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Review Article

RECENT TECHNOLOGIES FOR PREGNANCY DIAGNOSIS IN SHEEP AND GOAT: AN OVERVIEW

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Introduction

Heavy economic losses in milk and lamb production can result from failure in early pregnancy detection due to longer kidding and lambing intervals. So there is a need for early pregnancy diagnosis in sheep and goats. Palpation of the genital tract per rectum in larger animals, which is not possible in the smaller farm animals. Out of the many methods described to diagnose pregnancy in sheep and goats, most techniques are not adaptable to field conditions. The various techniques used for pregnancy diagnosis in sheep and goat include ultrasonography (A-scan (Watt *et al.,* 1984); Doppler ultrasonic (Trapp and Slyter, 1983); Real time, B-scope ultrasonics (Haibel, 1990; Bretzlaff *et al.,* 1993); estrone sulfate (Refstal *et al*.,1991); progesterone (Murray and New-stead, 1988); radiography (Barker and Cawley, 1967); rectal abdominal palpation (Ott *et al.,* 1981); vaginal biopsy (Richardson,1972a); abdominal palpation and ballottement (Pratt and Hopkins, 1975); palpation of the uterus via laparotomy (Smith,1980); pregnancy specific antigen (Ruder *et al.,* 1988). Methods of choice depend on the availability of equipment, number of days post-breeding, desired accuracy and experience of the examiner.

Methods of pregnancy diagnosis

Ultrasonic techniques: In living tissues ultrasonic techniques can be used to examine subsurface structures. One of the most important features of ultrasound, when used for tissue examination, is its safety to the operator and patient (Bishop, 1966). All three types of ultrasonographicals viz., amplitude-depth (A-scan), Doppler and Real time B-scan ultrasonics can be used to diagnose pregnancy in ewes and does under field conditions.

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A-Scan ultrasonic techniques: It involves the principles of echo amplitude or amplitude depth versus time. Diagnosis of pregnancy is based on detection of fluid filled uterus. These units are sensitive at a depth of 10 to 20 cm. A-scan ultrasound applied to the flank region has proven to be reliable from 50 to 120 days of gestation in sheep and goats (Watt *et al.,* 1984).In standing ewe or doe, the transducer is placed on the lower right flank in front of the udder. Clipping of hair or wool of this area facilitates optimal contact. Early reports revealed that an accuracy of 95% is possible between 60 and 80 days of gestation in ewes (Haibel, 1990) work with externally applied A- scan. However, Meredith and Madani (1980) reported that in ewes, a positive diagnosis of pregnancy can be made on the evidence of ultrasound reflections with an accuracy of 83% between 61 to 151 days post mating. As per the reports of Lindahl (1969a, b), earliest time at which pregnancy could be detected by using A-scan is between 40 and 50 days after mating. However, A-scan technique may be of particular importance in areas where transport or electricity may not be available.

Doppler ultrasonics: Diagnosis of pregnancy by the Doppler ultrasonics involves the principle of detecting the movements as an indication of pregnancy such as fetal heart beat, fetal circulation and fetal movements. In sheep, Doppler technique was first applied in sheep by Fraser and Robertson (1967). It detects maternal fetal tissue interfaces (Trapp and Slyter, 1983; Watt *et al.,* 1984). Fetal heart beat and fetal pulse which are faster than maternal pulse or fetal movement are taken as positive criteria of pregnancy (Lindahl, 1969 a,b). In ewes and does, external application of the ultrasonic Doppler has been used for detection of pregnancy and approaches an accuracy of 100% during last half of gestation (Fraser and Robertson, 1968) but is not effective 50 days or earlier (Lindahl, 1969 a, b).

Doppler ultrasonics - rectal: For diagnosing pregnancy, intra rectal Doppler technique is superior to the external technique during early second trimester (Ott *et al.,* 1981). It may also be used 25 to 30 days post-breeding but it is best to use between 35 to 40 days of gestation. Fetal viability can be detected but accurate detection of multiple fetuses is difficult with Doppler technique. The Doppler technique resulted in greater accuracy in ewes, which were at least 65 days pregnant (Lindahl, 1969b).

B-Scan ultrasonic techniques: Until recently, no satisfactory technique for determining fetal numbers in ewes and does was available. B-mode ultrasonic scanning developed in Australia appears to offer an accurate, rapid, safe and practical means of diagnosing pregnancy and determining fetal numbers. Real time ultrasound produces a two dimensional image on a screen which can be photographed by a Polaroid camera. It produces a moving image of the

uterus, fetal fluids, fetus, fetal heart beat and placentomes. For optimal image visibility, examinations should be performed away from direct sun light.

The scanning is performed on the standing ewe and doe. Trans abdominal scanning should be ideally performed between 40 to 75 days of gestation. In ewes, real time ultrasound systems used trans abdominally are reliable in determining pregnancy and fetal numbers from 50 days after breeding (Fowler and Wilkins,1984; White *et al.,* 1984). However, diagnosis of pregnancy would be accurate on days 25-30 by trans rectal approach (Haibel, 1990). Pregnancy is confirmed by imaging uterine fluid, by presence of placentomes or by identification of one or more fetuses (White and Russel, 1984; Buckrell *et al.,* 1986). Placentomes appear like echogenic densities in the uterine wall and are routinely found by 26-28 days post-breeding (Buckrell *et al.,* 1986). Between 45 and 90 days of gestation is the optimal time for counting fetal numbers (White and Russel, 1984; Wilkins and Fowler, 1984; Haibel, 1990). Various uterine pathological conditions such as hydrometra, pyometra and fetal mummification can be distinguished from pregnancy by real time ultrasonics (Haibel, 1990). Presence of a dense, hyperechoic image with no fluid indicates fetal mummification. By measuring width of the fetal skull, fetal age in ewe and doe can also be determined by the use of real time ultrasonics at 40-100 days of gestation (Haibel, 1988; Reichle and Haibel, 1991). Experienced person can expect an accurate diagnosis of 91-100% (White *et al.,* 1984; Buckrell, 1988). Rare false positives may be due to early embryonic death or unobserved abortion or sometimes misinterpreting the urinary bladder as the uterus (White *et al.,* 1984; Fowler and Wilkins, 1984; Haibel, 1990). Failure to image the tract early in gestation or inexperienced operator may result in false negatives (White *et al.,* 1984; Haibel, 1990). Real time ultrasonography can also be helpful in diagnosing diseases of the reproductive tract in addition to assess fetal viability, numbers, and age (Buck-rell, 1988).

Recommendations for diagnosis of pregnancy by ultrasonography: Buckrell, 1988 made following recommendations for pregnancy diagnosis using ultrasonography:

- Withheld feed and water for 12 h prior to diagnosis. Avoid unnecessary rectal scanning unless early diagnosis is essential, where it is better to use 5 MHz probe from day 25.
- 5 MHz head should be placed high in the fleeceless groin, close to the udder for trans abdominal scanning.
- Does vs ewes early diagnosis: Does are less cooperative than ewes when rectal probes are inserted. Trans abdominal diagnosis is easy and accurate when using a 5 MHz probe in

the standing doe at days 25-30. By using 5 MHz probe from day 40-50 and 3 MHz probe from day 50 to 100, 20 cm above the udder fetal counts are made on standing ewes or does.

- Advanced pregnancy diagnosis and fetal monitoring is recommended with a 3 MHz probe on the clipped ventral abdomen.

Hormone assay: Another method of pregnancy diagnosis in small ruminants is by measuring concentrations of steroid hormones such as estrone sulfate and progesterone at specific times post breeding (Murray and Newstead, 1988; Refstal *et al.,*1991). Sensitive tests developed by radioimmunoassay (RIA) have allowed detection of these hormones in the blood, milk and urine. Sheep and goat placenta produces estrone sulfate. In sheep plasma estrone sulfate can be detected around 70 days after conception and in does 40-50 days post breeding (Refstal et *al.,* 1991). A positive estrone sulfate test indicates a viable fetus. Enzyme linked immunosorbant assay (ELISA) for measuring concentrations of estrone sulfate in milk as an aid to diagnose pregnancy was used by Murray and Newstead (1988) who reported an accuracy of 82% for pregnancy and 83% for nonpregnancy.

Progesterone test: Plasma progesterone concentration is determined 18 days post breeding in ewes (Dobeli and Schwander, 1985) and does may be tested on 19-23 days after breeding with high accuracy. (Jain et al., 1980; Murray and Newstead, 1988). Accuracy of diagnosing pregnancy and non pregnancy was 85.7% and l00%, respectively as reported by Thibier *et al.* (1982) while measuring plasma progesterone concentration in 267 dairy does on days 2 l-22 post breeding. Concentration of progesterone in milk is much higher (Thibier *et al.,* 1982; Ozsar *et al.,* 1984; Murray and Newstead, 1988). Milk progesterone concentration above 10 ng/ml between 22 and 26 days after breeding was classified as positive (pregnant). A level of 7.25 ng/ml or above as indication of pregnancy between days 19 and 27 post breeding was reported by Jain *et al.* (1980). However milk progesterone concentration varies from day to day and also with the type of milk sample obtained (Bretzlaff *et al.,* 1989). Plasma progesterone concentrations tend to be more accurate than milk (Bretzlaff *et al,,*1989). Owing to a wide variation in the concentration of progesterone, multiple versus single birth predictions were only 67.4% accurate (Weigh et *al.,* 1975). Serum or plasma tends to give more reliable results than milk. Progesterone test in ewe and doe is a good test for non pregnancy but only a fair test for pregnancy.

Rectal abdominal palpation: Hulet. (1972) was first to describe rectal abdominal palpation technique for diagnosing pregnancy in the ewe, which has also been used in the doe (Ott *et al.,* 1981). A drenching gun is used for injecting enema of a soapy solution gently into the

rectum. 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. The accuracy of this method is about 97% at 60 days post mating and requires 30 s per ewe. Accuracy is greater for single than multiple fetuses. Rectal trauma, abortion and death have been reported following the examination (Hulet, 1972; Ott *et al.,* 1981). Does may require sedation prior to examination, whereas ewes are more submissive and do not require sedation.

Radiography: Radiography can be used to detect pregnancy and multiple births with an accuracy of 90% or more, provided ewes are examined later than day 90 of gestation (Ford et *al.,* 1963). In the smaller breeds of ewes, this technique could provide an accuracy of 100%. By this technique pregnancy may be diagnosed at 58 days after breeding in dairy goats (Barker and Caw-ley, 1967). Fetal skeleton is often radio opaque after 65 days of gestation. Uterine enlargement suggestive of pregnancy may be detected earlier than this but cannot be differentiated from hydrometra or pyometra. Under field conditions, this technique is not practical for examining large number of ewes and does, but may be useful for an individual animal when ultrasound equipment is not available.

Vaginal biopsy: For diagnosing pregnancy in ewes after 40 days, histological evaluation of vaginal biopsies have an accuracy of 97% (Richardson, 1972a). In pregnant ewes, vaginal mucosal cells and nuclei were half of the size than those in non pregnant animals, which have polygonal and squamous cells in more than 10 layers. Pregnant ewe's vaginal epithelium has few layers of columnar, cuboidal and prismodial cells. For biopsy samples must be taken from the anterior vagina.

Palpation of uterus via laparotomy: The gravid uterus can be palpated directly through a small incision in the abdominal wall. Direct palpation of the uterus provides an accuracy of 92% in diagnosing pregnancy in 4-5 weeks pregnant ewes (Hulet and Foote, 1968) whereas accuracy in does may approach 100% after 42 days of gestation (Smith, 1980). Uterine horns appear distended 4-5 weeks post breeding. Cotyledons become obvious and the horns are 5-10 cm in diameter after 6 weeks post breeding. Just cranial to the udder a small ventral paramedian incision is made large enough to permit entrance of 2-3 fingers. An enlarged thin walled uterus containing fluid is taken as positive evidence of pregnancy.

Abdominal palpation and ballottement: These techniques can be used for pregnancy diagnosis during late stages of pregnancy in ewes and does. It is easier in thin ewes and does than does in fat animals. These techniques have accuracies of 80 to 90% in ewes at 90-130 days of pregnancy (Pratt and Hopkins, 1975). By placing a hand on either side of the abdomen and squeezing or lifting upwards, the gravid uterus or fetus can sometimes be palpated through the relaxed abdominal wall. A fetus can sometimes be balloted low in the right flank during the last month of gestation. Examination becomes easy when feed and water are withheld for at least 12h before.

Increase in body weight: An increase of 13-16% body weight in ewes carrying twins has been recorded (Domanski and Lipecka, 1966), when precopulation weights and body weight at fourth month of gestation were compared. Single lamb carrying ewes had a gain of 6-12%. Weight changes were too variable to provide a reliable means of diagnosing pregnancy. Richardson, (1972b) described 24 pregnancy diagnostic methods in sheep since 1952. He repeated 17 tests on a flock of ewes and classified the methods into three groups according to relative merits:

***Group A methods*:** Include most reliable methods such as vaginal biopsy, ultrasonic fetal pulse detector and radiography with accuracies of over 80%.

***Group B methods*:** Provide useful aid to diagnose pregnancy particularly during third trimester of gestation and include use of raddle marking, ballotment and assessment of mammary development, enlargement of caudal uterine artery and plasma estrone level.

***Group C methods*:** These have yielded unsatisfactory or unreliable results and include vaginal smears, urine creatinine, viscosity of cervical mucus and increase in body weight.

Detection of pregnancy specific antigen: Detection of chorionic somatotrophin (a pregnancy specific antigen) in serum was utilized for pregnancy diagnosis in a commercial flock of 286 sheep 70 days after joining with rams (Robertson *et al.,* 1980). Ewes were pregnant between days 47 and 70. Positive diagnosis was 97% correct based upon lambing results. This test can be successfully used after day 55 of pregnancy. Radioimmunoassay is used for measuring concentration of ovine chorionic somatotrophin. Ewes having serum ovine chorionic somatotrophin values greater than 5 ng/ml were declared pregnant (Ruder *et al.,* 1988).

Palpation of the cervix: This technique involves digital palpation of OS cervix per vaginum at 50 or more days post breeding. Inability to reach the cervix or a very soft, blunted cervix is

suggestive of pregnancy while a firm conical-shaped cervix projecting into the vagina is suggestive of non-pregnancy (Richardson, 1972b).

Mammary secretion: Reports have revealed that ewes carrying their first lambs produce a sticky honey like mammary secretion after the third month of gestation and multiparous ewes produced a more watery secretion (Webb, 1942). Richardson (1972a) tested 11 ewes at 30-80 days of gestation and the stickiness was not observed. A honey like secretion was sometimes found in pregnant, non-pregnant, uniparous or multiparous ewes.

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