PREGNANCY AND ITS DIFFERENTIAL DIAGNOSIS

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An early and accurate diagnosis of pregnancy is also known as Cyesiognosis and is necessary for cost-effective animal husbandry.

For an economical dairy farm, cows must calve every year, and to maintain this sequence, identifying pregnant animals at an early date seems imperative.

Methods of pregnancy diagnosis in the domestic animals can be classified into visual/managemental, clinical and laboratory methods.

In most of the domestic animals clinical methods are currently used.

1. Visual/ Managemental methods:

A). Non return to estrus

B). Udder development

II. Clinical methods:

A). Trans-rectal palpation

B). Ultrasonography

C). Radiography

D). Abdominal ballottement /Palpation

E). Laparoscopy

III. Laboratory methods:

A). Vaginal biopsy

B). Immunological methods

i. Progesterone hormone assay

ii. Estrone sulphate

iii. PMSG

iv. Pregnancy associated glycoproteins (PAG)

C). Biological methods

1. Visual/ Managemental methods:

A). Non return to estrus:

When an animal is mated and it does not return to estrus the owner usually thinks that the animal has become pregnant and hence has not returned to estrus.

This happens because during pregnancy, the conceptus inhibits the regression of the corpus luteum and thus, prevents the animal from returning to estrus.

However, many a times the animal does not return to estrus because of non- regression of CL due to reasons other than pregnancy.

Moreover, in the seasonally breeding species the animal may not return to estrus when mating is done during the end of the breeding season because the season was over.

Anoestrus and the rare occurrence of gestational estrus in cattle and buffaloes can affect the reliability of non-return to estrus as a method of pregnancy diagnosis.

Moreover, difficulty in estrus detection and silent estrus render this method of pregnancy diagnosis unsuitable for the buffalo, Therefore, non-return to estrus is an unreliable procedure for pregnancy diagnosis in most domestic animal species.

Cocking of the tail

The pregnant female dromedary camels exhibit a characteristic behaviour when approached by a male or a person.

The female assumes a stiffened posture with the head held high and tail curled upwards. This is known as cocking of the tail.

This behaviour appears 14 to 15 days after fertile mating and known to be 95% reliable for pregnancy diagnosis in quiet and calm dromedary female camels.

However, many false positives can be obtained in agitated females if the observer is untrained. Tail cocking is also observed in the pregnant Bactrian camel, although not with the same intensity as in the dromedary female camel.

B) Udder development:

Besides the non-return to estrus a few of other visual signs of pregnancy appearing in late pregnancy include increase in the size of the abdomen, development of the udder specially in dairy heifers 4 months onwards slight vaginal discharge (from 4-5 months onward in dairy cows) and movements of the fetus visible externally (specially in fed cows on the right side of abdomen 6 months onwards).

However, the accuracy of these visual diagnostic symptoms is always low and a clinician must use them as a supplement to clinical diagnosis.

As parturition approaches, the mammary glands become grossly enlarged and oedematous and the teats take on a waxy, tumefied appearance.

II. CLINICAL METHODS

A). Recto-genital palpation/Trans-rectal/per-rectal Examination

Trans-rectal palpation is the oldest and most widely used method for early pregnancy diagnosis in dairy cattle.

In most large domestic animal species like cattle, buffaloes, mares and female camels recto-genital palpation (with some limitations) is the easiest, cheapest and fastest method of pregnancy diagnosis with little or nil harm to the animal and its fetus when performed carefully. To a limited extent this method is used for pregnancy diagnosis in pigs.

Trans-rectal palpation of the amniotic vesicle as an aid in determining pregnancy status in dairy cattle, whereas slipping of the chorioallantoic membranes between the palpator's thumb and forefinger starts between days 35-90.

Basic principle of rectal examination:

Examinations of cows under epidural anaesthesia usually result in ballooning of rectum that cannot be overcome until the anaesthesia wears off.

The genital organs lie usually on the pelvic floor during early pregnancy beneath the rectum in most species and in the abdominal cavity during late gestation.

The genital organs can thus be palpated indirectly by placing the hand in the rectum evacuated of the faeces.

The growth of the conceptus in either of the uterine horn leads to sequential increase in the size tenseness and palpable characteristics of the uterine cornua.

Thus, with experience the palpator can feel these changes in the uterus of a pregnant animal and with fair to good accuracy predict pregnancy depending upon the species, stage of gestation and his experiences.

Two bottlenecks appear to be significant while performing rectal palpation:

i) the peristalsis that occurs in the rectal musculature, which produces obstacles in palpation

ii) ballooning of the rectal wall due to entry of air inside.

The palpator must stop making movements of arm during a peristaltic wave (while still keeping his hand inside the rectum) wait for 1-2 minutes and then start palpation again when the peristalsis has subsided.

The ballooning of rectum can be easily appreciated, by the finding that the operator can move his hand up and down in the rectum without resistance when the rectum is ballooned.

The operator must move his hand back and forth (known as back racking) to evacuate the faeces. The faeces must be removed without taking out the hand completely.

After proper restraint and wearing of proper clothing and also proper lubrication, the operator must make a cone of his hand and push it inside the rectum.

Sufficient lubrication must be used while introducing the hand in the rectum. Non-irritating soap and water or liquid paraffin is a suitable lubricant. Since, the faeces of the mare are harder and the peristalsis stronger, more frequent lubrication is essential in the mare.

Tail bandaging is also advantageous in the mare to avoid tail hairs enter the rectum and cause damage to the rectal wall.

The anal sphincter dilates and the hand enters inside the rectum.

The cervix which is a hard round to oval or sometimes caudally enlarged disfigured structure is the land mark for location of genital structures in cattle and buffaloes.

This can be located by sliding the hand in an arc like fashion from dorsal to ventral side.

The cervix is followed further to locate the uterine body and the uterine horns. These structures can be pulled caudally when located at the pelvic brim or further, by retracting the broad ligament or hooking the inter-cornual ligament by the index finger.

When the pregnancy is beyond 60 day this cannot usually be done and the operator has to move his hand further in the rectum, so as to locate the intra-abdominally placed uterus and palpate other features diagnostic of pregnancy.

Rectal palpation is the most widely used method and it is non-invasive, accurate and results are quickly known.

During rectal palpation cervix and uterus and its contents are palpated and up to 120 days of pregnancy ovaries can also be palpated.

In herd where AI is used, the first palpation should be made between 35-42 days, of insemination. Even though, palpation at this stage requires a greater expertise, the main interest should be to identify those cows which are non-pregnant so that they can be observed more carefully.

In the **mare**, the cervix is not easily palpable and hence the ovaries are the land mark for rectal palpation especially for novice palpators.

They are located about 10-20 cm cranial to the shaft of the ilium bone and about 5 to 10 cm below the lumbar vertebrae in non-pregnant mares, and in mare during early pregnancy. After locating one ovary the hand is passed down the utero-ovarian ligament to locate the uterus.

Female **camels** are examined in a sitting position with both hind, and forelegs tied together separately with ropes. An assistant holds the head tightly. Vicious females often require pressure on the back by legs of persons standing on both sides to prevent side wise movements.

A striking feature of the genitalia of the female camel is the shortness of the right uterine horn. This is probably, because of the existence of exclusive left horn pregnancies in this species. Recto-genital palpation is similar to that described for cattle and buffalo as the cervix is easily palpable.

Rectal palpation is only possible to a limited extent in the large sized sows. It is barely possible in gilts and in small sized breeds of sows.

PRECAUTIONS DURING RECTAL PALPATION

When performed gently and carefully, rectal palpation is a non-invasive procedure. The following points would be helpful in minimizing damage to the animal and the examiner as well.

1) Ruthless movements of the hand in the rectum should be avoided. Avoid palpations during a peristaltic wave.
2) Examiners must trim their nails and avoid using dirty soiled sleeves.
3) Rectal examination without a sleeve must be avoided specially in mares to avoid contracting diseases and obnoxious odours. Sleeves must be replaced after examination of 2-5 animals or better after each examination.

1) Rectal palpation of an animal suffering from fever should be extremely gentle or better avoided as the blood vessels are more fragile and bleed easily. Similarly examining an animal with rectal tear or rectal fistula is hazardous. Whenever, a clinician notices such conditions he must bring them to the notice of the owner or else he would blame the clinician.
2) Compared to cattle rectal palpation in buffaloes must be gentle as the rectal mucosa is more fragile and bleed easily.
3) Clinicians must assure that even if the animal kicks it does not harm them, and so also the palpators must also be cautious that sudden sideways movement of the animal with the operators hand inside can cause fracture of the operators arm and hence due care must be exercised.
4) Uncareful palpation of the uterine horns with undue pressure can cause rupture of the amniotic vesicle and loss of an early pregnancy (iatrogenic embryonic mortality) and hence this must be avoided.

Palpable findings of pregnancy in cattle and buffaloes

Two things must be kept in mind by clinicians in making positive diagnosis of pregnancy by rectal palpations in cattle and buffalo.

The first is, that when the palpator in unable to detect any of the palpable characteristics mentioned herein, he must neither comment positively or negatively as both would be frustrating both to the clinician and the owner on a later date. He must better admit the fact that he is not able to detect out properly and the animal must be re-submitted for examination 15-30 days later preferably after a fasting.

The second thing that clinicians must keep in mind is the accuracy of the gestation period (this is especially applicable for the 5-8-month period in cattle and 5-9 months in buffalo). An approximation of the gestation period must be conveyed to the owner rather than an accurate period. Although experienced clinicians can more precisely comment on the gestation period after an examination it is usually safe to be approximate.

When pregnancy examinations are made early (Day 30-45) the possibility of an early embryonic death must be kept in mind and a confirmation of pregnancy must be done only after re-examination at a later period (60-90 days).

Owners often consult veterinarians on the fetal viability during mid to late gestation. It is often difficult to comment positively by a single rectal palpation. Until unless characteristic changes in the uterus and fetus are palpable negative comments must better be avoided.

The usual test for fetal viability during this period is the movement of the fetus in response to stimuli by the examiners hand (movement of a fetal leg when pressed by hand or suckling movements by the calf when a finger is touched in the mouth) however; this may be sometimes misleading especially when the fetus is depressed.

It is likely that the rectal examination of cows from 42 days of gestation is safe and reliable method when preferred carefully and skillfully.

Location of pregnant uterus

The pregnant uterine horn under goes sequential changes in size, location and morphology and they form the basis for pregnancy diagnosis.

The early pregnant uterus lies in the pelvic cavity in heifers, and just ahead of the pelvic bring in pluriparous large sized cows.

As it grows in size, its growth is forward so it starts descending into the abdominal cavity (approx 3½- 4 months). At approximately 5½-6 months it reaches the abdominal floor and at this time only cervix is palpable within the pelvic cavity which is also drawn forward. The growth is then forward and then again upwards.

. The entire uterus or the fetus is therefore barely palpable during the 5½-6½ months period and diagnosis has to be dependent on other features of pregnancy placentomes or fremitus.

After this period the fetal parts are usually palpable and clinicians find no difficulty in commenting whether the animal is pregnant or non-pregnant.

During early pregnancy (day 30-60) clinicians have to depend on finding of the fetal membrane slip or the palpation of the amniotic vesicle.

The definite signs of pregnancy in the cow as determined by rectal palpation are

i) palpation of enlarged uterine horn-containing the placental fluids-

ii) palpation of the amniotic vesicle

iii) slipping of the fetal membranes

iv) palpation or ballottement of the fetus

v) Palpation of the placentomes

vi) palpation of enlarged thin walled “whirring” middle uterine arteries.

Uterine changes.

The increase in the diameter of the uterine horns is characterized by a thinning of the uterine wall and the feeling of a fluid filled structure.

Unilateral enlargement of uterine horn and disparity in size, thinning of the uterine wall, fluid filled fluctuation of enlarged horn appears as early as on day 35.
By 40-90 days of pregnancy, the uterus feels like a thick rubber balloon nearly filled with water.
Around 90 days, tapping of distended horn with fingers may reveal the fetus rather like a piece of wood floating in the fluid beneath.

The volume of fluid increases rapidly the first 5 months of pregnancy and then increase slowly. With advancing pregnancy, the non-pregnant horn may also increase in size slightly.

The amniotic vesicle

The amniotic vesicle can be palpated with due care between 30-65 days of gestation as a movable oval object within the uterine lumen many a times at the apex of the cornua.

The vesicle is turgid, early in pregnancy but becomes flaccid with advancing gestation until days 65-70 when it is difficult to detect at all.

Shipping of the fetal membranes

The fetal membrane slip can be felt between 35-90 days of gestation.

The entire uterine horn must be grasped in the palm and allowed to slip while the fingers compress it gently.

The allantois- chorion, slip between the thumb and fingers before the uterine wall escapes. It is felt like a connective tissue band.

The pressure must be gentle. The entire diameter of each uterine horn must be palpated.

Palpation of placentomes

The presence of placentomes is another positive sign of pregnancy.

Placentomes are formed by 60-70 days of pregnancy and they can be detected from about 75-80 days to term. The period of pregnancy when the uterus has descended into the abdominal cavity and the fetus is not palpable,

Palpation of a placentomes is the surest indication that the cow/buffalo is pregnant.

Palpation of the fetus

The palpation of the fetus itself is a positive sign of pregnancy. Depending on the skill of the examiner and the location of the fetus, the fetus can be palpated from the time of amniotic softening (65 to 70 days) to term.

However, in large sized cows the abdomen should be lifted up by a bamboo held by two attendants on either side of the abdomen to palpate a fetus during mid gestation (4½-6½ months).

The whole of the fetus is palpable many a times only during early gestation (2 to 4 months).

The size of the fetus is approximately that of a mouse or rat at 2 and 3 months and it increases to the size of a small cat at 4 months, a large cat at 5 months and a beagle dog at 6 months respective.

Beyond 8 months of gestation, fetal parts (egs, head) are palpable within the pelvic cavity, or just cranial to the pelvic brim. Palpation of a fetal extremity is sufficient evidence for pregnancy if other uterine findings are normal.

Palpation of middle uterine arteries (fremitus)

The major supply of blood for the gravid uterus arrives, via the middle uterine artery, which enlarge considerably as pregnancy progresses.
The artery runs in the broad ligament along a tortuous course, passing downwards, forwards and towards the midline over the pelvic brim close to the junction between pubis and ilium.
Usually, it is identified 5-10 cm lateral to cervix. This can thus, be felt by the hand directed laterally towards the iliac shaft.

Enlargement of the uterine artery ipsilateral to the pregnant horn is detectable after 80 days of gestation.

By approximately 80-120 days the blood flow within the middle uterine artery increases to a point where the blood flow is palpable as a buzzing sensation, also called thrill', 'whirr' or "fremitus“.

By 7 to 8 months the fremitus often palpable on the side of the non-pregnant uterine horn also.

The detection of fremitus is a positive sign of pregnancy. If the fremitus was felt in early pregnancy and then disappear indicates death of fetus.

Palpation of ovaries

As the pregnancy advances ovaries may be dragged forward along with the pregnant uterus and may not be palpable beyond the four to five months. Cervix is landmark for pregnancy diagnosis in bovines.

Vaginal changes

During pregnancy the vagina usually develops a pale, dry sticky mucus membrane. The cervix is closed and the cervical (mucus) seal covers the external os by day 40 to 120 of pregnancy. Slight degree of vaginal discharge is evident in some cows beyond 5 months of pregnancy but the cervical seal liquefies only prior to parturition or abortion and is discharged in strings.

DIFFERENTIAL DIAGNOSIS OF PREGNANCY IN BOVINES

Owners are many times perplexed on the outcome of a pregnancy that did not progress normally and often present their animals for a definitive diagnosis to a clinician. Some pathological conditions mentioned in detail in subsequent chapters often should be identified to be different from a normal pregnancy. The palpable differences of these conditions are briefly described below.

a). Mummified fetus

A uterus with mummified fetus has thick uterine walls, absence of fluid and placentomes and hard palpable structure. The fetus is closely opposing the uterine wall. The fremitus is absent.

b). Maceration of the fetus

There is copious vaginal pus discharge. The uterine wall is thick and doughy. There is no dorsal bulging of the uterus and placentomes are not palpable Parts of bones are sometimes palpable separately floating tacked up. The fremitus is absent.

c). Pyometra/Mucometra

These two conditions are many times difficult to differentiate from normal pregnancy especially when the pus or mucus is present in enormous quantity (sometimes 20-40 litres) so that the uterus is largely enlarged and placed on the abdominal floor.

In pyometra the uterine wall is thick, uterus is doughy and placentomes or fetus is not palpable and the fremitus is absent.

However, it is always safer to make a re-examination 1 to 2 months later when the clinician is not sure, or make use of ultrasonography to differentiate the condition.

Sonographic evaluation would depict (anechogenic fluid without cotyledons and echogenic material accumulated.

A clinical therapy to terminate the Corpus luteum on an assumption that the condition is pyometra could be hazardous and loss to the owner, if unfortunately, the condition was a normal pregnancy.

In mucometra, the positive findings of pregnancy are absent, but, contrary to pyometra the uterine all is thin.

Ultrasonography can easily differentiate the condition from pregnancy.

When these two conditions are to be differentiated from early pregnancy a characteristic feature found most often is the bilateral enlargement of both uterine horns, which is not found in pregnancy.

The fetus is not palpable in these two pathologies, and often There is a history of vaginal discharge.

d). Urinary Bladder

Infrequently, full of urinary bladder creates confusion for the presence of an early pregnancy (60-90 days) in bovines. This can be easily differentiated by the absence of palpation of both uterine horns and the ease with which the animal urinates when the bladder is gently massaged leading to disappearance of the enlargement.

e). Rumen

Rumen feels doughy consistency, whereas gravid uterus gives a live fluid feeling sensation.

f). Tumours

Rarely tumours of the genital tract can create confusion, but their consistency and location is different.

PALPABLE FINDINGS OF PREGNANCY IN MARES

The early diagnosis of pregnancy in the mare has its own significance. A limited breeding season in some breed registries (for e.g. Thoroughbred) warrants that the non-pregnant mares are diagnosed early and steps be taken to breed the mare again in the same breeding season.

Diagnosis of an early pregnancy by rectal palpation is one of the easiest and accurate means for experienced personnel.

However, a diagnosis at day 18-20 should be confirmed at day 45-60 because of a late implantation of the equine embryo and chances of early embryonic death and reabsorption. The basis of pregnancy diagnosis in the equine species lies on the changes that follow in the cervix, uterus and ovaries.

Placentomes are not found in the equine species and the fremitus or fetal membrane slip is not marked, therefore some of the parameters used in cattle cannot be used in the equine species directly.

Cervix

As early as 16 to 18 days after ovulation, the cervix of the pregnant mare becomes tightly closed, firm, slender, and elongated. Between 16 to 30 days with the experience the cervix can be palpated on the floor of pelvis as a rigid firm structure.

Uterus

Most pregnancy diagnosis in the mare by rectal palpation is done by palpation of the uterus. The parameters used for a positive diagnosis are as follows:

a). Uterine tone and thickness

One of the positive sign that a mare has conceived to a breeding is the finding of increase in uterine wall thickness and a marked tone, The endometrial folds are no longer palpable as folds of tissue, The uterus becomes tubular, smooth and firm. The uterine tone appears at days 15-16 and continues up to day 48 to 55.

This is diagnostic except in cases of endometritis or in mares bred at the foal heat which are still undergoing uterine involution.

b). Uterine size and embryonic vesicle

The conceptus becomes positioned at the base of one of the uterine horns at the junction between uterine horn and body.

At 25-28 days, a small bulge may be palpable in maiden mares. The bulge progressively increases in size and is palpable in most mares by day 30-35 (approx 3-4 cm in diameter). A small notch can be readily appreciated on both sides of the bulges By day 42-45, the conceptus occupies approximately half of the gravid hornsand is 5 to 7 cm in diameter. The uterine wall over the bulge in thin.

At 60 days of gestation, nearly the entire gravid horn and half of the uterine body are filled with the conceptus but the non-gravid horn remains small. The pregnancy is like an elongated football and is nearly similar to a 60-day pregnancy in cattle.

The tonicity is markedly reduced at this time. The 60-day conceptus is approximately 8-10 cm in diameter and 12 to 15 cm in length.

c). Palpation of fetus

The fetus is active after 40 days and mobile after 70 days.

Palpation of the turgidity of the conceptus is absent by day 90, and the fetus is palpable, which feels like a small, heavy, submerged but floating object as the hand contacts it.

In most mares it is usually possible to palpate the fetus per rectum from the third month onward throughout the gestation. In a few deep bodied and large sized mares palpation of the fetus is difficult from the fifth to seventh months of gestation.

In these mares the location of uterus and ovaries would aid the diagnosis.

d). Location of the uterus

The uterus is located in the pelvic cavity or just at the pelvic brim until day 99.

At 100-200 days, the gravid uterus is positioned cranial to the pelvic brim in the abdominal cavity.

The ovaries are positioned cranial and ventral to their normal positions and closer together.

By 5 to 7 months the uterus is positioned low in the abdomen and it is difficult to thoroughly evaluate the conceptus by palpation per rectum.

During the sixth and seventh months, the horns are approximately perpendicular to the dorsal cranial aspect of the uterine body.

Beyond the seven to eight months the fetus is easily palpable by rectal palpation.

e). Ovarian palpation

Ovaries are landmark for pregnancy diagnosis in mare. Both ovaries should be palpated.

From 40 to 120 days, extensive ovarian activity with ovulations, luteinisation, and development of secondary corpora lutea is evident.

Follicular activity decreases from 120 days to term, and the ovaries become small and inactive.

The 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 when the uterus or other structures are difficult to palpate (3 to 5 months).

From 5 months of pregnancy onwards, the ovaries usually are not palpable as they are under the broad ligaments.

RECTAL PALPATION IN THE SOW

To a limited extent rectal palpation has been described as a method of pregnancy diagnosis in the sow. Sows are examined while standing in gestation crates or pens.

This technique is 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, the cervix and uterine walls are flaccid and thin.

The middle uterine artery is 5-8 mm in diameter and more easily identified. The uterus becomes progressively thin walled and fremitus can be identified at 37 days.

Beyond 60 days the fremitus is very strong, however, piglets can only be palpated towards the end of gestation.

The procedure is often difficult in gilts and small sized breeds because of a small pelvis and too small rectum.

False positive diagnosis is likely if the external iliac artery is mistakenly identified as the middle uterine artery. The technique is however, not popular at most locations.

RECTAL PALPATION IN THE CAMEL

The corpus luteum formed on the **camel** ovary ovulation is induced by mating persists and is necessary for the entire gestation.
The persistence of the CL is one of the earliest sign of pregnancy as otherwise; the luteal phase is very short. The CL is out of reach by day 90 of pregnancy. The left uterine horn is inherently longer than the right horn and this must be kept in mind when making pregnancy diagnosis in female camels.

The earliest detection of uterine change (increase in diameter and appearance of fluctuation) is palpable at about 40-45 days.

Between 60-70 days, the left uterine horn is increased about twice to its non-gravid size, has a thin wall and fluctuates.

The uterus becomes cranial and ventral after the third month of pregnancy.
The cervix is pulled forward and lies just at the pelvic brim by 4 months.
At 5 months of pregnancy, the uterus is completely in an abdominal position with a small degree of fluctuation but the fetus is not always palpable.
The fetus becomes palpable again beyond the 6th month of pregnancy, first with ballottement, and then, the head and legs become easily palpable by the 7-8 months as the fetus starts its ascent.
By the 9th month, movement can be observed by inspection of the right flank of the animal.
At around 11 months the fetal legs can be easily found in the pelvic cavity.
The precise estimation of the stage of pregnancy beyond 3 months, because of the absence of cotyledons and difficulty in reaching the fetus in this species is difficult. The fetal membrane slip is not seen in camels because of a diffuse placenta.

B). ULTRASONOGRAPHY

During the last couple of years, ultrasonography has gained popularity in veterinary medicine and has become the method of choice for diagnostic imaging of the various organs of the body, including reproductive organs.

Ultrasound is a high frequency sound wave. Sounds audible to the human ear vary between 20 to 20,000 Hertz (Hz) (Cycles per second) while ultrasound waves are of frequency higher than this, and for most diagnostic applications frequencies of 1-10 MHz are used.

Even the short distance between the transducer (which emits and receives ultrasound signals) and the patient must be bridged by a suitable coupling gel.

Basic principle

The ultrasound equipment basically consists of a transducer and a scan converter.

The transducer is the ultrasound producing part. It is fitted with a piezoelectric crystal (Lead-zirconate - titanate or others) which when stimulated by a high voltage current emits the ultrasound.

The ultrasound is transmitted to the patient from the transducer and propagates through the tissues.

The ultrasound beam is either reflected back, partially absorbed or entirely absorbed. The returning beam echoes meets back and deforms the crystals in the transducer. This mechanical energy is converted back to an electrical signal proportional to the strength of the echo and delayed by a time roughly proportional to the distance travelled.

The scan converter interprets the variations in brightness displayed on the cathode ray tube of a B-mode system (or as a variation in amplitude in A-mode oscilloscope screen) and also stores images when required.

The ultrasound is emitted in a pulse - echo manner. A pulse of ultrasound is emitted and its reflection perceived prior to emission of the next pulse.

Types of instruments and some definitions

For most diagnostic veterinary purposes B-mode, real time ultrasonography is used employing different types of transducers.

Transducers used commonly in veterinary reproductive practice are the linear trans-rectal transducer frequencies of 5-10 MHZ and the sector trans-abdominal transducer frequencies of 1-4.0 MHZ.

For most reproductive diagnostic work, linear array trans-rectal transducers are employed in cattle, buffaloes, mares and female camels.

Small sized trans-rectal transducers are also used for early pregnancy diagnosis in small ruminants (sheep and goat).

For bitches mostly trans-abdominal sector transducers are useful for pregnancy diagnosis with frequencies from 2 to 4.0 MHz.
The same transducers can be used for pregnancy diagnosis in sheep and goat beyond day 40 of gestation.
However, in order to visualize an early pregnancy or the non- pregnant bitch uterus trans-rectal transducers of high frequency (5-7.5 or 10.0 MHz) are essential.
A wide variety of transducers with single, dual, or multiple frequency and multiple functions are available and clinicians must decide what type of instrument and the transducer he must purchase depending upon his work.

Advancement to ultrasonography includes diagnostic imaging using color Doppler and magnetic resonance imaging.

When performing ultrasonography, it is important for the sonographer to have basic knowledge of the anatomic location of the different organs to be visualized and problems that can be encountered in obtaining and interpreting the images obtained.

A few of the common terms related to ultrasonography are described below:-

Anechoic (echolucent): In absence of any echo, the images seen on screen appear black. It is presented by complete transmission of sound waves such as through liquids like follicular fluid, chorionic or amniotic fluid.

Hyperechoic: It represents the bright echoes which appear as white on screen. Such images are given by highly reflective interfaces or dense tissues such as fetal bones, bovine cervix etc.

Hypoechoic: These appear as grey images on dark screen and are given by interfaces of moderate reflection such as soft tissues.

A-mode (Amplitude modulation) or 2D: A one-element (one dimensional) display with time (distance) on the horizontal axis.

B-Mode (Brightness modulation): A compound A-mode scan with amplitude translated into a brightness scale. Location on the display is related to position and depth. When an ultrasound beam meets a moving object the reflected ultrasound is either of increased or decreased frequency, depending upon whether the motion is towards or away from the transducer.

M-Mode (Motion mode): A rapidly updated one dimensional B-mode display with time on the second axis to allow study of moving structures. It is used in cardiology.

Echogenic: A structure causing a marked reflection of the ultrasound beam. A change in echogenicity in a homogeneous structure may indicate a pathological change.

Trans-rectal ultrasonography

The animal to be examined is properly restrained, the faeces are evacuated from the rectum and the perineum washed with water.

The coupling gel is applied to cover the transducer. The operator keeps the transducer in his gloved arm and takes it inside the rectum.

The uterine horn on one side is scanned to the entire length and the ovary of that side is also scanned.

The operator then moves his hand to the other uterine horn and ovaries.

If the pregnancy is advanced the operator may have to take his hand deeper.

When required the images seen may be frozen and the diameter of the structures measured by inbuilt callipers with the machine.

At 60 days the fetal crown length is approximately 6 cm. The amount of fluid and thickness can also be measured.

The transducer is then taken out and the perineum is washed again.

Trans-abdominal (Trans-cutaneous) ultrasonography

For reproductive trans-abdominal ultrasonography in **sheep and goats** the hair must be clipped from just above the udder and 15 to 20 cm ahead of the udder on both sides of the abdomen.

The transducer is placed above the udder between the thigh and abdomen preferably the left side and moved in W shape from one side of the abdomen to the other side.

The procedure can be performed with the animal standing (sector scan) or in lateral or ventral recumbency (linear scan) depending upon the type of transducer being used.

For pregnancy diagnosis, **sows** are examined in a standing position when using sector scanners 3.5-5.0 MHz frequency or Doppler instruments (which are more frequently used in pigs to detect fetal heart beat, fetal movements and uterine artery pulsations).

The transducers are placed over the abdomen just medial to the stifle skin fold, just at the level of second last teat.

Trans-abdominal imaging of the uterus of a **bitch** can be done with the bitch in the standing position after clipping the hair of the ventral abdomen.

However, dorsal and lateral recumbent positions may also be used. The uterus lies dorsal to the bladder, but its position may vary with the extent of bladder filling, and the size of the uterus.

During early pregnancy the uterus has a more dorsal position in the abdomen of a standing bitch, but with advanced pregnancy the uterus is closer to the ventral lower abdominal wall.

Sonographic findings during pregnancy

When the ultrasound strikes to a smooth and wide structure (e.g. a CL) there will be almost total return of echoes where the sound waves strike at right angles, giving an intensified signal that appears on the screen as a whiter shade of grey.

This is referred to as specular reflection, and is often seen in early pregnancy when imaging the embryonic vesicle.

With these basic things in mind one has also to keep in mind that fluid filled structures appear black (anechoic), hard structures (like the bone) appear white (hyperechoic) and other structures with their structure midway between the bone and fluid appear grey (hypoechoic).

The basic diagnosis of pregnancy lies in the identification of structures from black, grey or white scale.

The earliest sonographic finding for pregnancy in most animals is the appearance of anechoic fluid within the uterine lumen. This fluid goes on increasing to a stage when the embryo proper becomes visible as a hypoechoic structure floating within this fluid and progressively the fetal structures become more clearly visible along with the fetal membranes.

The viability of a growing fetus is ascertained when the fetal heart beat becomes visible as hypoechoic flickering structure.

With different probes the amniotic vesicle/fetal fluid is visible in most species between days 18-22 except in the mare in which it appears earlier (day 10-16).
The fetal heart beat can be seen between day 24-30 and the fetus itself between days 25-30 in most species.

The cotyledons are visible between day 30-40 and fetal extremity/bone by day 57-60 in cattle and day 70 in sheep.

It therefore, appears that the fetal fluid, fetal heart beat and the fetus become sonographically visible at nearly the same time in most species.

The most appropriate time for pregnancy diagnosis using ultrasonography with high accuracy in cattle, camel and buffaloes appears to be day 28-30 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.

Under field conditions a trans-abdominal probe (linear or sector 3.5 to 5.0 MHz frequency) is generally used and this can diagnose pregnancy earliest at day 40-50 in sheep and goats with reasonable accuracy as the fluid and cotyledons are easily visible sonographically at this time.

The diagnosis of pregnancy in the bitch requires a trans-abdominal probe (of frequency 3.5 to 5.0 MHz) to visualize pregnancy with accuracy from 25-30 days.

The visualization of earlier pregnancy or the visualization of a non-pregnant bitch, uterus necessitates the use of probes of higher frequency (7.5 to 10.0 MHz) as the uterus lie closer to the skin.

Doppler ultrasonography and A-mode probes are generally used in pigs for pregnancy diagnosis but B-mode probes with frequencies of 3.5 to 5.00 MHz can diagnose pregnancy with high accuracy between days 25-30 post mating.

C). RADIOGRAPHY

To a limited extent radiography has been used for pregnancy diagnosis in the small ruminants (sheep and goat), the companion animals (dog and cat) and rarely in pigs.

The technique is known to be good in evaluating fetal numbers in the bitch and cat, but is poor in evaluating fetal viability.

Moreover, the high cost and the hazards of exposure to growing foetuses to x-rays limit the use of radiography as a routine procedure, and warrant its use in specialized cases.

Mostly, a single radiograph taken with the animal in lateral recumbency is sufficient however; sometimes a dorsal or a dorso-ventral view may be required.

Radiography is suggested to be done only after day 90 in sheep and goat.

The 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. Because pregnancy can be diagnosed with high accuracy with other methods, radiography is seldom performed for pregnancy diagnosis in sows.

D). ABDOMINAL BALLOTTEMENT

Abdominal palpation and abdominal ballottement of the fetus is possible to some extent in bovines during late gestation 7 months onward.

In cattle abdominal ballottement is performed by placing the fist over the lower right abdominal wall and pushing it in an intermittent manner in a dorsal medial direction deeply. This is usually possible in lean cows after the 7th month of gestation.

The fetus can be felt as a hard solid object floating in fluid.

Palpation of foetuses through the abdomen is possible in sheep and goat only beyond 4 months of pregnancy by lifting the abdomen held between both hands and location of bony fetal structures.

E). LAPAROSCOPY

Laparoscopy can be used as a method of pregnancy diagnosis by directly visualizing the genitalia in animals however, the invasive nature of the technique, the high cost of equipment and clinic required, and the availability of non-invasive techniques limits the use of this technique as a means of pregnancy diagnosis in most animals.

III. LABORATORY METHODS FOR PREGNANCY DIAGNOSIS

A). 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 accuracy of this method between 18 and 22 days is 97% and 94% for the diagnosis of pregnancy and non-pregnancy respectively in the sow.

The basis for the test is the decrease in the layers of the stratum germinatum (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 limitations of such a diagnosis are the invasive nature of the test, poor results due to improper sampling and improper tissue processing.

B). IMMUNOLOGICAL METHODS:

Progesterone hormone assay:

The corpus luteum formed on the ovary subsequent to ovulation produces progesterone for maintenance of pregnancy for a reasonable time period in some species and for entire gestation in other species like the cow, buffalo, goat and sow.

In normally cycling cows and buffaloes, the CL is lysed because of the effects of prostaglandins from the uterus if the animal is not pregnant, and thus the progesterone level goes down. Therefore, low progesterone concentrations in maternal blood at 18 to 24 days post breeding can predict that the animal is non-pregnant and high progesterone gives an insight that probably the animal is pregnant.

The specificity of progesterone tests conducted between 18 and 24 days post breeding have shown a specificity of around 98% and is the easiest proven method for identifying non-pregnant (regularly cycling) animals post breeding.

However, for the pregnant animals the accuracy of the test is low (75%) because of early embryonic death which alter the results.

Commercially available ELISA, plasma or milk progesterone assay kits have not become popular due to their high cost and a low specificity.

Non pregnant cows not returning to estrus and pregnant cows in which embryonic death occurs at a later time can both give false results. Likewise, in mares, sheep, goats, buffaloes, camels and sows assay of plasma or milk progesterone is not very accurate for diagnosis of pregnancy.

Since progesterone concentration in the peripheral blood of pregnant bitches is similar to those of non-pregnant bitches, and since there is no placental progesterone produced in the pregnant bitch hence progesterone assay cannot be used to diagnose pregnancy in the bitch.

ii). Estrone sulphate:

The estrone sulfate is produced by the feto-maternal axis or the conceptus and therefore its presence in urine, milk, faeces or blood is an indicator of pregnancy.

The main oestrogens in the mare are estrone and a ketonic steroid equilin. However, oestradiol-17ß, oestradiol-17a and equilenin are also present.

The identification of estrone sulphate in the milk of a cow at 105 days of gestation later is a very reliable method of pregnancy diagnosis.

The appropriate day, at which detection of estrone sulphate detection is possible in the body fluids or secretions are mentioned in Table 1.

Table 1: Appearance of estrogen in domestic animals during pregnancy

Species	Day of detection
Mare	Maternal estrogen high after 60 days, conjugated urinary estrogens high after 150 days
Cow	Day 105 of gestation
Goat	High after day 50 of gestation
Sows	Rise start at 20 days peak at 25-30 days followed by a decline at45 days and again a rise at 70-80 days to term
Bitch	Slightly increased at implantation and remain constantly high for rest of gestation and decline 2 days pre-partum
Sheep	Detectable by day 70-rise thereafter till 2 days pre-partum
Buffalo	Appear at day 150 of gestation in the serum
Camel	Increase start at day 150 and peak from day 90-300

The detection of estrogens depends on the availability of suitable laboratory and availability of commercial assay kits.

Laboratories evaluating concentrations of estrogens in urine or serum usually are equipped with radioimmunoassay, enzyme immuno-assay or other more precise and specific diagnostic modalities for assay of steroids in urine, serum, faeces or other body fluids.

Evaluation of steroids like estrogen from faeces is especially helpful for zoo and feral species where faeces are the most easily collected specimens.

Commercial kits have been developed for pregnancy detection in mares by using on farm kits like Wee-Foal-Checker® or Equitest ES® which require urine or serum as the test material.

These commercially available tests are recommended to be performed only after 120 days of gestation and specially suggested for miniature horses and donkeys in which pregnancy diagnosis by rectal palpation or ultrasonography is extremely difficult.

Cuboni test: To detect urinary estrogen

This test was first developed by Cuboni (1934) and modified later (Galina and Cox, 1969). The test is performed in the mare for detection of pregnancy through assay of urinary conjugated estrogens. The Cuboni test is only effective between 150 and 300 days of gestation and also predicts fetal viability.

The test is performed as follows:

"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.

To this 18 ml of benzene is added and shaken vigorously for half a minute. The supernatant (mainly benzene) is collected in another tube and 3 to 10 ml of concentrated sulphuric acid, is added and the mixture heated in a water bath at 80°C for 5 minutes, and then allowed to cool.

A positive (pregnant) test is indicated by the appearance of a dark, only green fluorescent colour in the Tower sulphuric acid layer and a negative (non-pregnant) is characterized by absence of fluorescent colour and presence of a/brownish colour."

iii). PMSG (Pregnant Mare serum gonadotrophin)/eCG(Equine chorionic gonadotrophin) :

It was reported first time by Cole and Hart 1930 Endometrial cups are formed as early as day 35 and secrete the equine chorionic gonadotrophin (eCG) which appear in blood of pregnant mare as early as on day 68-42) and can be detected from serum of pregnant equine females.

The eCG continues to be secreted from day 40 to 140 days of pregnancy and disappear after day 150.

Once the endometrial cups are formed, they will persist and secrete eCG even if the fetus has died. They regress at the time that they would have done if the pregnancy had continued normally.

On farm testing: National Research Centre on Equines (NRCE), Hisar has developed a kit named as Pregmare Kit which detects eCG in serum samples of pregnant mare between day 35-125 post covering.

The kit requires 5 drops of blood collected, from the muzzle by using the lancet provided with the kit. This colour change test requires about one-hour completing.

iv). Early pregnancy/conception factor

This protein molecule was first identified in pregnant mice by Morton et al. (1976) and later in sheep (1979) and cattle by Nancarrow et al. (1981) by using the rosette inhibition bioassay.
An 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.

In cattle significant differences in rosette inhibition titer are observed between pregnant and non- pregnant cows on day 13-16 and 25 post breeding suggesting that measurement of EPF activity may be useful as an indirect method of pregnancy diagnosis.

In cow EPF has a molecular weight of 200000. Commercially available test kits are available that use the dipstick principle and can detect ECF in serum and milk from as early as 3 days after AI, although more accurate results are obtained if samples are taken later at 7-8 days.

v). Pregnancy associated glycoproteins (PAG)

Pregnancy specific proteins are known to be 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 by Butler et al. (1982).

Of these PSP-A was identified as a fetoprotein and PSP-B was found to be specific to the placental. These molecules appear in the maternal circulation and can be determined with accuracy from 24 days of gestation onwards.

The PAG continues to be existent in maternal blood for the entire pregnancy and up to 100-days post Partum. The assay involves radioimmunoassay on serum.

The limitations to the wide spread use of this test is non availability of the protein in urine, presence of PAG up to 100 days (i.e. false positive) and the non-availability of cow side commercially available kits for its detection.

Recently, the existence of PAG has been documented in bovine milk.

C). BIOLOGIC TESTS

For eCG, several biologic tests were developed for the detection of eCG including the Aschiem Zondek Test, the Friedman test (rabbit test) or the Frog or toad test.

Aschiem Zondek Test (A-Z test)

Ascheim and Zondek described a test (known as the A-Z test) which identified the presence of eCG in serum of pregnant mare wherein serum from mares is injected to rat 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 test was considered to be 95 percent accurate when performed between the 60 to 100 days of pregnancy in the mare.

Friedman Rabbit test

Serum from test mare is injected 2 ml given i/v to rabbits (14 to 20 weeks age) kept in isolation and laparotomy performed 24 h later. A positive test is indicated by the presence of corpus haemorrhagica and uterine edema.

Toad test

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

Two male toads are taken and their cloaca is wiped with normal saline and examined for presence of spermatozoa.

If no sperms are present, the cloaca is cleaned and 1 ml of test serum from a mare is injected into the dorsal lymph sac of 2 male toads thrice at an interval of 1 hour.

The cloaca is examined for the presence of the sperms. A positive test is indicated by the presence of sperms in the cloaca within 1 to 6 hours after the last injection.

PREGNANCY DIAGNOSIS IN BITCHES

More reliable methods include abdominal palpation at 30 days gestation), measuring the level of relaxin in the blood (after 26 days gestation), ultrasound of the abdomen (starting at 21-30 days gestation), and radiographs (after 45 days gestation). These methods vary in their ability to positively identify the number of fetuses and fetal viability.

1. Relaxin assay

Relaxin can be determined in the peripheral circulation of pregnant bitches at 20-30 days of gestation, whereas it is absent in non-pregnant bitches at all stages of the reproductive cycle.

This relaxin is known to be produced by the placenta the bitch and cat and is thought to contribute to its maintenance by inhibiting uterine activity.

In the domestic dog it has been established as a pregnancy- specific hormone. The site of synthesis in the bitch is primarily ascribed to the placenta, although the hormone can also be traced in the ovary and uterus

Note: Progesterone concentration in the peripheral circulation of pregnant bitches is similar to non -pregnant, therefore unlike other species progesterone can't be used to diagnose pregnancy.

2. Ultrasound

Ultrasound is the best method fused to visualize and evaluate the pups in the womb. Ultrasound is best done 21-30 days after breeding.

Ultrasound done prior to 21 day can give false negatives.

Ultrasound is a good method of distinguishing between pregnancy and pyometra.

Fetal viability can be determined by ultrasound through visualization of the fetal heart. The number of fetuses can be estimated, though it can be less accurate in bitches carrying larger litters, or when done later in gestation.

3. Radiographs

Twenty-eight days into pregnancy, the bones of the fetus begin to become calcified. A 42-45 days of pregnancy, radiographs can be taken to visualize the pups in-utero.

However, for best results, radiographs should be taken more than 47 days post breeding, when fetal skeletons are more readily visible.

Radiographs can help to determine the number of fetuses, though it is less accurate in bitches carrying large litters. Fetal viability cannot be determined by radiograph unless advanced lesions exist in the fetus, such as skeletal collapse or gas within the fetus.

In bitches the fetal skull is visible by day 45 and the entire fetal skeleton is visible by the end of seventh week of gestation.

The number and position of the fetuses can be detected easily by radiography at this time. 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).

4. Abdominal palpation

Abdominal palpation of pregnancy in bitches may be possible in small or medium sized bitches which are not too obese.

Moreover, bitches often tense their abdominal wall and respire too fast making abdominal palpation often too difficult to perform.

Beyond day 55, it is easy to diagnose pregnancy by the palpation of the fetuses. They may be palpable in the flank and also in the lower abdomen.

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Thank you