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## Ultrasonographical diagnosis of pregnancy in sheep at an organized farm

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### Abstract

The present study was aimed to assess pregnancy and embryonic mortality in ewes. Transrectal real-time B-mode ultrasonography (USG) was carried out on day 28 post-mating and pregnancy was confirmed by the presence of amniotic vesicle, or fetal image and embryonic heartbeat. Further, the USG was repeated on day 45 post-mating to estimate the embryonic mortalities and overall pregnancy rate. Out of 210 ewes on 28<sup>th</sup> day, 181 (86.19%) ewes were diagnosed as pregnant. On day 45<sup>th</sup> post-mating, The overall pregnancy rate was 82.85%, whereas the embryonic mortality was 3.86%. The transrectal real-time B- mode ultrasonography appeared to be safe, quick and convenient mean of pregnancy diagnosis in sheep.

**Keywords:** Pregnancy diagnosis, ewes, ultrasonography, embryonic mortality

### Introduction

Ewes are seasonally polyestrous animals, and a fertile ewe is desired to be pregnant as early as possible during the limited breeding season. Early diagnosis of pregnancy is of considerable economic value to the sheep industry. Improved control over the nutrition of ewes in late gestation would optimize birth weights, weaning weights and survivability of lambs (Scott, 1986) [6]. Early pregnancy detection may provide several important advantages in sheep flock management: (i) non-pregnant ewes can be quickly re-inseminated; (ii) it facilitates culling or selling decisions; (iii) it decreases feeding and managing expenses by detecting infertile ewes in flocks; (iv) feeding, vaccination, weaning, and marketing can be programmed more effectively; and (v) it provides an opportunity for assisted reproductive techniques such as laparoscopic artificial insemination and embryo transfer in valuable commercial sheep flocks (Ishwar, 1995) [3]. Ultrasound imaging is one of the most widely used powerful tools in animal diagnostics and research, which allows the real-time visualization of internal organs, tissues, structures or fluid accumulations and the investigation of blood flow without any radiation risk. It depends on the computerized analysis of reflected ultrasound waves. Due to its non- invasive character and apparent high safety, it has become one of the most rapidly advancing technologies in the clinical fields. An ideal pregnancy diagnosis method should be non- invasive, cheap, reliable, practical, repeatable, and easily applied during early pregnancy (Ishwar, 1995) [3]. Currently, the preferred method in field conditions is real-time transrectal/transabdominal ultrasonography. The main advantage of transrectal USG over transabdominal USG is earlier detection of pregnancy (Ishwar, 1995) [3]. After this first sign, the reliability of the ultrasound based diagnosis reaches 100% with observation of the embryo and its heart beat in the next few days (20 days after mating) (Schrack and Inskeep, 1993) [5].

### Materials and Methods

**Place of study:** This study was conducted at Central Sheep Breeding Farm, Hisar, (Haryana) India. The institute is located at latitude 29° N and longitude 75° E with average elevation of 215 m from the sea level. The institute is located at the place where mainly continental climatic conditions are present with a significant annual variation in the temperature during summers and winters. A total of 210 ewes in estrus detected by ram parading were subjected to natural mating with proven rams. Transrectal real-time B-mode ultrasonography (USG) was carried out on day 28 post-mating and pregnancy was confirmed by the presence of

amniotic vesicle, or embryo/fetal image and embryonic/heart-beat. Further, the USG was repeated on day 45 post-mating to estimate the embryonic mortalities and overall pregnancy rate.

**Ultrasonographic scanning:** For the ultrasonographic study of reproductive status and early pregnancy diagnosis, a real time B-mode portable Ultrasonography machine (SONPSCAPE S6/S6Pro/S6BW Portable Digital Color Doppler Ultrasound System) equipped with a linear array trans-rectal multi-frequency transducer using frequency of 5-7 MHz was used. The urinary bladder was the point of reference during trans-rectal ultrasonography.

#### Technique for Imaging

The ewes were restrained in dorsal recumbency. Corboxy gel (coupling gel) was applied on active portion of probe to minimize the air gap and also for lubrication. The rectal probe was inserted in to the rectum, and firstly, the urinary bladder was located that served as a point of reference for scanning of reproductive tract. The probe was rotated in clockwise and anti-clockwise direction to examine the both uterine horns and ovaries. Uterine horns were observed just at cranial aspect of urinary bladder. After proper imaging of scanned structures, the images were freezed and the pictures were taken using mobile phone. Hardly 2-3 minutes were taken per ewe for detailed scanning (Fig.1).

**Pregnancy rate (%):** Pregnancy rate (%) was calculated by calculating the number of pregnant animals divided by total number of animals multiplied by hundred (Paula *et al.*, 2020) [7].

**Embryonic mortality:** The embryonic mortality (%) was calculated as the difference in number of ewes that were diagnosed as pregnant on day 28 post-mating and non-pregnant on day 45<sup>th</sup> post-mating, multiplied by 100 and divided by the number of ewes pregnant on day 28<sup>th</sup> post-mating.

#### Results and Discussion

Out of 210 was on 28<sup>th</sup> day post-mating, 181 (86.19%) ewes were diagnosed as pregnant. Subsequently on day 45<sup>th</sup> post-mating, 174 (82.85%) ewes were reconfirmed pregnant and embryonic mortality rate was 3.86%. Pregnancy diagnosis at first schedule of examination (day 28<sup>th</sup> post-mating) was done by observation of circular anechoic areas in the uterine

lumen and embryo proper (Fig.4). At second schedule of examination (45 days), the fetuses were identified easily (Fig. 5, Table. 1). The diagnosis of pregnancy was very fast (about 2-3 minutes) by observation of fluid in the uterine lumen, embryo proper/fetus and concave placentomes

The ovine pregnancy could be diagnosed at an earliest day of 17-19 of gestation based on presence of anechoic intrauterine fluid by trans-rectal USG (5 MHz) while the embryo proper could be imaged between days 21 to 34 of gestation, with placentomes visualization on day 26-28 (Garcia *et al.*, 1993) [9]. The heart beat can be detected as early as the 18<sup>th</sup> or 19<sup>th</sup> day of pregnancy, heart beat may not be observed in all pregnant sheep until as late as day 23 (Martinez *et al.*, 1998) [8]. The position of the female will affect the distance between the uterus and the transducer, therefore, affecting the quality and accuracy of the scanned images. Compared with transabdominal application, transrectal application allows for earlier pregnancy diagnosis, due to the shorter distance between the uterus and probe. A transducer with high frequency generates a better image resolution, but has a shallower depth of penetration. Therefore, close proximity of the uterus to the rectal wall during transrectal ultrasonography will improve the visualization and scanning efficiency (Griffin and Ginther, 1992) [2]. It is established that embryos may be observed from day 28 (Picazo *et al.*, 1991) [10] and absence of embryonic vesicle, amniotic fluid and absence of detectable heart rate in fetuses on day 45 post-mating are diagnostic features for late embryonic mortality (Yadav *et al.*, 2020) [11].

Results of this study indicated that transrectal ultrasonographic examination in dorsal recumbency is a reliable method for pregnancy diagnosis in ewes, and give high accuracy reaching to 100% especially after 45 days of natural mating (Buckrell *et al.*, 1986) [1]. The accuracy of transrectal ultrasonography is higher than those of transabdominal ultrasonography (Kahn, 1992) [4]. Although factors causing early embryonic mortality in sheep are not well established, there is some evidence suggesting the involvement of luteal inadequacy, resulting from environmental factors such as heat stress or nutrition, has been shown to be a major cause of embryonic loss in sheep (Binelli *et al.*, 2001) [12]. The findings of the present study indicated that the early pregnancy diagnosis through transrectal ultrasonography could be a mean for assessment of late embryonic deaths occurring post maternal recognition of pregnancy.

Embryo proper with heart rate

Total number of animals subjected to natural mating	No. of ewes pregnant on 28 <sup>th</sup> day post mating	Percentage of pregnancy on day 28 <sup>th</sup> post-mating	No of ewes pregnant on 45 <sup>th</sup> day post-mating	Overall pregnancy percentage on day 45 <sup>th</sup> post-mating	Embryonic mortality detected on day 45 <sup>th</sup>	% Embryonic mortality
210	181	86.19	174	82.85	7	3.86



**Fig 1:** Trans-rectal ultrasonography in dorsal rectal



**Fig 5:** Embryo proper with heart rate



**Fig 2:** Image depicting non-pregnant uterine horn



**Fig 3:** Concave Placentomes



**Fig 4:** Image depicting pregnant uterine horn on day 28<sup>th</sup> post-mating

**Conclusion**

The study concluded that real-time B-mode ultrasonography is the earliest, most accurate, safest, faster and economical method of pregnancy diagnosis in sheep at farm level. The embryonic heart beat was first detected via transrectal scanning on day 28<sup>th</sup> and reconfirmation was attempted on day 45<sup>th</sup> post-mating. The characteristic cotyledonary placentation of ruminants was easily recognizable when examining sheep transrectally facilitated a rapid, accurate determination of pregnancy status.

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