Lecture title: Pregnancy diagnosis (cyesiogenosis)

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 Summary:

Clinical methods, Chemical tests, Biological tests, and other tests.

2. Clinical Methods

2.6. Fetal echocardiography

- used in the past to diagnose in cattle, sheep and mares

2.7. Vaginal electrical resistance

- Depend on calculate the conductivity of the vaginal mucous membranes changes. The change in estrus due to increased hydration, increased blood supply and other changes.
- vaginal electrical resistance is low at estrus.
- Limited use because false positive results.

2.8. Laparoscopy

- Directly visualizing the genitalia in animals.
- Need high cost, equipment and clinic required.

3. Laboratory tests for pregnancy diagnosis

- indirect methods of pregnancy diagnosis.
- Depending on calculation reproductive hormones or specific substances in maternal body or body fluids.

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1. Progesterone hormone assay

- Progesterone hormone responsible for maintenance of pregnancy.

- Progesterone hormone increase in all gestation in some species (cow, buffalo, goat, and sow, while for reasonable time in some species.

- Non pregnant animals, progesterone level goes down.

- Maternal blood progesterone concentration at 18 to 24 days after insemination, high level give indicate that probably pregnant.

- Specificity around 98% when tests between 18 and 24 days post breeding.

- In pregnant animals the accuracy of the test is low (75%) due to early embryonic death.

- Progesterone assay in plasma or milk in mares, sheep, goats, buffaloes, camels and sows is not very accurate for diagnosis of pregnancy.

- progesterone assay cannot be used to diagnose pregnancy in the bitch. progesterone concentration in both pregnant bitches and non-pregnant bitch is similar.

2. Estrone sulfate

- urine-based pregnancy test.

- The estrone sulfate is produced by the feto maternal axis.

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- Presence in urine, milk, feces or blood is an indicator of pregnancy.

- Can detection pregnancy in gestation period after 100 days in cow, 70 days in sheep, and 150 days in buffalo.

- The test need laboratory and availability of commercial assay kits (radioimmunoassay, enzyme immuno-assay or other more precise and specific diagnostic modalities).

- Analysis of feces is especially helpful for zoo animals.

- Commercial kits for pregnancy detection in mares and using in farm by test urine or serum is available.

- Commercial kits recommended to use after 120 days of gestation.

Chemical test

1-Cuboni test

- Use in mare for diagnosis pregnancy.
- The test affective after 150 days of gestation.

2- vaginal cornification, mouse or rat tests.

- Detection of urinary estrogens in mares.
- using ovariectomised female rats or mice, and the phenol sulphonic acid test.
- Injected urine or serum from mare in the ovariectomised mouse or rats would induce vaginal edema, appearance of cornified cells and mucus discharge due to presence of estrogens in the pregnant mare's serum or urine (induce estrus).

University of Mosul
Lecture No.: 2
College of Veterinary Medicine
Date: 2025
Unit of Scientific Affairs
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3- **Phenolsulphonic acid test.**

- 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
- The accurate of the test are 70-80% and suitable between the 120 to 250 days of pregnancy.

4- **Mucin test**

- The vaginal mucus from a pregnant mare showed dark staining columnar epithelial cells (pregnancy cells).
- Efficacy of the test around 94% from day 70 to end of gestation.

5- **Barium chloride test:**

- A test for pregnancy diagnosis in the bovine species.
- 5 ml of urine from cows a few drops of 1% barium chloride is added and warmed slightly.
- Non-pregnant cows a white precipitate is formed, and, the urine remains clear in pregnant animals.
- The accuracy of the test was 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.

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6- Milk alchohol coagulation test

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

7- Copper sulfate test:

- 1 mL of milk when mixed with a few drops of 3% copper sulfate coagulates if the animal is pregnant.
- The accuracy of these tests is considered to be low (52.0 to 64.2%).

8- Costa's test:

- for testing of pregnancy in human females.
- based upon sedimentation of haematin in the presence of a solution of novocaine.
- The test was experimented in cows and the test was known to be positive after 38 days of insemination with 65.3% accuracy in cows and inaccurate in camels.

9- Assay of gonadotrophins

- Use in human pregnant women by appear of gonadotrophins in urine.
- Gonadotrophins appear only in Equine, therefore we can use for pregnancy diagnosis in mare.
- Gonadotrophins equine chorionic gonadtrophin (eCG) secretion from endometrial cups from day 35.
- Detected gonadotrophins from serum and urine.
- The eCG continues secretion from day 40 to 120 days of pregnancy.

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Biologic tests

1-Aschiem Zondek Test (A-Z tests)

- Identified the presence of hCG in human urine.
- Urine was injected into an immature rat or mouse.
- No reaction in non-pregnant urine, and in the pregnant, the rat would show an estrous reaction (be in heat) despite its immaturity.
- A similar test in mares by injected serum from mare to mice and the results are read later.
- The serum (0.5 ml SC daily for 2 to 4 days or 5 ml intraperitoneally) from test mare is injected to 2 to 3 rats (22 days of age) and rats are killed (72 hr when injected intraperitoneally and 96 to 120 h later when injected subcutaneously) and a positive test is indicated by the presence of multiple corpora haemorrhagica on the ovaries and uterine edema.
- The accurate of the test around 90% when performed between the 60 to 100 days of pregnancy in the mare.
- In camels revealed no changes in the mice due to lack of any gonadotropic molecule.

2- Friedman Rabbit test

- Serum from test mare is injected (2 ml given IV) to rabbits (14 to 20 weeks age) kept in isolation and laparotomy performed 24 h later.

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Date: 2025
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- Positive test is indicated by the presence of corpus haemorrhagicum and uterine edema.

Other methods

3- Milk ejection by low dose prostaglandin

- Low dose of prostaglandin F2 alpha injection (non-luteolytic dose) in animals two weeks after breeding resulting into milk ejection.

4- Pregnancy associated glycoproteins (PAG)

- Pregnancy specific proteins produced in various ruminant species including cattle, buffalo, sheep and goats.
- There are two pregnancy specific proteins A and B have been isolated from bovine fetal membrane extracts.
- A protein was identified as a a-fetoprotein and B was found to be specific to the placenta.
- PAG can determine in blood with accuracy from 29 to 30 days post breeding and stay up to 100 days post partum.
- The sensitivity of the test 92.0% from 29 to 30 days post insemination.
- Now simple ELISA available to detect the PAG molecule in the serum of cows.

5- Early pregnancy factor

- protein molecule
- calculation occur by using the rosette inhibition bioassay

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- Faster method to diagnosis pregnancy, the factor detected in the serum of all mammals within 24 to 48 h of fertilization and disappeared within 24 to 48 h after death or removal of embryo.
- A commercially marketed kit is available.

6- Relaxin assay

- Relaxin can be determined in the blood of pregnant bitches at 20-30 days of gestation, whereas it is absent in non-pregnant bitches at all stages of the reproductive cycle.
- produced by the placenta in the bitch and cat.
- Act to inhibiting uterine activity.
- Relaxin calculate at 25 days and in the cat it appears during the third week of pregnancy.

7- Vaginal Biopsy

- Calculation histologically the number of layers of the stratified squamous epithelium of the vaginal mucosa.
- used as a method of diagnosing pregnancy in the sow and limited use in sheep.
- The accuracy of this method between 18 and 22 days is 97% for pregnant and 94% for non-pregnant in the sow.
- During pregnancy decrease in the layers of the vaginal epithelium cells: to 3 to 4 layers at 18-25 days of pregnancy due to influence of progesterone hormone.
- In estrus, the number of layers is high (around 20 layers) due to influence of estrogen hormone.

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- The accuracy of the test in sheep the technique was 91% in diagnosing pregnancy after 40 days and increased to 100% after 80 days of gestation.

Reference

1- Govind, P. 2010. Methods Of Pregnancy Diagnosis In Domestic Animals: The Current Status