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# Color-Doppler signals of blood flow in the corpus luteum and vascular perfusion index for ovarian and uterine arteries during expansion of the allantochorion in *Bos taurus* heifers

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A B S T R A C T

Hemodynamics of the CL and each main uterine artery during expansion of the allantochorion from the ipsilateral side (CL side) to the contralateral side were studied in heifers (n = 8 nonbred, 9 pregnant). Progesterone concentration, vascular perfusion index for each uterine artery (by spectral ultrasonography), extent of blood flow in the CL (percentage of CL tissue with color-Doppler signals of blood flow), and intrauterine location of the expanding allantochorion (by gray-scale ultrasonic imaging) were determined daily from Days 14–60 (Day 0 = ovulation). In the pregnant group, but not in the nonbred group, the percentage of CL tissue with blood-flow signals increased ($P < 0.003$) from Days 16 ($66.7 \pm 4.2\%$) to 23 ($79.4 \pm 2.5$) and then more slowly increased ($P < 0.02$) from Days 24 ($76.7 \pm 2.9\%$) to 50 ($85.0 \pm 2.0\%$). The volume of CL increased ($P < 0.0001$) progressively from Days 26 ($6.1 \pm 0.4$ cm$^3$) to 60 ($7.3 \pm 0.7$ cm$^3$). The vascular perfusion index in the ipsilateral uterine artery did not change in the nonbred group and progressively increased in the pregnant group beginning on Day 17 in approximate temporal association with the increase in luteal blood-flow signals. Functional increases in the CL during early pregnancy were attributed to the greater uterine arterial blood flow on the ipsilateral or CL side and the reported prominent anastomosis from a branch of the uterine artery to the ovarian artery that supplies the CL ovary. After an initial significant decrease in vascular perfusion index in each uterine artery in the pregnant group, the index began to increase on Day 17 in the ipsilateral artery and on Day 18 in the contralateral artery. The perfusion continued in the ipsilateral artery but discontinued in the contralateral artery on Day 21. Perfusion and diameter of the uterine artery were greater for the ipsilateral side until the vesicle entered the contralateral horn on Day 33 and increased for the contralateral horn between vesicle entry and filling of the horn on Day 44. During Days 35–60, the perfusion index ($P < 0.04$) and artery diameter ($P < 0.001$) were lower in the contralateral than in the ipsilateral artery. Results supported the hypotheses that (1) CL function increases during early pregnancy in temporal association with an increase in blood flow in the ipsilateral uterine artery and (2) blood flow in each of the ipsilateral and contralateral uterine arteries increases as the allantochorion expands in each uterine horn.



## 1. Introduction

Pregnancy establishment is an orchestrated event with physiologic interactions between the conceptus, corpus luteum (CL), and uterus. Crucial events in the establishment of pregnancy in ruminants include secretion of interferon tau by the conceptus [1], suppression of the endometrial luteolytic mechanism [2], and appropriate progesterone concentration for placentation or adhesion of the embryonic vesicle to the endometrium beginning on Day 19 (Day 0 = ovulation) [3]. Progesterone concentration in pregnant cattle is maintained on the day that luteolysis occurs (eg, Day 17) in nonpregnant cattle [4,5]. However, blood flow in the CL and blood flow through the ovarian and uterine arteries in response

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to expansion of the conceptus into the ipsilateral (CL side) and contralateral uterine horns need study.

Transrectal B-mode (gray scale) ultrasonic imaging is a noninvasive tool for the evaluation of temporality among morphologic changes in the ovaries and uterus [6]. Conceptus development has been described in detail based on ultrasonic imaging from Days 10–60 in heifers [7,8] and from Days 20–70 in the excised uterus in cows [9]. Color-Doppler ultrasonography is a noninvasive method for assessing blood flow to and within the reproductive organs during the interovulatory interval (IOI) and pregnancy [6,10–13]. The assessment of CL and uterine blood flow can be done by studying percentage of tissue with color-Doppler signals of blood flow and blood flow indexes in an individual artery [10]. Blood flow in the CL has been described until about Day 21 in nonpregnant and pregnant cattle [14–17] and to the uterus in pregnant heifers from Days 0–18 [17] or 21 [18].

Based on electromagnetic blood-flow probes, some blood flow to the CL ovary during diestrus is supplied by the ipsilateral uterine artery through an anastomosis between a branch of the uterine artery and the ovarian artery [19]. Blood flow in the anastomosis reverses direction and is toward the uterus during estrus apparently from a dilatory effect of estradiol on the uterine vascular bed. Blood flow in the uterine arteries during the IOI and pregnancy has been studied in cattle by Doppler ultrasonography [10]. Monthly evaluations of blood flow in a uterine artery indicated a continuous increase in pregnant cows [11], and evaluations every 3 days indicated greater uterine blood flow in pregnant than in nonbred cows 18 days after estrus [18]. Based on scores of 1–4 (nil to maximum) for color-Doppler signals of blood flow in the endometrium of pregnant heifers, an increase in score in the horn ipsilateral to the CL ovary was first detected on Day 18 [17]. The increase occurred between Days 18 and 22 in each uterine horn but with less intensity on the contralateral side. The contralateral horn had a more prominent increase on Day 32 and reached a similar intensity as on the ipsilateral side by Day 42. Expansion of the allantochorion was not studied. A detailed study is needed on the dynamics of blood flow in the ovaries and uterine arteries in parallel with the extension of the conceptus into various portions of the uterus.

The present study evaluated the effects of pregnancy on the CL based on evaluation of concentrations of progesterone, CL volume, and percentage of CL tissue with blood-flow Doppler signals. Blood flow in the ovarian artery to the CL ovary and to the ipsilateral and contralateral uterine arteries during expansion of the allantochorion were also assessed. The following hypotheses were tested: (1) CL function increases during early pregnancy in temporal association with an increase in blood flow in the ipsilateral uterine artery and (2) blood flow in each of the ipsilateral and contralateral uterine arteries increases as the allantochorion expands in each uterine horn.

## 2. Material and methods

### 2.1. Animals

Holstein heifers (n = 23) aged 17–20 months were used from December 2015 to April 2016 in the northern temperate zone. Heifers were selected with no abnormalities of reproductive tract as determined by ultrasound examination, kept under natural light, and given *ad libitum* access to a mixture of alfalfa and grass hay, water, and mineralized salt. Handling of heifers was in accordance with the United States Department of Agriculture Guide for Care and Use of Agricultural Animals in Research.

### 2.2. Experimental design

Heifers were randomly assigned to nonbred (n = 8) and bred (n = 15) groups. Before breeding, heifers were observed for estrus and examined daily by transrectal B-mode (gray scale) and color-Doppler ultrasonography beginning on Day 16. The presence of a follicle larger than 10 mm, decreased CL blood-flow signals to less than 30% of luteal tissue, and endometrial edema were used as indicators of impending estrus. Artificial insemination was done in the presence of estrous signs, and heifers were scanned for ovulation the next day. If ovulation did not occur, heifers were reinseminated until ovulation was detected 12–24 h after insemination.

Ultrasound scans and blood sampling were done daily from Day 14 until ovulation in the nonbred heifers and until Day 35 in heifers that became pregnant. Thereafter, scanning and sampling in the pregnant group were done every 5 days from Days 35–60. Heifers in the pregnant group in which the CL regressed before Day 60 were excluded from the analyses and considered separately. The experimental design is illustrated (Fig. 1).

### 2.3. Ultrasound scanning

Transrectal ultrasonography used a scanner (Aloka SSD-3500; Aloka America, Wallingford, CT, USA) that was equipped with a 7.5 MHz transrectal linear-array transducer. Morphologic CL changes were assessed by volume as determined from the maximal cross-sectional area. The value for area was displayed by the scanner from a tracing of the periphery. The conversion of area to volume of a sphere was done using the formula: $volume = 0.75 \times \sqrt{area^3}$. If the CL contained a cavity, the cavity volume was subtracted from the entire volume. Vascular changes in the CL were assessed in color-Doppler mode as the estimated percentage of the CL tissue with signals of blood flow [10,17]. The validity of estimating percentage of CL with blood-flow signals has been documented by computerized pixel analyses and by agreement between operators [10].

The extent of blood flow in the ovarian artery to the CL ovary and in each uterine artery was determined in spectral-Doppler mode. The main uterine artery on each side was located by beginning at the mesometrial attachment and moving the probe away from a horn until the branches of the artery began at the main artery (Fig. 2). Resistance index (RI) [10,11] was obtained by placement of the cursor at the most prominent branch of the ovarian artery just before entry into the ovary or at each main uterine artery before branching. Considering that RI is negatively correlated with vascular perfusion in the tissues distal to the placement of the cursor, RI was converted to vascular perfusion index as represented by 1 minus RI. This was done as described [20] for ease of interpretation and communication. In addition, the transverse diameter

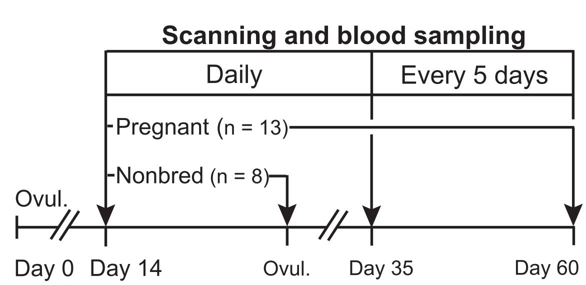


**Fig. 1.** Diagram of protocol showing days of ultrasound scanning and blood sampling in nonbred and pregnant heifers. Ovul. = ovulation.