Reproductive Ultrasonography

OvaGenix, LP
Charles R. Looney, PhD
What is Required

- US console w/5.0 or 7.5mHz linear transducer

- Facilities:
  - appropriate chute (w/squeeze? w/incline?)
  - visibility & lighting (reduce glare, increase contrast)

- Animals:
  - keep restrained, not immobilized; calm, not excited
Tringa by PieData

Easi-Scan by Steuart Labs
Aloka 500V Settings

- **Menu 1:**
  - FRM-CO = AUTO
  - CONT = 4
  - AGC = 1 or OFF

- **Menu 2:**
  - DT-DSP = ON
  - IMG-DI = “top down”

- **Menu 3:**
  - TM-FRZ = OFF
  - IMG-PO = POSI
  - PUNC = ON/OFF 1/0.5

- **FOCUS = All OFF or**
  - F1 for Ovaries, <35 d
  - F2 for <60 d
  - F3 for >60 d

- **Near Gain = 19 – 25**
- **Far Gain = 1.7 – 2.4**
- **Master Gain = 82 – 90**
- **MODE = “B”**
- **Magnification x1.0, x1.5**
- **Image Direction  R or L**
Acquisition of Images

- Scanning technique
  - Dorsal (overhand) vs. Ventral (underhand) preference?
  - Retraction of uterus helpful or required?
  - Ballottement of fetus helpful or required?
Acquisition of Images (con’t)

- **Sectional Views**
  - Transverse (cross-sectional)
  - Sagittal (profile)
  - Frontal (dorso-ventral)
Reproductive Anatomy & Stage of Cycle

- Determine landmarks & relative sizes of:
  - Cervix, uterine body, uterine horns, ovaries, follicles, corpora lutea

- Use all information available concerning:
  - Reproductive history, parity, status, cyclicity, recent behavioral activity, synchronizations, bull exposure, AIs, ETs, etc.
Estrus Uterus
Pre-Ovulatory Follicle
Corpus Luteum & Small Follicles
Corpus Luteum & Medium Follicles
Corpus Luteum & Large Dominant Follicle
Pregnancy Determination

- Determine landmarks & relative sizes of:
  - Fluid volume, uterine diameter, fetus, amnion, cotyledons

- For fetal sexing, determine orientation of:
  - head, neck, vertebrae, ribs, heart, umbilicus, legs, tail
### Sonographic Fetometry of Bovine Conceptus

<table>
<thead>
<tr>
<th>CHARACTERISTIC</th>
<th>MEAN</th>
<th>RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryo proper</td>
<td>20.3</td>
<td>19 to 24</td>
</tr>
<tr>
<td>Heartbeat</td>
<td>20.9</td>
<td>19 to 24</td>
</tr>
<tr>
<td>Allantois</td>
<td>23.2</td>
<td>22 to 25</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>29.1</td>
<td>26 to 33</td>
</tr>
<tr>
<td>Forelimb buds</td>
<td>29.1</td>
<td>28 to 31</td>
</tr>
<tr>
<td>Amnion</td>
<td>29.5</td>
<td>28 to 33</td>
</tr>
<tr>
<td>Eye orbit</td>
<td>30.2</td>
<td>29 to 33</td>
</tr>
<tr>
<td>Hindlimb buds</td>
<td>31.2</td>
<td>30 to 33</td>
</tr>
<tr>
<td>Placentomes</td>
<td>35.2</td>
<td>33 to 38</td>
</tr>
<tr>
<td>Split hooves</td>
<td>44.6</td>
<td>42 to 49</td>
</tr>
<tr>
<td>Fetal movement</td>
<td>44.8</td>
<td>42 to 50</td>
</tr>
<tr>
<td>Ribs</td>
<td>52.8</td>
<td>51 to 55</td>
</tr>
</tbody>
</table>

¹Adapted from Curran et al., 1986.
Crown-Rump Length (CRL)
Cranial Width (CW)
Sonographic Fetal Aging

- **Crown-Rump Length**
  - ¼ inch = ~ 30 days
  - ½ inch = ~ 35 days
  - ¾ inch = ~ 40 days
  - 1 inch = ~ 45 days
  - 1½ inches = ~ 50 days
  - 2½ inches = ~ 55 days
  - 3 inches = ~ 60 days
  - 4 inches = ~ 65 days
  - 5 inches = ~ 70 days

- **Cranial Width**
  - NA
  - NA
  - ¼ inch
  - ½ inch
  - ¾ inch
  - 1 inch
  - 1¼ inch

- **Vessicle Diameter**
  - ½ inch
  - ¾ inch
  - 1 inch
  - 1½ inch
  - 2 inches
  - 2½ inch
  - 3 inches
  - 3½ inch
  - 4 inches
Crown-Rump Length in Developing Calves

Days

Length (cm)

0 1 2 3 4 5 6

20 30 40 50 60
Early Pregnancy Determination
Bovine 35 Days
Bovine 40 Days

OvaGenix, LP
Bovine 45 – 50 Days
Bovine 65 Days
Crown-Rump Length in Developing Calves

Length (cm)

Days

60 75 90 105 120 135 150 165 180

0 10 20 30 40 50 60
Bovine 70 – 75 Days

OvaGenix, LP
Bovine 3 – 4 Months
Gestational Abnormalities

- Abnormal (smaller) volume of fluid
- Lack of detectable fetal heartbeat
- Separation of chorion from uterine wall
- Separation of amnion from fetus
- Appearance of echogenic particles

- May require re-evaluation within 2 - 7 days
Early Embryonic Death
GENDER DETERMINATION

“FETAL SEXING”
with Transrectal Ultrasound
**Procedure**

- **Acquire clear images w/distinguishable gender-specific anatomy:**
  - Determinable window is b/t 58 and ~90 days
  - Optimum window is b/t 65 and 75 days

- **Factors:**
  - equipment, environment, stage of gestation, etc.

- **Interpret results after independent observations**

- **Factors:**
  - Presence or absence of gender-specific genitalia
Fetal Development in Males

Genital Tubercule (Prepuce):

- 1st seen as hyper-echogenic, bi-lobed structure ~45 days in neutral position, b/t hind legs

- ~55 to 58 days Male GT migrate to normal anatomical position, posterior to the umbilicus

- Prepuce swells & becomes more tubular over next 2 weeks; more definitive outline, but less echogenic
Fetal Development in Males (con’t)

- Scrotum
  - 1st seen as echogenic, bi-lobed structure ~62 to 65 days in normal anatomical position, b/t hind legs
  - Characterized by 3 echogenic parallel lines
Fetal Development in Females

- **Genital Tubercule (Vulva):**
  - 1st seen as hyper-echogenic, bi-lobed structure at ~45 days, in neutral position b/t hind legs
  - at ~55 to 58 days, Female GT migrate to normal anatomical position, ventral to the tail
  - GT shrinks slightly in size over next 2 weeks, thus, more difficult to visualize
  - characterized by 2 echogenic parallel lines
Fetal Development in Females (con’t)

- Mammary (teats)
  - 1st seen as 4 small echogenic dots ~72 to 75 days in normal anatomical position, b/t hind legs
  - Characterized by normal array of 4 individual teats, w/ anterior pair being slightly wider spaced
**Interpretation of Results**

- **Scientific method approach:**
  - Independent observations for EACH subject:
    - Bull? Yes or No (ask & answer 3 times)
    - Heifer? Yes or No (ask & answer 3 times)
    - Non-determinable is an acceptable answer
    - Removes bias & increases accuracy

- **Improving proficiency**
  - Practice, practice, practice
  - Review training tapes and/or video-taped sessions
  - Re-scan non-determinable results
Multiple Pregnancy Determination
Ovarian Superstimulation
Ovarian Response to Superovulation
ADVANCED REPRODUCTIVE PROCEDURES

with Transvaginal Ultrasound
In-Vitro Embryo Production

- Gamete recovery (follicle aspiration)
- In-vitro maturation of oocytes (IVM)
- In-vitro fertilization of oocytes (IVF)
  - Individual embryos vs. small groups
  - Sperm co-incubation vs. ICSI
- In-vitro culture of embryos (IVC)
  - Static vs. dynamic (stage specific) media
Requirements

- Convex Linear Transducer & Ultrasound Console
- Stable cart or gurney
- Vacuum pump w/filtered vacuum line
- Needles, tubing, & collection vessel
- Collection media (TL Hepes w/BSA & Heparin)
Aloka 500V TV Probe & Equipment
SonoVet 600 TV Probe
SonoSite 180 TV Probe
Oocyte Recovery or Ovum Pick-Up (OPU)

- **Bovine OPU**
  - Harvest intact oocyte-cumulus complexes from ovarian follicles for IVF (IVM / IVF / IVC)

- **Equine OPU**
  - Harvest intact oocyte-cumulus complexes for oocyte transfer or gamete intra-fallopian transfer (GIFT) procedures
Transvaginal Oocyte Recovery
Cyst Removal / Drainage

- Persistent cystic structures
  - Luteal vs. follicular cysts
    - Best if accompanied by appropriate hormone therapies and monitoring every 3-4 days
**Dominant Follicle Removal or Ablation (DFR)**

- Puncture, rupture, or drainage of dominant ovarian follicular, sufficient to induce atresia

- Removes negative endocrine feedback from DF affecting FSH synthesis & release at hypothalamic & pituitary level

- Induces FSH release and artificial control of ovarian follicular waves
FSH Release Post-DFR

![Graph showing FSH release post-DFR over 96 hours. The graph indicates a peak around 36 hours post-ablation.]
Transvaginal Twin Reduction by Allocentesis or Fetal Puncture

- **Bovine**
  - Results: < 35% singletons retained after pinching
  - Experimental evaluation w/TV reduction at ≤35 days

- **Equine**
  - Results: 32 - 57% singletons retained after endometrial cup formation by intercardiac, abdominal or thoracic injection of KCl or PPG
Experimental Procedures

- **Intra-Follicular injection**
  - e.g. - LH, hCG or other hormone to induce OV

- **Follicular fluid sampling**
  - e.g. - for serial sampling and measuring intra-follicular fluid concentrations of hormones
Transvaginal Amniocentesis

- Determination of fetal sex (or other genetic markers) by PCR using amniotic fluid samples
Acknowledgment to Dr. Brad Lindsey