

# Pregnancy Diagnosis in domestic farm and pet animals



**Prof G N Purohit**



# Need for pregnancy diagnosis?

1. Improving reproductive performance
2. Earlier the pregnancy diagnosis performed, the more profitable is the return for dairy cows and buffaloes
3. Management
4. Feeding
5. Sale
6. Monitor development of pregnancy
7. Confirm absence of twin
8. Monitor early embryonic death
9. Improve record keeping data
10. Inform decisions on individual cows

# **The goal of any method used to do the pregnancy examination is to determine the pregnancy status with:**

- 100% accuracy
- No false positives
- No false negatives
- Determine the pregnancy as early as possible
- The ability to age the conceptus
- Be able to determine the viability of the conceptus
- Possibly determine the sex of the fetus
- And have the results immediately

# Methods Direct or Indirect

## Management/ Visual methods

- Non return to estrus
- Cocking of the tail [appears 14 to 15 days after fertile mating and known to be 95% reliable female camel
- By assessing enlargement of abdomen, udder and vulva.
- based on breeding history

## Clinical methods

- Recto-genital palpation.
- Ultrasonography.
- Radiography.
- fetal ballotement.

## Laboratory tests

- Assay of hormones
- Chemical tests
- Biological methods

## Visual methods

### **Non return to estrus**

Return to estrus from 18 to 24 days after AI is often considered by dairy farmers the easiest and least costly method for determining non-pregnancy in dairy cattle early post breeding.

### Problems with this method

Estrus detection efficiency is low at most farms and individual animals

Poor estrus behavior of parturient cows

Estrus cycle length varies from 21-24 days

Early embryonic deaths after Day 17 would prolong the cycle length

Cocking of the tail [appears 14 to 15 days after fertile mating and known to be 95% reliable female camel

By assessing enlargement of abdomen, udder and vulva.

Based on breeding history



CLINICAL METHODS (DIRECT METHODS)

TRANSRECTAL PALPATION

TRANSRECTAL ULTRASONOGRAPHY

ABDOMINAL BALLOTEMENT

LABORATORY METHODS (INDIRECT)

# TRANSRECTAL PALPATION

One of the oldest methods.

Determining pregnancy by transrectal palpation in cattle is not very difficult, but it requires experience, practice, and a thorough knowledge of the cow's reproductive system.

# RESTRAINT



# Protective Clothing

- Disposable long arm sleeves
- Lubrication

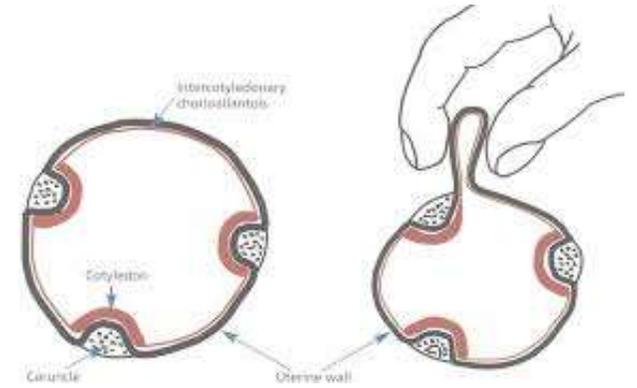
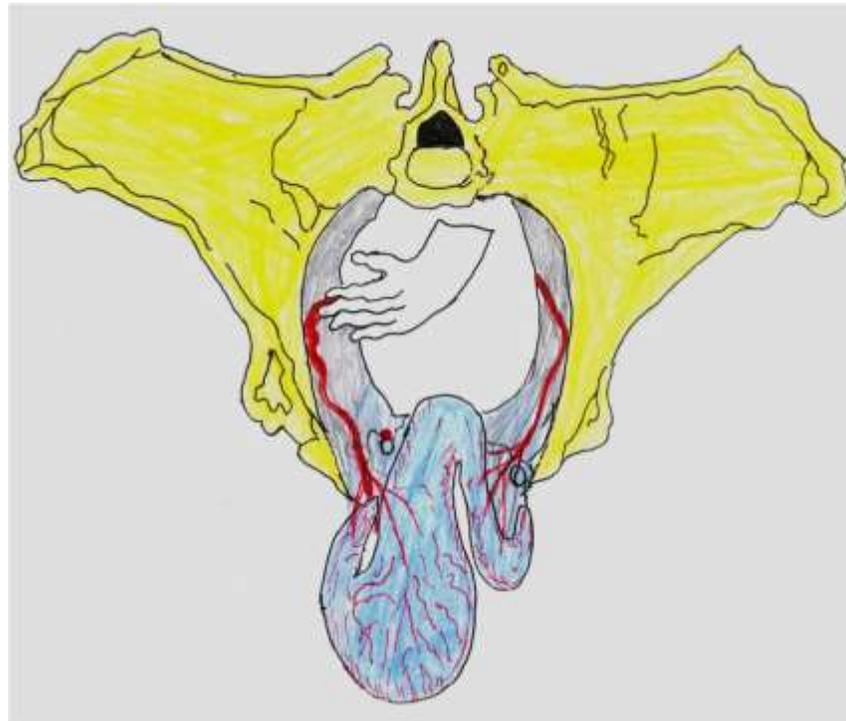


# Procedure for palpation



# Transrectal palpation (cattle, buffalo, mares, camel, sow)

- **Four Positive Signs in cattle:**
- Fetal membrane slip
- Amniotic vesicle
- Placentomes
- Fetus
- Fremitus (middle)
- Uterine artery)

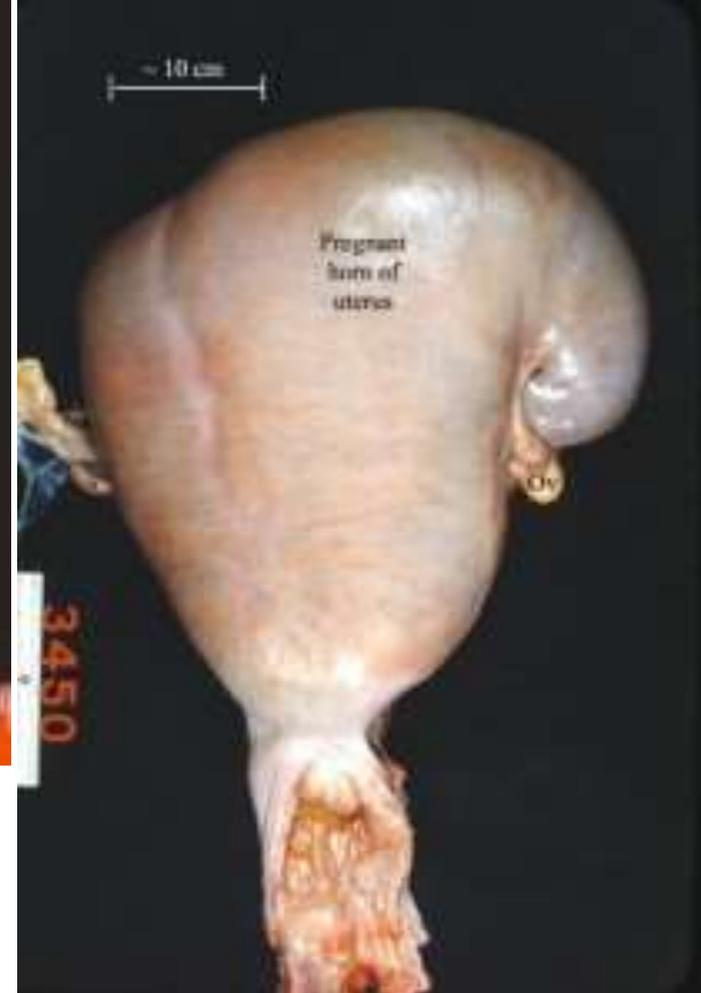




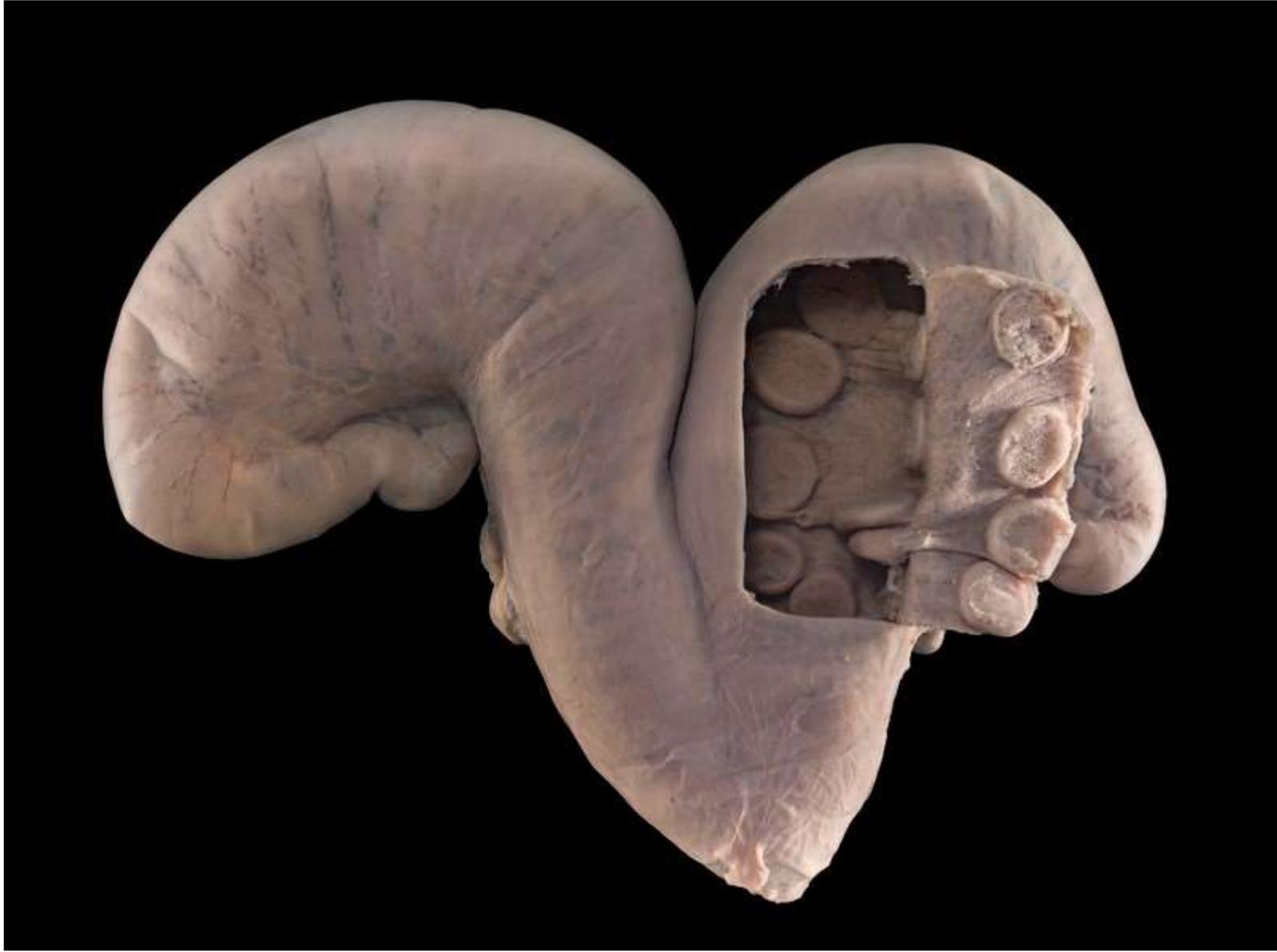
30 days

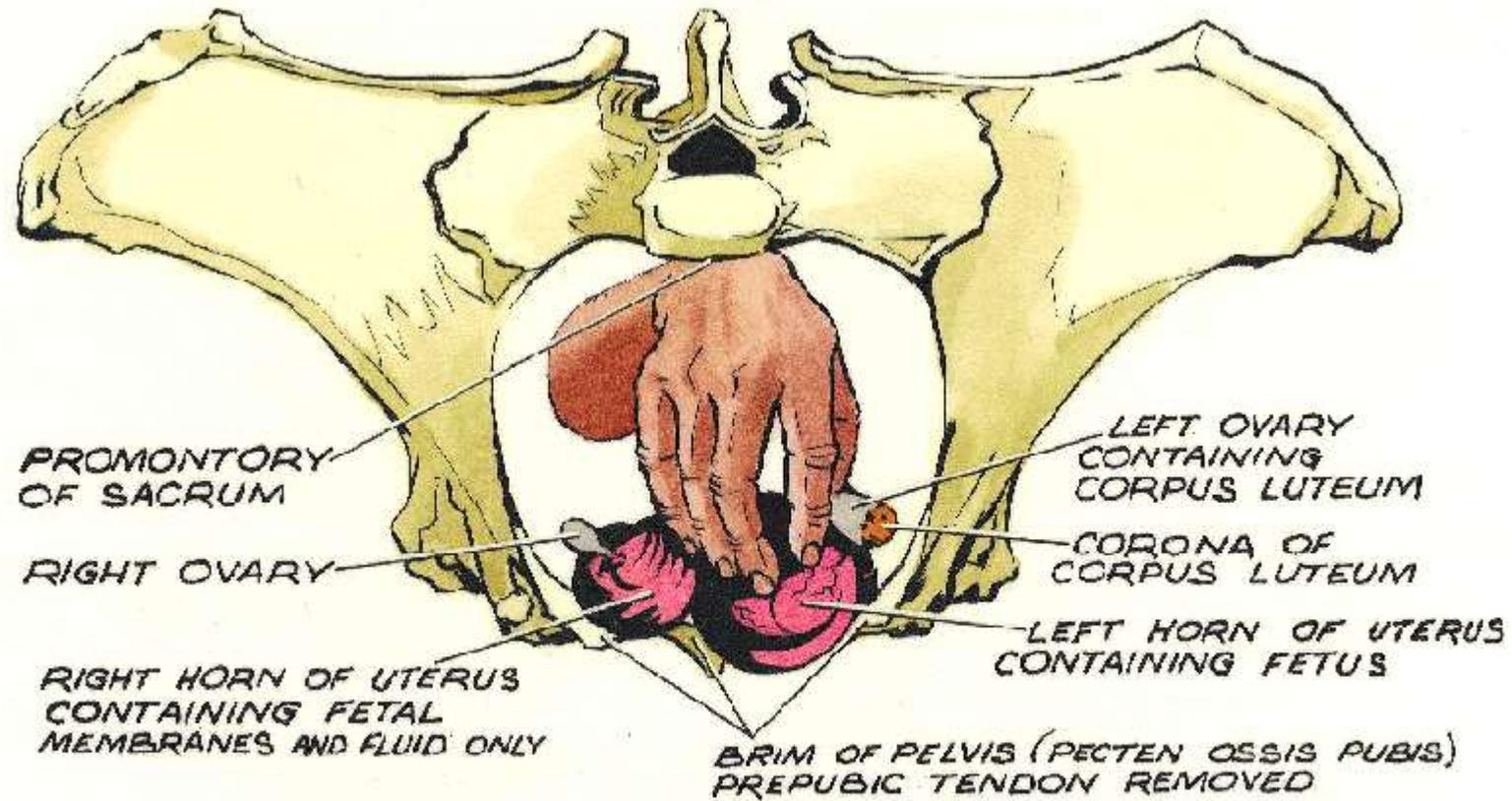


72 days

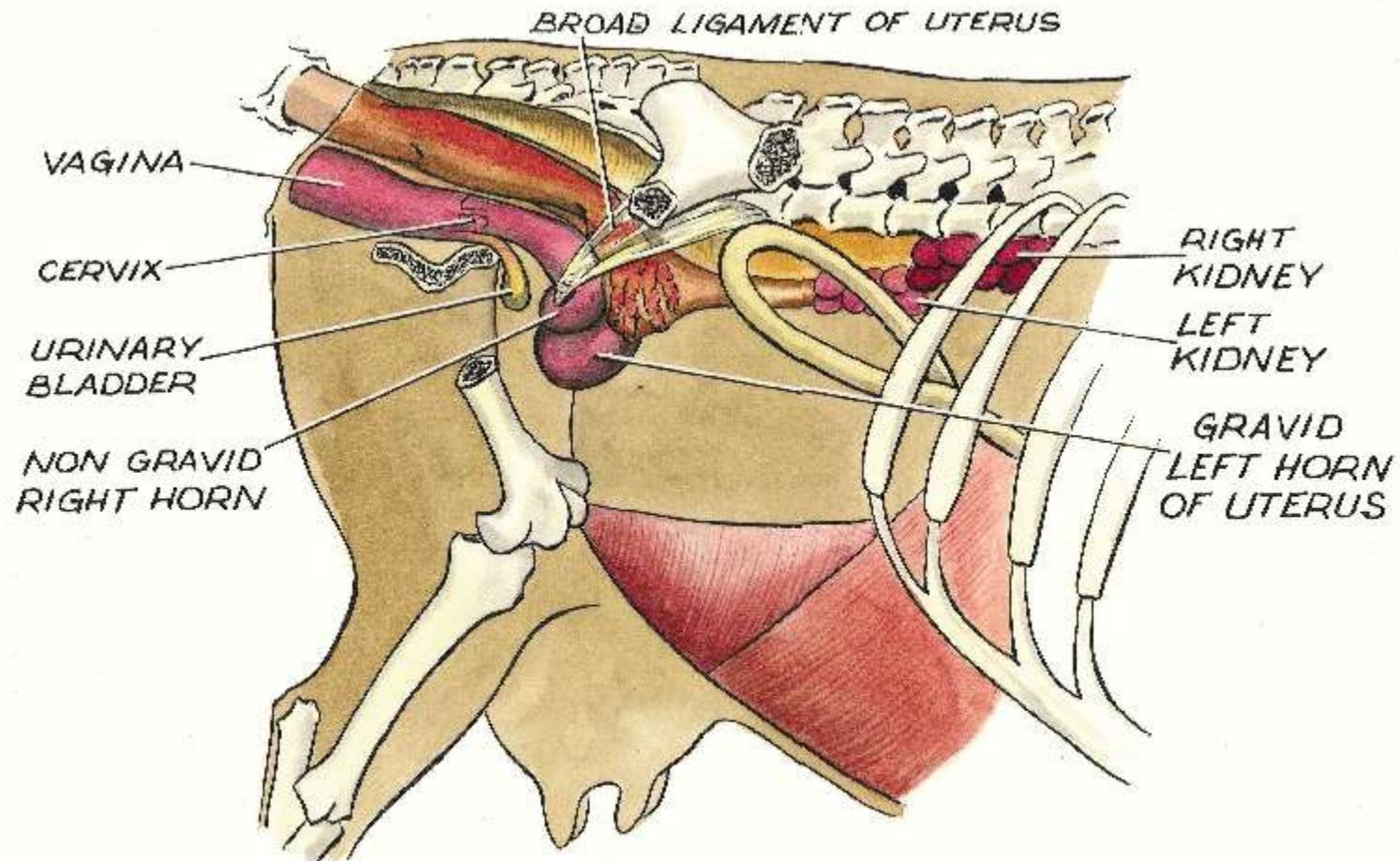


4.5 months

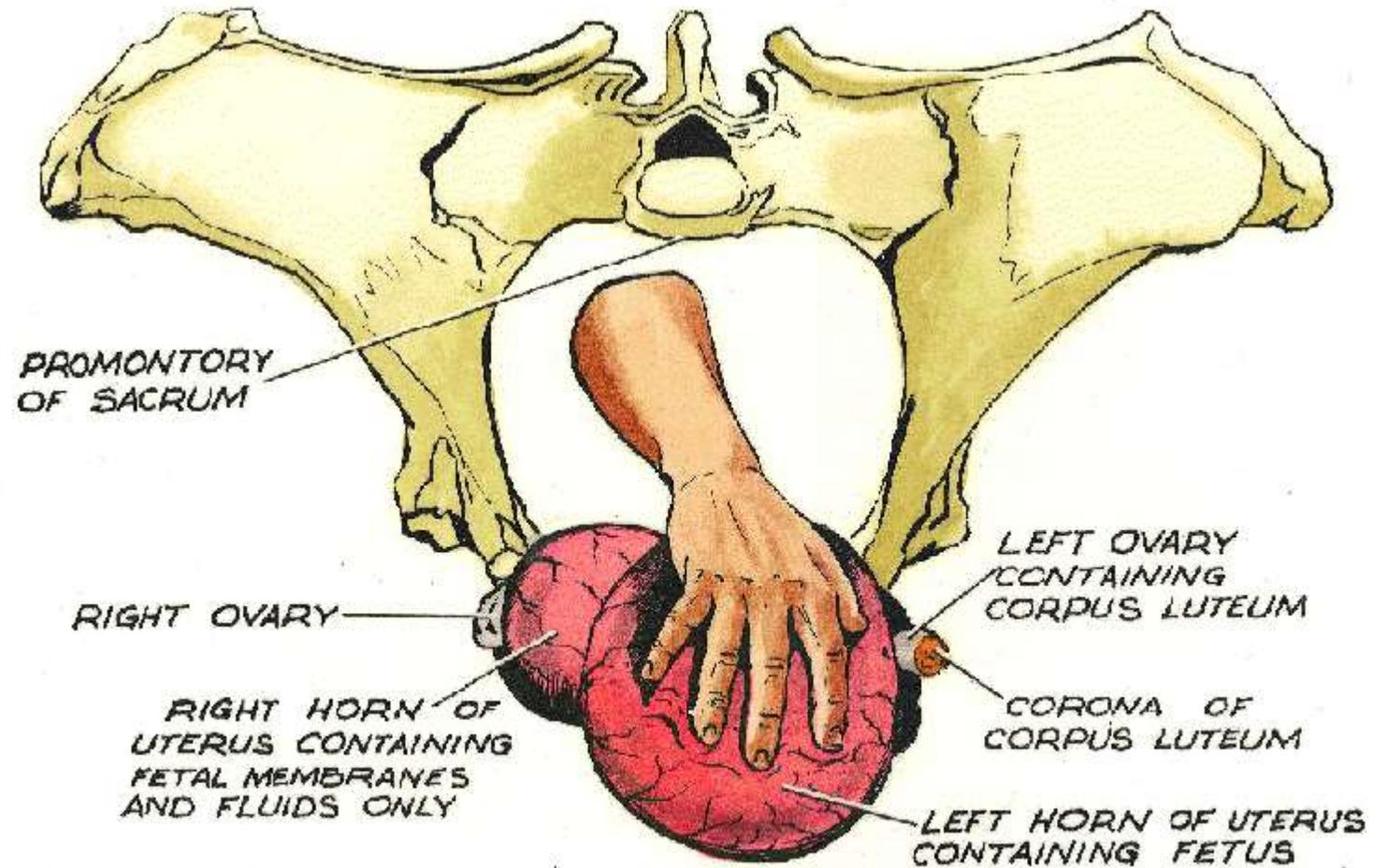




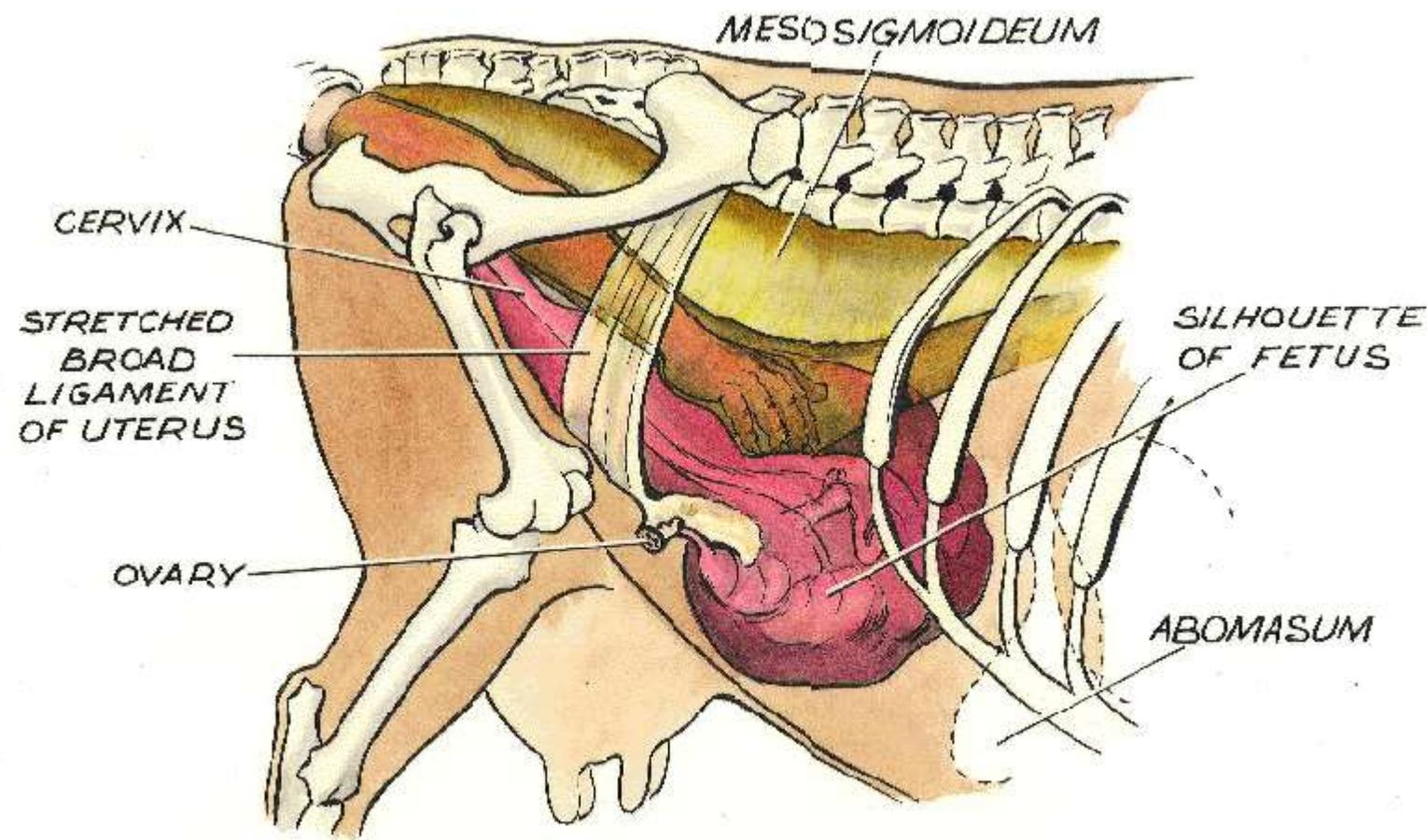
**RECTAL EXAMINATION OF PREGNANT COW.  
GRAVID UTERUS - 70 DAYS.**



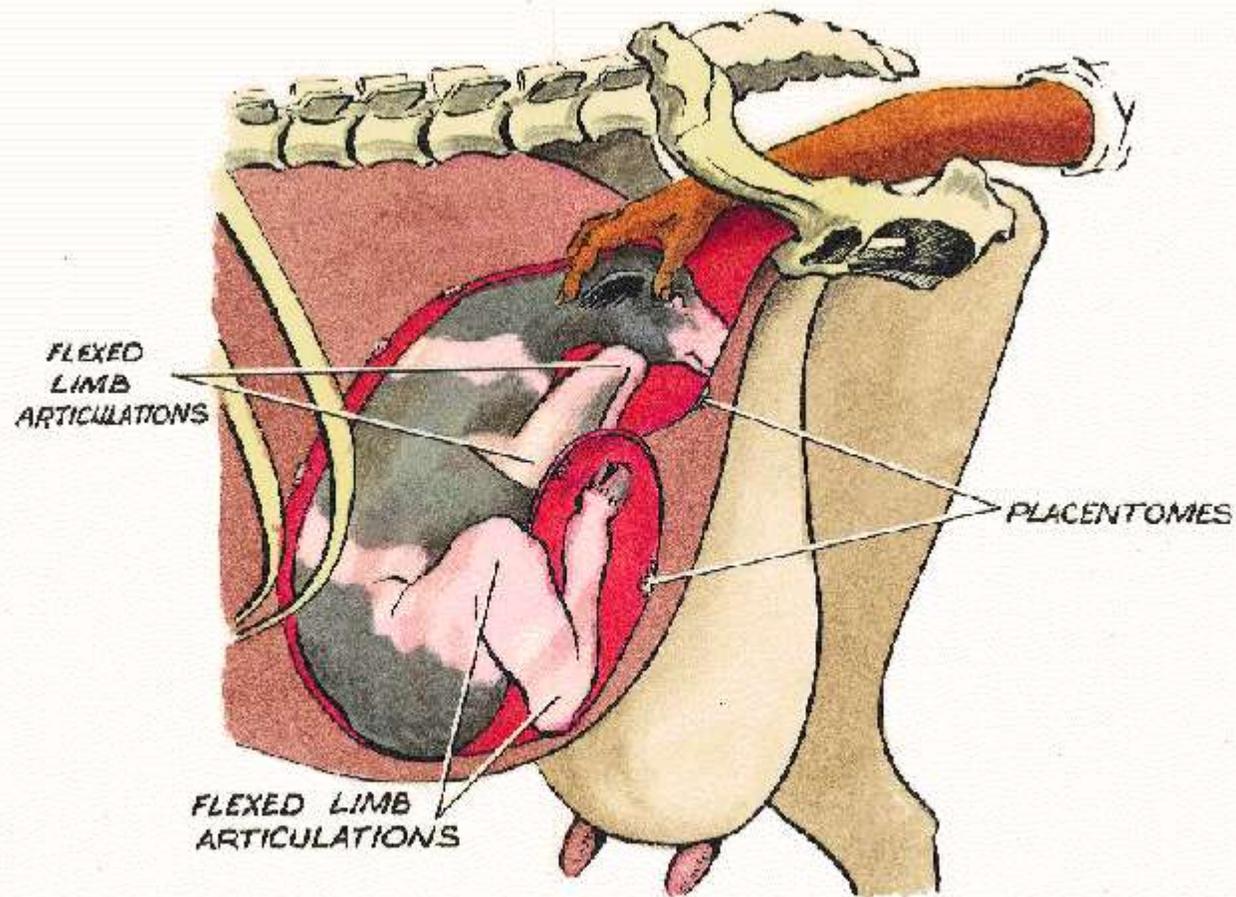
**RECTAL EXAMINATION OF A COW AT THE  
END OF THE THIRD MONTH OF GESTATION.**



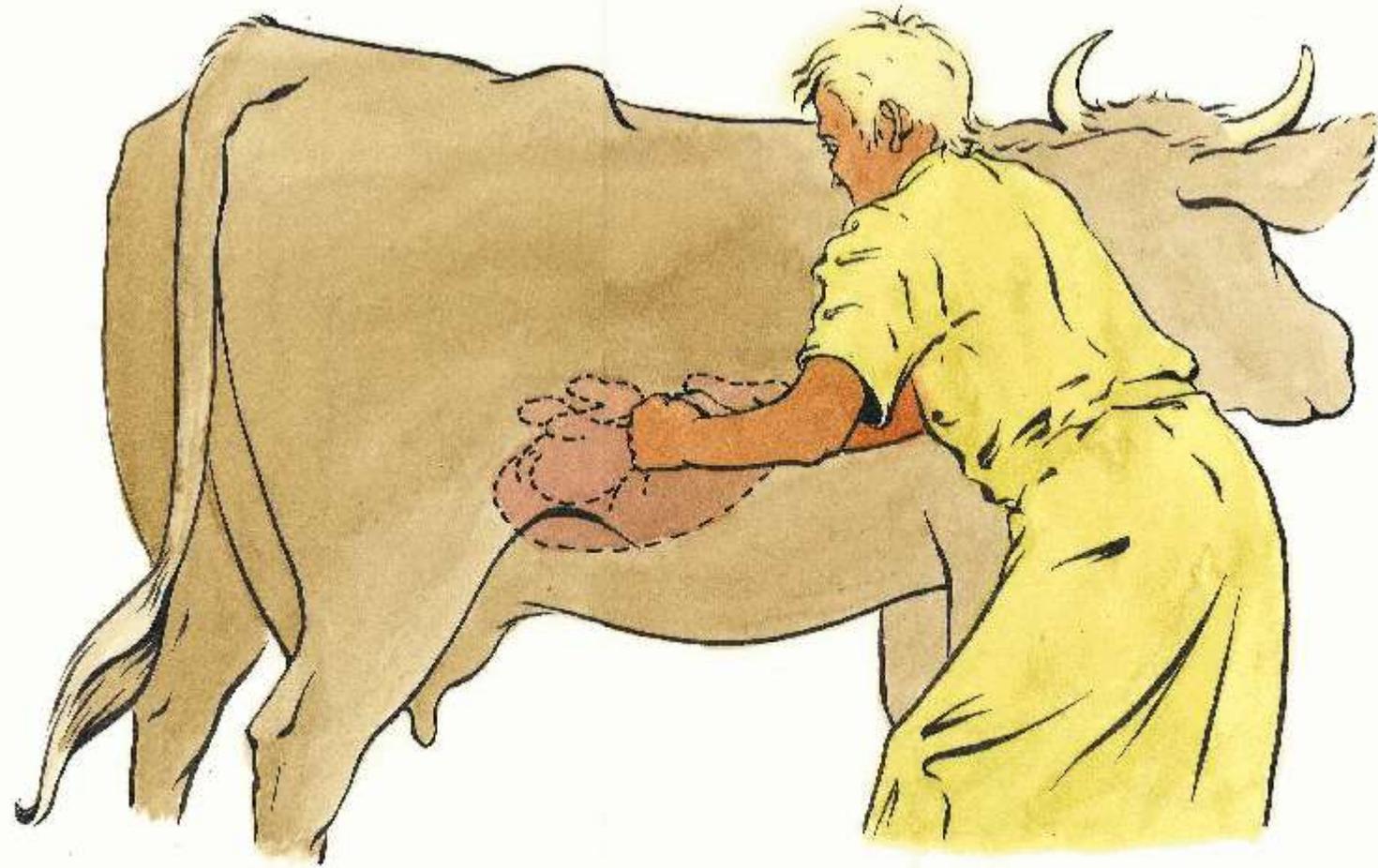
**RECTAL EXAMINATION OF PREGNANT COW.  
GRAVID UTERUS - 110 DAYS.**



**RECTAL EXAMINATION OF A COW AT THE SIXTH MONTH OF GESTATION.**



***RECTAL EXAMINATION OF A COW APPROACHING TERM. RUMINANT STOMACH REMOVED.***



*EXTERNAL METHOD OF DIAGNOSING  
PREGNANCY IN THE COW.*

# Table 1 Days of palpation of fetal and maternal structures during pregnancy

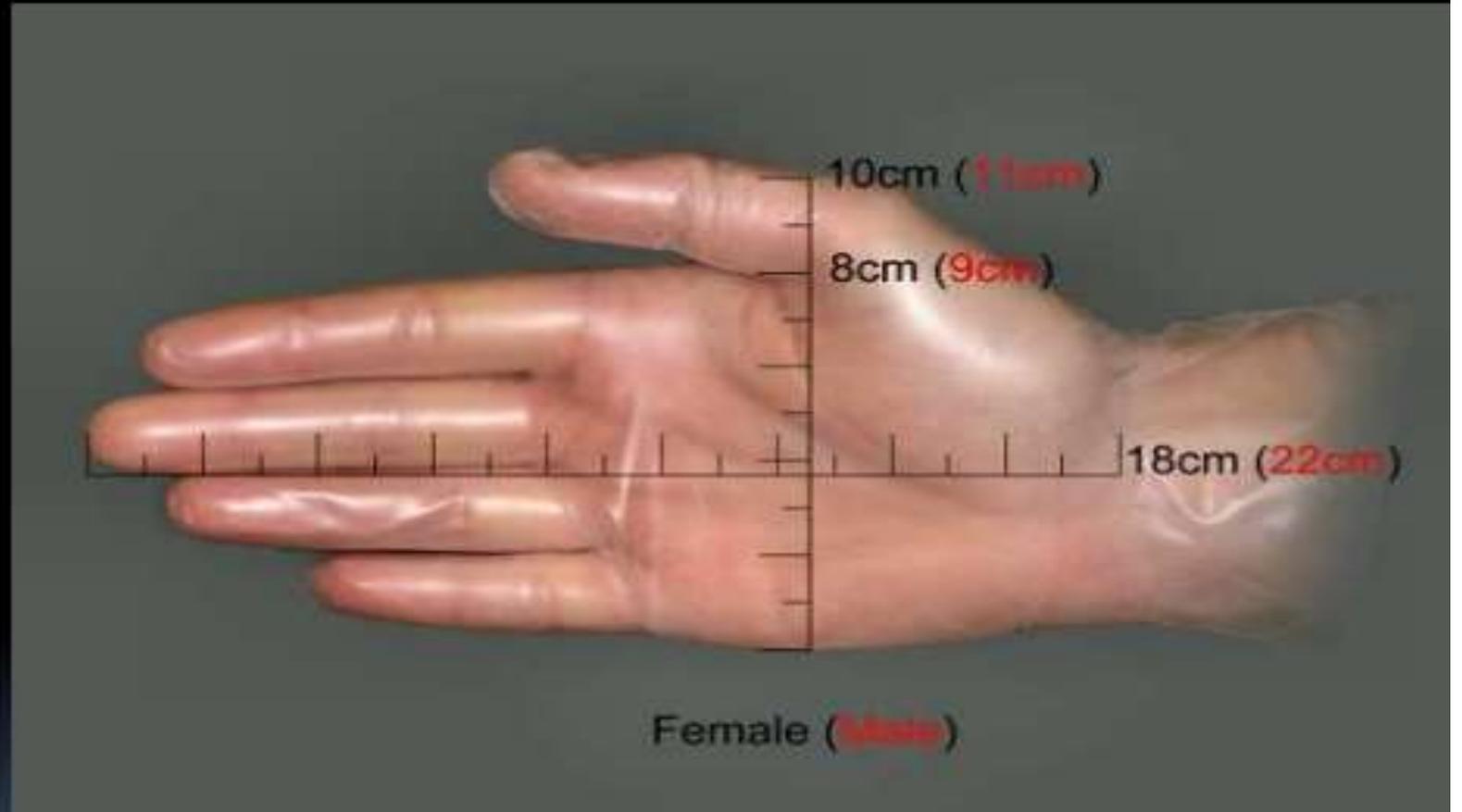
Gestation Days	Palpable Findings
Palpation of amniotic vesicle	30-65 days
Asymmetry of uterine horns and fluctuation of uterine contents	35-90 days
Palpation of fetal membrane slip	35-90 days
Palpation of fetal bump	65-150 days
Palpation of cotyledons	70 days to term
Fremitus in middle uterine artery	90 days to term
Fetus and its movement	180 days to term

## Amniotic Vesicle

Days Gestation	Width in mm	Width in fingers
35	7	1/2
42	15	1
48	35	2
52	55	3
58	75	4
62	90	4+
65	105	5 (can feel the fetus)

# Crown Rump Length (CRL)

- ✓ 9 cm at 2 months
- ✓ 22 cm at 4 months
- ✓ 44 cm at 6 months
- ✓ 80 cm at 8 months
- ✓ 100 cm at 9 months



✓ (Löfstedt RM, 2010)

To estimate an aborted fetus they are:

2 months mouse

3 months rat

4 months small cat

5 months large cat

6 months beagle dog

## Fetus

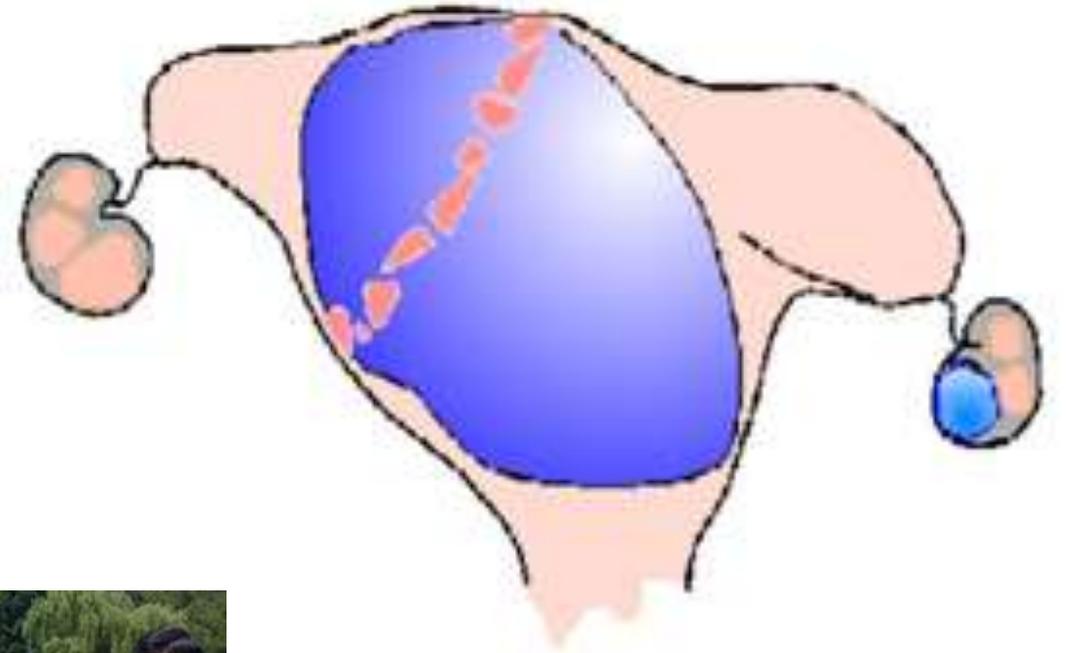
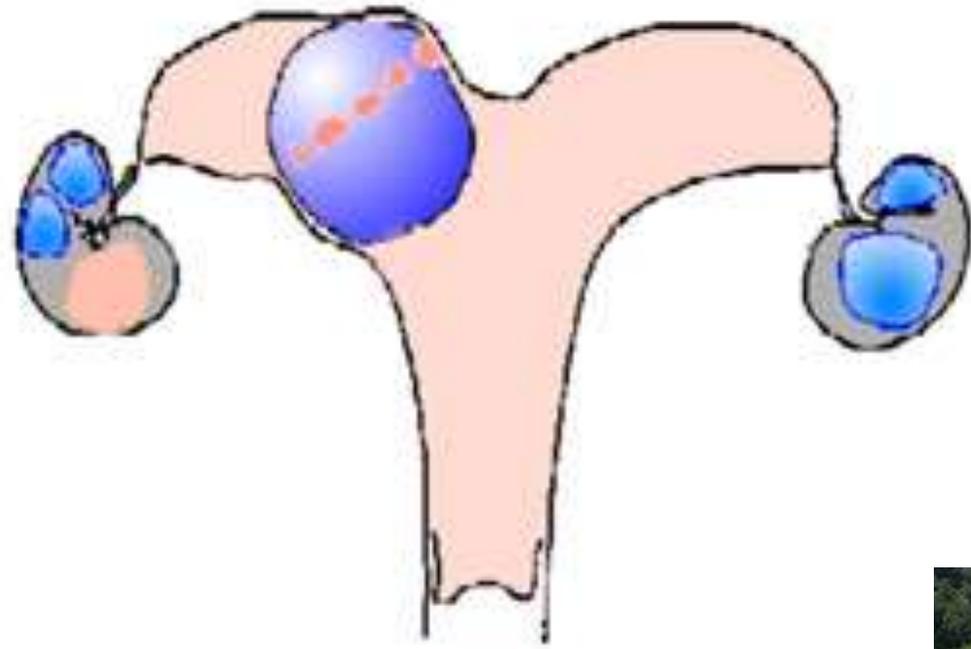
- ✓ 2 months mouse
- ✓ 3 months rat
- ✓ 4 months small cat
- ✓ 5 months large cat
- ✓ 6 months beagle dog



Drost M , 1982)

# Transrectal palpation (Mare)

- The **uterine tone** appears at **days 15-16** and continues up to **day 48 to 55** .
- The bulge in uterus appears and progressively increases in size and is palpable in most mares by day 30-35 (approx 3-4 cm in diameter).
- By day 42-45, the conceptus occupies approximately half of the gravid horn and is 5 to 7 cm in diameter. The uterine wall over the bulge is thin.
- At 72 days the pregnancy is like an elongated football and is nearly similar to a 60 day pregnancy in cattle.



- **Palpation of fetus**

- After 40 days and mobile after 70 days.

- **Location of the uterus**

- The uterus is located in the pelvic cavity or just at the pelvic brim until day 90.
- At 100-200 days, the gravid uterus is positioned cranial to the pelvic brim in the abdominal cavity.

- **Ovarian palpation**

- Position of the ovaries up to 60 days of pregnancy is similar to that for the non-pregnant mare.
- From then on, they are drawn cranially and medially but remain dorsal to the uterus.
- The finding of both ovaries nearer to each other and close to the pelvic floor is a positive indication for pregnancy (3 to 5 months).
- From 5 months of pregnancy onwards, the ovaries usually are not palpable as they are under the broad ligaments.

Day of Gestation	Palpable Characteristics
20	Embryonic vesicle: 30–40 mm in diameter at the base of one horn Good overall uterine tone, slight decrease in tone over the embryonic bulge
30	Embryonic vesicle: 40–50 mm in diameter; noted as a discrete fluid swelling ventrally in the uterus More obvious decrease in uterine tone over the bulge
40	Fetal bulge: 65 mm in diameter Decreased tone over the bulge and decreased tone of the pregnant horn Excellent tone of the cervix: rod-like consistency
50	Fetal bulge: at least 8 cm in diameter; the fluid-filled vesicle extends into the uterine body
60	Fetal bulge: 10–13 cm in diameter; nearly the entire gravid horn and uterine body are filled, the non-gravid horn remains small More obvious loss of tone over the bulge Uterus starts to be pulled over the pelvic brim Ovaries are moving closer together Note: the urinary bladder may be mistaken for the conceptus
85	Fetus itself is palpable due to decreased turgidity of the conceptus
90	Conceptus fills the entire uterus Uterus is located further ventrally in the abdomen making direct palpation of the fetus more difficult Ovaries are pulled closed together in a more ventral position
90–150	Uterus in the abdominal position Fetus can usually be easily palpated or balloted within the uterus
150–200	Uterus further ventrally in the abdomen Ovaries are pulled ventrally under the broad ligament and are not accessible Thorough evaluation of the fetus is more difficult
200–270	Uterus expands further dorsally Fetus is more accessible
270 to parturition	Fetus is easily accessible and is usually in cranial presentation, dorsal recumbancy with head and forelimbs usually flexed, and hindlimbs pointing towards the pelvis Note: several minutes might be needed for detection of fetal movements
Parturition	Cervical relaxation occurs causing the cervix to soften and shorten Increased uterine tone and myometrial contractions present Fetus extends the head, neck, and forelimbs and positions itself for parturition Cervical dilation will occur

# Advanced pregnant mare



# Rectal palpation in the sow

- Based on examination of the cervix and uterus, together with palpation of the middle uterine artery to assess size, degree of tone, and type of pulse.
- At around 21-30 days of gestation the bifurcation of the cornua is less distinct.
- Cervix and uterine walls are flaccid and thin.
- Fremitus can be identified at 37 days.
- Middle uterine artery is 5-8 mm in diameter and more easily identified.
- Uterus becomes progressively thin walled and ill defined by 31-60 days and Beyond 60 days the fremitus is very strong.
- Piglets can only be palpated towards the end of gestation.

# Rectal palpation in female camels

- Persistence of the CL is one of the earliest sign of pregnancy as otherwise luteal phase is very short.
- Left uterine horn is inherently longer than the right horn.
- Earliest detection of uterine change (increase in diameter and appearance of fluctuation) is palpable at about 40 days.
- Between 60-70 days left uterine horn is increased about twice to its non gravid size has a thin wall and fluctuates.
- Fetal membrane slip and placentome not seen.

- The uterus is more cranial and ventral after the 3<sup>rd</sup> month of pregnancy
- The cervix is pulled forward and lies at the pelvic brim by 4 months
- At 5 months uterus is completely abdominal and fetus is not palpable.
- Fetus becomes palpable after 6 months.
- By the 9<sup>th</sup> month the fetal movements can be seen in the right flank
- External signs of pregnancy (enlarged abdomen and udder are visible from the 11<sup>th</sup> month.
- Precise estimation of fetal age beyond 3 months is not possible.

# Ultrasonography

Transducer and a scan converter



Basic principle: Piezo electric crystal when stimulated electrically emit ultrasound

- Ultrasound: Sound frequency above 20,000 Hz    Infrasound: below 20 Hz
- Ultrasound Frequencies: 2 MHz – 12 MHz
- Types of transducers: Sector, linear, curvilinear
- **Trans-abdominal- Sector convex, curvilinear**
- **Trans-vaginal- Sector**
- **Transrectal- Linear**

## Echotexture terminology

**echogenic**

**anechogenic**

## Echotexture terminology of a tissue structure relative to the surrounding tissue

**Hyperechogenic**

**Isoechogenic**

**Hypoechogenic**

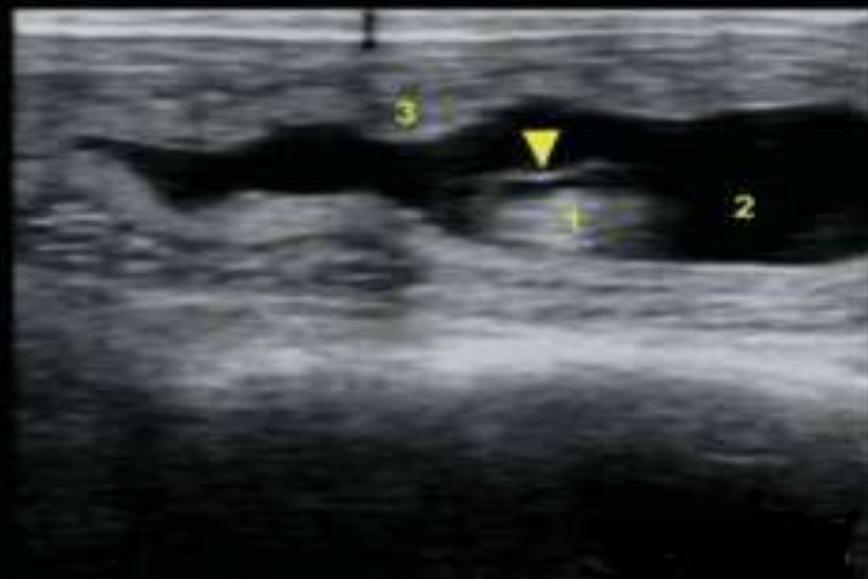
# Probe placement for transabdominal ultrasonography



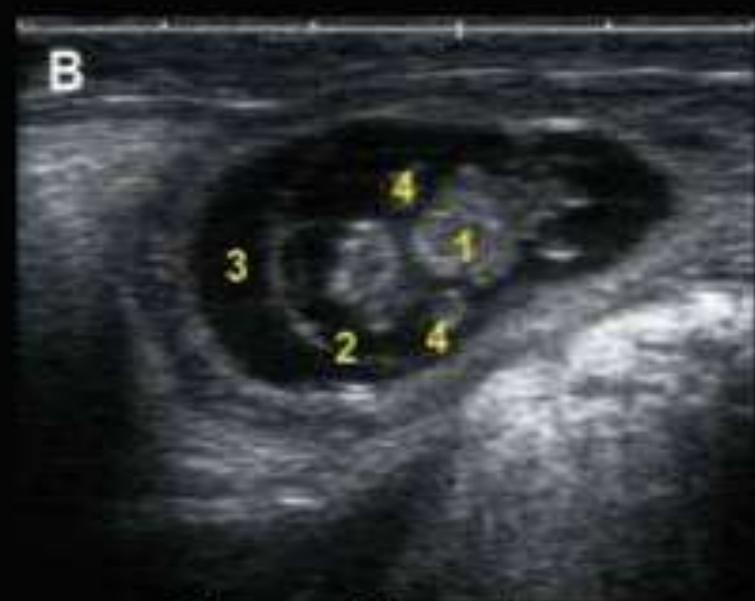
- Vesicle/fetal fluid is visible in most species between days 18-22 except in the mare in which it appears earlier (day 10-16).
- Fetal heart beat can be seen between day 24-30 and the fetus itself between days 25-30 in most species.
- Cotyledons are visible between day 30-40 and fetal extremity/bone by day 57-60 in cattle, day 70 in sheep; however it is visible earlier (42-50 days) in the bitch.
- The most appropriate time for pregnancy diagnosis using ultrasonography with high accuracy in cattle, camel and buffaloes appears to be day 28-30 days using a trans rectal linear array probe of 5.0 to 7.5 MHz frequencies.
- Using the same probe pregnancy can be diagnosed with sufficient accuracy a little earlier (24-25 days) in the mare.
- Bitch requires a trans-abdominal probe of frequency 3.5 to 5.0 MHz) to visualize pregnancy with accuracy from 25-30 days.

<b>Ultrasonographic features of early pregnancy in various species</b>								
<b>Sonographic Structure Appearance (days post mating)</b>	<b>Cow</b>	<b>Buffalo</b>	<b>Mare</b>	<b>Sheep/ Goat</b>	<b>Sow</b>	<b>Bitch</b>	<b>Camel</b>	<b>Cat</b>
Fetal fluid	18-20	18-22	10-16	20-25	18-20	18-20	17-18	10-16
Fetal Heart beat	24	30	24-25	21-23	-	24	28-30	16-18
Fetus	28-30	20-26	20-22	25-30	25-30		23-25	16-20
Cotyledons /Allantois	35-40	30-35	20-22	40-50	-	-	-	25
Fetal bones/ fetal buds	57-60	70-75		70	-	42-50	40	30-33
Fetal sex determination	57-60	60-70	60-70	60-90	-	-	-	38-43
Fetal movement	42-50	47-51	40-45	-	60	-	-	30-34

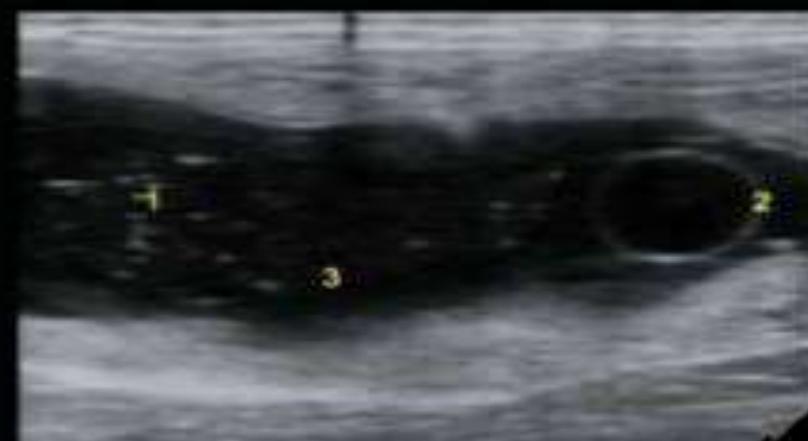
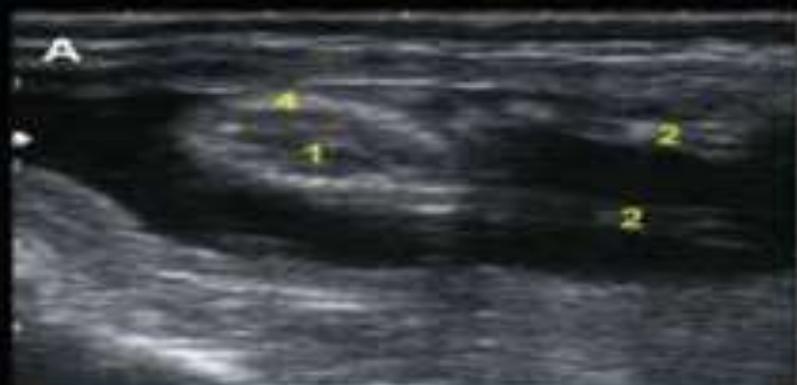
# Cow



30 - day - old embryo



40 - day - old embryo



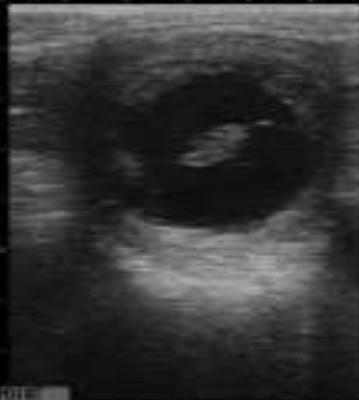
Fetus on day 59 with the first view of the ribs

embryonic mortality observed at day 30

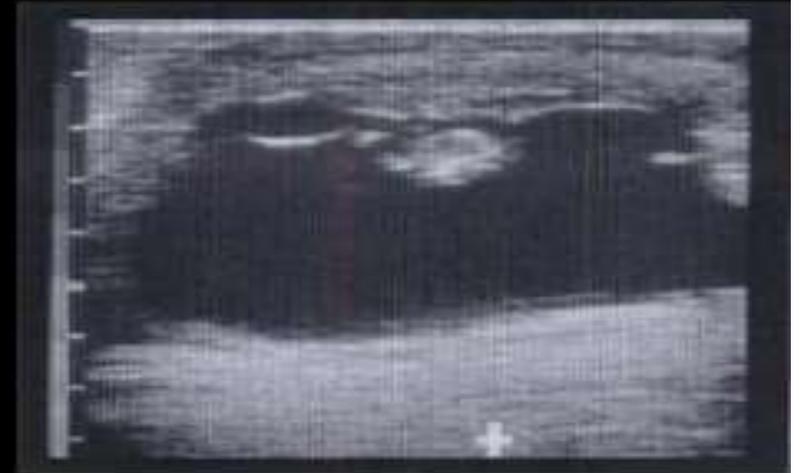
# Mare



Day -10



Day 30:



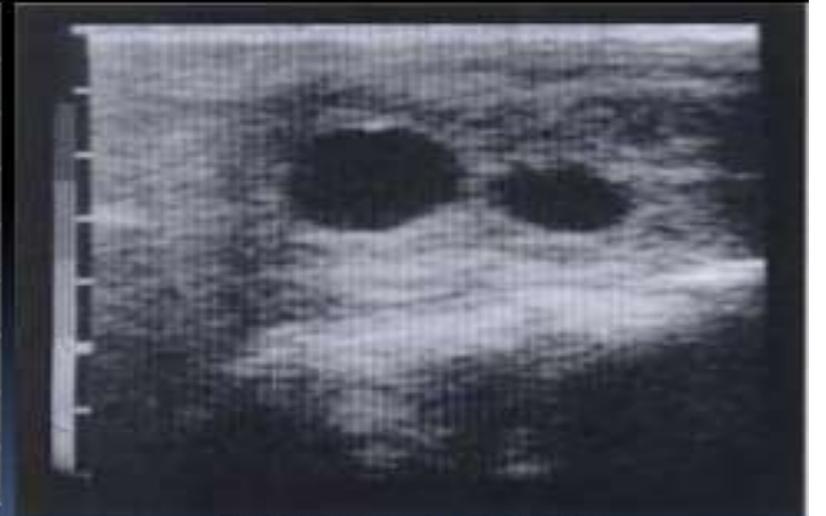
Day 35



Day 48:



Day 60



Twin pregnancy

# Bitch



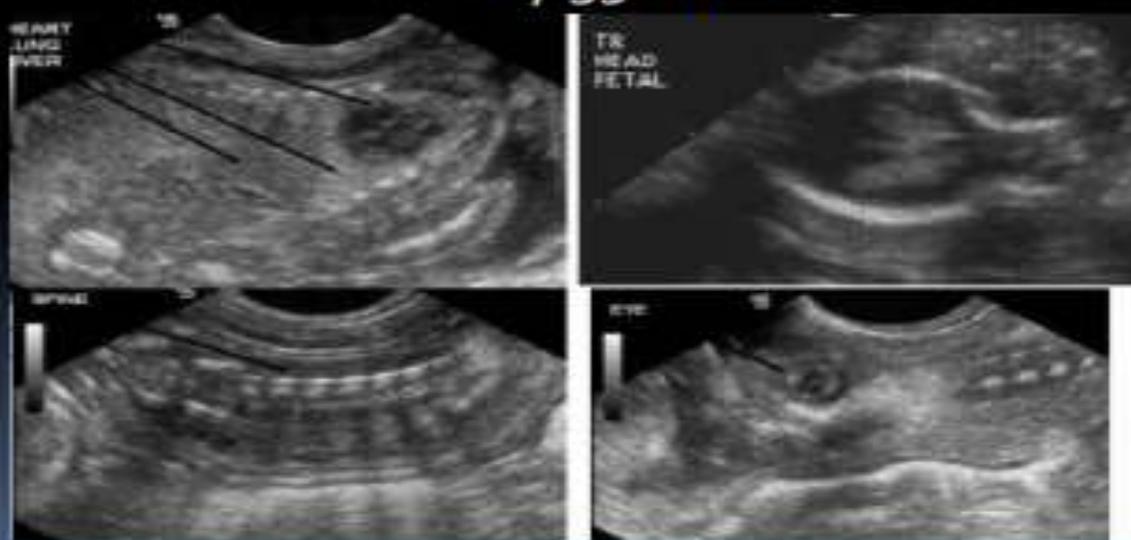
Day 25, head



Day 35 Yolk sac



Day 41 stomach



Day 55

# Radiography (Small ruminants, bitch)

- Sheep and goat, fetuses are visible by day 70 of gestation
- Bitches fetal skeletons are visible with high accuracy only by the sixth week of pregnancy.
- Fetal skulls are visible by day 45 and the entire fetal skeleton is visible by the end of seventh week of gestation.
- Signs of fetal death as seen by radiography include the spalding sign, (which is the overlapping of the cranial bones), gas shadows in the fetal heart and stomach and tightly flexed spine (seen in fetuses died for long time)
- Fetal skeletons begin to calcify only after the sixth week in sows and hence radiography should be performed only after this time for pregnancy diagnosis in sows.



**Radiographic Pregnancy Diagnosis**  
Verstegen J (2009)

**Radiography of Fetuses.**  
Shille VM (1982)

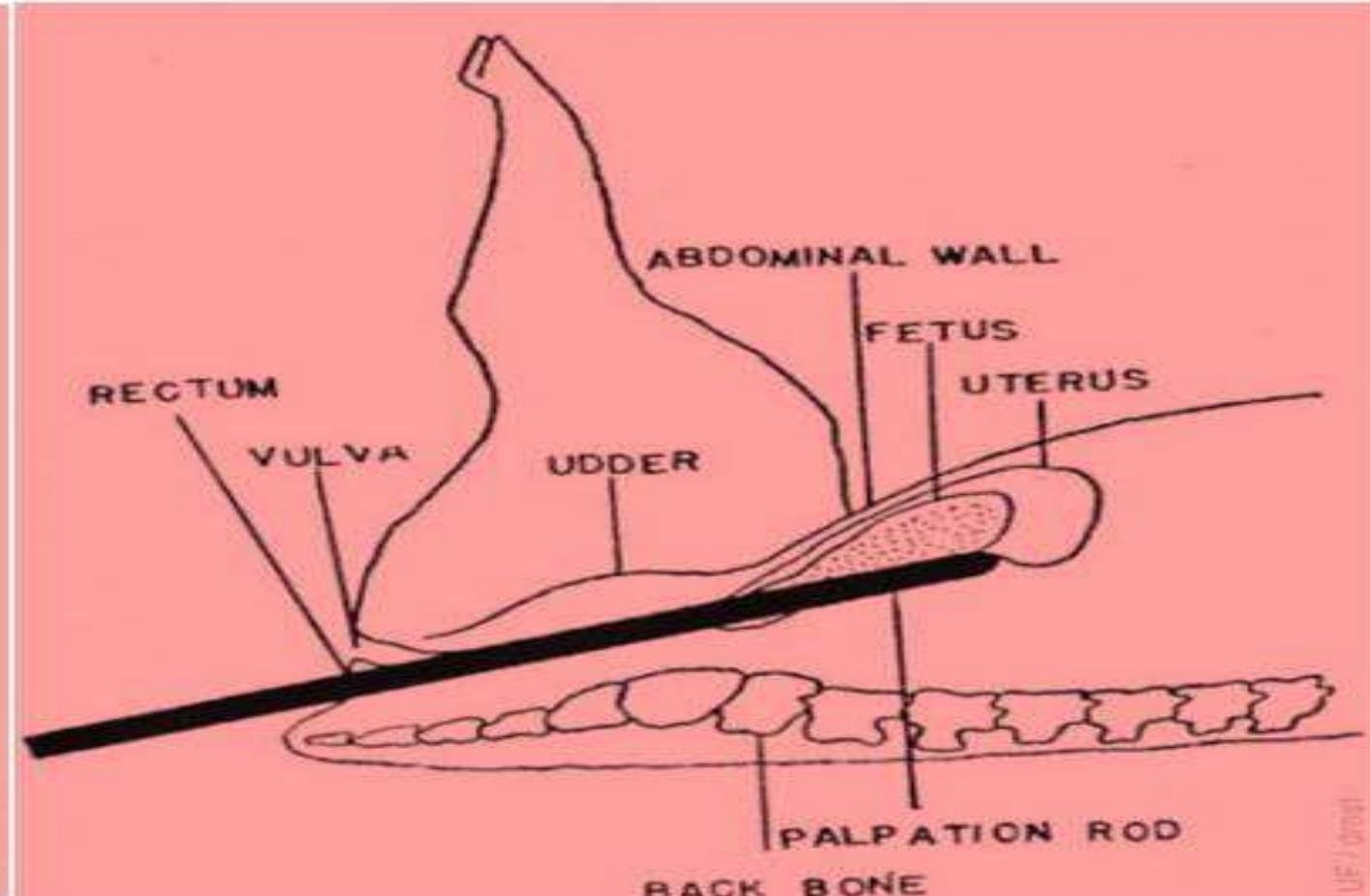
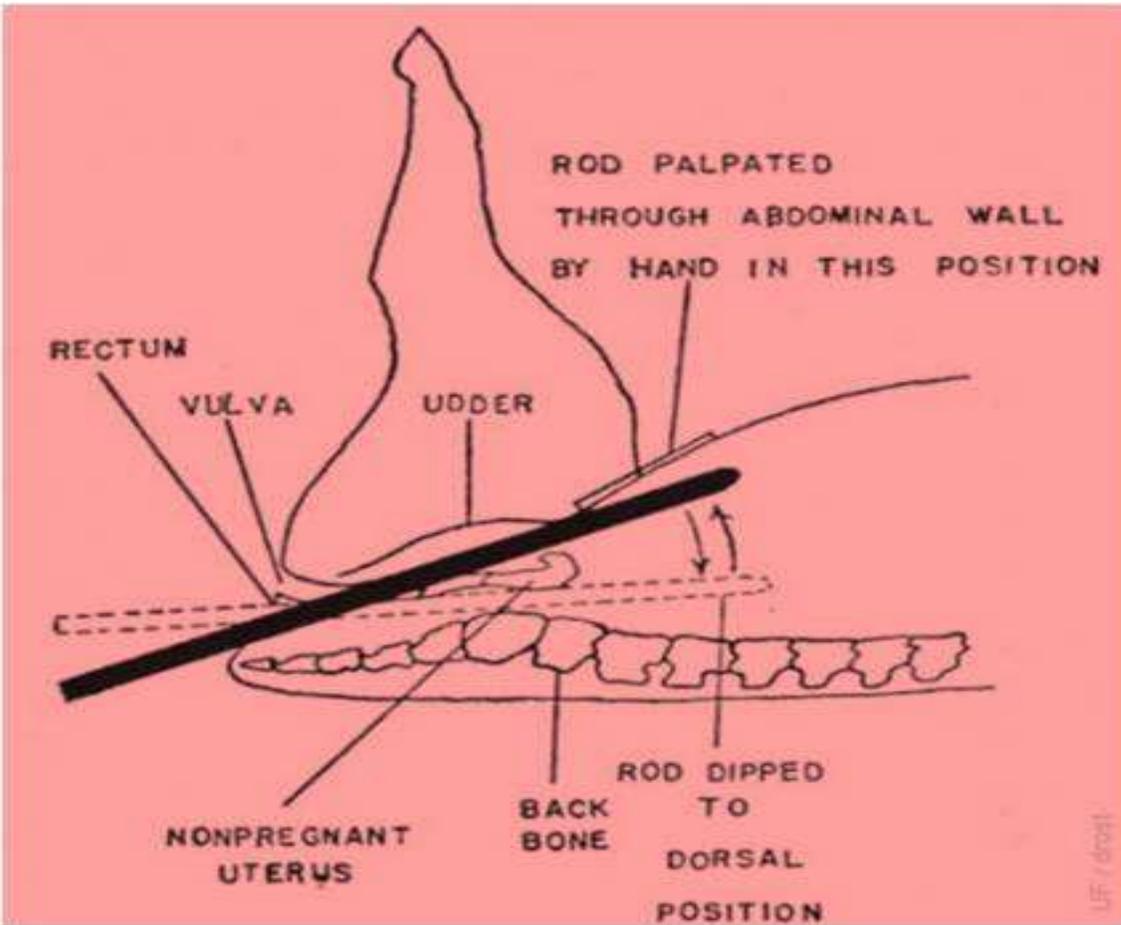
# Abdominal Ballottement and palpation

- Possible to some extent in cows during late gestation (7 months onward).
- Sheep and goats rectal abdominal palpation (by using a glass rod placed in the rectum to lift the uterus which is palpated through abdomen) has been suggested
- Similarly bimanual palpation for pregnancy diagnosis (palpation of uterus through fingers in the rectum and lifting the abdomen) has been reported for small ruminants.
- Palpation of fetuses through the abdomen is possible in sheep and goat only beyond 4 months of pregnancy
- In bitches beyond day 55 it is easy to diagnose pregnancy by the palpation of the fetuses
- At about 30-35 days the accuracy is high (87%) and depends on the palpation of tense conceptual swellings (6 to 30 mm in diameter)

# Recto-abdominal palpation

- It is a technique used for pregnancy diagnosis in sheep and goat.
- A hollow lubricated plastic rod (1.5×50 cm) with a rounded tip is inserted gently into the rectum to a depth of 30 to 35 cm.
- One of the hands is placed on the posterior abdomen while the rod is manipulated with other hand. Rod is moved up and down and from side to side until an obstruction is encountered and palpated against the abdominal wall or a decision is reached that the ewe or doe is not pregnant.

# Recto-abdominal palpation



# Laboratory tests for pregnancy diagnosis (Indirect methods)

- Irritable animals
- Rectal palpation not preferred by owner
- Lack of availability of ultrasound
- **Progesterone hormone assay**
- Specificity of around 98% (Less specific)
- Progesterone concentrations in milk or serum can be quantified using a laboratory RIA or ELISA procedures.
- Usually, samples are collected at 21 and 24 days after insemination.

- **Based on estrogen**
- One of the earliest written records of a urine-based pregnancy test can be found in an ancient Egyptian document. Wheat seeds when they are soaked in urine from pregnant cows which inhibits germination compared to urine from non-pregnant cows which stimulate germination has been described to be known as **punyakoti test** .
- The estrone sulfate is produced by the feto-maternal axis or the conceptus and therefore its presence in urine, milk, feces or blood is an indicator of pregnancy.
- In cows, concentrations of estrone sulfate detectable in the whey fraction of milk are similar to those in maternal plasma and increase from about 60 days.

# Appearance of estrogen in domestic animals during pregnancy

Species	Day of detection	Reference
Mare	Maternal estrogen high after 60 days Conjugated urinary estrogens high after 150 days	Sist et al. (1987); Cox (1971); Bhavnani and Woolever, 1978
Cow	Day 100 of gestation	Hamon et al. (1981); Robertson et al., 1978
Goat	High after day 50 of gestation	Refsal et al (1991) Chaplin and Holds worth (1982)
Sow	Rise start at 20 days peak at 25-30 days followed by a decline at 45 days and again a rise at 70-80 days to term	Cunningham, 1982 Seren et al.(1983) Robertson et al.(1978); Guthrie and Deaver, 1979
Bitch	Slightly increased at implantation and remain constantly high for rest of gestation and decline 2 days prepartum	Concannon et al. (1975)
Sheep	Detectable by day 70; rise thereafter till 2 days prepartum	Illera et al., 2000; Worsfold et al., 1986
Buffalo	Appear at day 150 of gestation in the serum	Prakash & Madan (1993); Kamonpatana, 1984
Camel	Increase start at day 50 and peak from day 90-300	Skidmore et al., 1996

- **Cuboni test (Detects conjugated urinary estrogens)**
- To 15 ml of urine 3 ml of concentrated hydrochloric acid is added and heated in a water bath for 10 minutes and then cooled under a tap
- Positive -dark, only green fluorescent color in the lower sulfuric acid layer
- Negative- absence of fluorescent color and presence of a brownish color effective beyond 150 days of gestation and also predicts fetal viability

# Commercial kits For urinary estrogens in mare

- *Wee-Foal-Checker*® or *Equitest ES*

**Not Pregnant**



**Pregnant**



## Allen Diosey-test

Injection of pregnant mares urine contain estrogen and this when injected to spayed mice produce estrous like changes in the female tract.

## Barium chloride test

- Take 1 percent barium chloride solution and add urine in equal parts
- Non pregnant cows a white precipitate is formed
- Pregnant animals the urine remains clear
- Accuracy of the test was described to be 70-95% from 15 to 210 days of pregnancy
- In Camels the test was considered to be 85% accurate between days 50-90 of pregnancy

## Milk alcohol coagulation test

In this test there is coagulation of milk from pregnant cows when mixed with equal quantities of alcohol and allowed to stand for 1-3 hours

## Copper sulfate test

1 ml of milk when mixed with a few drops of 3% copper sulfate coagulates if the animal is pregnant.

## Mucin test

Vaginal mucus from a pregnant mare showed dark staining columnar epithelial cells as early as 20 day of coitus. (pregnancy cells)

Efficacy of 94% from day 70 to end of gestation

- Phenolsulphonic acid test

- The phenolsulphonic acid test utilizes ether extraction of urinary estrogens subsequent to removal of urinary pigments by hydrolysis and their conjugation with phenolsulphonic acid reagent after evaporation of the ether. The final reaction gives a pink to cherry red color if the urine is from pregnant mares.
- 70-80% accurate when performed between the 120 to 250 days of pregnancy.

# Biological tests

The only animal species that secrete sufficient quantities of gonadotrophins that can be used as a marker molecule for pregnancy diagnosis is the equine.

eCG continues to be secreted from day 40 to 120 days of pregnancy

## **Aschiem Zondek test**

Friedman rabbit test

## **Toad test**

Basis of this test is the concept that the sperm cells are emitted by toads/frogs only when stimulated with female frogs or gonadotrophins.

On farm tests some commercial kits are currently available which can detect the presence of eCG in blood of mares between 40-100 days of pregnancy with 96-98% accuracy.



# Milk ejection by low dose prostaglandin

- the injection of low dose prostaglandin F2 alpha (non-luteolytic dose) in animals two weeks after breeding resulting into milk ejection. The animals detected further as pregnant showed an increase in the pressure in the milk ejection and alveolar milk volume collected by a teat probe in comparison with the non-pregnant cows.
- Cows
- Ewes
- Directly correlated to the plasma progesterone profiles

# Pregnancy associated glycoproteins (PAG)

- Produced in various ruminant species including cattle, buffalo, sheep and goats.
- Two pregnancy specific proteins (PSP) A and B have been isolated from bovine fetal membrane extracts.
- PSP-A was identified as a fetoprotein and PSP-B was found to be specific to the placenta.
- These molecules appear in the maternal circulation and can be determined with accuracy from 29 to 30 days post breeding.
- PAG continues to be existent in maternal blood for the entire pregnancy and up to 100 days post partum.
- Assay involves radioimmunoassay on serum.

- Sensitivity and specificity of PSPB based on RIA is known to be 92.0% and 82.6 to 91.9% from 29 to 30 days post insemination.
- ELISA techniques have been developed that detect the PAG molecule in the serum of cows.
- Limitations to the wide spread use of this test is non availability of the protein in milk or urine presence of PAG up to 100 days postpartum (which interfere with subsequent detections).
- Non availability of cow side commercially available kits for its detection.
- Existence of PAG has been documented in bovine milk.

# Early pregnancy factor

- Protein molecule was first identified in pregnant mice
- Then found in Sheep and cattle
- Using the rosette inhibition bioassay EPF was detected in the serum of all mammals tested within 24 to 48 h of fertilization and disappeared within 24 to 48 h after death or removal of embryo.
- Developing embryo bears antigens foreign to the mother; hence immune rejection of the early embryo may occur
- Immunosuppressive early pregnancy factor (EPF) appears as early as 6 to 48 h of mating which functions to suppress the maternal immune response thereby allowing for pregnancy to proceed
- Measurement of EPF activity may be useful as an indirect method of pregnancy diagnosis.
- Commercially marketed kit is available in the US (ECF test, Concepto Diagnostics Knoxville, TN)
- Reliability is known to be poor and need to be substantially improved

# Relaxin assay

- Pregnant bitches at 20-30 days of gestation
- Produced by the placenta in the bitch and cat and is thought to contribute to its maintenance by inhibiting uterine activity.
- Established as a pregnancy-specific hormone
- Cat it appears during the third week of pregnancy, with concentrations declining just before parturition
- Molecule has also been detected and used successfully for pregnancy diagnosis
- Commercially available canine relaxin enzyme-linked immunoassay (ELISA) (Reprochek, <sup>®</sup>a synbiotics corporation, San Diego, CA, USA)

# Canine Pregnancy Test Kit

Trouse de détection de la grossesse chez la chienne



**WITNESS RELAXIN**

Kit de détection pour 1 test

Manufactured and Distributed by  
Merial, Inc. 2015  
10000 North Central  
Expressway  
Kenilworth, NJ 07033 USA

**SYNBIOTICS**  
LABORATORIES



# Vaginal Biopsy

- Histological assessment of the number of layers of the stratified squamous epithelium of the vaginal mucosa obtained by biopsy can be used as a method of diagnosing pregnancy in the **sow** and to a limited extent in **sheep**.
- The basis for the test is the decrease in the layers of the stratum germinativum (vaginal epithelium cells: to 3 to 4 layers at 18-25 days of pregnancy) under the influence of progesterone).
- The number of layers is high at estrus (around 20 layers) due to influence of estrogen hormone.

- The above lectures are also explained in video lectures at my YouTube Channel Govind Narayan Purohit
- Kindly share the videos and subscribe to my channel if you like them
- Thanks