Applying ultrasonographic evaluation of antral follicle count to improve reproductive management in heifers
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Abstract
Ultrasonography is a powerful technology that can be used to improve reproductive management in heifers. By counting the number of antral follicles observed on an ultrasound screen the practitioner can gather additional information when reproductive tract scoring, because the number of antral follicles is predictive of the status of the ovarian reserve. The number of antral follicles is also predictive of response to exogenous gonadotropins and can be used as a screening tool to remove poor responders before time and money is squandered trying to recover oocytes or embryos.

Introduction
Palpation of the ovaries and reproductive tract of the cow has been used by veterinarians for decades to evaluate reproductive status. Advantages of this technique are that the reproductive tract is easily accessible, examinations can provide information on ovulatory status and size of the ovaries and the uterus, results are instantaneous allowing management decisions to be made at time of examination, and labor is used efficiently because a relatively large number of heifers can be evaluated in a relatively short period of time. These advantages led to development of the Reproductive Tract Score, a formalized method of evaluating reproductive tract development and ovulatory status in heifers1 and cows.2 Especially in the beef industry where access to the heifers can be limited under some management practices, this has provided a method for evaluating replacement heifers that did not require observation for behavioral estrus over several months to determine pubertal status. Nevertheless, adoption by producers has been slow because of need for highly-trained individuals with palpation skills, costs associated with procuring services of such trained individuals, need for suitable working facilities, and need to gather heifers and bring them into the working facilities. From a veterinarian’s perspective, strain on the practitioner’s arm and shoulder deters enthusiasm for providing such a service. In cases where an extension arm can be used, such as pregnancy diagnosis, ultrasonography can eliminate this strain on the shoulder. Furthermore, ultrasonography can provide additional information not acquired by traditional palpation, such as antral follicle count and blood flow, leading to better decision making about females to retain for breeding purposes.

Ultrasound and its application to reproductive management in bovine females
Ultrasound machines are composed of a primary electronic processing unit and a piezoelectric transducer that emits and detects high frequency sound waves. Based on density of tissue being scanned, the sound waves emitted by the transducer either reflect back to the transducer or continue to penetrate the tissue. Returning sound waves are detected by the transducer and converted to a real-time image displayed on the screen of the processing unit. Fluid filled structures such as the antrum of a follicle or a gravid uterus do not reflect sound waves and appear on a standard screen as a black area.

Transducers may comprise linear or sector (convex) crystal arrays. Linear transducers have crystals arrayed in a single plane and emit waves at a ninety degree angle from the transducer. Sector or convex transducers have a crescent-shaped array of crystals and emit waves across a broader area. In the past, transducers had a fixed frequency and there was a trade-off because a greater frequency gave a better image quality but decreased depth of tissue penetration. In newer machines, frequency for a transducer can be changed and this allows greater flexibility in the information that can be obtained from a single examination.

Compared with traditional palpation, ultrasonography increases the information that can be collected as part of an examination process. Ultrasonography can be useful in detecting multiple...
ovulations, size of corpora lutea, number and gender of fetuses, heart beat of fetuses, abnormalities of the genital tract, and other indicators of fertility. For example, analysis of gray scale patterns of uterine images may provide some indication of fertility. Researchers began using ultrasonography to measure diameters of antral follicles as a method to understand follicular development and ovulation. From this research, they discovered that diameter of the ovulatory follicle is related to estradiol production and overall fertility; however, follicle diameter measurements must be taken at consistent times during the follicular phase to generate measurements that can be compared across cows and be useful for making decisions. Counting the number of antral follicles that are visible on the ultrasound screen can be used as an indicator of the number of microscopic follicles within an ovary. This measurement is less dependent on stage of the estrous cycle because small (< 5 mm) antral follicles comprise the majority of the count and do not fluctuate during follicle wave progression.

Color Doppler ultrasonography makes use of the Doppler effect to determine direction and rate of movement relative to the position of the transducer. It is most commonly used to measure blood flow with the colors (blue and red) simply indicating orientation of the flow to the transducer and having nothing to do with arterial or venous blood flow. Some researchers feel that it has application for early pregnancy diagnosis in cows by focusing on the function of the corpus luteum (CL), because during luteal regression blood flow to the CL decreases. Therefore, by examining blood flow at days 18 to 19 after breeding the technician may be able to identify corpora lutea that are regressing in the absence of pregnancy. It is still difficult to time this correctly because bovine estrous cycles with three waves of follicular development will average 24 days in length. Color Doppler ultrasonography can also be used to measure blood flow to a gravid uterus and understand the role of blood flow in fetal development. The diagnostic uses of this for reproductive management of cows have yet to be established.

Relative distance from the surface of the transducer to the surface of the tissue being examined along with the frequency of the sound waves being emitted affect the size of structures measured. For pregnancy diagnoses in cattle, curved handles that enclose the probe and connecting cable can be inserted per rectum to decrease strain on the arm and shoulder that are characteristic of palpation. In contrast, the transducer must be held and manipulated by hand to insure consistent measurements of ovaries, diameters of follicles or CL, and cross-sections of uterus. There may be future solutions for this requirement, but the current technology can provide very informative data that helps veterinarians and producers make profitable decisions.

Ultrasound machines have become more compact and mobile with greater computing capabilities. As computing capabilities and image analysis software improve, applications of the machines to improve reproductive management will continue to increase. Ability to store digital video clips of reproductive tract scans of individual animals will create permanent records that are managed, stored, and queried more easily than with the video tapes used in the past. Imaging software will attain a level of sophistication to instantaneously provide pixel densities of the ovaries as a proxy for antral follicle count and blood flow measurements to better evaluate CL and reproductive tract function. This should improve diagnostic capabilities and perhaps decrease the amount of time a diagnostician has to spend with their arm inserted into a rectum. Wireless technologies and adoption of electronic identification will eliminate need for a practitioner to record animal identifications by hand, thereby decreasing the error rate and the time required to complete an examination of an animal.

**Antral follicle count as an indicator of fertility**

Compared with traditional palpation, ultrasonography allows a practitioner to visualize and count the number of antral follicles present on an ovary. By the early 1990’s, human medicine had moved in this direction as a method to assess reproductive status of women with fertility issues. Women that were approaching menopause or that suffered from primary ovarian insufficiency had lower numbers of antral follicles detectable by ultrasonography. Because basic mechanisms controlling numbers of follicles in the ovaries are similar in all mammalian females, we began to investigate the role of antral follicles counts in bovine reproduction, and it has spread as a tool to a number of mammalian species as a way of
addressing reproductive management in endangered species. Antral follicle counts provide practitioners with a non-invasive estimate of the number of follicles in an ovary, because histological studies have demonstrated that there is a positive relationship between the number of microscopic follicles and the number of antral follicles in a bovine ovary.11-13,19

Antral follicle counts are associated with a number of important reproductive traits. Heifers that give birth early in their first calving season have greater numbers of antral follicles at pre-breeding ultrasonographic examination than those that give birth later in the first calving season.20 Similarly, dairy cows with high numbers (≥ 25) of antral follicles required fewer inseminations per conception than those with low numbers (≤ 15) of antral follicles.21 Because beef heifers that give birth early in their first calving season have greater reproductive longevity,22 it is tempting to speculate that differences in germ cells numbers may contribute to this increased reproductive longevity. However, there is little information on the mechanisms influencing the rate of depletion of the follicles. If a heifer with high numbers of follicles has a high rate of depletion and a heifer with a low number of follicles has a low rate of depletion, they could still both reach reproductive senescence at the same age. Further research is needed to understand the mechanisms controlling depletion of the ovarian reserve.

Dairy heifers with high numbers of antral follicles have greater serum progesterone concentrations during the luteal phase and greater endometrial area during the first 6 days of the estrous cycle than those with low numbers of antral follicles.23 Serum follicle stimulating hormone (FSH) concentrations are decreased and follicular fluid estradiol concentrations are increased in cows with high numbers of antral follicles compared to cows with low numbers of antral follicles.24 Furthermore, ovaries from cows with high numbers of antral follicles are larger, but this is not just due to the increased volume of the antral follicles, because these ovaries have more microscopic follicles on a per gram of tissue basis.13,19 Thus, there are clearly differences in hormonal milieu and reproductive tract function that contribute to improved conception in cows with high numbers of antral follicles.

Whether there is a difference in oocyte competence between cows with high and low numbers of antral follicles is unclear. The majority of studies have reported increased numbers of blastocysts produced in vitro, but no difference in percentage of blastocysts produced in vitro between cows with high numbers of follicles and cows with low numbers of follicles.24,25 However, there is one study that reported an increase in percentage of blastocysts produced in vitro from oocytes collected from cows with high numbers of follicles compared to those with low numbers of antral follicles.26

Response to exogenous gonadotropins

The use of exogenous gonadotropins either for multiple ovulation embryo transfer or ultrasound-guided oocyte pick up (OPU) is a way to rapidly increase the number of progeny of a genetically superior heifer while decreasing the generation interval. The decision to use a heifer in such a program should not be based solely on genetic merit, but should also be based on reproductive capacity. We demonstrated that the number of microscopic and antral follicles in one ovary was predictive of the response to exogenous FSH in the other ovary.12 Cows with low numbers of primordial follicles had fewer antral follicles and poor ovulatory response to exogenous gonadotropins while cows with greater numbers of primordial follicles had more antral follicles and a strong ovulatory response to exogenous gonadotropins.

These results led others to investigate the use of ultrasonography to determine antral follicle numbers as a method to predict response to exogenous gonadotropins in cows.27 One hundred and forty one cows were submitted for ultrasonography and based on total number of follicles ≥2 mm that were observed at follicle wave emergence, cows in the top 10% and cows in the bottom 10% were chosen. The cows in the high group had more than twice the number of follicles >5 mm after treatment with FSH for three days than the cows in the low group. The authors concluded that screening of cows prior to the start of treatment with exogenous gonadotropins was a useful way to remove poor responders. Similar results were reported for OPU,28 demonstrating that cows with high numbers of follicles had greater numbers of cumulus-oocyte-complexes recovered after treatment with exogenous gonadotropin. A follow-up study proposed the need for individual protocols based on follicle numbers, but giving greater doses of FSH in the low follicle number cows did not improve response.29 This is most
likely because others have demonstrated that cows with low numbers of follicles already have greater circulating concentrations of FSH due to less negative feedback.24

Conclusions

Ultrasonography is a powerful tool that can contribute greatly to reproductive management in heifers and cows. The ability to visualize physiological functions that would not be palpable such as heart beat or blood flow and to record these measurements for future diagnostic reference provide advantages beyond traditional palpation. It provides a method for early and accurate pregnancy detection and diagnosis of ovarian cysts. One advantage that it has over traditional palpation for evaluating reproductive capacity is the ability to visualize and count the number of antral follicles. As a predictor of the number of microscopic follicles in the ovaries, antral follicle counts can help to identify replacement females that may not produce enough calves to pay for their development costs or identify poor responders to treatments with exogenous gonadotropins. Continued research on antral follicle counts and the ovarian reserve will identify the mechanisms that contribute to decreased fertility and provide better decision making tools for reproductive management.

References


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