Ultrasound Imaging and Use in Assessment of Reproductive Conditions

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Advantage:

Repeated, noninvasive visualization:

Internal anatomy of the organs and the events occurring there can be visualized without any interference.
Principle:

Sound frequency

- Greater than 20,000 Hz is ultrasound

Diagnostic ultrasound

- 1 to 10 MHz
- 10 – 25 MHz

Ophthalmology

- Different abilities of tissues to either reflect or propagate sound waves
- Echogenic / nonechogenic tissues

- Piezo electric crystals
- Liquid containing structures like ovarian follicles, urinary bladder Non echoic- appear black in the ultrasound screen. gall bladder, yolk sac etc

- Dense tissues like fetal bones, cervix etc. Echoic- appear white on the screen

> Various tissues in the body are seen in various shades of grey depending on their echogenicity, - GREY SCALE IMAGING

- Artifacts/ distortions - interpreting an ultrasound image.

- Thus, interpretation of ultrasound images is very intricate and requires knowledge of the relationships between tissues and echoes and the ability to differentiate between true and artifactual responses.
A Mode

B Mode

M mode

Doppler

Real time

[Diagram of ultrasonic imaging with labeled A and H]
**B-mode real-time scanners**

Ultrasound instruments used presently, for veterinary reproductive studies

B-mode refers to brightness modality in which the image is produced

Real time imaging refers to the fact that events can be visualized ‘live’ i.e., as they occur, like the movements of the fetus, heart beats etc. two other modes are available for study of soft tissues.
The A-mode

In the past, was used for pregnancy diagnosis in ewes.

However, now with suitable modifications, the B-mode itself has been adapted for this purpose.

The M-mode (motion mode)

It is an adaptation of the B-mode and is used for evaluating moving structures.

Doppler ultrasound systems are used to monitor fetal heartbeat and blood flow in large vessels.
Console (Monitor/Display)

Transducer (Probe)
Probe (Transducer)

Surface (percutaneous) & Internal probes

Linear
Sector
Convex
Curvilinear

Different frequencies
Different kinds of ultrasound probes: L=Linear; S: Sector; CL: Curved Linear
Ultrasound technique can be used in evaluating and monitoring various aspects of reproduction.

To give just a few examples:

- Determination of attainment of puberty.
- Monitoring follicular development.
- Detecting ovulation and diagnosing failure of ovulation.
- Monitoring development, function and regression of corpus luteum.
- Examination of the endometrium during the oestrous cycle.
• Evaluating time and suitability for breeding
• Monitoring uterine involution.
• Pregnancy diagnosis including detecting twin pregnancy at a very early stage.
• Differentiating pregnancy and pseudo-pregnancy
• Interactions between embryo and uterus.
• Diagnosing early embryonic death.
• Diagnosing ovarian, reproductive tract pathology.
Days of estrous cycle

2-3 waves ovulatory follicle Selection Deviation
Dominant & Subordinate follicles
OPU
Ultrasound guided ovum pick up.
- ultrasound scanner (Aloka SSD 500, Japan)
- 5 MHz convex array transducer (Aloka, Japan)
- ovum aspiration needle (17 G, 50 cm)
- connected to an aspiration line made of silicon tubing (Cook, Australia)
- vacuum pump – pressure (80-120 mm Hg) / flow rate (20 ml /mn)
TRANSVAGINAL OOCYTE RECOVERY

Collection Needle

Ultrasound Probe

Fluid Recovery

Vagina

Ovary

Rectum
Transvaginal Ultrasound Guided Follicle Aspiration (OPU)
Sagittal section through a non-pregnant uterus. Arrows demarcate the large curvature of uterus.
Transverse section of non-pregnant uterus (Horns demarcated by arrows)
A. Ultrasound image of the corpus luteum with a cavity at a frequency of 5.0 MHz. The outline of corpus luteum is indicated by arrows.

The same corpus luteum as in A imaged at a frequency of 3.5 MHz.

Size of CL = 36.5 X 26. MM

Size of cavity = 25x 18 mm.
Cranial half of the urinary bladder (fundus and body)

Above the fundus is the corpus luteum)

Caudal half of the urinary bladder (U)
Paramedian section through the pelvic floor (Arrows); parallel to the initial echo; stretching into the depth of the image are the series of echoes caused by multiple reflections (reverbration artifacts between the sound probe and pelvic floor).

Sagittal section through the uterine cervix; ventrally to the cervix lies the urinary bladder (U). Arrows represents several cervical rings.

Centre of cervix shows bright linear echo of the cervical canal.
Follicle (F) in a cow on the day of estrus. Diameter of Follicular antrum=17 mm
Thickness of wall= 1-2 mm
U= Urinary Bladder

Ovary with an estrus follicle on the day of ovulation.
Small arrow (Echoic spot) at floor of follicle (at 5MHz sector scanner)
Fa = Estrous follicle at 5 MHz

Fb = Same follicle in A at 3.5 MHz.
Ovary with 4 developing follicles at 5 days after start of superovulation FSH treatment.

Multiple follicle son an ovary at 9 d after start of ECG induced superovulation (3000 IU after 0.5 mg cloprostenol 7 d earlier)
A. Ovary with a mature compact corpus luteum. Compared to the surrounding ovarian parenchyma, the corpus luteum (arrow) is less echoic.

Cross section of the solid corpus luteum analogous to the sectional plane in Fig A.
Ovary containing three theca follicular cysts. Two cysts have a straight dividing wall.

(taken in water bath)
Left: Conceptus with embryo and amnion (2 arrows) on day 33 of pregnancy. The amnion forms a thin membrane which surrounds the embryo.

Right: An embryo with surrounding amnion and adjoining (Al) allantochorion on day 37 of pregnancy.
Uterus on Day 47 of pregnancy. The amnion (A) with the embryo drifts in the dorsal part of the embryonic vesicle. The head (H) lies on the right.
Ultrasound image of fetal head; diameter 2.0 cm; estimated age 69 days.

Ultrasound image of crown-rump; length 3.5 cm; estimated age 48 days.
Ultrasound images of a male fetus (68 days; frontal).

Left panel shows hindlimbs (HL) and penis (P).

Right panel shows scrotum (S).
Ultrasound images of the bovine fetus at various stages of development

25 Day Pregnancy

30 Day Pregnancy

35 Day Pregnancy

43 Day Pregnancy

50 Day Pregnancy

100 Day Pregnancy
Ultrasound image of bovine ovaries prior to emergence of a follicular wave (note two small follicles [< 5 mm]; Panel A), during proestrus (note preovulatory follicle[13 mm]; Panel B), and after development of a follicular cyst (note delamination of granulose layer into the antrum; Panel C). Images were taken using a 7.5 Mhz transducer (Lamb, 2001).
Thanks
Major Applications in Cattle

Ultrasound evaluation of ovarian follicles:
Ultrasound is a more sensitive method than palpation per rectum for detecting and measuring ovarian follicles, especially those within the ovarian stroma.

Correlation coefficients between ultrasound measurements and actual counts obtained by slicing ovaries after slaughter ranged from .80 to .92 for number of follicles detected in various size categories and was .97 for diameter of the largest follicle.

Ultrasound monitoring of ovarian function has been used to determine that bovine follicular development occurs in two, three or four coordinated waves throughout the estrous cycle and that follicular waves continue at approximately 10-day intervals during pregnancy.

The echotexture characteristics of the dominant follicle may be correlated with the functional and endocrine status of the follicle.
After the dominant follicle reaches its peak diameter, referred to as the static phase, granulosa cells are sloughed into the antrum. The debris increases the **echogenic heterogeneity** of the antral fluid.

The changes in follicular echotexture measured by computer-assisted echotexture analysis coincides with the ovulatory potential of the follicle and steroid content of follicular fluid.

At present, however, there is no method to determine the physiological status of a large follicle without serial examinations and retrospective analysis.

Future use of computer assisted image analysis may improve the diagnostic potential of ultrasound to determine the health of a large follicle in a single examination.
• Several studies have been conducted to test the effects of a dominant follicle present at the initiation of treatment on the superovulatory response. Dominant follicles were defined in that study as being >9mm in diameter and either in a growth or static phase.

• The removal of a dominant follicle was followed by an increase in circulating FSH and a subsequent increase in small follicles.

• Ovulation is detected by ultrasonography as the acute appearance of a large follicle (9-20 mm) that was present at a previous examination.

• The site of ovulation is visible on the day that the large follicle disappears, and the CL may develop as a solid or fluid-filled structure.

• The cavities of fluid-filled corpora lutea are distinguished from follicles by a non-spherical, often lobulated, appearance and by the surrounding border of luteal tissue.

• Several researchers have demonstrated that a CL with a fluid-filled cavity is a normal condition and that the cavity is usually replaced by a dense, solid core of luteal tissue late in the estrous cycle or during the first 25 days of pregnancy.
The echogenicity of the corpus luteum (CL)
The echogenicity of the corpus luteum (CL) depends on the stage of CL development. The corpus hemorrhagicum is visible from ovulation to day 3 post-ovulation as it is less dense than the surrounding stroma and often has an anechoic, fluid-filled center. The growth of the CL is most extensive between days 3 and 4 of the estrous cycle (day 0 = ovulation) and it reaches maximal diameter between days 12 and 16 of the cycle. Ultrasonic detection of corpora lutea may be more sensitive than detection by palpation, but this is dependent on the experience of the individual performing rectal palpation.

Detection of a CL with ultrasound is based on the differences in echogenicity between the stroma and the luteal tissue; whereas, CL detection by palpation is based on the presence of a crown protruding from the ovary, a discernible, defined structure within the ovary and/or total ovarian size.

The ability to discern CL from the stroma depends on the quality of the ultrasound equipment and the skill of the ultrasound technician. Occasionally it can be difficult to differentiate the CL from the stroma due to the size of the CL and the area of the ovary occupied by the corpus luteum.

Usually the stroma can be differentiated from the CL by the presence of numerous small follicles dispersed throughout the stroma (21). Ultrasound machines with expanded gray scale capabilities enhance the ability to differentiate ovarian structures due to subtle differences in echogenicity.
Embryo transfer practitioners often reject recipients presented for transfer based on the absence of palpable luteal tissue or the presence of a small, irregular, fluid-filled or soft CL.

Follicular cysts are non-echogenic structures with a thin wall (2mm or less) and are 25mm or larger.

In addition to their large size and absence of luteal tissue, follicular cysts may be distinguished by coincident estrous behavior and low plasma progesterone concentrations.
Ultrasonic appearance of the bovine uterus
Ultrasonic appearance of the bovine uterus is dependent on stage of the estrous cycle.

Variation in the appearance of the uterus involves changes in endometrial thickness, vascularity and the presence of intraluminal fluid.

The changes in endometrial echotexture are attributed to development of edema which increased in uteri of non-bred heifers beginning around day 16 and continued until day 20 of the estrous cycle.

During estrus the endometrium is noticeably echogenic, the endometrial/myometrial border is obvious and small accumulations of fluid occur throughout the uterine lumen (29).
The echogenicity and “puffy” appearance of the uterine endometrium decreases by 4 or 5 days after ovulation.

The uterine horns are extended during and shortly after estrus, but become highly coiled during the luteal phase.

Real-time, B-mode ultrasonography has been reported to detect pregnancy in cattle as early as 9 or 12 days into gestation.

Other reports, however, have disputed those claims and emphasized that accuracy of ultrasound diagnosis of pregnancy on day 10 through 16 was not significantly better than a random guess (≤50%).

Accuracy of diagnosis improved, however, by day 18 (85%), 20 (100%) and 22 (100%) of pregnancy.
Presence and vitality of the embryo
Presence and vitality of the embryo can be confirmed by the detection of a heartbeat as early as 19 to 24 days of gestation. The embryo initially appears as a short, straight echoic line (20-22 days), later becomes C-shaped (22-30 days) and finally, by 30-32 days of gestation assumes an L shape.

The potential advantages of using ultrasonography for pregnancy diagnosis are that the presence of an embryo can be detected earlier than by palpation per rectum and that direct physical manipulation of the gravid reproductive tract is unnecessary with ultrasonography.

The latter fact should reduce the risk of inducing embryonic mortality or atresia coli. An association between early, vigorous palpation per rectum of the amniotic vesicle and atresia coli in calves has been proposed.

Use of ultrasonography rather than palpation per rectum may also improve consistency of early (<45 days) pregnancy diagnosis by reducing the variation in accuracy among practitioners.
The efficiency (speed with accuracy) of detecting early pregnancy with ultrasound is markedly increased when the embryo can be detected more easily.

Although the embryo can first be detected between the days of 19 and 24 of gestation, when scanning large numbers of cattle, it is most practical to scan females which are expected to have embryos >24 days of age.

The ability to identify open cows with ultrasonography earlier than by rectal palpation can be an economic benefit to beef and dairy producers.

Some have reported using ultrasound as early as 21 days after insemination to identify open cows (36). The negative predictive value (the accuracy of a negative diagnosis) reached 100% by day 28 post insemination or by day 33 in another study.
Table 2. Formulas for Determination of Fetal Age from Ultrasonic Measurements

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Day of Gestation</th>
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<tbody>
<tr>
<td>Head diameter (cm)</td>
<td>(log of head dia.) x (45.23) + 37.7</td>
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<td>Trunk diameter (cm)</td>
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<td>(log of crown-rump) x (16.73) + 27.5</td>
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1 formula requires natural log (ln) function available on most pocket calculators.
Determination of fetal viability and age
Subjective evaluation of anatomical traits can be used to age bovine fetuses, but the most accurate estimate of gestational age is derived from actual measurements of specific features.

The growth of the embryo proper from 20 through 60 days of gestation can be determined when the characteristics such as the heartbeat (day 22), spinal chord (day 28), placentomes (day 35), split hooves (day 44), and ribs (day 52) first became detectable.

Measurements of crown rump length, head diameter and trunk diameter are the easiest predictive measurements to use for estimation of gestational age. In addition, the use of these measurements in formulas to estimate age results in the least variation between the estimated and actual ages.

Early embryonic death rate between days 25 and 90 was estimated at 10% ± 5% when based on uterine fluid alone but the estimate decreased to 6% ± 5% when the diagnosis of pregnancy was based on visualization of an embryo.
Others have reported early embryonic mortality rates of 6% (34), 8% (44) and 15% (36, 37) during the first 90 days of gestation. Knowing this, it is recommended that the pregnancy status of all cows diagnosed pregnant before 35 days be reconfirmed after 60 days. Macerated fetuses may appear as distorted images surrounded by purulent fluid characterized by anechoic background fluid containing echogenic particles.

Degenerating embryonic tissues within the vesicle increases the echogenicity of the amniotic fluid surrounding the embryo which also may appear distorted. Frequently, these changes are too subtle to be detected by palpation. Sometimes, the fetus may retain its shape but a heartbeat cannot.)
Results

• The oocyte recovery rate - 39.2%
• IVEP suitable oocytes - 46.7%
• IVEP was carried out as per the standard procedure
• Cleavage rate - 58.6% and
• Transferable embryo production rate - 31%
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