Principle, procedure and application of Ultrasonography in Farm and pet animal reproduction

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Points under discussion

- Background
- Introduction
- Different modes
- Principles/terminologies of USG
- USG probes
- Applications in Animal reproduction
- Advances in USG
- Conclusion

Background

Jacques & Pierre Curie

- **Curie and Curie (1880)**: The piezo-electric properties of certain crystals where by the application of electricity resulted in their deformation and the production of sound waves with a frequency greater than **20 KHz**, known as ultrasound.

- Piezein - Greek for “squeeze”

- This technique was first developed as a shipping aid to identify submerged objects, allow fog bound ships to identify potential hazards and to determine water depth

- It was also employed in unsuccessful attempts to locate the sunken Titanic (Curry et al., 1990).
- First used in animals as a means of determining back fat thickness in relation to carcass quality (Temple et al., 1956).
- In sheep for pregnancy diagnosis (Lindahl, 1966)
- USG in bovine reproduction started in 1980's

"I wish I could see what's going on there" a lament for the farm animal reproductive biologists and clinicians throughout most of the 20th century.

Introduction

- **Ultrasonogram**: a flattened **two-dimensional image** of a finely-cut section of tissue

- **Image**: on the ultrasound screen represents a **fine section** of an organ, grossly resembling a weakly-magnified histological cut
- **The probe**: simulates the passage of a knife, slicing through an organ or tissue from top to bottom.

Radiography: a 2-dimensional superimposed view of the entire thickness of an animal or of a limb under observation

Different modes

- **"A" (Amplitude) Mode**:

Simplest mode in which transducer scans a line through the body with the echoes plotted on screen as a function of depth.

- **"M" (Motion) Mode:**

Records temporal changes in echoes from continuously moving hollow organs (fetal heart), towards and away from the transducer.

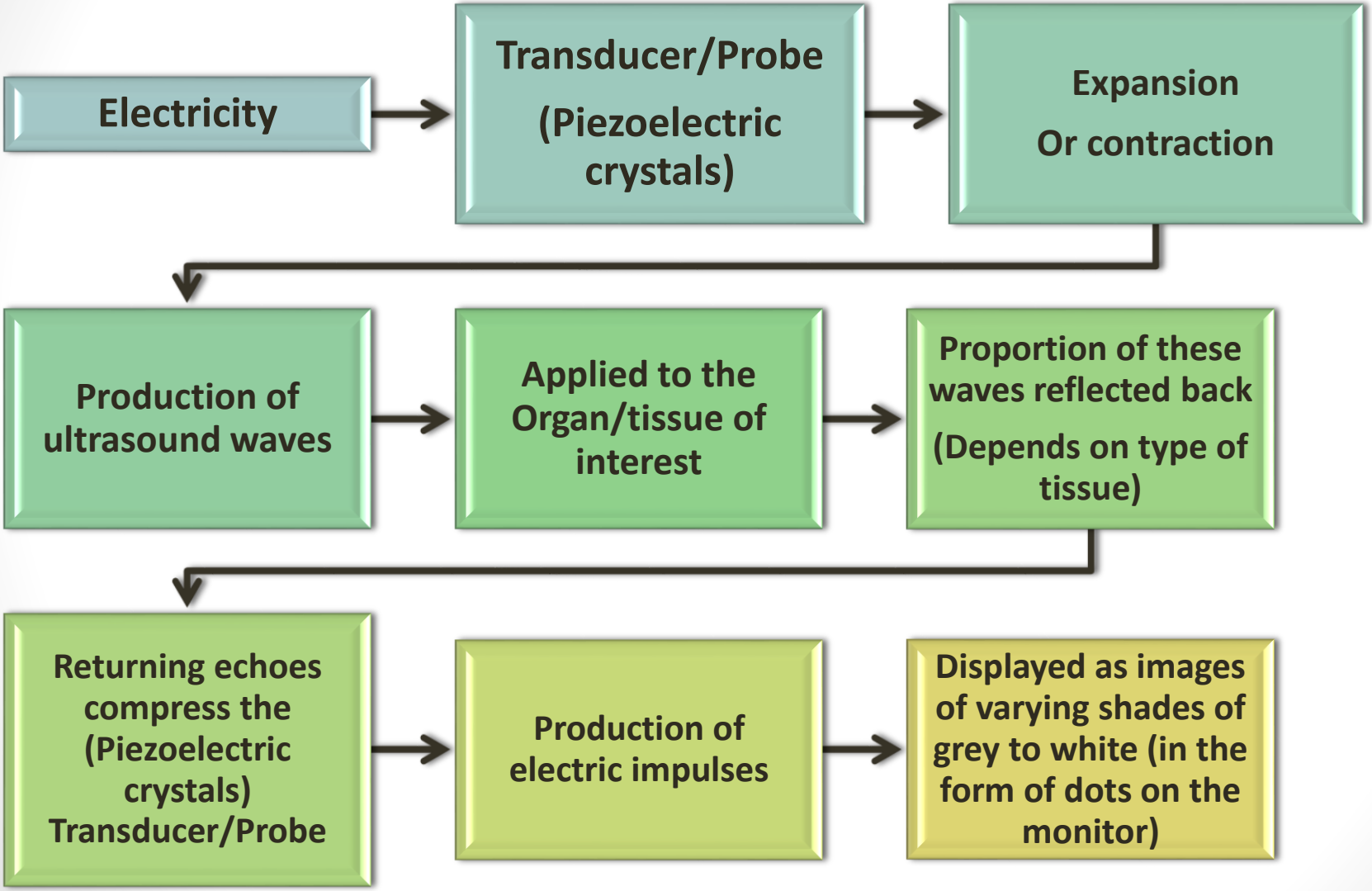
- **"B" (Brightness) Mode: Also K/as Real time 2-D**

Most commonly used and popular in veterinary practice.

- **Duplex imaging**

Modern scanners which combine real-time imaging (B-mode) with Doppler or with the M-mode.

Principle (B-mode/2-D)

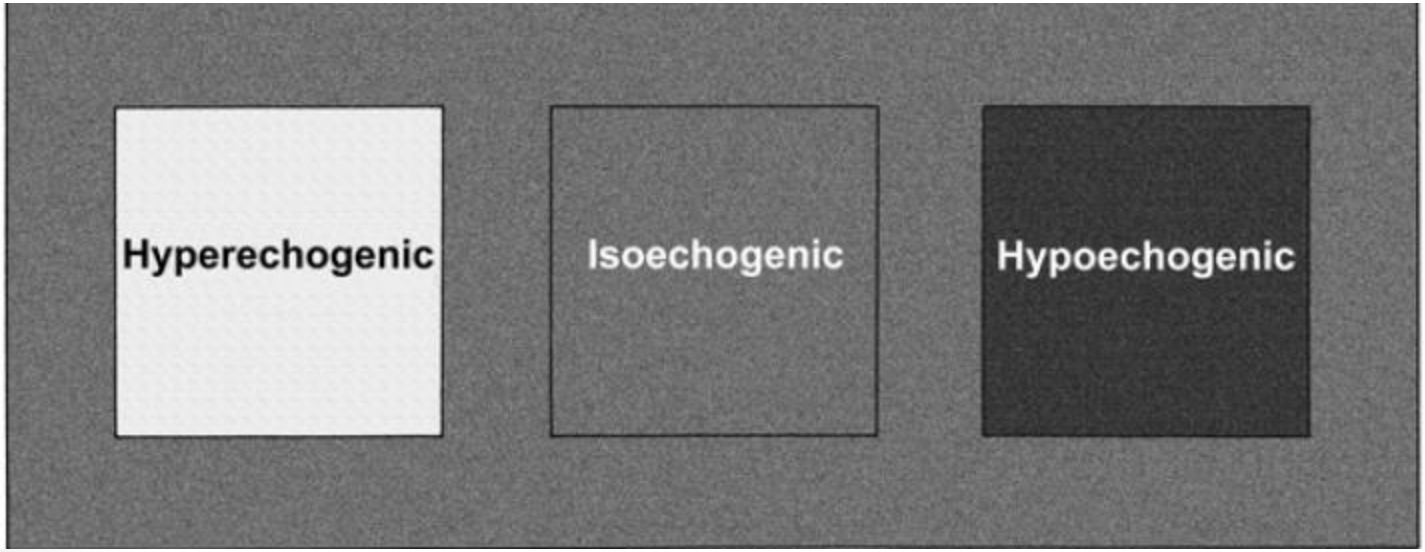


Terminologies and interpretation of the images

- **Echogenic/Echoic:** Reflects majority of waves back to the probe; appears from **white to shades of grey** on the screen (e.g. Tissue)

- **Anechogenic/anechoic:** does not produce echoes; transmits the waves to more deep tissues; **appears black** (e.g. Follicular fluid)

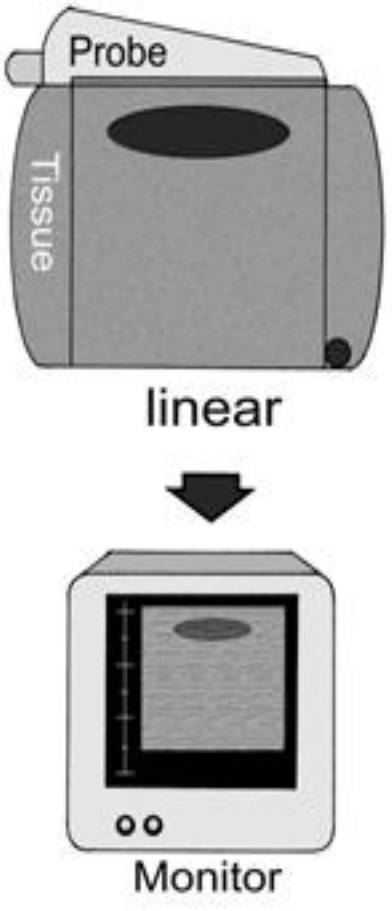
- **Hyperechogenic/Hyperechoic**: an **increase** in echogenicity
- **Isoechogenic/Isoechoic**: **Similar** echogenicity
- **Hypoechogenic/Hypoechoic**: **Decrease** in echogenicity in comparison to the surrounding tissue



Type of probes/transducers

❖ **The probe is the most fragile component of the ultrasound apparatus**.

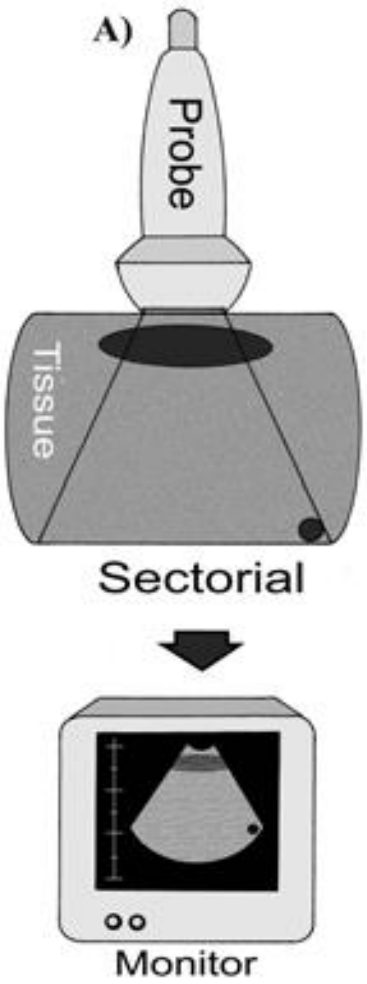
Linear array transducers: piezoelectric crystals arranged side by side in linear fashion electronically to form a **2D-rectangular image**



- ✓ Preferred for trans-rectal ultrasound examinations of the ovaries and uterus
- ✓ Gives good resolution for tissues located close to the probe
- ✓ Needs more area of contact for scanning
- ✓ Cheaper, more robust

Sectorial/Sector transducers: one or several crystals which oscillates or rotates to produce a beam in the shape of a pie slice/fan shaped.

- ✓ Ideal for viewing the small ruminant fetus by trans-abdominal, trans-vaginal aspiration of bovine oocyte.
- ✓ doesn't require a large surface of contact, and it scans a greater overall surface.
- ✓ Resolution is comparatively poor.



Curved array transducers: Modified linear array with curved surface

Transducer/probe

- ✓ Properly lubricated, Hairs should be clipped/area shaved
- ✓ Air (99% of ultra-sound waves may be reflected) should not be trapped b/w tissue and transducer
- ✓ Suitable coupling medium/gel (methyl cellulose)
- ✓ Should be small enough to be cupped in hand
- ✓ Smooth in contour, Water proof, Easy to clean
- ✓ Good biosecurity for scanning the different animals

- In the veterinary market, usual probes have a frequency of 3.5, 5.0 or 7.5 MHz.

Choice of shape & frequency of a transducer depends on the type of investigation to be performed

- Lower the frequency (3.5 MHz) : better tissue penetration, poor resolution. (e.g. trans-abdominal approach in small ruminants)

- Higher the frequency (5-7.5 MHz): reverse is true (Trans-rectal approach)

3.5 MHz (Low frequency)	**5 - 7.5 MHz (Intermediate-high frequency**
High display depth (0-20 cm)	Intermediate display depth (0-12 cm)
Post-partum uterus Advanced pregnancy	Follicles, corpus luteum, Pregnancy diagnosis Fetal sexing

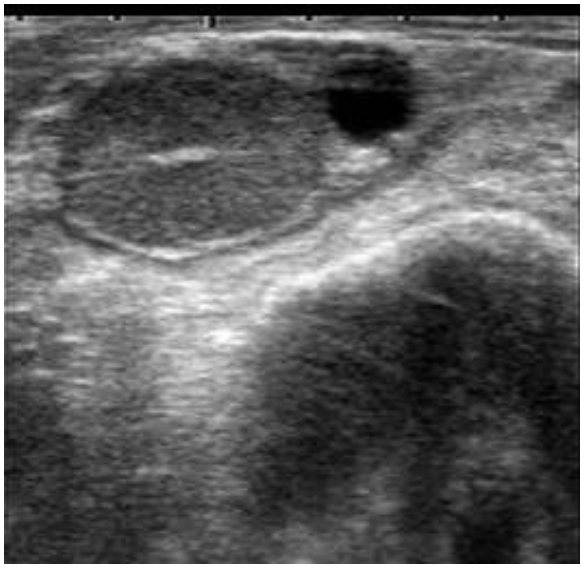
Applications in animal reproduction

"Gray-scale diagnostic ultrasonography is the most profound technological advance in the field of large-animal research and clinical reproduction "
O.J. Ginther, 1986

- ✓ **Pregnancy diagnosis/images of fetus/ovaries/follicle/CL**
- ✓ Fetal abnomalities
- ✓ Follicular dynamics
- ✓ Ovum Pick-up
- ✓ Fetal sex determination
- ✓ Twin pregnancy
- ✓ Uterine pathology, etc.

Ovaries

- Advent of bovine ovarian ultrasound in 1984.



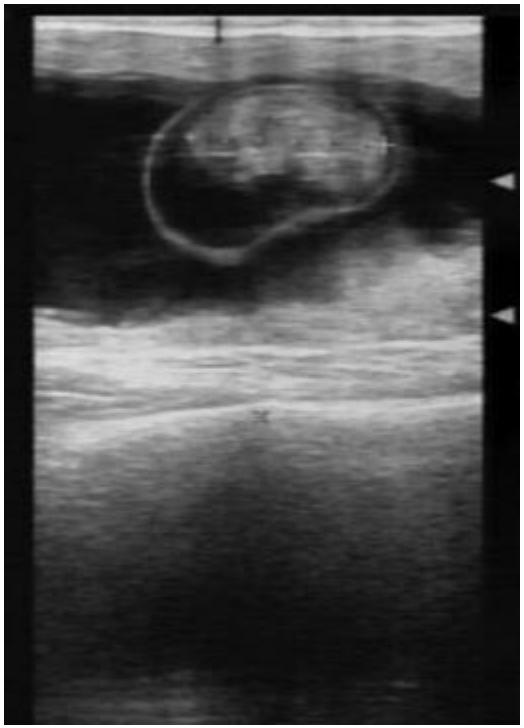
- **Follicles:** Spherical anechoic/black structures of variable size and clear demarcation between wall and antrum
- **Ovarian stroma:** mottled echotexture
- **Corpus luteum:** well defined borders and **mottled echogenic** appearance (**less echogenic than stroma**)
- **Ovarian blood vessels:** anechoic/black elongated structures

Early pregnancy diagnosis

- Important part of dairy herd management program.
- **Trans-rectal palpation**: risk of embryonic death in early phases (day 25-45)
- **USG**: less traumatic, more precise, reliable and easy-to-use tool for diagnosing early pregnancy in dairy cows
- Result of the test is known immediately
- Early pregnancy diagnosis in between days 27 and 35 post-insemination is advantageous and economic

Appearance of embryo

- Echogenic structure floating within anechoic/black fluid filled cavity
- A 25 day, bovine embryo 1 cm in length, with a relatively straight shape
- Modifies gradually into a C-shape by approximately day 30 post-insemination.



A day 42 bovine embryo

Day 28 and 38 bovine conceptus

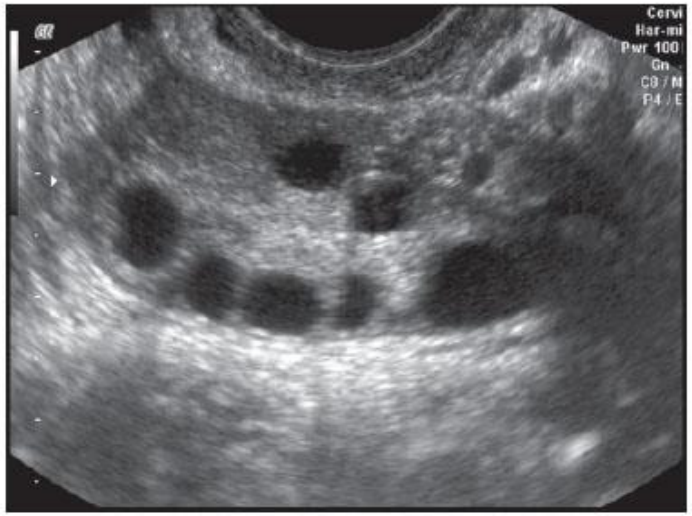
Day of first detection of ultrasonographically identifiable characteristics of the bovine conceptus

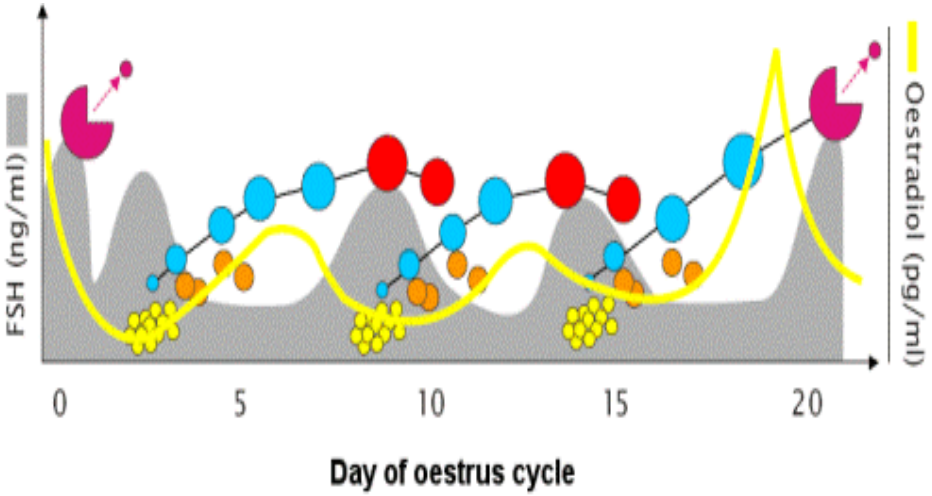
Characteristic	First detected (days)
Embryo proper	19-24
Heart beat	19-24
allantois	22-25
Spinal cord	26-33
Fore limb buds	28-31
Amnion	28-33
Eye orbit	29-33
Hind limb buds	30-33
Placentomes	33-38
Split hooves	42-49
Fetal movements	42-50
Ribs	51-55

(Source: curran et al., 1986)

Ovarian follicular dynamics

- **Follicular dynamics**: Ultrasound enables to describe the dynamics of follicular growth in follicles greater than 1 mm in diameter.

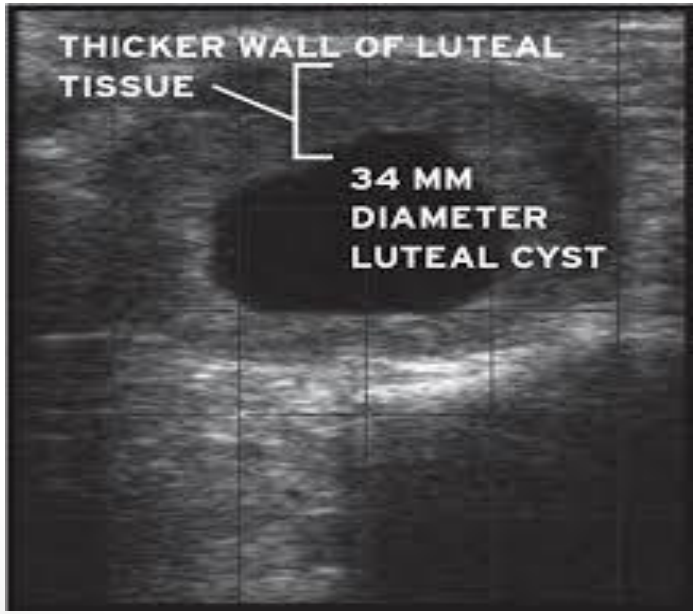


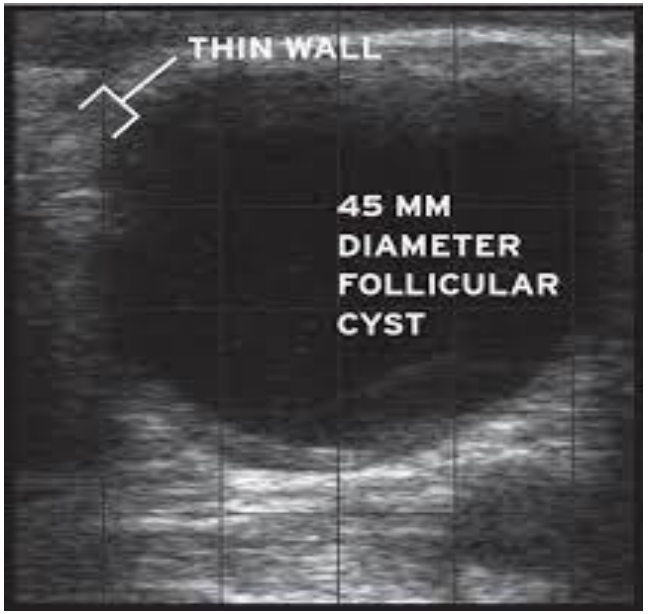


Cystic ovaries

- **Follicular & luteal cysts**

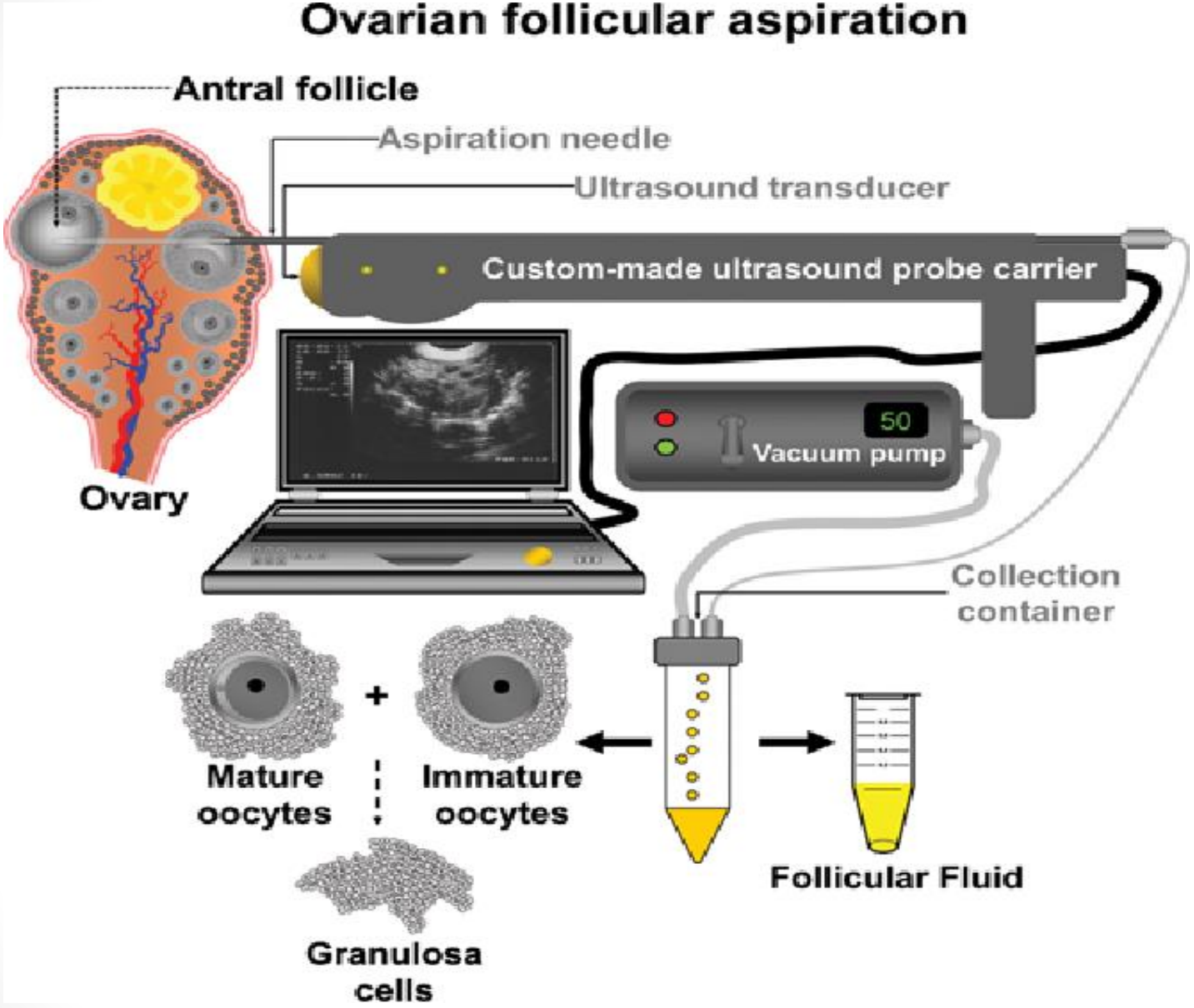
Follicular cyst can be differentiated from a luteal cyst by its **thin wall** and **uniformly anechogenic follicular fluid**





Ovum Pick-up (OPU)

- Transvaginal-ultrasound guided oocyte aspiration.
- Rapid, minimally invasive technique for retrieving oocytes repeatedly from a live donor (oviductal occlusions/poor superovulatory response/heifers).
- Principle: a 5-7.5 MHz transducer incorporated into a 17 G aspiration needle (60cm).
- Inserted into the vagina of animal while other hand in rectum holding ovary and pulled caudally towards the transducer needle.



(Velazquez et al., 2014)

Uterus

Metritis complex

- ✓ Uterus may vary in appearance as **anechogenic fluid in black**, to the presence of **echogenic material floating** in a black background
- ✓ A **purulent** exudate is **echogenic** or **isoechogenic** in appearance.
- ✓ Increase in the thickness of uterine wall.

Mucometra: Resembles a pregnancy, except for the fact that it is **impossible to view the embryo or its adjacent membranes**.

Embryo viability

It is also very important to evaluate the embryo's viability

- ✓ **Heart Beat**: at the center of embryo starting at day 25 of pregnancy, **twinkling light** (140-160 beats/min.)
- ✓ **Umbilical cord**: day 40-45 between uterus and embryo
- ✓ **Fetal movements**: day 45 onwards

Indications of embryo degeneration/death observed through USG

- ❖ **Presence of echogenic particles/debris in uterus, amniotic fluid**
- ❖ **Irregular contours of the embryo and presence of surrounding particles**

Fetal sex determination

- Transrectal ultrasound can be used to detect the sex of bovine fetuses in utero
- Based on **morphology** and **location** of the **genital tubercle** using ultrasound
- Fetuses at **48** to **119** days of age have been sexed successfully
- Reliable and accurate (92 to 100%) with Ideal window of opportunity is between **days 60** and **80** of gestation

- **Male**: Genital tubercle usually a bilobed located just caudal to umbilicus as hyperechoic bright structure

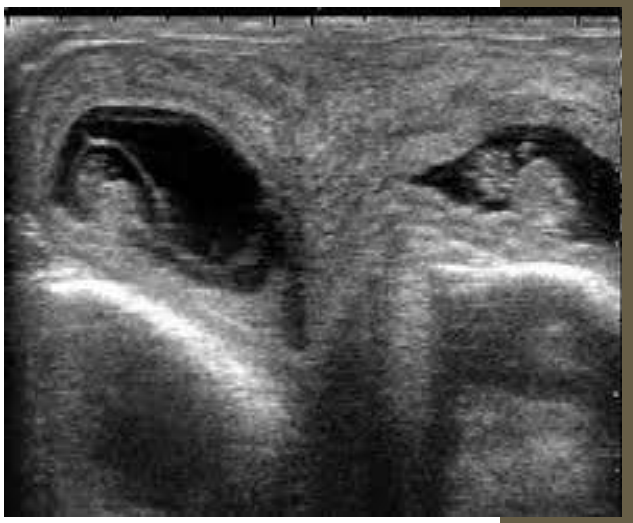
- **Female**: Genital tubercle is just ventral to the tail

Practical utility

✓Increased sale value of pregnant animal carrying heifers
✓Full fill the bull contracts to plan embryo transfer
✓Prediction of replacement needs

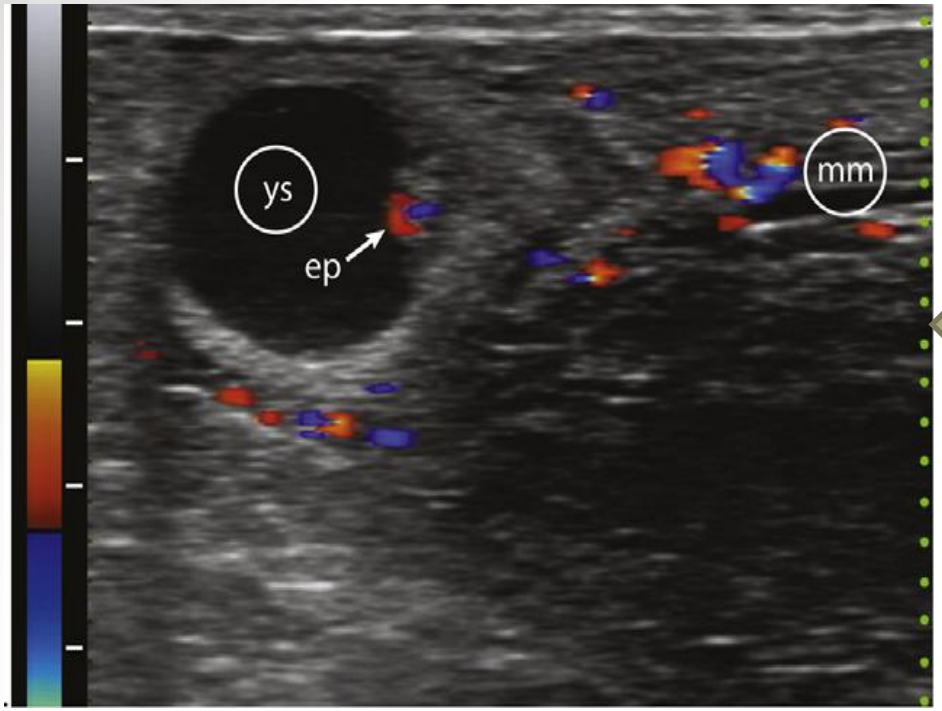
Detection of multiple fetuses

- Twinning : an **unavoidable outcome of reproduction** and is **undesirable too**.
- **Cows carrying twins**: at greater risk of embryonic loss
- Reduction in overall dairy farm profitability reproductive efficiency
- It should be detected at the same time as fetal sex determination (60-70 days of gestation)
- To avoid the risk of obtaining a freemartin heifer, if the fetuses are male and female



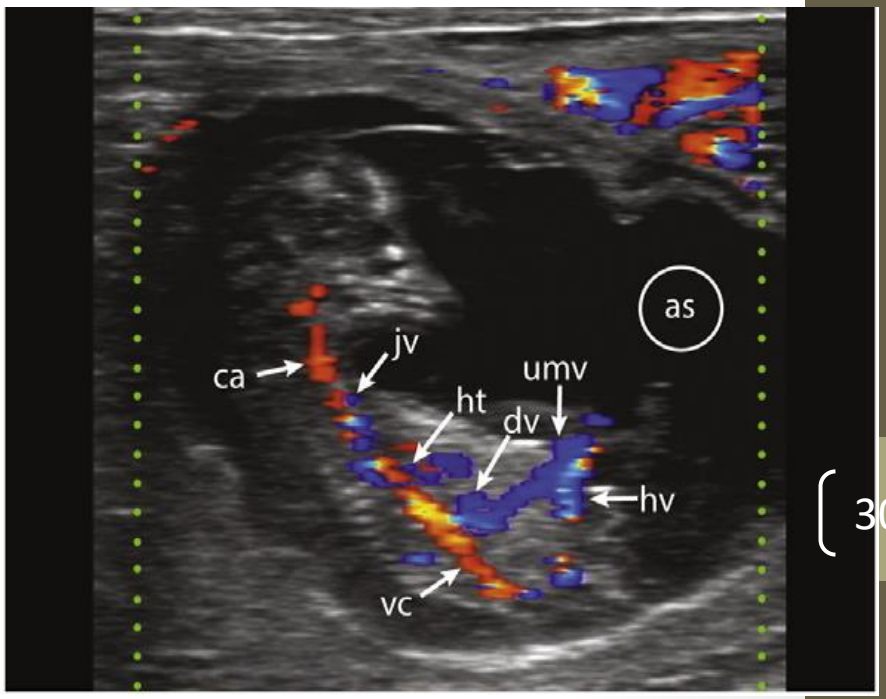
Advances in USG

- **Color Doppler:** Based on the **Doppler principle for sound waves** to assess the **blood flow to different** tissues.
- Based on Doppler-shift frequencies.
- Frequency of echoes from moving red cells is **increased** (**Positive shift**) or **decreased (Negative shift)** as the cells move toward or away from the transducer.
- Direction of blood flow relative to transducer represented by different colors on the screen display.



Day 25 bovine conceptus

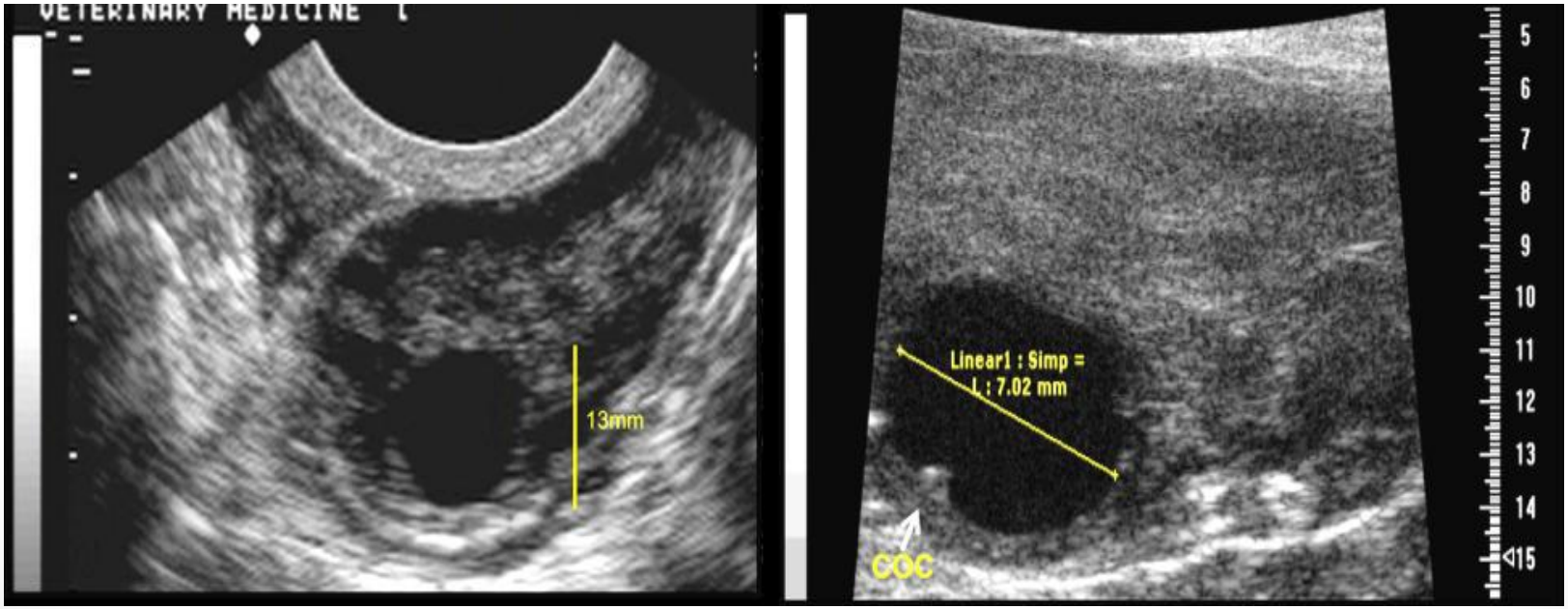
Day 52 bovine conceptus



3-D/4-D USG: In addition to 2D, a third dimension of the tissue can be visualized and rotated in all the planes.

Ultrasound biomicroscopy

- "**Ultrasound biomicroscope**" uses a **single crystal** to emit a sound frequency of 25 to 70 MHz, resolution of 30-50 µm.



Conclusion

- ✓Transrectal gray-scale UI, color-Doppler, expanded and indeed revolutionized research in large animal reproduction.
- ✓Ultrasonography is particularly useful for early pregnancy diagnosis, assessing fetal health, and fetal sex diagnosis
- ✓Imaging the ovaries in cattle has led to an understanding of follicular wave dynamics to new protocols for ovarian synchronization and superovulation.

THANK YOU