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Doppler sonography of the uterine arteries during a superovulatory regime in cattle
Uterine blood flow in superovulated cattle

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Abstract

Transrectal color Doppler sonography was used to investigate the effects of a gonadotropin treatment to induce superovulation on uterine blood flow and its relationship with steroid hormone levels, ovarian response and embryo yield in dairy cows. The estrous cycle of 42 cows was synchronized by using $PGF_{2\alpha}$ during diestrus and GnRH 48 h later (Day 0). Cows were examined on the day of eCG (2750 IU)-administration (Day 10), 3 days after eCG (Day 13) and 7 days after artificial insemination (Day 22), including the determination of total estrogens (E) and progesterone (P_4) in peripheral plasma. Eight days after insemination (Day 23) the uterus was flushed and the number of total ova and embryos as well as transferable embryos was determined. The ovarian response was defined by the number of follicles > 5.0 mm in diameter on Day 13 and the number of corpora lutea on Day 22. Uterine blood flow was reflected by the blood flow volume (BFV) and the pulsatility index (PI) in the uterine arteries. Both variables showed distinct changes throughout the superovulatory cycle: BFV increased by 94% and PI decreased by 30% between Days 10 and 22 ($P < 0.0001$). On Day 13, BFV but not PI correlated with follicle numbers ($r = 0.35$; $P < 0.05$); no correlation was found with E and P_4 ($P > 0.05$). On Day 22, BFV correlated positively and PI correlated negatively with the number of corpora lutea ($r = 0.45$ and $r = -0.37$; $P < 0.05$) and P_4 ($r = 0.39$ and $r = -0.30$; $P < 0.05$). The number of transferable embryos was solely related to BFV measured on Day 13 ($r = 0.32$; $P < 0.05$). Our results show for the first time that in cows a superovulatory treatment is associated with a marked increase in BFV and a marked decrease in PI in the uterine arteries, concurrent with the development of multiple follicles and corpora lutea. However, transrectal color Doppler sonography of the uterine arteries does not facilitate the prediction of embryo yields following superovulatory treatment.

Keywords: Cow; Color Doppler sonography; Embryo yield; Superovulation; Uterine blood flow

1. Introduction

The high variability and unpredictability in embryo yields following superovulatory treatment in cows still create problems affecting both the efficiency and profitability of conventional embryo transfer (ET) programs in cattle [1–4]. Since the introduction of transrectal B-mode sonography in bovine reproduction

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approximately 20 years ago, several approaches including the determination of the follicular status before hormonal stimulation [5–7] and the elimination of the suppressive effects of a dominant follicle [8–10] have been used to reduce the unpredictability of the bovine superovulatory response and to increase the number of transferable embryos. The mean response following superovulatory treatment in terms of transferable embryos can be increased by using specific FSH preparations [3,11]. However, these methods did not eliminate the variability in superovulatory response and a better prediction is still critical for the application of MOET programs [2,12]. The variation in the number of transferable embryos per superovulatory cycle, ranging from 0 to 50 [13], is mainly attributed to the variability in ovulation and fertilization rates [1,11], but also the impact of a poor uterine environment, which could be incompatible with normal embryo development, has not been ruled out [14–16]. In human medicine, similar problems have been encountered in infertile women undergoing gonadotropin treatment for in-vitro fertilization (IVF) and ET programs, showing low predictability of conception rates due to variations in uterine receptivity. Attempts, that have been made to identify reliable markers for an adequate uterine development and thus a good receptivity, evaluated the resistance to uterine blood flow as an indirect parameter for endometrial receptivity [17–24]. Using transvaginal color Doppler sonography of the uterine arteries, it could be discriminated between conception and non-conception cycles in patients: at different time points of the IVF cycle they found lower resistance indexes (RI) or pulsatility indexes (PI) in women who conceived than women who did not. However, since these results could not be confirmed in other studies [25–29], the clinical usefulness of Doppler ultrasound of the uterine arteries in the assessment of endometrial receptivity and the prediction of the IVF outcome remains controversial.

During the last few years transrectal color Doppler sonography has been shown to be a useful tool for assessing uterine blood flow in cattle by non-invasive means [30–32]. The usage of this technique enabled to determine uterine blood flow during the estrous cycle, pregnancy and puerperium in clinical studies [32]. For example, a typical blood flow pattern has been shown during the estrous cycle in temporal relationship with plasma concentrations of estrogen and progesterone [31]. To the best of our knowledge uterine blood flow has not yet been studied during a superovulatory regime in cows, and no attempts have been made to correlate uterine blood flow with the subsequent embryo yield.

The goal of the present study was to assess uterine blood flow in cows undergoing a gonadotropin treatment and to correlate uterine blood flow with ovarian response and steroid hormone levels. In addition, we tested whether or not transrectal color Doppler sonography of the uterine arteries allows predicting embryo yields.

2. Materials and methods

The study was carried out at the Institute of Farm Animal Genetics in Mariensee, Germany. A total of 42 cows, including 14 Holstein Friesian, nine German Black Pied and 19 cross-bred cows were investigated. On average, cows were 4.4 ± 1.7 years old and had calved 1.7 ± 1.1 times. The animals were at least 6 weeks post partum. Twenty-nine of the cows were lactating and 13 were non-lactating. The cows were kept in tie-stalls and fed a mixed ration and additional concentrate based on milk yield. Water was available ad libitum.

At the start of the experiment, the estrous cycle of all cows was synchronized using a $PGF_{2\alpha}$ analogue (657.5 μg cloprostenol, Estrumate®, Essex, Munich, Germany i.m.) during diestrus and a GnRH analogue (10 μg Buserelin, Receptal®, Intervet, Unterschleißheim, Germany i.m.) 48 h later. The day of the GnRH treatment was defined as Day 0. On Day 10, 2750 IU eCG (Intergonan®, Ch-B.: 24616, Intervet, Unterschleißheim, Germany i.m.) were applied to stimulate follicular growth. Seventy-two hours later (Day 13), luteolysis was induced using the $PGF_{2\alpha}$ analogue mentioned above. At 48, 60 and 72 h after the second $PGF_{2\alpha}$ administration, the cows were artificially inseminated with 15×10^6 frozen/thawed semen from two fertile Holstein bulls (Day-56 non-return rates of 72% and 73%). Eight days after the first insemination (Day 23), ova and embryos (total ova/embryos) were collected by standard non-surgical procedures using 500 ml PBS supplemented with 1% new-born calf serum [8]. The quality of the recovered embryos was morphologically evaluated according to IETS guidelines [33], embryo qualities 1 to 2 were classified as transferable.

Transrectal Doppler sonography of the uterine arteries was performed immediately before the administration of eCG (Day 10) and the second injection of $PGF_{2\alpha}$ three days later (Day 13) as well as 7 days after the first insemination (Day 22). Additionally, transrectal B-mode imaging was used to examine the ovaries for the presence of functional structures. On Day 10, the ovarian status prior to hormonal treatment was