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Uterine blood flow during the first 3 weeks of pregnancy in dairy cows

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Abstract

Transrectal color Doppler sonography was used to compare changes in uterine blood flow between cyclic and early pregnant lactating dairy cows. Examinations were carried out in 53 multiparous lactating cows on days 3, 6, 9, 11, 13, 15, 18 and 21 (day 0 = estrus). Fourteen cows were examined during the estrous cycle and thirty-nine cows after insemination with frozen/thawed sperm. Uterine blood flow was reflected by the time-averaged maximum velocity (TAMV) and the pulsatility index (PI) in the uterine artery ipsilateral to the corpus luteum. Twenty-one cows that were not pregnant on day 25 were excluded from the study. There was high inter-individual variability in PI- (CV: 22–62%) and TAMV-values (CV: 22–42%) at all examinations. In cyclic cows, TAMV values increased between days 13 and 18 and PI values decreased between days 15 and 21 ($P < 0.05$). In pregnant cows ($n = 18$), TAMV values increased from days 9 to 11 and then decreased to minimal values by day 18 ($P < 0.05$). The PI values decreased between days 3 and 11 and then increased to maximum levels on day 18 ($P < 0.05$). On day 21, both variables reached ($P < 0.05$) values that did not differ ($P > 0.05$) from those on day 11. The changes in TAMV were correlated with estrogen and progesterone concentrations ($r = 0.69$ and -0.70, respectively; $P < 0.05$) in cycling cows, but not in pregnant cows ($P > 0.05$). The PI values did not correlate with steroid hormone levels ($P > 0.05$). Differences in uterine blood flow between cycling and early pregnant cows were observed only on day 18 ($P < 0.05$). The results show that in pregnant cows changes in uterine blood supply can be detected already in the second week after insemination; these changes do not occur in the second week of the cycle.

Keywords: Cow; Color Doppler sonography; Early pregnancy; Uterine blood flow

1. Introduction

Successful establishment of pregnancy involves interactions between the conceptus and the maternal environment [1]. Therefore, a number of studies have been carried out on embryo-maternal communication during early pregnancy. Although several endocrine, molecular and cellular factors have been shown to be involved in the process of the establishment of pregnancy, the mechanisms responsible for maternal recognition of pregnancy and implantation are not fully understood [1–3]. Previous investigations indicated that the conceptus may have a local effect on uterine blood flow that could be involved in the complex process of establishment of pregnancy. Using invasive methods a transient rise in uterine blood flow during maternal recognition of pregnancy was observed in cows [4,5], ewes [6,7] and sows [8,9], followed by a second rise

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associated with implantation [4,6,8]. These alterations in blood flow were limited to the uterine horn containing a conceptus. In pluriparous swine, blood flow was increased in uterine segments that were in direct contact with conceptuses compared to segments lying between them on day 13 after estrus [10]. The effect on uterine blood flow by the horse conceptus was investigated using transrectal color Doppler sonography [11,12]. The results obtained with this method indicated that the horse embryo locally stimulates endometrial perfusion in association with location changes of the embryonic vesicle during the mobility phase and after fixation.

In earlier experiments involving a limited number of cows, blood flow was measured using electromagnetic probes implanted surgically around the uterine artery. Seven [4] and three [5] pluriparous Hereford cows were used but uterine blood flow could be analyzed in only four pregnant cows; no information was provided whether or not the cows were lactating. More recently, color Doppler sonography has been established as a non-invasive method of measuring uterine blood flow in cows [13,14]. This method is characterized by a high reproducibility [14] and can be carried out non-invasively in a large number of cows.

The primary goal of this study was to compare for the first time changes in uterine blood flow during the first 3 weeks of pregnancy and during an estrous cycle in lactating German Holstein and German Black Pied cows using transrectal color Doppler sonography. Secondary goals were to investigate the variability in uterine blood flow between individual cows and the relationship between uterine blood flow and the plasma concentration of total estrogen and progesterone.

2. Materials and methods

2.1. Animals

The studies were carried out at the Institute of Farm Animal Genetics in Mariensee, Germany, between April 2005 and January 2006. A total of 53 German Holstein ($n = 11$), German Black Pied ($n = 17$) and cross-bred cows ($n = 25$) were used. Their mean age was 5.2 ± 0.2 years (range: 2.9–11.3) and they had calved 2.4 ± 0.2 (range: 1–7) times. All the cows had been lactating a minimum of 6 weeks. The cows were housed in tie-stalls from October to May and pastured from June to September. They were fed a total mixed ration supplemented with concentrate, based on milk yield, and had free access to water. Estrus detection was carried out for 40 min twice daily in the morning and late afternoon.

2.2. Study design

Cows were randomly divided into two groups. One group ($n = 14$) was examined during the estrous cycle (cycling cows), and the other ($n = 39$) after insemination. Cows of the second group were inseminated 12 h after they were first observed in estrus using 20×10^6 frozen/thawed sperm from two fertile Holstein bulls. The presence and location of the corpus luteum was determined on day 3 (estrus = day 0) using B-Mode sonography. Transrectal color Doppler examinations of the uterine arteries ipsilateral to the ovary bearing the corpus luteum were carried out on days 3, 6, 9, 11, 13, 15, 18 and 21, in each cow always at the same time of the day between 8:00 h and 14:00 h. The examinations lasted up to 30 min for each cow. On day 25, pregnancy diagnosis was performed by ultrasound in all inseminated cows. All the cows in which an embryo proper with a heartbeat was identified were diagnosed pregnant, and the remaining cows were excluded from further analysis.

2.3. Doppler examinations

All Doppler measurements were carried out by the same operator (CV) using a LogiqTM Book XP ultrasound device (General Electrics Medical Systems, Jiangsu, P.R. China), equipped with a 7.0 MHz linear probe (General Electrics Yokogawa Medical Systems, Tokyo, Japan). The uterine artery ipsilateral to the corpus luteum was examined using a method with a high intra-observer reproducibility, as described earlier [14]. In brief, the uterine artery was identified within the mesometrium near its origin at the umbilical artery and near the external iliac artery. At this location uterine blood flow waveforms were obtained by activating the pulsed Doppler function and placing the Doppler gate over the uterine artery, adjusted to the diameter of the vessel. The blood flow waveforms were derived at an insonation angle between the Doppler ultrasound beam and flow direction between 20° and 50° and were recorded digitally in DICOM-format. To prevent influences on uterine blood flow by transrectal manipulation, the uterus was not touched before and during measurements.

Doppler analysis was performed off-line on a personal computer using the software PixelFlux (Chameleon-Software, Leipzig, Germany). For the analysis of uterine blood flow, two representative consecutive waveforms with a maximum ratio between diastolic and systolic frequency shift were selected for each investigation. Waveforms were defined as repre-