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Research Article

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Diagnosis of Pregnancy and Embryonic Development by 2D Ultrasound in Kurdish Sheep

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Abstract

Background and aim: The objective of this study was to obtain the first time of pregnancy diagnosis and the images of fetal development in different times of pregnancy period using ultrasonography.
Material and Methods: Two-dimensional ultrasound from day 25 to 120 of pregnancy was performed twice a week from days 25 to 65 and once a week from days 65 to 120 of pregnancy on ten Kurdish sheep. The ultrasonographic images were obtained Sonosite Titan (USA) 2D ultrasound machine.
Results: On the 25th day, the pregnancy was diagnosed. On 39th day, clear pictures of pregnancy like amniotic membrane, and umbilicus were seen. On 75th day of gestation, internal organs of fetus like heart, kidney, liver, urinary bladder, and stomach were seen in the images. The scrotum in the male fetus was identified on the 89th day of gestation. Between the 110th and 120th days of gestation, complete details of internal organs were seen in ultrasonographic images.
Conclusion: The transrectal probe to detect pregnancy is better than transabdominal probe. The accuracy of 2D ultrasound was 100% for detecting pregnancy. The shape of fetus changes in the images from days 25 to 42 in each ultrasound scan, and on the 42nd day of pregnancy, the fetus is fully identified.

Key words: *Pregnancy, Ultrasound, Kurdish sheep*

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Introduction

Kurdish sheep is considered as a meat breed among Iranian sheep. Kurdish sheep are of a medium body size with long arms and legs. The color of their body wool is sugar white and their hands, feet and head are brown or black. This breed has a large tail (Nasirian *et al.,* 2010). Pregnancy diagnosis in sheep usually is done using some form of ultrasonography, since rectal palpation is impractical due to the size of the animals (Griffin & Ginther, 1992; Romano & Christians, 2008; Karen *et al.,* 2004). Linear-array 7.5 MHz 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.,* 2004). 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 bipari- etal 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 (Lee *et al.,* 2005; Padilla-Rivas *et al.,* 2005). 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 sheeps of Kurdish 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 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 was 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 animals 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. The transducer 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

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fetus and related images in sheeps 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 $p<0.05$ were considered to be statistically significant. All statistical analysis were performed using the SPSS (21.0) 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 0.73 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 31th 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 ultrasonography as circular echoic structure facing toward the fetus was made on 35th 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 34 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 42nd 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 49th 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 55th 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 65th day of gestation in 2D

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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-3).

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 81nd day of gestation. In earlier studies, genital tubercle has been reported by various investigators. Between 110 and 120 days of gestation complete details of the fetal stomach, heart, liver, gall bladder, kidney, and urinary bludder were seen in 2D ultrasonographic images (Figure 3).

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 in uterus.

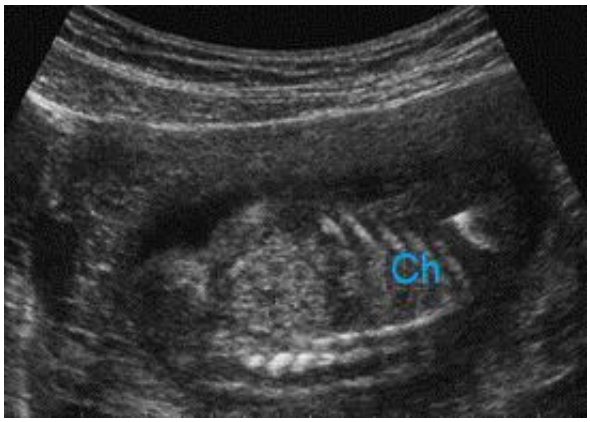

Figure 2. Images of fetal chest (Ch) at day 49 pregnancy.

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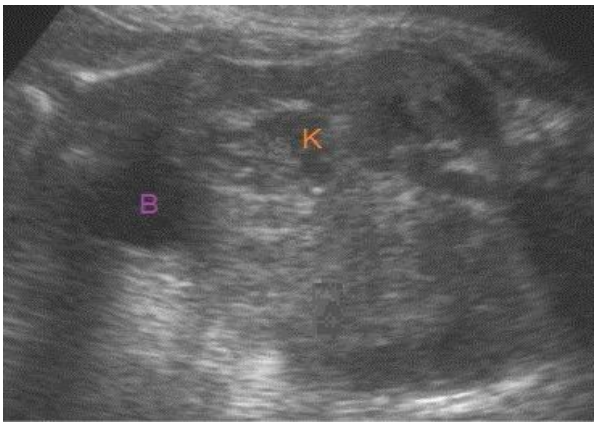

Figure 3. Images of fetal organs: (B) bludder and (K) Kidney is visible in 2D ultrasonogram at day 120 of pregnancy.

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 0.73 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 non-pregnant cases.

The 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 (Medan *et al.,* 2004).

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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. Similarly, no parallel study is available in goat regarding the development of fetal organs in utero (antenatal) using B-mode ultrasonography. 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 but there is no previous parallel study in this area.

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 27. Conceptus changed its shape from 25 to 42 days of gestation, and full identifiable conceptus took its shape on day 42. 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.

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مقاله پژوهشی

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بررسی تشخیص آبستنی و تکامل جنینی با استفاده از دستگاه اولتراسوند دوبعدی در گوسفند نژاد کردی

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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: سونوگرافی دو بعدی از روز ۲۵ تا ۱۲۰ بارداری بصورت دو بار در هفته از روزهای ۲۵ تا ۶۵ و یک بار در هفته از روزهای ۶۵ تا ۱۲۰ بارداری بر روی ده گوسفند کردی انجام شد. تصاویر سونوگرافی با دستگاه سونوگرافی دو بعدی سونوسایت تیتان (ساخت کشور آمریکا) انجام گردید.

یافته‌ها: ابتدا از همان روز ۲۵، آبستنی تشخیص داده شد. درادامه در روز ۳۹ تصاویر واضحی از مراحل آبستنی همانند تشخیص پرده آمنیوتیک و بند ناف مشاهده گردید. در روز ۷۵ از آبستنی اعضای داخلی جنین مانند قلب، کلیه، کبد و مثانه در تصاویر دیده شدند. در روز ۸۹ هم اسکروتوم در جنین نر مشاهده شد. در ادامه کار بین روزهای ۱۱۰ تا ۱۲۰ جزئیات اندامهای داخلی جنین در تصاویر اولتراسوند مشاهده شد.

نتیجه‌گیری: پروب ترانس رکتال جهت تشخیص آبستنی بهتر از پروب ترانس آبدومینال است. دقت تشخیص آبستنی با اولتراسونوگرافی دوبعدی ۱۰۰٪ بود. شکل جنین در تصاویر روزهای ۲۵ تا ۴۲ در هر بار سونوگرافی تغییر میکند و در روز ۴۲ آبستنی جنین به صورت کامل شناسایی می شود.

واژه‌های کلیدی: *آبستنی، اولتراسوند، گوسفند کردی*

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