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Research Article

Islamic Azad University
Kazerun Branch
Fars, Iran

# The Study of Pregnancy Detection and Fetal Growth by Ultrasound in Shin Bash Sheep

**Foad Sadi*, Salahaddin Khodamoradi**

Department of Clinical Sciences, Faculty of Veterinary Medicine, Mahabad Branch, Islamic Azad University, Mahabad, Iran

Received: 13/Sep/2023 Revised: 22/Nov/2023 Accepted: 29/Nov/2023

**Abstract**

**Background and aim:** The aim of this study was to obtain first time diagnosis of pregnancy and study of fetal development in different times of pregnancy period.
**Materials and Methods:** 2D ultrasound was performed from day 25 to 120 of gestation in 10 Shin Bash sheep, twice in week from day 25 to 65 and once in week from day 65 to 120 of gestation on eight sheeps. The ultrasonographic images were obtained Sonosite Titan (USA) 2D ultrasound machine.
**Results:** On the 25th day of gestation, earliest diagnosis of pregnancy was done. On 37th day, clear pictures of conceptus, amniotic membrane, and umbilicus were seen. On 76th day of gestation, internal organs of fetus heart, kidney, liver, urinary bladder, and stomach was seen in image. The scrotum in the male fetus was identified on the 85th day of gestation. Between 109 and 118 days of gestation complete details of internal organs were seen in ultrasonographic images.
**Conclusions:** Transrectal probe performed better than the transabdominal probe to detect pregnancy. The accuracy of ultrasound was 100% for detecting pregnant and non-pregnant cases. Conceptus changed its shape from 25 to 41 days of gestation, and full identifiable conceptus took its shape on day 41.

**Keywords:** *Pregnancy, Shin Bash, Ultrasound*

***Cite this article as:*** Foad Sadi, Salahaddin Khodamoradi. The study of pregnancy detection and fetal growth by ultrasound in Shin Bash sheep. J Altern Vet Med. 2024; 7(22): 1314-1320.



**** Corresponding Author***
*Department of Clinical Sciences, Faculty of Veterinary Medicine, Mahabad Branch, Islamic Azad University, Mahabad, Iran.*
*E-mail: foadsadi@yahoo.com, Orcid: https://orcid.org/0000-0001-4717-3332*

## Introduction

Shin Bash sheep are breed in the south of West Azerbaijan province, especially in the cities of Mahabad and Piranshahr, and its population is about 200,000. It seems that the Shin Besh is of great economic importance for local breeders due to its large size in terms of milk and meat production, but due to the unknown production performance and also mixing with other breeds in the region, its population is decreasing. Shin Bash in Kurdish means blue, white forehead, so the color of this breed is dark blue and has a white forehead. Because this mountainous region has very cold winters, this breed is resistant to cold weather and has hands and the feet are suitable for walking in mountainous areas (Javanrouh & Khodamoradi, 2022**).**

Pregnancy diagnosis in sheep usually is done using some form of ultrasonography, since rectal palpation is impractical due to the size of the animals. Linear-array ultrasonography is a common method because it allows the operator to view the fetus, determine fetal number and collect measurements of the fetus to determine fetal age (Griffin & Ginther, 1992; Romano & Christians, 2008; Karen *et al.,* 2008). One measurement that is commonly used to estimate fetal age is crown-rump length (CRL). In addition to CRL, other measurements used to estimate fetal age in sheep include head width and thoracic sheep include head width and thoracic depth and biparietal skull and body trunk diameter (Sergeev *et al.,* 1990; Aiumlamai *et al.,* 1992). Neither of these studies indicated that there was an effect of fetal number on the estimated age of the fetus based on the measurements collected. Diagnostic ultrasonography is a valuable alternating image system that can provide more accurate information about pregnancy and reproductive disorders in comparison to all traditional methods (Lee *et al.,* 2005; Padilla-Rivas *et al.,* 2005). Early pregnancy diagnosis and fetal quantification through ultrasonography contribute to rationalize management and bring financial benefits to sheep production (Martinez *et al.,* 1998; Medan et al., 2004).

## Materials and Methods

Ten healthy pregnant sheep of Shin Bash breed approximately 4 years of age having a history of normal reproductive performance were selected for the study. In all the animals, pregnancy was through natural mating. They were kept on grazing as well stall feeding. Ultrasonography was conducted from days 25 and continued till 120 days of gestation. The ultrasonography was conducted 2 times in a week from day 25 to day 65 and after this; the scanning was done once in a week. 2D ultrasonography were performed on each examination. No sedation was given to animals. The lower ventral and lateral abdomen area around teats of the sheeps were shaved, and the sheeps were positioned in lateral recumbency. There was no period of fasting before transrectal or transabdominal scannings. Ultrasonographic examinations were conducted using real-time ultrasound scanner equipped with a linear array 7.5 MHz transrectal scanner and a convex 5.0 MHz transabdominal.

The ultrasound machine used for this study was 2D ultrasound machine Sonosite Titan Equipment (USA). The 7.5 MHz transducer was well-lubricated attaed to the tip of a rigid extension rod was introduced. The transducer was inserted gently until the urinary bladder was identifiable. Probe was moved gently forward and back ward rotating it 90 degrees clockwise and counter clockwise. In conducting transabdominal ultrasonography, the contact fluid (lubricant) was applied to the test side, area of 150 to 200 cm2 on the right flank above the under after removing the hairs over it. Then, the transducer was placed at the right side of the sheep, 5.0 cm in front of the rear leg and 2.5 cm above the teat. Pregnant and non-pregnant sheeps were determined using real-time monitor by fetal heart, spinal cord, limbs, and other fetal structures. The study was carried out to identify images of fetus and related images in goats throughout pregnancy using both probes and to compare the efficacy of 7.5 MHz tranrectal and 5.0 MHz transabdominal probes. The efficacy of both probes was determined by the frequency of occurrence of the observed structures. Differences at a $p<5\%$ ($p<0.05$) were considered to be statistically significant. All statistical analysis were performed using the SPSS (26.0) system for windows.

## Results

In the current study, it was found that 2D ultrasonography was easily applicable without any significant risk to conduct study in pregnant uterus. Pregnancy was assessed as positive on day 25 to 30 of

gestation by observing a small non-echogenic vesicle of 0.75 cm diameter with the help of per-rectal probe using 7.5 MHz frequency, however, on this day only uterus was enlarged and accumulation of fluid was seen, but there was no sign of conceptus (Figure1). On 32th day of pregnancy, the conceptus was seen as oblong shaped in 2D scanning, showing attachment to side of membrane.

It was easily identified as anechoic structure with beating heart. The uterine layers were also clearly visible. The earliest detection of placentome by transrectal sonography as circular echoic structure facing toward the fetus was made on 36th day and in 2D scan, the amount of uterine fluid increased showing wavy margins of endometrium. Details of fetal attachment were clearly seen, on this day in matters of details of the uterus. The earliest detection of placentome by transrectal ultrasonography as circular echoic structure facing toward the fetus was made on day 33 and measured up to day 95 with the help of transabdominal approach. The placentome diameter was increased significantly during whole observation ($p<0.05$). On the 39th day, clear pictures of the fetus and hyperechoic amniotic membrane were seen.

Umbilicus was seen on day 37 of gestation. On 41nd day of pregnancy, the fetus was seen in the uterine lumen with easily identifiable head, ear-buds, folded forelimbs, and proper trunk in 2D scanning.
Dorsal side of the fetus was surrounded by a thick band of the placenta from head to tail. Head, ear, forelimbs, umbilicus, and forelimbs were seen. Full details of the uterine structures including placenta and fetal attachments were seen. On 48th day of gestation, skull, rib cage, the spinal cord of the fetus, forelimbs, hind limbs, and other bony structures were seen in the 2D image. To view the full fetus, the transducer was positioned between thighs and udder of the dam, however, with a slight pressure of transducer, fetus quickly changed its position. The fetus was mobile at this stage and by focusing on the fetus; rumination like the movement of mouth parts of the fetus was seen. Fetus and its body parts were identifiable with the help of 2D scanning on this day. The details of placenta toward limb and thickness of endometrium on one side of the fetus were seen while a broadsheet of placenta was seen on other side of the fetus. Depression of concave shaped cotyledons was also seen on the flat placenta.

On the 54th day of gestation, placenta, fetus, and the endometrium were clearly visible. In 2D scan, fetus showed movements in the fetal fluid. Fetus enlarged in size, and it was difficult to get full fetus in one frame. Therefore, the fetus was imaged in parts.

The head of fetus and rest of body were embedded in anechoic fetal fluid and to image the fetal head, transducer needed to focus on head area for quite some time. Details of placental attachment to the endometrium were also seen. The endometrium was contracted on this structure in wavy form. At the 66th day of gestation in 2D scanning, it was found difficult to get full fetus in one scan, therefore, fetal trunk area or head was focused at one time. Further in this figure, surface top-view of the fetus showed fetal limb, abdomen, hind limbs, and part of the head. It appeared that the attachment of the fetal membrane to endometrium was not simple, but complicated. There were elongated and round projections on membranes that extended from endometrium over to the head.

The endometrium was also wavy, leaving spaces in between. The 2D image showed greater details of organs on the 76th day of gestation. On this day internal organs *viz* heart, kidney, urinary bladder, stomach, and liver of fetus were easily identifiable (Figure 2).

In the present observations, there was a rapid growth of internal organs around and after day 76 of gestation. The scrotum in the male fetus was identified on the 85nd day of gestation. In earlier studies, genital tubercle has been reported by various investigators. Between 109 and 118 days of gestation complete details of the fetal stomach, lung, heart, liver, gall bladder, kidney, and urinary bludder were seen in 2D ultrasonographic images (Figure 2).

**Discussion**

In most pregnancies, ultrasounds are positive experiences and pregnancy care providers do not find any problems. The stage of gestation at the time of examination was calculated from the date of mating. In the present study, pregnancy was observed as a small non-echogenic vesicle of 0.75 cm diameter on day 25 of gestation with the help of per-rectal probe with 7.5 MHz frequency. This is in agreement with Padilla-Rivas et al. (Padilla-Rivas *et al.,* 2005). The investigators reported a small non-echogenic vesicle of about 1 cm in diameter in the uterine lumen by day 28 (Martinez *et al.,* 1998). Similarly, Medan et

al. reported the appearance of a circular or elongated gestational sac in the uterine lumen on days 20.2±0.6 of pregnancy (Medan *et al.,* 2004). It is concluded that the early detection of pregnancy in sheep is possible around day 25. In the present study, the accuracy of ultrasound was 100% for detecting pregnant and

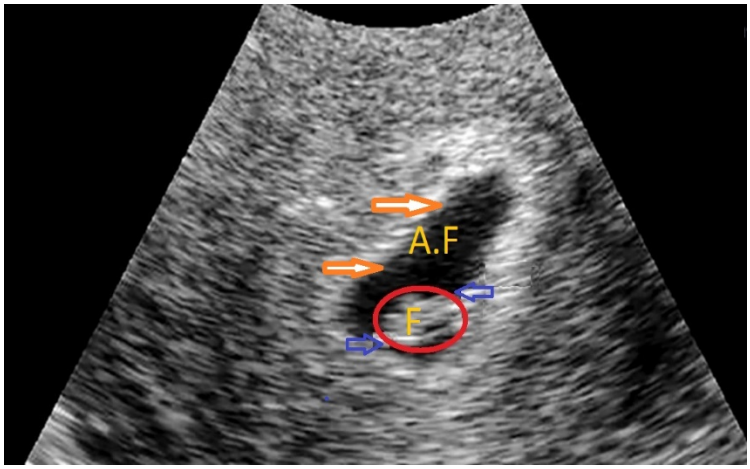


**Figure 1.** Transrectal ultrasonogram of uterus of sheeps at day 25 to 30 of gestation with a 7.5 MHz transrectal transducer showed accumulation of anechoic fluid (A.F) and fetus (F) in uterus.

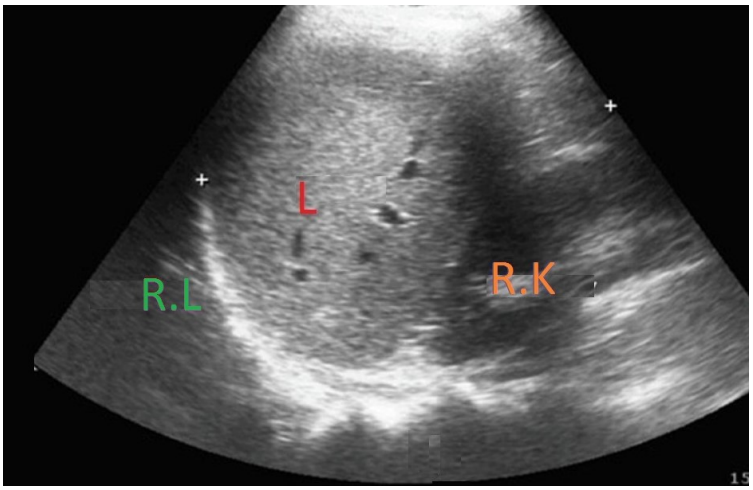


**Figure 2.** Images of fetal organs: (B) Liver, (R.K) Right Kidney and (R.L) Right Lung is visible in 2D ultrasonogram at days 109 to 118 of pregnancy.

non-pregnant cases. Almost all previous investigators have used transabdominal ultrasonography for pregnancy detection in sheep and goat except Martinez et al. and Sadi used transrectal transducer for pregnancy diagnosis (Martinez *et al.,* 1998; Sadi, 2019).

This transabdominal detection in the present study was 4 days earlier than observation of Martinez et al (Martinez *et al.,* 1998).

Heartbeats were detected on day 25 in the present study, which is in agreement with the study of Medan et al., Martinez et al. and with most of other authors who reported heart beats around this time of pregnancy in sheeps (Padilla-Rivas *et al.,* 2005; Martinez *et al.,* 1998; Medan *et al.,* 2004; Omontesea *et al.,* 2012). On the 30th day of pregnancy, the fetus was surrounded by anechoic fluid and its location was toward the cranial side of urinary bladder, which is in agreement with the study conducted by Martinez et al (Martinez *et al.,* 1998). The earliest detection of placentome by transrectal ultrasonography as circular echoic structure facing toward the fetus was made on

day 35 and measured up to day 98 with the help of transabdominal approach. Other investigators reported that placentomes appear first as small echogenic densities in the wall of the uterus at days 26-28 of gestation (Buckrell *et al.,* 1986; Kasikci *et al.,* 2011). In some studies, placentomes were recognized clearly at 7 weeks of gestation or at day 38 of gestation (Padilla-Rivas *et al.,* 2005). These investigators reported the presence of umbilical cord on the 30th day of gestation. On 49th day of gestation, skull, rib cage, the spinal cord of fetus, forelimbs, hind limbs, and other bony structures were seen in 2D image which was 1-week earlier than Suguna et al (Suguna *et al.,* 2008). Medan et al. also reported that skeletal structures were obvious at 2 months of pregnancy, almost 12 days later that present observations (Padilla-Rivas *et al.,* 2005). The fetus was mobile at this stage and by focusing on the fetus; rumination like the movement of mouth parts of the fetus was seen.

Also reported movement of body part of the fetus on day 39 after conception in the sheep (Chandolia *et al.,* 2005). In The 2D images had reported that the placentome increases in size and appeared as a 'C' or 'O' shaped (Lee *et al.,* 2005). The 2nd month of pregnancy has been reported to be the best period for imaging placentomes (Suguna *et al.,* 2008). On 75th day of gestation, both 2D images showed greater details of organs *viz* heart, kidney, urinary bladder, stomach, and liver of the fetus. There is no parallel study, reported on 3D ultrasonography. Considering that goat and sheep are both small ruminants, then the results of the research conducted by Sadi in 2019 on the Markhoz goat are close to the results of the current research (Sadi, 2019). Matsas had also reported that the fetal skeleton grows rapidly between second and 3rd month of gestation (Matsas, 1997). The scrotum in the male fetus was identified on the 82nd day of gestation. In earlier studies, genital tubercle has been reported by various investigators.Ramphal has reported genital tubercle in ram at day 53 of gestation and scrotum on day 90 of gestation (Ramphal, 2000). Lack of more reports on this area could be due to less work on advanced pregnancy. Also reported that the fetal sex can be determined best in sagittal or cross-sectional position in buffaloes (Yotov *et al.,* 2011).

Between 110 and 120 days of gestation complete details of fetal stomach, heart, liver, gall bladder, kidney, and urinary bladder were seen in 2D ultrasonographic images. These findings are similar to the research conducted by Sadi in 2022 on Kurdish sheep but there is no previous parallel study in this area (Sadi, 2022).

## Conclusion

Transrectal probe performed better than the transabdominal probe to detect pregnancy.Images of internal organs of the fetus were viewed in details in 2D images, which might be used as a future guide for antenatal assessment of normal conceptus.

## Conflict of interest

There is no conflict of interest.

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# بررسی تشخیص آبستنی و رشد جنین به وسیله اولتراسونوگرافی در گوسفند نژاد شین باش

**فواد سعدی*، صلاح الدین خدامرادی**

گروه علوم بالینی، دانشکده دامپزشکی، واحد مهاباد، دانشگاه آزاد اسلامی، مهاباد، ایران

تاریخ دریافت: ۱۴۰۳/۰۶/۲۳ اصلاح نهایی: ۱۴۰۳/۰۹/۰۲ تاریخ پذیرش: ۱۴۰۳/۰۹/۰۹

## چکیده

**زمینه و هدف:** هدف از این مطالعه به دست آوردن اولین زمان تشخیص آبستنی و تصاویر حاصل از پیشرفت حاملگی در زمانهای مختلف در طول دوره حاملگی به وسیله اولتراسونوگرافی بود.

**مواد و روش‌ها:** این مطالعه به وسیله اولتراسونوگرافی دو بعدی از روز ۲۵ تا ۱۲۰ آبستنی در ۱۰ راس گوسفند شین باش انجام گردید که از روز ۲۵ تا ۶۵ آبستنی به صورت دو بار در هفته و از روز ۶۵ تا ۱۲۰ به صورت یک بار در هفته انجام گردید. این بررسی به وسیله دستگاه اولتراسونوگرافی دوبعدی به نام سونو سایت تیتان آمریکا انجام گردید.

**یافته‌ها:** از روز ۲۵ آبستنی تشخیص داده شد. سپس در روز ۳۷ تصاویر مناسبی مانند پرده آمینیوتیک و بند ناف مشاهده شد. در ادامه در روز ۷۶ حاملگی تصاویر اعضای داخلی جنین مانند قلب،کلیه،کبد و مثانه رویت گردید. در روز ۸۵ تصویر اسکروتوم جنین نر مشاهده گردید. سپس در روزهای ۱۰۹ تا ۱۱۸ جزئیات اندام های داخلی جنین رویت گردید.

**نتیجه‌گیری:** پروب ترانس رکتال جهت تشخیص آبستنی عملکرد بهتری نسبت به پروب ترانس آبدومینال دارد. دقت تشخیص آبستنی با اولتراسونوگرافی دوبعدی ۱۰۰٪ بود. شکل جنین در تصاویر روزهای ۲۵ تا ۴۱ در هر بار سونوگرافی تغییر می کند و در روز ۴۱ آبستنی جنین به صورت کامل شناسایی می شود.

**واژه‌های کلیدی:** *آبستنی، اولتراسوند، شین باش*

فواد سعدی، صلاح الدین خدامرادی. بررسی تشخیص آبستنی و رشد جنین به وسیله اولتراسونوگرافی در گوسفند نژاد شین باش. مجله طب دامپزشکی جایگزین. ۱۴۰۳؛ ۷(۲۲): ۱۳۱۴-۱۳۲۰.



* نویسنده مسئول: *گروه علوم بالینی، دانشکده دامپزشکی، واحد مهاباد، دانشگاه آزاد اسلامی، ، مهاباد، ایران.*
*Orcid: https://orcid.org/0000-0001-4717-3332 ،Email: foadsadi@yahoo.com*