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BOVINE REPRODUCTIVE ULTRASOUND

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INTRODUCTION

Historical research of the bovine reproductive tract has relied upon examination of the tissue by rectal palpation, during surgery or after removal. Real-time ultrasound imaging provides a noninvasive technique to monitor ongoing reproductive phenomenon *in situ*. While the use of ultrasonography has increased in the past decade, this technology is still not utilized to its full potential in veterinary practice. Following this short review is a list of references if more in depth information is desired.

NORMAL OVARIAN STRUCTURES

Ovarian follicles contain fluid and appear ultrasonically as black (nonechogenic), roughly circumscribed areas within the borders of the ovary. Follicles can be round to irregular shaped. Irregular shapes are attributable to neighboring structures (additional follicles and corpora lutea) impinging on the follicle. Follicles as small as 2 mm can be discretely identified with high quality equipment and a 5 MHz transrectal linear array transducer. These follicles can be counted and their diameters measured. The dominant (pre-ovulatory) follicle can be predicted one to three days prior to ovulation and the time of ovulation determined by disappearance (or greatly reduced size) of the dominant follicle. Care must be taken to differentiate follicles from other nonechogenic structures, such as blood vessels and the central cavity of the corpus luteum.

After ovulation, young corpora lutea can be difficult to differentiate from ovarian stroma. A corpus luteum becomes visible approximately 3 to 4 days post ovulation and reaches maximal size around day 12. It has a granular echogenic appearance with distinct borders which differentiates from ovarian stroma. A corpus luteum can appear various shades of gray on the ultrasound image as opposed to a follicle, which appears black, and ovarian stroma, which appears virtually white. A central cavity may or may not be present but does not appear to affect total luteal tissue area or plasma progesterone concentration.

NORMAL UTERINE APPEARANCE

The ultrasonic characteristics of the non-pregnant bovine uterus changes profoundly with different stages of the estrous cycle. These characteristics include thickness of the uterine body, ultrasonic texture (evidence of edema), accumulation of intravaginal and intrauterine fluid, uterine shape and number of cross sections of the uterine horn visible in a single 5 MHz field.

The thickness of the uterine body begins to increase approximately 3 to 4 days prior to ovulation and decreases rapidly after ovulation. The increase in thickness is relatively uniform from dorsal to ventral uterus. Ultrasonic texture of the uterus becomes more heterogeneous prior to ovulation, corresponding to the time of increased uterine thickness. With approaching estrus, the uterus becomes less tightly curled and the number of cross sections of uterus in a single field decreases as the horn uncurls. The increases in both uterine thickness and heterogenicity and change in uterine shape and number of cross sections are likely due to the development of uterine edema as ovulation approaches. Endometrial folds can be more easily distinguished during estrus. Intrauterine fluid present at this time aids in the identification of these folds. During diestrus, the uterine diameter remains relatively constant, its appearance returns to a more homogenous texture.

Intrauterine and intravaginal fluid accumulations are often present during the period surrounding ovulation, beginning approximately 3 to 4 days prior to ovulation. During diestrus, small discrete fluid accumulations may be present in the uterus, which make pregnancy diagnosis prior to day 22 in cattle very difficult.

EARLY PREGNANCY

Real time ultrasound provides a noninvasive and nondisruptive technique to determine early pregnancy in cattle. The chorionic vesicle increases rapidly in length but remains approximately 2 mm in height from Days 10 through 19, postovulation. This is at the limit of resolution of a 5 MHz probe, making identification of the conceptus difficult at this time. Accuracy of pregnancy diagnosis has been shown to be no better than a guess during this period of elongation. Beginning approximately 19 days postovulation, a localized bulge appears in the chorionic vesicle. This appears as an irregular nonechogenic area in the lumen of the uterine horn. The embryo proper can be detected within this bulge during the next 5 days. The embryo proper appears as an echogenic linear structure 3 to 4 mm in length. Both the fluid dilation and the embryo proper continue to increase rapidly in size as the pregnancy progresses. A heartbeat can first be detected on Day 21, confirming viability of the embryo. While these parameters have been demonstrated under research conditions (darkened examination room, high quality equipment, animals trained to tolerate the procedure with minimal movement and an experience operator), it has been suggested under field conditions most practical to scan animals which are expected to have at least a 26 day old embryo.

As the pregnancy continues beyond Day 26, various embryonic and fetal structures can be ultrasonically identified. Structures include forelimbs, (Day 29 postovulation), hindlimbs (Day 31), placentomes (Day 35), split hooves (Day 45), and ribs (Day 53). Fetal movement can first be detected on the average by Day 45. Structures that can be identified in the older fetus using both a 5 MHz and 3.5 MHz probe include head, neck, vertebral column, thorax, stomach, liver, urinary bladder, limbs, scrotum, teats, umbilical cord and fetal membranes. The age of the early fetus can be estimated by measurement of fetal parameters. The diameter of the fetal trunk or eye can be estimated and fetal age determined between approximately 30 and 120 days.

Twins can be identified using ultrasonography. In one study, multiple fetuses were most accurately detected between 49 and 55 days post insemination. A deliberate effort must be made to look for multiple fetuses or they can be easily overlooked. Ultrasonic findings that suggest multiple fetuses may be present include excessive amount of placental fluid, the presence of extra membranes within the uterine lumen and multiple corpora lutea. The extra membranes are likely the apposition of the two allantochorions. Identification of twin pregnancies may not change the outcome of the pregnancy but may affect the management of the dams throughout their gestations.

Advantages of ultrasonography for diagnostic purposes include earlier determination of pregnancy than by rectal palpation, determination of embryonic viability by identification of a heartbeat, determination of the number of embryos present and evaluation of the embryo or fetus for gross abnormalities. In addition, manual manipulation of the reproductive tract is usually not necessary for a thorough ultrasonic examination of the reproductive tract.

FETAL GENDER DETERMINATION

Prior to day 50, the genital tubercle (forerunner of the clitoris or penis) can be ultrasonically identified but it is located between the hind limbs in both male and female fetuses. In both genders, the genital tubercle appears as a hyperechogenic bilobar structure. As differentiation occurs, the relative location of the genital tubercle moves toward the umbilicus in males and the tail in females. The genital tubercle is first located immediately caudal to the umbilical cord on mean day 56 in male fetuses and under the tail on mean day 54 in female fetuses. This technique can be used to predict fetal gender with a high degree of accuracy between 55 and 101 days postovulation.

PATHOLOGICAL CONDITIONS

Cystic ovaries: Ultrasonic appearances of ovarian cysts are similar to that of follicles, differing primarily in size. Follicular cysts are thin walled structures $\geq$25 mm in diameter filled with nonechogenic fluid. Various shapes are possible, depending on surrounding structures, although singular cysts are usually spherical. If pressure is equal between two neighboring cysts, the wall (seen as an echogenic line) between the two is straight. If the pressure of one cyst is greater than the other, it appears to bulge into the lower pressured cyst.

Fluid cavitations can also be present in corpora lutea. One study has shown ultrasound to be more effective than rectal palpation for diagnosing ovarian cysts as follicular or luteal (based on serum progesterone levels) in cows. The presence of either follicular or luteal cysts does not necessarily imply a pathological condition. Follicular cysts may be present during a normal pregnancy and luteal cysts do not necessarily affect serum progesterone levels.

Ovarian tumors: Although ovarian tumors are rare in cattle, ultrasound has been used to identify the physical characteristics of a tumor. Confirmation of diagnosis must be made histologically. Additional research is required to develop a diagnostic method for tumor analysis.

Endometritis: Ultrasonic diagnosis of endometritis can be made by the appearance of nonechogenic uterine fluid accumulations containing echogenic particles. This gives a "snowy" appearance to the uterine fluid as viewed on the ultrasound screen. The amount of fluid varies depending on the severity of the endometritis. In many cases, a thickened uterine wall can also be identified.

Pyometra: When large quantities of fluid are present causing distention of the uterus and a corpus luteum is present, a diagnosis of pyometra can be made. Ultrasonic characteristics of the fluid varies depending on the consistency of the uterine fluid. A very thick uterine fluid, full of leukocytes and tissue debris, will appear similar in echogenicity to the uterine wall. A thinner uterine fluid will have the "snowy" appearance of echogenic particles in nonechogenic fluid. The uterine wall may be either thickened or thinned. As with endometritis, the entire uterus must be examined to rule out the possibility of pregnancy.

Fetal death: Embryonic and fetal death can be identified by lack of a heartbeat. Research using ultrasound has shown that while the placental fluid may be resorbed following embryonic death, the tissues are lost through the open cervix at estrus. Also, the corpus luteum can be maintained for long periods following embryonic or fetal death.

Macerated or mummified fetus: Dense (hyperechogenic) bone fragments in purulent fluid contained within thickened uterine walls are the typical ultrasonic appearance for a macerated fetus. Mummified fetuses can be more difficult to identify. Uterine fluids are usually not present and the uterine wall is thickened but the mummified fetus itself may or may not be discerned.

Fetal anomalies: Gross fetal anomalies can be identified during routine pregnancy examination of the older fetus. These conditions are rare but can cause abortion or dystocia at term. Conditions such as schistomsomus reflexus, compact fetal mass, fetal hydrops, a two-headed fetus, and umbilical dilations or abnormalities have been identified.

ADDITIONAL APPLICATIONS

Embryo transfer program: Ultrasound can be used to assess follicular status and identify the presence of a dominant follicle prior to superovulation. This may help to determine if the animal is a good candidate for flushing or if the procedure should be discontinued before additional money is spent. Research suggests that the presence of a dominant follicle at the time of superovulation decreases the response and potential yield of embryos by a donor animal and ablation of the dominant follicle leads to increased numbers of embryos collected per donor flush. A single examination can determine the presence of a dominant follicle by counting the number of follicles from 3 to 8 mm. If <10 follicles between 3 and 8 mm diameter are present, it can be assumed that an active dominant follicle is present. If ≥10 follicles of this size are present, it can be assumed that there is not an active dominant follicle present.

Transvaginal follicular aspiration is used to collect oocytes for *in vitro* fertilization in problem breeders. This requires additional equipment and expertise. The biggest benefit of ultrasonography to embryo transfer programs is the increased understanding of folliculogenesis by ultrasonic monitoring of ovaries. This has lead to improved protocols for reproductive cycle manipulation.

Male reproductive tract: B-mode ultrasonography can be used to enhance the breeding soundness examination of the bull. The normal bull testicular parenchyma has a homogenous and moderately echogenic texture. The head and tail of the epididymis can also be easily identified but the epididymal body and ductus deferens are more difficult to consistently distinguish. Within the testis, the mediastinum testis was a normally visible structure and measured between 0.1 and 0.33 cm but could not be used as a reliable predictor of breeding soundness score.

SUMMARY

Real-time ultrasound imaging provides a noninvasive technique to monitor ongoing reproductive phenomenon *in situ*. Repeated or continuous scanning allows the study of dynamic reproductive events with minimal disruption or discomfort to the animal. This technique expands the previous limits of information available.

Ovarian and uterine tissue can be easily identified with good quality ultrasound equipment. Ultrasonography can be used to discern follicular and luteal structures in detail during the estrous cycle. It is used to study follicular dynamics and intraovarian relationships among follicles. Follicular wave patterns have been described during the estrous cycle and early pregnancy. Ovarian status prior to and following superovulation in an embryo transfer program can also be assessed.

The ultrasonic appearance of the non-pregnant and pregnant uterus can be described and studied. Profound changes in uterine characteristics occur during the bovine estrous cycle. Pregnancy can be diagnosed and an embryonic heartbeat detected as early as 21 days postovulation. Growth of the conceptus can be monitored throughout pregnancy. Real-time ultrasound also provides a unique method to study spontaneous and induced embryonic death.

Additional uses of ultrasonography in bovine reproduction include determination of fetal gender, assessment of pathological conditions of the ovaries, uterus and fetus, transvaginal follicular aspiration for collection of oocytes and dominant follicle ablation and examination of the male reproductive tract. The potential of this technology is not full utilized yet in practice or research. maximal utilization requires high quality equipment, good examination conditions, keen observational skills and a creative mind.

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