly after insemination, but could suffer embryonic death and return to estrus or be found non-pregnant when examined later. Thus, chemical tests for pregnancy such as those to detect PAG's or any of a number of as-yet undiscovered markers for pregnancy should properly be thought of as "tests for openness" rather than "tests for pregnancy". Another factor that may influence a decision to use an early pregnancy test is the necessity that cows diagnosed as "pregnant" during the first few weeks of gestation be re-examined at a later stage (perhaps 45 to 60 days) by some other method such as ultrasound or transrectal palpation to detect those cows that have lost their pregnancies during the interval between examinations.

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Pregnancy diagnosis in sheep is an important management practice because of the impact of reproductive performance on economic return from the flock. Early pregnancy diagnosis, determination of fetal numbers and estimation of day of gestation, and recognition of abnormalities of pregnancy can provide producers with significant management opportunities to enhance reproductive efficiency. Historically, producers of small ruminants have had few options with regard to pregnancy diagnosis.