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Vijaylaxmi Gundalli
Medical officer, Department of
veterinary medicine muttagi
bagewadi.

Shivanand Gundalli
Assistant professor, Department
of Pathology, S N Medical
College, Bagalkot

Vikrant S. N.
Assistant professor, Department
of surgery sdurms kolar

Sunitha V. N.
Assistant professor, Department
of OBGY sdurms kolar

Rutuja Kolekar
Senior Resident, Department of
OBG, S N Medical College,
Bagalkot

Amit K.
Assistant professor, Department
of surgery DR AVBRH
Sawangi wardha

Correspondence:
Vijaylaxmi Gundalli
Medical officer, Department of
veterinary medicine muttagi
bagewadi.

Comparative efficacy of various techniques to detect early pregnancy in kenguri ewes

Vijaylaxmi Gundalli, Shivanand Gundalli, Vikrant S. N., Sunitha V. N., Rutuja Kolekar, Amit K.

Abstract

The study entitled "Comparative efficacy of various techniques to detect early pregnancy in Kenguri ewes" was carried out in 24 ewes belonging to Department of LPM, Veterinary College, Bidar. These ewes were randomly and equally divided into 4 groups. These ewes were monitored for estrous cycle, were bred in estrus period with natural service, and were subjected for various pregnancy diagnostic techniques from day 24 post breeding. Finally, these early pregnancy diagnostic techniques were compared with lambing. The cervical mucus samples were collected from ewes on days 24, 34 and 44 with sterilized cotton swab using vaginal speculum. The collected mucus was smeared on two sets of glass slides and air-dried. First set of smear slides were observed for crystallization pattern under low power objective of phase contrast microscope and second set of slides were subjected for Geimsa staining for vaginal cytological study for early pregnancy diagnosis in Kenguri ewes. The accuracy of pregnancy diagnosis was highest (100%) with non-return rate and cervical swab smear and staining on days 24, 34 and 44 whereas accuracy of transabdominal ultrasonography and Punyakoti test was also 100% on day 44 post breeding in Kenguri ewes. The accuracy of pregnancy diagnosis was 66.67% by Punyakoti test on day 34 and 44 trans-abdominal ultrasonography on day 34 and 50.00% on day 24 in Kenguri ewes.

Keywords: Estrous, Diagnosis, Trans-abdominal ultrasonography.

Introduction

Indian breeds of sheep are essentially monotonous and having inherent low fertility rates. Sheep with its utility for wool, meat, skin and manure form an important component of rural economy particularly in arid, semi-arid and mountainous areas of the country. Kenguri is a purely mutton purpose breed of sheep, also known as Tengury (Tengury after the name of coat color, "Teng" meaning coconut). Distribution of this breed is in hilly tracts of Koppal, parts of Gulbarga and Bagalkot districts of Karnataka. Kenguri breed is known for its medium body size and longer legs. Their body color is mostly dark brown along with fleece. The breed is known to thrive well under scarcity condition and sparse vegetation.

The intensive sheep management and wide spread application of the controlled breeding techniques, such as artificial insemination and out of season breeding; increase the need for an accurate test for pregnancy diagnosis. Separation of herd into pregnant and non-pregnant ewes might reduce reproductive loss like abortions, stillbirths and production of weak lambs (Wani *et al.*, 1998). Abdominal palpation and ballottement are only effective during late pregnancy and do not give always-reliable information.

Early pregnancy detection by progesterone estimation or ultrasonography would be profitable for sheep breeders; it enables them to adjust nourishment of pregnant ewes according to the individual needs and good management. Early detection of pregnancy is of considerable economic value in sheep industry. Non-pregnant ewes could be sold, reducing field expenses also marketed at higher prices than they would bring as mature ewes (Gearhart *et al.*, 1988).

The basic methods for pregnancy diagnosis are non-return to estrus, abdominal palpation, abdominal ballottement, palpation of middle uterine artery, laparotomy, peritonoscopy, the rosette-inhibition test (Ishwar, 1995) and cocking of tail; this behavior appears 14 to 15 days after fertile mating.

Non-return to estrus and abdominal ballottement are traditional methods that are not satisfactory and non-return to estrus is affected by anestrus and gestational estrus. In sheep, estrus detection methods are not properly designed to make efficient use of non-return rate to

estrus as a method of pregnancy diagnosis. Failure to return to heat is commonly used as a cheap and simple method of pregnancy diagnosis. Non-return to estrus following breeding may be suggestive of pregnancy, but pathological conditions of the uterus or ovaries, physiological anestrus late in the breeding season and out of breeding season may cause anestrus in non-pregnant ewes and does. In addition, non-return to estrus is an unreliable method when ewes or does are synchronized and bred during the non-breeding season. Among all these pregnancy diagnosis techniques, ultrasonography has special application in Veterinary practice in research and diagnosis (Yeager *et al.*, 1992) [14]. Unlike radiography and laparoscopy, ultrasonography method does not cause any negative effects on fetus or dam. Among this ultrasonography, A-mode and Doppler techniques are past methods and they are considered as non-imaging system (Lindhal, 1969). B-mode ultrasonography is preferred and it is considered as imaging ultrasound system in small ruminants for the diagnosis of pregnancy as well as the fetal numbers (Fowler and Wilkins, 1980 and Tainturier *et al.*, 1983). The use of transabdominal ultrasonography for the detection of pregnancy is preferred in field. The technique of transabdominal ultrasonography has been used with great accuracy as a means for pregnancy diagnosis and estimation of fetal numbers in sheep (Buckrell, 1988; Garcia *et al.*, 1993). Prediction of the number of the fetuses allow appropriate nutritional management of the ewes in late gestation that will prevent the occurrence of pregnancy toxemia (Ford *et al.*, 1963), minimize pre lambing feeding costs, optimize birth weight, survivability of lambs and reduces the incidence of dystocia (Gearhart *et al.*, 1988). Based on ancient Egyptian knowledge, a simple non-invasive bioassay has been developed to diagnose pregnancy called Punyakoti test during 2200 BC (Veena *et al.*, 1997). At that time, this was practiced to diagnose the pregnancy in women by treating wheat and barley seeds with urine of women suspected for pregnancy. Urine samples of pregnant and non-pregnant cattle were collected randomly. Germination tests for Mung beans were performed by diluting the urine with distilled water at the ratio of 1:4, 1:10 and 1:14. Germination is due to the presence of plant hormone known as Abscisic acid which is higher in the urine of pregnant cows (170.62 nanomoles/ml of urine) than non-pregnant cows (74.46 nanomoles/ml).

The cervical mucus examination (Kawase, 1956; McDonald and Rascside, 1958; Hashimoto, 1961) has limited value in sheep flock. The pregnancy associated glycoproteins (PAG) have been isolated from domestic ruminant placenta (Zoli *et al.*, 1992 and 1995 and Garbayo *et al.*, 1998) and radioimmunoassay has been developed for their determination in the maternal plasma progesterone (Zoli *et al.*, 1992, Rannila *et al.*, 1994 and Perenyi *et al.*, 2002) or in the milk (Gonzalez *et al.*, 2000).

By considering the available methods of pregnancy diagnosis, present investigation was planned to study efficacy of four methods for pregnancy diagnosis for its accuracy and safety with following objectives.

1. To detect and compare various methods to diagnose early pregnancy by non-return rate, transabdominal

ultrasonography, Punyakoti test and cervical swab smear and staining.

2. To compare early pregnancy diagnostic techniques with lambing.

Materials and Methods

3.1 Experimental ewes and management

The study was carried out on 24 Kenguri ewes that had previously lambed, in the age group of 3-4 years belonging to sheep unit, ILFC, Veterinary College, Bidar. All the ewes were monitored under uniform management conditions and reared under the semi-intensive housing system. All these Kenguri ewes were divided equally into 4 groups and were subjected to various pregnancy diagnostic methods *Viz.* non-return rate, transabdominal ultrasonography, Punyakoti test and cervical swab smear and staining on days 24, 34 and 44 post breeding.

3.2 Estrus detection and mating

The estrus was detected in all the ewes by parading rams twice in a day early in the morning and evening hours. All the experimental ewes were bred using pen mating system. The ewes detected in the estrus were kept in tupping pens with healthy, fertile, breeding rams. The day of the mating was recorded as day 0, for calculating the approximately gestational stage of the ewe that was also later confirmed from the lambing date.

3.3 Pregnancy diagnosis

Pregnancy diagnosis was initiated on days 24, 34 and 44 of post mating using the respective diagnostic methods until the onset of lambing. The various methods of pregnancy diagnosis used in the ewes are described as below.

3.4 Non-return rate to estrus (Day 24, 34 and 44 after breeding)

All the naturally served Kenguri ewes (n=6) were monitored for estrus exhibition using vasectomised rams on day 24, 34 and 44 post breeding and the ewes which did not show estrus were considered as pregnant.

3.5 Punyakoti test (Day 24, 34 and 44 after breeding)

About 15 seeds of wheat were placed in Petri dish containing filter paper with 15 ml diluted urine (1 ml urine +14 ml water) from the ewes whose pregnancy was to be diagnosed. Similarly, a set of Petri dish containing seeds treated with 15 ml water was served as control. After 5 days, the ewe was diagnosed as pregnant if the seeds have not germinated and have turned blackish in color. This test was carried out on Kenguri ewes (n=6) at days 24, 34 and 44 after breeding in Kenguri ewes.

3.6 Transabdominal ultrasonography (Day 24, 34 and 44 after breeding)

Food and water was withheld overnight for 12 hours before scanning. All the ewes (n=6) were scanned transabdominal thrice on days 24, 34 and 44 by using scanner equipped with probe of 3.5 MHz sector probe (SAMSUNG Sync Master E 1720 Logic Book XP). The ventral abdominal wall was shaved closely and transducer was applied at inguinal

regions of both sides after adding coupling gel. A ewe was designated as pregnant by imaging apparent conceptus (anechoic, elongated structures) within uterine fluid. Accuracy of pregnancy tests was determined by comparing the pregnancy status with lambing.

3.7 Cervical swab smear and staining (Day 24, 34 and 44 after breeding)

Cervical mucus samples were collected from ewes (n=6) on days 24, 34 and 44 after breeding with sterilized cotton swab and vaginal speculum. The clear cervico-vaginal mucus samples with typical fern pattern from 6 animals were subjected for Geimsa staining and the vaginal mucus samples having slight cloudiness or flakes of pus and samples from post estrus bleeding animals were discarded. The collected mucus was smeared on glass slide and air-dried. The microscopic crystallization pattern for each mucus samples was studied, directly after preparation of smear and evaluated as per the method described by Ghannam and Sorenson (1967). The mucus discharge was spread uniformly in thin layers on a glass slide and dried at room temperature. Its shape, appearance and arborization pattern were observed under the low power of the microscope and samples were classified as typical, atypical and nil fern leaves pattern.

A cervico-vaginal mucus smear sample was subjected to Geimsa staining by methanol fixation for 1 minute. After methanol fixation Geimsa stain was added and it was washed after 15-20 minutes. The smear was observed under low and high power magnification and cytology was studied.

3.8 Comparison of different methods of pregnancy diagnosis

The data obtained from all the techniques of pregnancy diagnosis methods was compared for its efficacy and was

confirmed after actual lambing in Kenguri ewes by using standard statistical procedure.

Results

The study entitled "Comparative efficacy of various techniques to detect early pregnancy in Kenguri ewes" was carried out in 24 ewes belonging to Department of ILFC, Veterinary College, Bidar. These ewes were randomly divided into 4 groups. These ewes were monitored for estrous cycle and were bred in estrus period with natural service by rams and were subjected for various pregnancy diagnostic techniques *Viz.* Non-return rate, transabdominal ultrasonography, Punyakoti test and cervical swab smear and staining on days 24, 34 and 44 post breeding. Finally, these early pregnancy diagnostic techniques were compared with actual lambing. The results of various pregnancy diagnostic techniques in Kenguri ewes are presented below.

4.1 Non-return rate to estrus (day 24, 34 and 44 after breeding)

All the naturally served Kenguri ewes (n=6) were monitored for estrus exhibition using vasectomised rams on day 24, 34 and 44 and the ewes who did not show estrus were considered as pregnant.

Out of 6 Kenguri ewes subjected for early pregnancy diagnosis using non-return rate, all six ewes did not exhibit estrus on days 24, 34 and 44 and were considered as pregnant (100%). All the six Kenguri ewes (100%) lambled after completion of gestation period. The sensitivity of the non-return rate and positive predictive value was 100% whereas specificity and negative predictive value was 0% (Table)

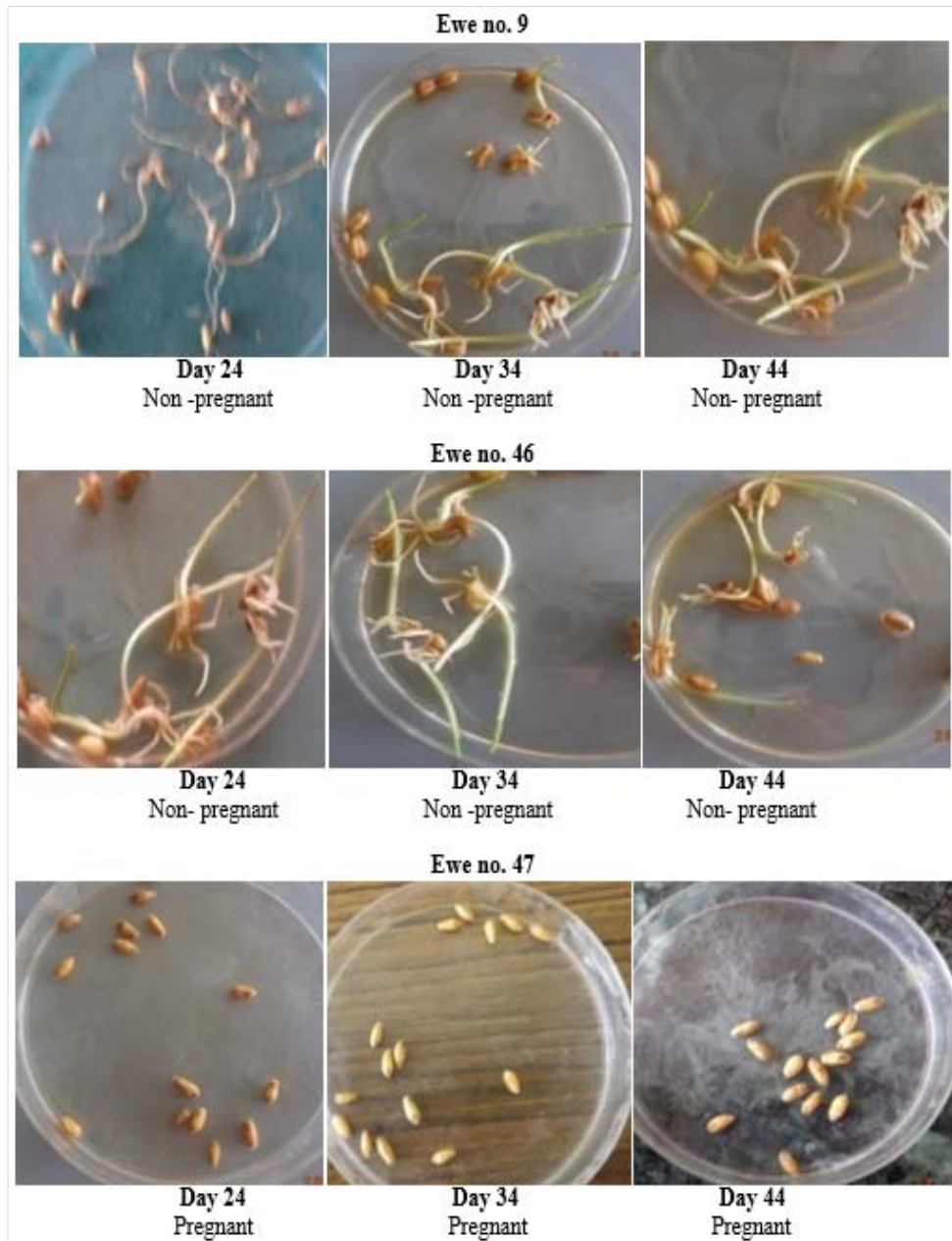


Plate 1 : Efficacy of Punyakoti test on days 24, 34 and 44 post breeding for detection of early pregnancy in Kenguri ewes no. 9, 46 and 47

4.2 Cervical swab smear and staining (Days 24, 34 and 44 post breeding)

The cervical mucus samples were collected from ewes on days 24, 34 and 44 with sterilized cotton swab using vaginal speculum. The collected mucus was smeared on two glass slides and air-dried. First set of smear slides were observed for crystallization pattern under low power objective of phase contrast microscope and second set of slides were subjected for Geimsa staining for cytological study for early pregnancy diagnosis in Kenguri ewes.

Out of 6 Kenguri ewes, 3 were confirmed pregnant (50%) and 3 as non-pregnant (50%) on day 24 post breeding as there was typical fern leaf pattern but there was negative fern leaf pattern on days 34 and 44 post breeding. Whereas, in pregnant Kenguri ewes there was negative fern leaf pattern on days 24, 34 and 44 post breeding. (Plate 3 and 4).

Out of 6 Kenguri ewes, 3 were confirmed pregnant (50.00%) and 3 ewes as non-pregnant (50.00%) on day 24 post breeding as there were clumps of spermatozoa, anuclear cells, superficial cells, intermediate cells and parabasal cells. It indicated that, these ewes were returned to estrus and hence mating with rams has occurred.

On day 24 post breeding in Kenguri ewes, the anuclear cells varied from 60.00% to 90.00% (Av. 75.00%) in pregnant and 2.00% to 10.00% (Av. 5.33%) plus sperm cells in non-pregnant; superficial cells 5.00% to 25.00% (Av. 15.00%) in pregnant and 31.00% to 50.00% (Av. 40.33%) in non-pregnant; intermediate cells 1.00% to 15.00% (Av. 7.33%) in pregnant and 40.00% to 65.00% (53.33%) in non-pregnant and mean parabasal cells 4.00% in pregnant and 3.00% in non-pregnant ewes respectively (Table 4, 5 and Plate 5, 6).

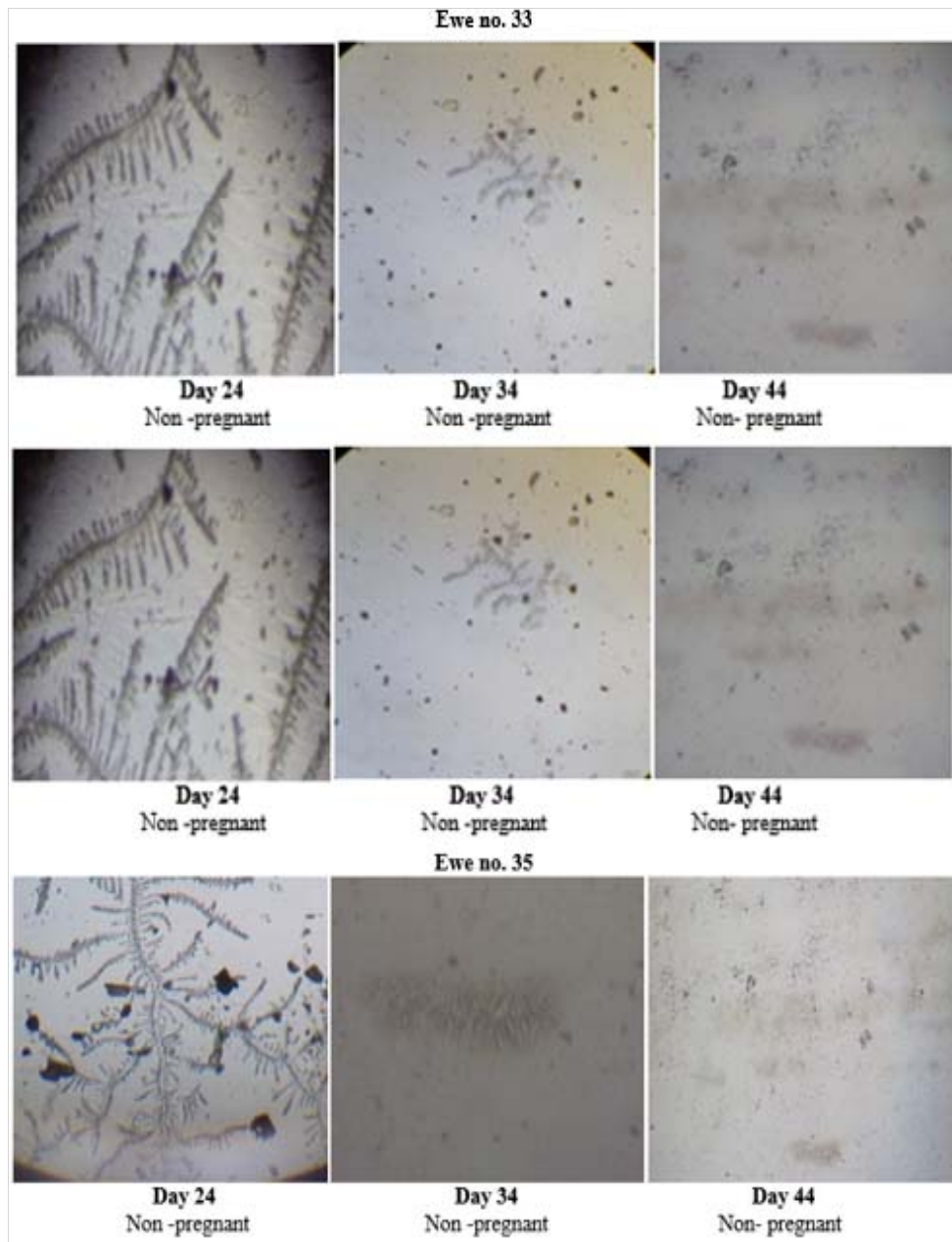


Plate 2: Efficacy of pregnancy diagnosis with cervical swab smear staining on days 24, 34 and 44 post breeding in Kenguri ewes no. 33, 34 and 35

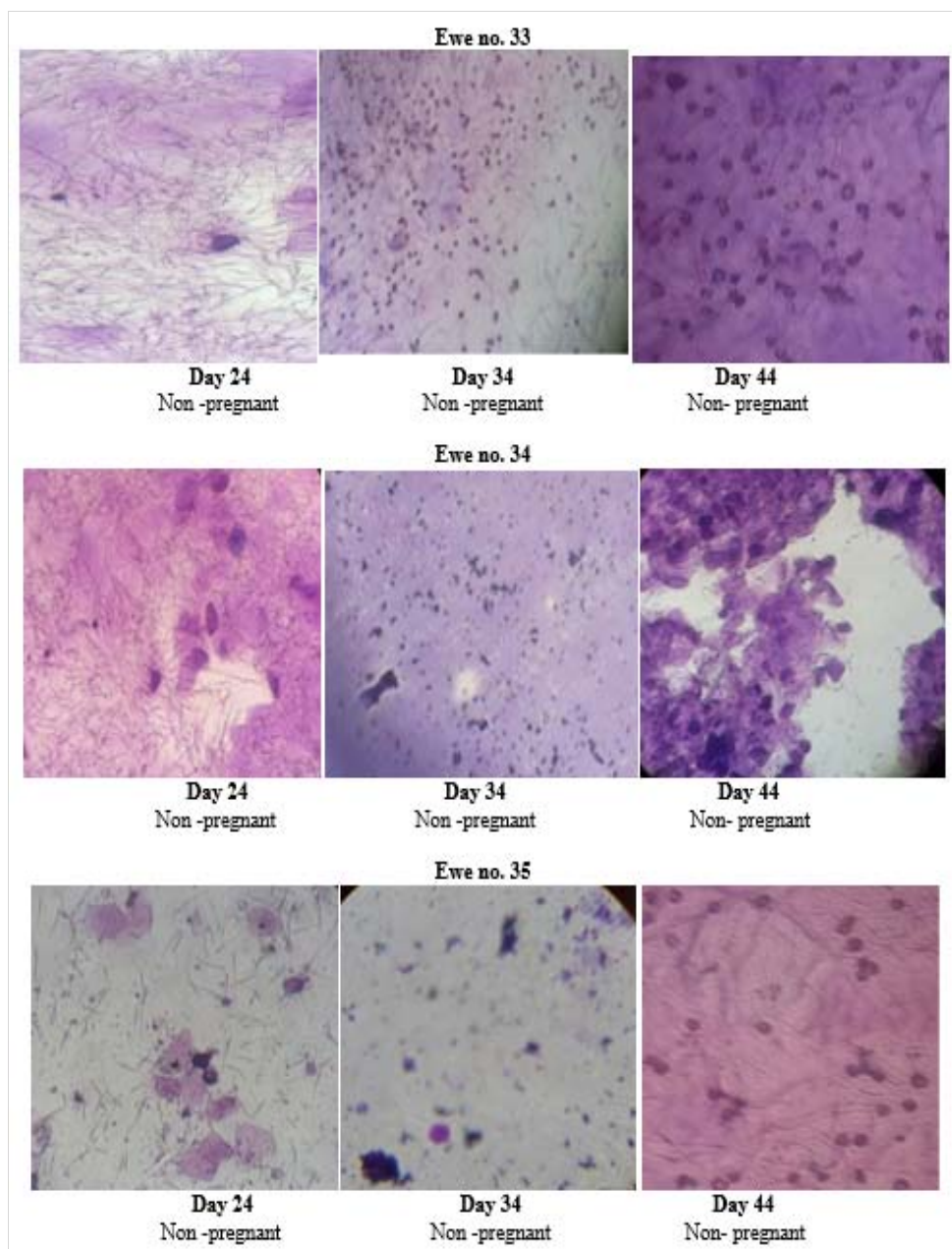


Plate 3: Efficacy of pregnancy diagnosis with cervical swab smear staining on days 24, 34 and 44 post breeding in Kenguri ewes no. 33, 34 and 35

Discussion

The present study entitled “Comparative efficacy of various techniques to detect early pregnancy in Kenguri ewes” was carried out in 24 ewes belonging to Department of ILFC, Veterinary College, Bidar. These ewes were equally and randomly divided into 4 groups. These Kenguri ewes were monitored for estrous cycle and were bred during estrus period with natural service with rams and were subjected for various pregnancy diagnostic techniques *Viz.* Non-return rate, transabdominal ultrasonography, Punyakoti test and cervical swab smear and staining on days 24, 34 and 44 post breeding. Finally, these early pregnancy diagnostic techniques were compared with actual lambing.

5.1 Non-return rate (NRR) to estrus Days 24, 34 and 44 post breeding)

All the naturally served Kenguri ewes (n=6) were monitored for estrus exhibition using vasectomised rams on days 24, 34 and 44 and the ewes which did not show estrus were considered as pregnant.

Out of 6 Kenguri ewes subjected for early pregnancy diagnosis using non-return rate, all six ewes did not exhibit estrus on days 24, 34 and 44 and were considered as pregnant (100%). All the six Kenguri ewes (100%) lambled after completion of gestation period. The sensitivity of the non-return rate and positive predictive value was 100% whereas specificity and negative predictive value was 0%.

The findings of the present study are in line with the report of Das *et al.* (2011) who stated that, non-return to estrus is still the easiest and cheapest method applicable at field and

farm level in sheep. The results of the present investigation are also supported by Martinez *et al.*, (1999) who reported accuracy for detecting pregnancy was greater ($P < 0.05$) for non-return to estrus (89.4%) and then ultrasonography on day 60 (73.7%) then the ultrasonography on day 30 (47.3%) and the abdominal palpation (47.3%). Further, he concluded that, non-return to estrus and doppler ultrasonic on day 60 post-estrus are the most accurate techniques for field determination of pregnancy in Pelibury sheep.

Thompson and Salisbury (1947) stated that, for commercial A.I. an inexpensive method of estimating fertility based on cows not returning for insemination was developed as an essential component of the A.I. programme.

When the animal is mated and it does not return to estrus then the owner usually thinks that the animal has become pregnant. This happens because during pregnancy, the conceptus inhibits the regression of corpus luteum and then, 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. Estrus detection methods used for sheep, goat and mare needs to be properly designed to make efficient use of non-return to estrus as a method of pregnancy diagnosis in these species.

5.2 Trans-abdominal ultrasonography (day 24, 34 and 44 after breeding)

All naturally served Kenguri ewes ($n=6$) were subjected for transabdominal ultrasonography using 3.5 MHz probe on day 24 revealed an enlarged uterine lumen as an anechoic zone located cranially or ventrally to the urinary bladder. Ultrasonography on day 34 revealed fetal heart rate and placentomes. On day 44 post breeding, fetal vertebral column was evident in ewes suggestive of pregnancy.

Out of 6 Kenguri ewes subjected for transabdominal ultrasonography on day 24 post breeding, 3 ewes were confirmed pregnant (50.00%). The sensitivity and positive predictive value was 50.00% whereas the specificity and negative predictive value was 0%. From the initial 3 negative pregnancy diagnoses, were later confirmed pregnant during the next ultrasonography (on days 34 and 44) and actual lambing.

The sensitivity was 66.67% on day 34 while 100% on day 44 of gestation length whereas the positive predictive value was 66.67% on day 34 and 100% on day 44 of gestation period respectively. The specificity and negative predictive value was 0% on days 24, 34 and 44 of gestation using ultrasonography. All the ewes that were confirmed pregnant on day 44 post breeding lambed (100%) after completion of gestation length.

The present findings are in agreement with several researchers (Azzarini, 1998, Buckrell, 1988) reported that, diagnosis of pregnancy can be made about 90% correctly after 45th day of pregnancy using B-mode real time ultrasonography through transabdominal route in sheep. The reasons for false negative results could be from fetal and placental structures escape from notice before 45 days of pregnancy in sheep (Gearhart *et al.*, 1988), displacement of uterus in the pelvis especially in the early pregnancy period in sheep and goat (Haibel, 1990). Furthermore, false negative

diagnosis reported to occur more often during 30-60th days of pregnancy in sheep (Gearhart *et al.*, 1988; Haibel, 1990).

However, Karen *et al.* (2004) concluded that, the accuracy of transabdominal ultrasonography for determination of the fetal numbers in Awassi X Merino crossbred ewes is too low. Garcia *et al.* (1993) concluded that, real time transrectal ultrasonography scanning of sheep between days 24 and 34 gestations often a safe, accurate and practical means for diagnosing pregnancy. In addition to this, Anwar *et al.* (2008) reported that, a 100% accuracy of pregnancy diagnosis in Balkhi ewes can be achieved at day 42 of gestation by transabdominal ultrasonography using a 3.5 MHz probe. Placentomes and leg buds were visible in 100% pregnant ewes between 45 and 50 days of gestation and vertebral column was apparent in 100% ewes between 51 and 55 days of gestation.

The false negative findings could be attributed to the early period of the study (Day 20) when the embryo is commonly not visualized and the enlarged uterine lumen could be due to accumulation of oestral secretion or due to some kind of uterine pathology in cattle (Michel, 1998). Transabdominal ultrasonography with a 5 MHz transducer could be used for detection of early pregnancy in the Stara Zagora dairy sheep breed and its crossbreeds with accuracy of 98% 35 days post insemination. The visualization of enlarged uterine lumen and an embryos size with detection of fetal heart rate were the most accurate criteria for pregnancy diagnosis (Yotov, 2005).

By using transabdominal approach, pregnancy was first verified at day 25 (Gearhart *et al.*, 1988) or day 30 after breeding in sheep and goat (Bretzlaff *et al.*, 1993). The sensitivity and specificity of the technique were high after day 29 of gestation (Taverine *et al.*, 1985) reaching approximately hundred percent from days 46 to 106 of gestation in sheep (White *et al.*, 1984; Fowler and Wilkins, 1984; Davey, 1986; Gearhart *et al.*, 1988). However, Logue *et al.* (1987) reported a lower specificity on days less than 40 to 100 after mating.

5.3 Punyakoti test (Day 24, 34 and 44 after breeding)

All the naturally served Kenguri ewes ($n=6$) were subjected for Punyakoti test for early pregnancy diagnosis by wheat seed germination inhibition technique on days 24, 34 and 44 post breeding.

Out of 6 Kenguri ewes, 2 ewes were confirmed pregnant on days 24 and 34 while 3 ewes were confirmed pregnant on day 44. The sensitivity and positive predictive value was 66.67% on day 24 and 34 while 100% on day 44 post mating. The specificity and negative predictive value was 75% on day 24 and 34 while 100% on day 44 of gestation length. From the initial one negative pregnancy diagnosis that was later confirmed pregnant during the Punyakoti test on day 44 and actual lambing.

Out of 6 Kenguri ewes confirmed pregnant on day 44, 3 ewes lambed (50.00%) after completion of gestation length.

These values appear to be slightly higher compared to those reported by Swamy *et al.* (2010); Dilrukshi and Perera in Malnad Gidda cattle (2009). There is paucity of references in ewes on Punyakoti test hence discussion is related with other animals.

The present findings are in line with Dilrukshi and Perera (2009) who stated that, the germination percentage and shoot length of Mung beans treated with pregnant cow urine reduced significantly to those treated with water. Urine of pregnant cows dramatically inhibited the germination and shoot growth of Mung beans than the non-pregnant cows. This inhibitory effect persists throughout the pregnancy. In cattle urine, apart from the normal urinary constituents such as urea and uric acid, a plant hormone known as Abscisic acid (ABA) has been identified. Its main effect on seeds to maintain their dormancy. A high concentration of ABA acid is found in urine of pregnant cattle. This simple test is based on this hormone. This modified seed germination test is simple, non-invasive, from the animal point of view, and does not require any chemicals or sophisticated instruments. Swami *et al.* (2010) concluded that, the mean germination percentage inhibition and reduced shoot length in positive group of Malnad Gidda cattle was indicative of pregnancy state. Seed inhibition technique is useful to detect pregnancy in Malnad Gidda cattle as a simple and economical method. The mean germination inhibition percentage was 73.65 ± 2.81 , 27.90 ± 2.56 and 21.48 ± 2.69 respectively in positive, negative and control groups.

5.4 Cervical swab smear and staining (days 24, 34 and 44 post breeding)

The cervical mucus samples were collected from ewes on days 24, 34 and 44 with sterilized cotton swab using vaginal speculum. The collected mucus was smeared on two sets of glass slides and air dried. First set of smear slides were observed for crystallization pattern under low power objective of phase contrast microscope and second set of slides were subjected for Geimsa staining for cytological study for early pregnancy diagnosis in Kenguri ewes.

Out of 6 Kenguri ewes, 3 were confirmed pregnant (50%) and 3 as non-pregnant (50%) on day 24 as there was typical fern leaf pattern but there was negative fern leaf pattern on days 34 and 44 post breeding. Whereas, in pregnant Kenguri ewes there was negative fern leaf pattern on days 24, 34 and 44 post breeding.

Out of 6 Kenguri ewes, 3 were confirmed pregnant (50.00%) and 3 ewes as non-pregnant (50.00%) on day 24 as there were clumps of spermatozoa, anuclear cells, superficial cells, intermediate cells and parabasal cells. It indicated that, these ewes were returned to estrus and hence mating with rams has occurred.

On day 24 post breeding in Kenguri ewes, the anuclear cells varied from 60.00% to 90.00% (Av. 75.00%) in pregnant and 2.00% to 10.00% (Av. 5.33%) plus sperm cells in non-pregnant; superficial cells 5.00% to 25.00% (Av. 15.00%) in pregnant and 31.00% to 50.00% (Av. 40.33%) in non-pregnant; intermediate cells 1.00% to 15.00% (Av. 7.33%) in pregnant and 40.00% to 65.00% (53.33%) in non-pregnant and mean parabasal cells 4.00% in pregnant and 3.00% in non-pregnant ewes respectively.

On day 34 after breeding in Kenguri ewes, the anuclear cells varied from 70.00% to 90.00% (Av. 81.66%) in pregnant and 10.00% to 15.00% (Av. 11.66%) in non-pregnant; superficial cells 1.00% to 15.00% (Av. 8.00%) in pregnant and 60.00% to 65.00% (Av. 63.00%) in non-pregnant; intermediate cells

2.00% to 15.00% (Av. 8.5%) in pregnant and 15.00% to 25.00% (Av. 20.00%) in non-pregnant and parabasal cells 14.00% in pregnant and 1.00% to 15.00% (Av. 6.6%) in non-pregnant ewes respectively.

On day 44 post breeding in Kenguri ewes, the anuclear cells varied from 69.00% to 95.00% (Av. 84.66%) in pregnant and 5.00% to 8.00% (Av. 6.33%) in non-pregnant with sperm cells; superficial cells 1.00% to 10.00% (Av. 5.00%) in pregnant and 45.00% to 54.00% (Av. 50.33%) in non-pregnant; intermediate cells 1.00% to 25.00% (Av. 13.00%) in pregnant and 38.00% to 46.00% (Av. 42.00%) in non-pregnant and parabasal cells 5.00% and 4.00% in pregnant and non-pregnant ewes respectively.

As there is paucity of literature in respect of cervical swab and staining in ewes, the present findings of the research work are compared with other animals. The variation in the crystallization pattern of cervical mucus may be due to variation in length of estrus period in non-pregnant and in silent estrus and infections of genital tract, this may be the reason for incorrect diagnosis of pregnancy in cattle (Rebeiro, 2012).

Summary and Conclusion

The study entitled "Comparative efficacy of various techniques to detect early pregnancy in Kenguri ewes" was carried out in 24 ewes belonging to Department of ILFC, Veterinary College, Bidar. These ewes were randomly and equally divided into 4 groups. These ewes were monitored for estrous cycle, were bred in estrus period with natural service, by rams and were subjected for various pregnancy diagnostic techniques *Viz.* Non-return rate, transabdominal ultrasonography, Punyakoti test and cervical swab smear and staining on days 24, 34 and 44 post breeding. Finally, these early pregnancy diagnostic techniques were compared with lambing.

All the naturally served Kenguri ewes (n=6) were monitored for estrus exhibition using vasectomised rams on day 24, 34 and 44 and the ewes which did not show estrus were considered as pregnant.

Out of 6 Kenguri ewes subjected for early pregnancy diagnosis using non-return rate, 6 ewes did not exhibit estrus on days 24, 34 and 44 and were considered as pregnant. All the 6 Kenguri ewes (100%) lambled after completion of gestation period. The sensitivity of the non-return rate test and positive predictive value was 100% whereas specificity and negative predictive value was 0%.

All the naturally served Kenguri ewes (n=6) were subjected for transabdominal ultrasonography using 3.5 MHz probe on day 24 revealed an enlarged uterine lumen as an anechoic zone located cranially or ventrally to the urinary bladder. Ultrasonography on day 34 revealed heart rate and placentomes on day 44, fetal vertebral column was evident in ewes suggestive of pregnancy.

Out of 6 Kenguri ewes subjected for transabdominal ultrasonography on day 24, 3 ewes were confirmed pregnant (50.00%). The sensitivity and positive predictive value was 50.00% whereas the specificity and negative predictive value was 0%. From the initial 3 negative pregnancy diagnoses, 3 were confirmed positive for pregnancy during the next ultrasonography (on day 34 and 44) and actual lambing.

The sensitivity was 66.67% on day 34 while 100% on day 44 of gestation length whereas the positive predictive value was 66.67% on day 34 and 100% on day 44 of gestation period respectively. The specificity and negative predictive value was 0% on days 24, 34 and 44 of gestation using ultrasonography. All the ewes that were confirmed pregnant on day 44 post breeding lambled (100%) after completion of gestation length.

All the naturally served Kenguri ewes (n=6) were subjected for Punyakoti test for early pregnancy diagnosis by wheat seed germination inhibition technique on days 24, 34 and 44 post breeding.

Out of 6 Kenguri ewes, 2 ewes were confirmed pregnant on days 24 and 34 while 3 ewes were confirmed pregnant on day 44. The sensitivity and positive predictive value was 66.67% on day 24 and 34 while 50% on day 44 post mating. The specificity and negative predictive value was 75% on day 24 and 34 while 0% on day 44 of gestation length. From the initial one negative pregnancy diagnosis was later confirmed pregnant during the Punyakoti test on day 44 and actual lambing.

Out of 6 Kenguri ewes confirmed pregnant on day 44, 3 ewes lambled (50.00%) after completion of gestation length. The cervical mucus samples were collected from ewes on days 24, 34 and 44 with sterilized cotton swab using vaginal speculum. The collected mucus was smeared on two sets of glass slides and air-dried. First set of smear slides were observed for crystallization pattern under low power objective of phase contrast microscope and second set of slides were subjected for Geimsa staining for cytological study for early pregnancy diagnosis in Kenguri ewes.

Out of 6 Kenguri ewes, 3 were confirmed pregnant (50%) and 3 as non-pregnant (50%) on day 24 as there was typical fern leaf pattern but there was negative fern leaf pattern on days 34 and 44 post breeding. Whereas, in pregnant Kenguri ewes there was negative fern leaf pattern on days 24, 34 and 44 post breeding.

Out of 6 Kenguri ewes, 3 were confirmed pregnant (50.00%) and 3 ewes as non-pregnant (50.00%) on day 24 as there were clumps of spermatozoa, anuclear cells, superficial cells, intermediate cells and parabasal cells. It indicated that, these ewes were returned to estrus and hence mating with rams had occurred.

On day 24 post breeding in Kenguri ewes, the anuclear cells varied from 60.00% to 90.00% (Av. 75.00%) in pregnant and 2.00% to 10.00% (Av. 5.33%) plus sperm cells in non-pregnant. Superficial cells 5.00% to 25.00% (Av. 15.00%) in pregnant and 31.00% to 50.00% (Av. 40.33%) in non-pregnant. Intermediate cells 1.00% to 15.00% (Av. 7.33%) in pregnant and 40.00% to 65.00% (53.33%) in non-pregnant. Mean parabasal cells 4.00% in pregnant and 3.00% in non-pregnant ewes respectively.

On day 34 after breeding in Kenguri ewes, the anuclear cells varied from 70.00% to 90.00% (Av. 81.66%) in pregnant and 10.00% to 15.00% (Av. 11.66%) in non-pregnant. Superficial cells 1.00% to 15.00% (Av. 8.00%) in pregnant and 60.00% to 65.00% (Av. 63.00%) in non-pregnant. Intermediate cells 2.00% to 15.00% (Av. 8.5%) in pregnant and 15.00% to 25.00% (Av. 20.00%) in non-pregnant.

Parabasal cells 14.00% in pregnant and 1.00% to 15.00% (Av. 6.6%) in non-pregnant ewes respectively.

On day 44 post breeding in Kenguri ewes, the anuclear cells varied from 69.00% to 95.00% (Av. 84.66%) in pregnant and 5.00% to 8.00% (Av. 6.33%) in non-pregnant with sperm cells. Superficial cells 1.00% to 10.00% (Av. 5.00%) in pregnant and 45.00% to 54.00% (Av. 50.33%) in non-pregnant. Intermediate cells 1.00% to 25.00% (Av. 13.00%) in pregnant and 38.00% to 46.00% (Av. 42.00%) in non-pregnant. Parabasal cells 5.00% and 4.00% in pregnant and non-pregnant ewes respectively.

The accuracy of pregnancy diagnosis was highest (100%) with non-return rate and cervical swab smear and staining on days 24, 34 and 44 whereas accuracy of transabdominal ultrasonography and Punyakoti test was also 100% on day 44 post breeding in Kenguri ewes. The accuracy of pregnancy diagnosis was 66.67% by Punyakoti test on day 34 and 44 trans-abdominal ultrasonography on day 34 and 50.00% on day 24 in Kenguri ewes.

The following conclusions were drawn from the present research investigation.

1. Non-return rate is cheap, simple and effective method of early pregnancy diagnosis in Kenguri ewes. Furthermore, the measurement of non-return rate can provide reliable up-to-date information for evaluating sheep flock fertility. The ewes that returned to estrus can be identified and mated immediately.
2. Transabdominal ultrasonography on day 44 post breeding was accurate, rapid, non-invasive, potential and safe technique than day 24 and 34 for early pregnancy diagnosis in Kenguri ewes. The visualization of an enlarged uterine lumen and an embryo's size with detection of fetal heart beats was the most accurate criteria.
3. Punyakoti test is less accurate in detecting early pregnancy in Kenguri ewes on days 24 and 34 post breeding. However, it may serve as a simple, non-invasive, dependable, user friendly, economical and doorstep technique on day 44 for early pregnancy diagnosis in Kenguri ewes.
4. Increased percentage of anuclear cells and decreased percentage of superficial and intermediate cells in cervical mucus on days 24, 34 and 44 post breeding is indicative of pregnancy whereas increased percentage of superficial and intermediate cells was indicative of non-pregnancy in Kenguri ewes.
5. Cervical swab smear and Geimsa staining are also accurate in detecting early pregnancy on days 24, 34 and 44 post breeding in Kenguri ewes.

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