Early Pregnancy Diagnosis by Means of Ultrasonography as a Method of Improving Reproductive Efficiency in Goats

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Abstract. Eighteen cyclic Shiba goats were used in this study. Estrus was synchronized with a single injection of 125 µg of a synthetic analogue of prostaglandin F2α (PGF2α) after detection of at least one corpus luteum by B-mode ultrasonography. Blood samples were collected from each animal on days 0, 7 and 21 post-mating for progesterone assay. Animals in estrus were either allowed to be mated by fertile bucks twice during estrus (group I; n=12) or not at all (group II; n=6). Ultrasonographic examinations were performed transrectally or transabdominally using a real-time B-mode scanner equipped with a 7.5 or 5 MHz transducer. All animals exhibited estrus 56.0 ± 2.7 h after injection of PGF2α. The results show that the accuracy of the progesterone assay in diagnosing pregnancy on day 21 after mating was 80% for pregnancy and 100% for non-pregnancy, retrospectively. Ultrasonographic examinations showed that gestational sac and embryos heartbeats were detected on days 20.2 ± 0.6 and 24.3 ± 0.7 of gestation, respectively. Placentomes were detected on day 35.4 ± 1.0 of gestation as small nodules (0.7 ± 0.2 cm in size). At two months pregnancy, skeletal structures like skull, thorax and long bones were clear. Biparietal diameter of the skull and length of long bones could be used as an estimate of gestational age. The accuracy of detection of fetal number using real-time B-mode ultrasonography was 91.7% on day 60 of gestation. In conclusion, progesterone assay at day 21 after mating was 80% for pregnancy and 100% for non-pregnancy, retrospectively. Ultrasonographic examinations showed that gestational sac and embryos heartbeats were detected on days 20.2 ± 0.6 and 24.3 ± 0.7 of gestation, respectively. Placentomes were detected on day 35.4 ± 1.0 of gestation as small nodules (0.7 ± 0.2 cm in size). At two months pregnancy, skeletal structures like skull, thorax and long bones were clear. Biparietal diameter of the skull and length of long bones could be used as an estimate of gestational age. The accuracy of detection of fetal number using real-time B-mode ultrasonography was 91.7% on day 60 of gestation. In conclusion, progesterone assay at day 21 post-mating (cut-off value, 1 ng/ml) can be used for pregnancy diagnosis in goats. However, B-mode transrectal ultrasonography was more efficient due to detection of embryo and confirmation of its viability by heartbeats. In addition, fetal number and gestational age could be determined only by ultrasonography.

Key words: Pregnancy diagnosis, Fetal number, Ultrasonography, Progesterone, Goats


The early and accurate diagnosis of pregnancy in addition to determination of fetal number is essential for the maintenance of high levels of reproductive efficiency [1, 2]. A reliable technique for early pregnancy diagnosis would allow early culling or rebreeding of barren does. A variety of techniques have evolved over the years. Ultrasonography [3], progesterone assay [4] and radiography have emerged as the most useful methods utilized today. Older described methods of laparotomy, abdominal palpation, and rectal-abdominal palpation with a rod [5] have limited utility. Ultrasonography is quicker and less stressful for the animals than either laparoscopy or
laparotomy [6]. Ultrasonic imaging of the heartbeat is regularly used to detect the embryo and to evaluate embryo viability [7]. Heartbeats were first detected between days 19 and 29 in heifers [8, 9] and ewes [6]. Ultrasonography has been used for pregnancy diagnosis in goats [10, 11]; however, this approach has not been used widely to detect fetal number in goats.

The objective of the present study was to diagnose pregnancy at an early stage by progesterone assay and real-time B-mode ultrasonography. Another important objective was to determine fetal number by real-time B-mode ultrasonography as a means of improving reproductive efficiency.

Materials and Methods

Eighteen cyclic Shiba goats were used in this study. Their average age was 2.9 years and they had an average of 1.4 pregnancies. Estrus was synchronized with a single i.m injection of 125 µg of a synthetic analogue of PG F2α (Estrumate, Schering Plough Animal Health, New Jersey, USA) after detection of at least one corpus luteum by ultrasonography. Estrous behavior was evaluated every 6 h by using mature fertile bucks. Animals in estrus were either allowed to be mated twice during estrus (group I; n=12) or not at all (group II; n=6). The Day of estrus was designated as day 0 (day of 1st mating). Ultrasonographic examinations were performed either transrectally or transabdominally. Transrectal ultrasonography was performed daily using a real-time B-mode scanner equipped with a 7.5 MHz transducer starting from day 15 post-mating to day 35. Transabdominal ultrasonography was performed on days 40, 50, 60, 70, 80 and 90 post-mating using a 5 MHz transducer. The main events observed were recorded by a thermal-video printer (Hitachi EUZ-VP7, Hitachi Medical Corporation, Tokyo, Japan). The transducer was lubricated with carboxymethylcellulose gel (Hijelly, Aqueous coupling agent for ultrasound transmission, Hitachi, Tokyo, Japan). In transrectal examination, the transducer was fastened to a plastic rod (30 cm length and 20 mm in diameter) by adhesive tape to manipulate it externally into the rectum. The transducer was inserted into the rectum until the urinary bladder was displayed on the screen. The uterine horns were observed cranial to the bladder and the transducer was rotated laterally 90° clockwise and then 180° counterclockwise to scan the ovaries (for presence of CL). Transabdominal examination was done according to Haibel [3]. Determination of fetal number by ultrasound was compared with the actual number of kids born or delivered by cesarean operation.

Blood samples were collected from the jugular vein into heparinized vacutainer tubes (Terumo Venoject II, Tokyo, Japan) on days 0, 7 and 21 post-estrus. Blood samples were centrifuged at 1200 g for 15 minutes and plasma was separated and stored at –20°C until assayed for progesterone. Plasma concentrations of progesterone were determined by a double antibody radioimmunoassay (RIA) system using 125I-labeled radioligand as described previously [12]. Antisera against progesterone (GDN 337) were kindly provided by Dr. G.D. Niswender (Animal Production and Biotechnology, Colorado state University, Fort Collins, Co. USA). The intra and inter-assay coefficients of variation were 8.2% and 9.2%, respectively.

Statistical analysis

The data are expressed as means ± SEM. Student’s t-test was used to compare progesterone concentrations in group I and group II with the SAS computer package [13]. The accuracy of determination of fetal number was estimated from the number of cases determined correctly by ultrasonography (as compared to the actual kids born or delivered by cesarean section) divided by the total number of pregnant goats.

Results

After injection of PGF2α, all goats exhibited estrus. The interval between injection of PGF2α and estrus was 56.0 ± 2.7 h. All goats in the mated group (group I) were pregnant as confirmed at kidding or by cesarean section. Plasma concentrations of progesterone on days 0, 7 and 21 after estrus are shown in Table 1. There was no significant difference between groups I and II on days 0 and 7. However, there was a significant decrease (P<0.01) in the concentration of progesterone in group II on day 21 after estrus. The accuracy of progesterone concentrations in
diagnosing pregnancy on day 21 after mating was 80% for pregnancy and 100% for non-pregnancy as shown in Table 2. The progesterone cut-off value between pregnancy and non-pregnancy used in this study was 1 ng/ml.

Ultrasound examination of non-pregnant does showed that the uterus was observed immediately anterior to the urinary bladder. The urinary bladder is easily recognized as an anechogenic sphere (Fig. 1), appearing immediately after probe introduction into the rectum. In non pregnant does, the uterus appeared as a spherical structure with a medium echogenic density with or without a central cavity less than 2 mm in diameter (Fig. 2). In pregnant does, the first sign of pregnancy was the appearance of a gestational sac, which was circular or an elongated anechoic structure located in the uterus cranial to the bladder (Fig. 3). The gestational sac could be detected first on day 20.2 ± 0.6 of gestation. The embryos were detected at 24.3 ± 0.7 days of gestation, all pregnant animals

Table 1. Mean (± SEM) plasma progesterone concentrations in goats on days 0, 7 and 21 after estrus

<table>
<thead>
<tr>
<th>Group</th>
<th>Progesterone concentration</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 7</td>
<td>Day 21</td>
</tr>
<tr>
<td>Group I (mated; n=12)</td>
<td>0.5 ± 0.1 ±0</td>
<td>6.8 ± 0.4a</td>
<td>7.4 ± 0.5a</td>
</tr>
<tr>
<td>Group II (not mated; n=6)</td>
<td>0.6 ± 0.1 ±0</td>
<td>6.7 ± 0.3a</td>
<td>1.5 ± 0.5b</td>
</tr>
</tbody>
</table>

* Within columns, values with different superscripts are significantly different (P<0.01).

Table 2. Accuracy of early pregnancy diagnosis in goats by plasma progesterone assay or real-time B-mode ultrasonography and detection of fetal number

<table>
<thead>
<tr>
<th></th>
<th>No. correct</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone assayb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td>15</td>
<td>12/15 (80)</td>
</tr>
<tr>
<td>Non-pregnant</td>
<td>3</td>
<td>3/3 (100)</td>
</tr>
<tr>
<td>Ultrasonographyc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td>12</td>
<td>12/12 (100)</td>
</tr>
<tr>
<td>Non-pregnant</td>
<td>6</td>
<td>6/6 (100)</td>
</tr>
<tr>
<td>Fetal numberd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 40 after mating</td>
<td>12</td>
<td>8/12 (66.7)</td>
</tr>
<tr>
<td>Day 50 after mating</td>
<td>12</td>
<td>10/12 (83.3)</td>
</tr>
<tr>
<td>Day 60 after mating</td>
<td>12</td>
<td>11/12 (91.7)</td>
</tr>
</tbody>
</table>

* Determined at kidding or cesarean section.

* Measured at day 21 after estrus and the cut-off value used was 1 ng/ml.

* Confirmed by the detection of embryo and its heartbeats at 24.3 ± 0.7 days of gestation.

* Diagnosed by ultrasonography by finding more than one heart, skull and sets of ribs.
showed at least one embryo (Fig. 4), typically observed as an area of high echographic density located on the basal zone of the gestational sac. The presence of embryos was confirmed by detection of heartbeats. The accuracy of pregnancy diagnosis and detection of fetal number by real-time B-mode ultrasonography are shown in Table 2. Fetal number (Fig. 5) could be detected on day 40 after mating, and reached maximum accuracy (91.7 %) on day 60. The fetal number detected by ultrasonography was compared with the actual number of kids (5 singles, 6 twins and one triplet in the respective 12 pregnant goats) at delivery or cesarean operation. Only one case (triplet) was not diagnosed correctly by ultrasound. Placentomes were visible as small nodules $0.7 \pm 0.2$ cm in size at $35.4 \pm 1.0$ days of gestation. As pregnancy progressed, placentomes increased in size and appeared as C-shaped or O-shaped gray image (Fig. 6). At 2 months pregnancy, skeletal structures were obvious. The ribs appeared as groups of white dots. In between ribs, there was an anechoic area which is the heart (Fig. 7). The length of long bones (Fig. 8A) and biparietal diameter of the skull (Fig. 8B) could be measured.

Fig. 2. Ultrasound image of non-pregnant uterus (U) in goats obtained using a 7.5 MHz transrectal transducer. The uterus appears as a spherical structure with a medium echogenic density. Scale bar represents 10 mm.

Fig. 3. Ultrasound image of early pregnant uterus using a 7.5 MHz transrectal transducer on day 21 post-mating (notice the dark, fluid-filled sacs (arrows). The fluid-filled sacs were round or oblong in shape). Scale bar represents 10 mm.

Fig. 4. Ultrasound images of a goat embryo on days 30 (A) and 35 (B) of gestation obtained using a 7.5 MHz transrectal transducer. The embryo (E, arrow) is observed as an area of high echogenic density. Image B shows the umbilical cord (arrow). Scale bars represent 10 mm.
Discussion

Accurate pregnancy diagnosis in goats would provide essential information for effective herd management practices such as culling of non-pregnant females. Determination of fetal number would allow producers to separate goats carrying singles, twins or triplets for differential management. Analysis of hormones or proteins in milk has been proposed to help breeders diagnose pregnancy. Among these systems, determination of progesterone on Days 20 to 22 after mating is accurate for detecting non-pregnant goats [14, 15], but it is inaccurate for detecting pregnant ones when the CL lifespan is extended for reasons other than pregnancy [16]. Moreover, the interpretation of a progesterone assay requires precise information about the date of mating or artificial insemination, and sample collection at certain times after mating [17, 18]. In the present study we found a significant decrease in the concentration of progesterone in group II on day 21 after estrus indicating luteolysis of CL. However, CL lifespan was extended in 3 goats in group II (progesterone concentrations remained high on day 21 after estrus). Ultrasonography is an important tool for early pregnancy diagnosis in goats [19]. In this study, we found that the technique is easily applicable without significant risk for does, which could be examined in a standing position in a wooden chute. No distress, no rectal bleeding or interruption of pregnancies was seen after ultrasonography examinations. The results show that pregnancy diagnosis in does could be carried out from 20.2 ± 0.6 days after mating, with 100% accuracy on day 24.3 ± 0.7 as confirmed by detections of the heartbeats. During evaluation of the initial stages of pregnancy, the observation of the embryo and its heartbeat in the gestational sac is important. In this study the presence of an embryo heartbeat was detected as early as 24.3 ± 0.7 days of gestation. This is in agreement with that reported in goats by Martinez et al. [10]. Studies of

Fig. 5. Ultrasound image of twin pregnancy (arrows) on day 40 of gestation obtained using a 5 MHz transabdominal transducer. Scale bar represents 10 mm.

Fig. 6. Ultrasound images of placentomes in goats (arrows) obtained on day 35 (A) using a 7.5 MHz transrectal transducer, and on day 60 (B) of gestation using a 5 MHz transabdominal transducer (note that placentomes are visible as small nodules on day 35 and become C or O shape later and increased in size as in image B). Scale bars represent 10 mm.
ewes have reported the detection of an embryo heartbeat from 18 [6] to 30 days of gestation [20]. The ability to identify multiple fetuses with real-time ultrasonography is a clear advantage over other techniques. In the present study, fetal number could be detected at day 40 post-mating; the best time was day 60 after mating. Another advantage of real-time ultrasonography is the ability to estimate gestational age through determination of the crown rump length of the embryo, size of placentomes, biparietal diameter of the skull and length of long bones. Moreover, viable pregnancies can be distinguished from hydrometra, pyometra and fetal mummification by ultrasonography [21]. In the present study, no cases of embryonic mortality were recorded.

In conclusion, we found that both the plasma progesterone assay and real-time B-mode ultrasonography were reliable methods for early pregnancy diagnosis. However, the determination of fetal number in goats and viability of the fetuses were clear advantages for real-time B-mode ultrasonography over the progesterone assay. Therefore, the use of real-time B-mode ultrasonography might help to improve the reproductive efficiency of goats.

Acknowledgments

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