



Iranian Veterinary Surgery Association

# IRANIAN JOURNAL OF VETERINARY SURGERY

Journal homepage: [www.ivsajournals.com](http://www.ivsajournals.com)

## ORIGINAL ARTICLE

### Study of Diagnosis of Pregnancy and Fetal Development by 2D Ultrasound in Markhoz Goat

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Received: 21 November  
2018  
Accepted: 20 April 2019  
Available Online: 20 April  
2019

#### Keywords:

Markhoz goat;  
Pregnancy;  
Ultrasound.

#### Abstract

**Objective-** The aim of this study was to obtain first time diagnosis of pregnancy and study of fetal development in different times of pregnancy period.

**Design-** Descriptive study.

**Animals-** 8 pregnant Markhoz goats.

**Procedure-** 2D ultrasound was performed from day 25 to 130 of gestation, twice in week from day 25 to 70 and once in week from day 70 to 130 of gestation on eight goats. The ultrasonographic images were obtained Sonosite Titan (USA) 2D ultrasound machine.

**Results-** On the 25<sup>th</sup> day of gestation, earliest diagnosis of pregnancy was done. On 37<sup>th</sup> day, clear pictures of conceptus, amniotic membrane, and umbilicus were seen. On 78<sup>th</sup> 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 88<sup>th</sup> day of gestation. Between 115 and 130 days of gestation complete details of internal organs were seen in ultrasonographic images.

**Conclusions and clinical relevance-** The accuracy of ultrasound was 100% for detecting pregnant and non-pregnant cases. Conceptus changed its shape from 25 to 44 days of gestation, and full identifiable conceptus took its shape on day 44.

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## 1. Introduction

Ultrasonography is quicker and less stressful and good tool for early pregnancy diagnosis. Two approaches of ultrasonography (transabdominal and transrectal) have been used with great accuracy as a means for pregnancy diagnosis and estimation of fetal numbers in goat.<sup>1,2</sup> Previously, several studies used two-dimensional (2D) ultrasonography to diagnose pregnancy and for measuring fetal dimension.<sup>2,3</sup> 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.<sup>4,5</sup> Early pregnancy diagnosis and fetal quantification through ultrasonography contribute to rationalize management and bring financial benefits to goat production.<sup>6,7</sup>

## 2. Materials and Methods

Eight healthy pregnant goats of Markhoz breed approximately 3 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 20 to 25 and continued till 130 days of gestation. The ultrasonography was conducted 2 times in a week from day 25 to day 70 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 goats were shaved, and the goats 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 attached 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 cm<sup>2</sup> on the right flank above the under after removing the hairs over it. Then, the transducer was placed at the right side of the goat, 5.0 cm in front of the rear leg and 2.5 cm above the teat. Pregnant and non-pregnant goats 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 (21.0) system for windows.

## 3. 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 20 to 25 of gestation by observing a small non-echogenic vesicle of 0.7 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 (Figure 1a). On 28<sup>th</sup> day of pregnancy, conceptus was seen as oblong shaped in 2D scanning, the conceptus was also seen as oblong shaped, 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 37<sup>th</sup> 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 37 and measured up to day 97 with the help of transabdominal approach. The placentome diameter was increased significantly during whole observation ( $p < 0.05$ ). On the 38<sup>th</sup> day, clear pictures of the fetus and hyperechoic amniotic membrane were seen. Umbilicus was seen on day 38 of gestation. On 44<sup>th</sup> 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 52<sup>th</sup> day of gestation, skull, rib cage, the spinal cord of the fetus, forelimbs, hindlimbs, and other bony structures were seen in the 2D image (Figure 1b). To view the full



**Figure 1.** Typical images of fetal development: (a), Transrectal ultrasonogram of uterus of goats at day 20 to 25 of gestation with a 7.5 MHz transrectal transducer showed accumulation of anechoic fluid (F arrow) in uterus. (b), On day 52 eye ball (Eb arrow), forelimbs (F.l arrow) and fetal trunk (F.t arrow) are seen. (c), On day 52 spinal cord (S.P arrow) is seen.

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 broad sheet of placenta was seen on other side of the fetus. Depression of concave shaped cotyledons was also seen on the flat placenta.

On the 58th 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.

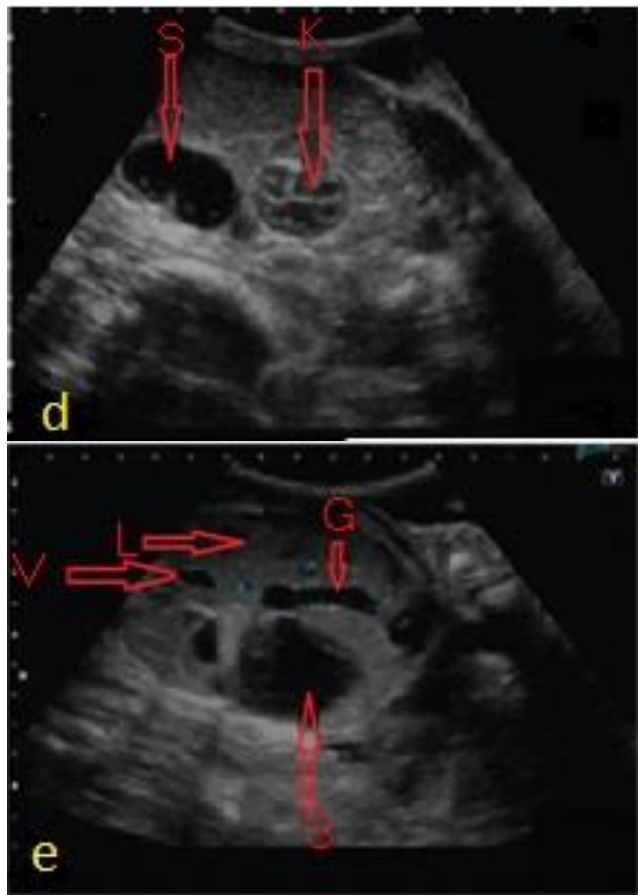
At the 70<sup>th</sup> day of gestation in 2D scanning, it was found difficult to get full fetus in one scan, therefore, fetal trunk area or head was focused at one time. It appeared that the attachment of the fetal membrane to endometrium was not simple, but complicated. There were elongated and round 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 78th day of gestation. On this day internal organs viz heart, kidney, urinary bladder, stomach, and liver of fetus were easily identifiable.

In the present observations, there was a rapid growth of internal organs around and after day 78 of gestation. The scrotum in the male fetus was identified on the 88nd day of gestation. Between 115 and 130 days of gestation complete details of the fetal stomach, heart, liver, gall bladder, kidney and urinary bladder were seen in 2D ultrasonographic images (Figure 2c and d).

#### 4. Discussion

Ultrasonography is an important tool for early pregnancy diagnosis. 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 0.7 cm diameter on day 20 to 25 of gestation with the help of per-rectal probe with 7.5 MHz frequency. This is in agreement with Padilla-Rivas *et al.*<sup>8</sup> The investigators reported a small non-echogenic vesicle of about 1.1 cm in diameter in the uterine lumen by day 23.<sup>9</sup>

Reported the appearance of a circular or elongated gestational sac in the uterine lumen on days  $20.2 \pm 0.6$  of pregnancy. It is concluded that the early detection of pregnancy in goats is possible around day 25. In the present study, the accuracy of ultrasound was 100% for detecting pregnant and non-pregnant cases. Almost all previous investigators have used transabdominal ultrasonography for pregnancy detection in goat except Martinez *et al.* used transrectal transducer for pregnancy diagnosis.<sup>9</sup>



**Figure 2.** Images of fetal organs: (d), Stomach with echogenic particles (S arrow) and kidney (K arrow) is visible in 2D ultrasonogram at day 125 of pregnancy. (e), Liver (L arrow), gall bladder (G arrow), stomach (S arrow) and hepatic vessel (V arrow) in 2D ultra sonogram at day 130 of pregnancy.

Heartbeats were detected on day 25 in the present study, which is almost agreement with the study of Medan *et al.*<sup>10</sup>, Martinez *et al.*<sup>9</sup>, and with most of other authors who reported heartbeats around this time of pregnancy in goats.<sup>8,11</sup> On the 30<sup>th</sup> 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.*<sup>9</sup> The earliest detection of placentome by transrectal ultrasonography as

circular echoic structure facing toward the fetus was made on day 37 and measured up to day 97 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 25-28 of gestation.<sup>12,13</sup> In some studies, placentomes were recognized clearly at 7 weeks of gestation or at day 38 of gestation.<sup>8</sup> These investigators reported the presence of umbilical cord on the 30<sup>th</sup> day of gestation. On 52<sup>th</sup> day of gestation, skull, rib cage, the spinal cord of fetus, forelimbs, hindlimbs, and other bony structures were seen in 2D image which was 3 day earlier than Suguna *et al.*<sup>2</sup> Medan *et al.*<sup>10</sup> also reported that skeletal structures were obvious at 2 months of pregnancy, almost 10 days later that present observations. 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. Chandolia *et al.*<sup>14</sup> also reported movement of body part of the fetus on day 38 after conception in the goat. In The 2D images, Lee *et al.*<sup>7</sup> had reported that the placentome increases in size and appeared as a 'C' or 'O' shaped. The 2<sup>nd</sup> month of pregnancy has been reported to be the best period for imaging placentomes.<sup>2</sup>

On 78<sup>th</sup> day of gestation, both 2D images showed greater details of organs heart, kidney, urinary bladder, stomach, and liver of the fetus. Matsas<sup>15</sup> had also reported that the fetal skeleton grows rapidly between second and 3<sup>rd</sup> month of gestation. The scrotum in the male fetus was identified on the 84<sup>th</sup> day of gestation. In earlier studies, genital tubercle has been reported by various investigators.

Ramphal<sup>16</sup> has reported genital tubercle in ram at day 53 of gestation and scrotum on day 90 of gestation. Lack of more reports on this area could be due to less work on advanced pregnancy. Yotov *et al.*<sup>17</sup> reported that the fetal sex can be determined best in sagittal or cross-sectional position in buffaloes. Between 115 and 130 days of gestation complete details of fetal stomach, heart, liver, gall bladder, kidney and urinary bladder were seen in 2D ultrasonographic images but there is no previous parallel study in this area.

In conclusion, for early pregnancy diagnosis, the transrectal approach was better than the transabdominal approach. Early pregnancy could be detected at 25 days of gestation with 7.5 MHz transducer. Fetal development in great details, a particularly segmented form of the embryo could be observed in 2D ultrasonography on day 25. Conceptus changed its shape from 25 to 44 days of gestation, and full identifiable conceptus took its shape on day 44. 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 or abnormal conceptus.

## Conflict of interest

None declared.

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نشریه جراحی دامپزشکی ایران  
سال ۲۰۱۹، جلد ۱۴ (شماره ۱)، شماره پیاپی ۳۰

چکیده

## بررسی تشخیص آبستنی و تکامل جنینی با استفاده از دستگاه اولتراسوند دوبعدی در بزهای آبستن نژاد مرخز

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**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.

**طرح مطالعه-** بررسی توصیفی

**حیوانات:** ۸ رأس بز مرخز آبستن

**روش کار-** این بررسی از روز ۲۵ حاملگی به صورت دو بار در هفته در مدت زمان روزهای ۲۵ تا ۷۰ آبستنی و یکبار در هفته در روزهای ۷۰ تا ۱۳۰ آبستنی در ۸ رأس بز نژاد مرخز به وسیله دستگاه اولتراسوند دوبعدی سونوسایت تیتان (ساخت کشور آمریکا) انجام گردید.

**نتایج-** ابتدا از همان روز ۲۵، آبستنی تشخیص داده شد. در ادامه در روز ۳۷ تصاویر واضحی از مراحل آبستنی همانند تشخیص پرده آمنیوتیک و بند ناف مشاهده گردید. در روز ۷۸ از آبستنی اعضای داخلی جنین مانند قلب، کلیه، کبد و مثانه در تصاویر دیده شدند. در روز ۸۸ هم اسکروتوم در جنین نر مشاهده شد. در ادامه کار مابین روزهای ۱۱۵ تا ۱۳۰ جزئیات اندام‌های داخلی جنین در تصاویر اولتراسوند مشاهده شد.

**نتیجه‌گیری و کاربرد بالینی-** پروب ترانس‌رکتال جهت تشخیص آبستنی بهتر از پروب ترانس‌آب‌دومینال است. دقت تشخیص آبستنی با اولتراسونوگرافی دوبعدی ۱۰۰٪ بود. شکل جنین در تصاویر روزهای ۲۵ تا ۴۴ در هر بار سونوگرافی تغییر می‌کند و در روز ۴۴ آبستنی جنین به صورت کامل شناسایی می‌شود.

**واژه‌های کلیدی-** آبستنی، اولتراسوند، بز مرخز