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Effects of a shortened preovulatory follicular phase on genital blood flow and endometrial hormone receptor concentrations in Holstein-Friesian cows

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Abstract

The goal of this study was to determine the effects of the length of the preovulatory phase on genital blood flow and mRNA expression of endometrial hormone receptors in cattle (*Bos Taurus*). Ovulation was synchronized in 50 Holstein-Friesian cows using a modified Ovsynch (ovulation synchronization) protocol, in which the second gonadotropin-releasing hormone (GnRH) administration was given 40 h (G40, n = 17) or 60 h (G60, n = 16) after the prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) administration. The third group (S, n = 17) did not receive a second GnRH administration. Transrectal color Doppler examinations were carried out 24 h before (Day –1) and on Day 7 after ovulation (Day 0). Follicular size (FS) and luteal size (CLS) were quantified by measuring the areas of these structures on cross-sectional B-mode ultrasound images. Follicular blood flow (FB) and luteal blood flow (CLB) were quantified by determining the colored areas of these structures. Uterine blood flow was measured using the time-averaged maximum velocities (TAMVs) and the pulsatility indices (PIs) of both uterine arteries. Endometrial mRNA transcript abundance of estrogen receptors α and β as well as oxytocin and progesterone receptor were determined on Days –1 and 7 in G40 and G60 cows. In all cows, plasma progesterone (P_4) values were measured on Day 7. On Day –1, FS and FB values were lower ($P \leq 0.05$) in G40 cows compared with those in S cows but did not differ ($P > 0.05$) between G60 cows on the one hand and G40 cows and S cows, respectively, on the other hand. On Day 7, CLS and P_4 did not differ ($P > 0.05$) between cows of Groups G40, G60, and S; CLB was lower ($P \leq 0.05$) in G40 cows than in G60 and S cows, but no difference ($P > 0.05$) occurred between G60 and S cows. The uterine TAMV and PI values did not differ among the three groups ($P > 0.05$). Gene expression of hormone receptors did not differ ($P > 0.05$) between Groups G40 and G60 on Days –1 and 7. Results of this study indicate that a shortened preovulatory follicle phase primarily affects ovarian but not the measured uterine events in cows undergoing synchronization of ovulation.

Keywords: Corpus luteum; Endometrial receptors; Follicle; Genital blood flow; Ovsynch

1. Introduction

Synchronization of ovulation with gonadotropin-releasing hormone (Ovsynch) and fixed-time insemination without observation of estrus in dairy cows has been used for more than 10 yr [1]. The conception rates of this protocol, however, are often considerably lower than those achieved with insemination after spontaneous ovulation [2–4]. Thus, numerous modifications of the original Ovsynch protocol have been investigated in an attempt to increase pregnancy rates [5–7]. One modification involved an increased interval between

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prostaglandin and the second gonadotropin-releasing hormone (GnRH) administration from 48 h to 52 and even 60 h, which was associated with improved pregnancy rates [4,8]. The underlying physiologic mechanisms for the enhanced fertility remain unknown; it has been speculated that the increased time interval has a positive effect on maturation of the preovulatory follicle and, subsequently, on oocyte developmental competence [9] and the corpus luteum [10–13]. It has also been postulated that extending the preovulatory follicular phase has a positive effect on the concentration of steroid hormone receptors in the endometrium [13,14].

The blood supply of the internal reproductive organs plays a crucial role in fertility [15,16]. In human medicine, a series of color Doppler sonographic studies has demonstrated a positive relationship between follicular [17–19], luteal [20], and uterine perfusion [21–23] and important fertility parameters. Color Doppler sonography studies in cows focused mainly on the investigation of changes during the estrous cycle and pregnancy [24–28]. Recent studies showed a positive relationship between follicular blood flow and embryo development after in vitro fertilization [29] as well as conception after artificial insemination in heifers [30].

The goal of the current study was to investigate the effects of an induction of early ovulation on genital blood flow and endometrial expression of selected hormone receptors in cows undergoing synchronization of ovulation without artificial insemination. This should aid in determining whether the above-mentioned increase in fertility seen by extending the prostaglandin-GnRH interval during the Ovsynch protocol is associated primarily with the ovarian or endometrial milieu, or both.

2. Materials and methods

2.1. Animals

Fifty Holstein-Friesian cows (*Bos Taurus*) from the Institute of Farm Animal Genetics (Mariensee, Germany) were used. The animals were 30 to 113 mo ($\bar{x} \pm s = 58 \pm 20$ mo) old and had calved one to five ($\bar{x} \pm s = 1.7 \pm 1.1$) times. All cows were lactating for 45 to 180 d ($\bar{x} \pm s = 95 \pm 46$ d) prior to the start of the study. The herd average milk production was 23.4 to 27.1 L ($\bar{x} \pm s = 25.3 \pm 1.7$ L) per cow during the study. The cows were kept in tie stalls, bedded with straw, and fed primarily corn and grass silage and a balanced grain ration.

2.2. Study design

Clinically healthy cows that had no reproductive disorders and had a corpus luteum with a minimum diameter of 20 mm were included into the study. Initially, ovulation was induced in all the cows with a synthetic GnRH analogue (10 μg buserelin; Receptal; Intervet, Unterschleißheim, Germany). Seven days later, a prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) analogue (150 μg (+)-cloprostenol; Dalmazin; Selectavet, Weyarn/Holzolling, Germany) was administered to all cows to induce luteolysis. A second administration of GnRH was given 40 h after $PGF_{2\alpha}$ in the G40 group (n = 17) and 60 h later in the G60 group (n = 16). Cows in the S group (n = 17) did not receive a second administration of GnRH. Cows in Groups G40 and G60 were monitored for ovulation 24 and 36 h after the second GnRH administration. Cows in Group S were monitored for spontaneous ovulation every 12 h starting 60 h after prostaglandin administration. The time point defined as ''24 h before ovulation/Day –1'' was determined retrospectively. Transrectal B-mode and color Doppler sonography were used to compare follicular size (FS) and follicular blood flow (FB) on Day –1 and luteal size (CLS) and luteal blood flow (CLB) on Day 7 in all three groups. Follicular perfusion was determined in Groups G40 and G60 immediately before the second administration of GnRH. In Group S, follicular blood flow was determined 60 h after administration of prostaglandin and every 12 h thereafter until spontaneous ovulation had occurred. Luteal blood flow was determined in all the cows on Day 7. At each ovarian examination, uterine blood flow was also assessed by Doppler sonography of both uterine arteries in Groups G40 and G60. The concentration of P_4 was determined in all cows on Day 7. Endometrial biopsy samples for determination of hormone receptor mRNA expression were collected on Days –1 and 7 from the cows in Groups G40 and G60.

2.3. Ultrasonography

Sonographic measurements were carried out using a portable LOGIQ Book XP ultrasound machine (General Electric Healthcare, Solingen, Germany), equipped with a linear probe (6 to 10 MHz; model 1739-RS; General Electric Medical Systems, Ltd., Yokogawa, Japan). All examinations were conducted by the same investigator (D.P.). During each investigation, images of both ovaries were recorded, with a focus on follicles with a diameter of at least 5 mm during the preovulatory phase and on luteal tissue on Day 7. The largest follicle was identified by B-mode, and its ultrasonic image was