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Noninvasive color Doppler sonography of uterine blood flow throughout pregnancy in sheep and goats

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ABSTRACT

In contrast to cattle or horses, uterine blood flow in small ruminants has been investigated predominantly after surgical intervention and chronic instrumentation. The objective of the present study was to investigate the clinical applicability of noninvasive color Doppler sonography to characterize blood flow in the maternal uterine artery of sheep, n = 11 (18 pregnancies) and goats, n = 11 (20 pregnancies). The following parameters were measured transrectally or transabdominally: blood flow volume, time-averaged maximum velocity (TAMV), resistance index (RI), pulsatility index (PI), Time-averaged mean velocity, impedance of blood flow (AB or systolic/diastolic [S/D] velocity ratio), peak velocity of blood flow and blood flow acceleration. Examinations started 2 weeks after breeding and continued at 2-week intervals until parturition. Outcomes for sheep and goats were similar and will be discussed together. Based on noninvasive color Doppler sonography, blood flow volume increased (approximately 60-fold, $P < 0.0001$) until the end of pregnancy, with a rapid increase early in gestation, and a slow increase after week 18. Time-averaged maximum velocity in the uterine artery increased (approximately 4-fold; $P < 0.0001$) throughout pregnancy in sheep and goats. Furthermore, for uterine artery blood flow, there was an effect of stage of pregnancy on PI and RI ($P < 0.001$ and $P < 0.0001$, respectively), both indices decreased until the end of gestation. Time-averaged mean velocity decreased from week 18 to 20 in both species. The blood flow acceleration increased ($P < 0.0001$) until week 16 and week 14 in sheep and goats, respectively, and then decreased until parturition. Similar to PI and RI, vascular impedance of the uterine decreased ($P < 0.0001$) throughout pregnancy. This is apparently the first study using noninvasive color Doppler sonography of uterine blood flow throughout physiological pregnancy in small ruminants. Clearly, this technology facilitates repeated, noninvasive assessments, with great potential for future studies.

1. Introduction

Advances in Doppler ultrasound technology allowed the noninvasive estimation of uterine blood flow in human, horse, and cattle pregnancies. In human obstetrics, those Doppler ultrasound studies have been increasingly used as a noninvasive means to assess low- and high-risk pregnancies and to monitor fetal well-being [1,2]. Pregnancy-related veterinary studies in large domestic animal species are mainly concentrated on horses [3–5] and cattle [6–10]. Although sheep have been frequently taken as biomedical model organisms in human obstetrics, especially related to fetal growth restriction and other pathologic conditions, invasive techniques for the assessment of maternal and fetal vessels are still the rule. Although the fetal compartment in small ruminants has been monitored

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transabdominally [11–13] and after surgery and instrumentation [14], invasive techniques have been chosen for the investigation of blood flow parameters in uterine arteries [15–19]. Only recently, a transabdominal approach in anesthetized animals after surgical mobilization of the arteries was reported [20,21]. Sequential noninvasive investigations throughout pregnancy are missing.

The objective of the present study was to investigate the clinical applicability of noninvasive Doppler sonography and to characterize blood flow in the maternal uterine artery throughout pregnancy in sheep and goats.

2. Material and methods

2.1. Animals

Studies were carried out at the Institute of Reproductive Biology, Hannover, during three physiological breeding seasons (2008–2011). As described in Table 1, 38 pregnancies (clinically healthy, nonlactating pluriparous female German Merino sheep [n = 11; 18 pregnancies; 5.5 ± 2.4-years old; body condition score {BCS} 3–4 according to Russel 1991 [22]] and Boer × German–improved Fawn goats [n = 11; 20 pregnancies; 7 ± 1.9-years old; BCS 3–4 according to Villaquiran 2005a [23]; Villaquiran 2005b [24]]) were investigated during the period of this study. Animals were kept on pasture, except during late-autumn and winter, when they were housed in a barn (3–5 female per box) with access to outside runs. The animals had *ad libitum* access to hay, mineral supplementation, and water and were also given pellets according to National Research Council 1985 [25] (amount commensurate with the stage of pregnancy).

2.2. Experimental design

Females were mated with a proven fertile ram or buck during behavioral estrus in November. The day of estrus in sheep and the second day of estrus in goats were designated as Day 0 of gestation. Pregnancy was verified ultrasonically (Hitachi EUB 405; B-Mode, 7.5 MHz, Hitachi Medical System, Tokyo, Japan) on Day 18 ± 2.8 in sheep and Day 14 ± 3.5 in goats. Fetuses were counted on Day40 to Day 60 of gestation. In sheep, nine singleton, eight twin, and one triplet pregnancies were observed, whereas in goats twin pregnancies were predominant (three singleton, 14 twin, and three triplet pregnancies). No fetal loss was detected throughout the whole study. The gestation periods averaged 150 ± 3.4 and 151 ± 3.1 days in sheep and goats, respectively.

At 2-week intervals from mating throughout pregnancy, Doppler sonography was carried out at the same time of day (between 3:00 PM and 7:00 PM) in standing, unsedated animals. To avoid unnecessary stress, examinations were conducted in a close proximity to pen mates. There was no period of fasting before transrectal or transabdominal scans. All examinations were done by a single experienced operator (ME).

Table 1
Experimental sheep and goats' numbers (n), age, body condition score (BCS), and pregnancy numbers.

Species/n	Age	BCS	Number of pregnancies
Sheep (11)	5.5 ± 2.4	3–4	18
Goats (11)	7 ± 1.9	3–4	20

2.3. Mean distance between uterine artery and anus in goats and sheep

Before conducting studies on live animals, I had the opportunity to participate in Gross Anatomy Course at the Institute for Anatomy (supervisor: Prof. C. Staszyk) and to observe autopsies in the Autopsy Room of the Institute of Pathology, both at the University of Veterinary Medicine Hannover, Foundation. This was an extraordinary opportunity to study the location of vessels supplying the reproductive tract in small ruminants (Fig. 1A, B). Especially, the approximate location of the uterine arteries in relation to the anus was investigated in nonpregnant and early pregnant animals postmortem (seven goats: Institute for Anatomy, one of these animals with Biodur marked vessels; three sheep: Institute for Pathology).

2.4. Color Doppler sonography

A LOGIQ 5 Pro ultrasound device (General Electrics Healthcare, Kranzbuehler/Medizin Systeme Solingen, Germany) equipped with a linear multifrequency (5–10 MHz) transducer was used for the investigations. For transrectal scanning, the transducer was fixed to a plastic guide with color markings in 5-cm intervals to facilitate intrarectal manipulation and orientation (Fig. 1C). Ultrasound gel (15 mL) was introduced intrarectally, and the transducer was generously lubricated with this gel before it was gently introduced into the rectum. After the 12th week of gestation, examinations were conducted transabdominally, as the localization of uterine arteries transrectally takes more time because of ventral displacement of the gravid uterus.

The transducer was first rotated 180° dorsally to locate the abdominal aorta and then by 90° clockwise or counter clockwise to follow the external iliac artery and to locate the uterine artery, according to the side.

Preliminary to the studies on living animals, the approximate location of the uterine arteries in relation to the anus had been assessed in nonpregnant and early pregnant small ruminants postmortem (sheep: Institute for Pathology, autopsy findings (Fig. 1A); goats: Institute for Anatomy, gross anatomy course, Fig. 1B). Uterine arteries were found in a distance of 10.7 ± 1.5 cm in sheep and 6.5 ± 1.3 cm in goats, which was also true in living animals at the start of investigations on Day 14 of pregnancy. Uterine arteries were located cranio-lateral to the bladder, close to the external iliac artery (Fig. 1A, B). The urinary bladder was taken as landmark during transrectal examinations, and the color Doppler function of the ultrasound device was used for detection and visualization of the uterine arteries and to adjust the insonation angle.