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# Effects of exogenous oxytocin on uterine blood flow in puerperal dairy cows: The impact of days after parturition and retained fetal membranes

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ABSTRACT

The objective of this study was to examine whether an oxytocin challenge test (OCT), evaluated by measuring the changes in uterine blood flow using transrectal colour Doppler ultrasonography, is a suitable non-invasive method to determine uterine contractility in puerperal dairy cows. The changes in uterine blood flow during uterine contractions induced by oxytocin were evaluated on days 2 and 5 postpartum (pp). Twelve clinically healthy Holstein cows were randomly assigned into two groups: (1) oxytocin group ($n = 7$), 50 IU oxytocin injected IM and (2) control group ($n = 5$), 5 mL saline injected IM. Blood flow volume (BFV) and pulsatility index (PI) in the uterine arteries were determined before and after injection for 120 min on days 2 and 5 pp.

BFV declined and PI increased rapidly after oxytocin injection on day 2 ($P < 0.05$), whereas oxytocin on day 5 pp did not cause changes in blood flow parameters. The result confirmed that uterine responsiveness to oxytocin decreases with time postpartum in healthy cows. The same OCT was applied in cows with retained fetal membranes ($n = 6$) on day 2 pp, however uterine blood flow showed no change after oxytocin injection. The results showed that an OCT on day 2 pp may be a useful method for investigating the uterine contractile response to oxytocin (reflected as the decrease of uterine blood flow) and the potential pathophysiology of uterine involution in cows.



## Introduction

In postpartum dairy cows, rapid uterine involution is a prerequisite for a high conception rate and short interval from calving to conception (Opsomer et al., 2000). The uterus shows a distinct size reduction during the first 3 weeks postpartum (pp), with uterine contractility crucial in the process of uterine involution, particularly with regard to the removal of excessive fluid and debris and the elimination of bacteria from the uterine lumen during the early postpartum period (Zerobin and Sporri, 1972; Paisley et al., 1986; Arthur et al., 1996).

There are only limited data on uterine contractility in postpartum cows because of the difficulties in evaluation. Methods that have been used to assess uterine activity include palpation per rectum (Lindell and Kindahl, 1983), measurement of intrauterine pressure by balloon catheters (Zerobin and Sporri, 1972), and determination of electrical activity of the myometrium (Gajewski et al., 1999). However, these methods tend to be subjective or invasive. B-mode ultrasonography allows non-invasive visualisation of the uterus and ovaries (Ginther, 1998), but cannot provide information on organ function, such as vascular perfusion. However, combined with colour Doppler sonography it can be used to investigate uterine blood flow and this has been increasingly used for studies of uterine blood flow in cattle (Bollwein et al., 2000; Ginther, 2007; Herzog and Bollwein, 2007).

During uterine contraction, the spiral arteries in the endometrium constrict proportionally to the intensity of the contraction. This constriction can be visualised using colour Doppler as an increase in vascular flow resistance and as a decrease in blood flow velocity (Fleischer et al., 1987; Janbu and Nesheim, 1987). Exogenous oxytocin stimulates uterine contractions (Wu et al., 1996) and decreases uterine blood flow during those contractions (Li et al., 2003, 2004; Tahara et al., 2009). Colour Doppler could therefore be used as a non-invasive method of measuring the response to oxytocin during an oxytocin challenge test (OCT). However, to date no studies have evaluated the suitability of colour Doppler for obtaining such information.

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Retained fetal membranes (RFM) are a major risk factor for delayed uterine involution during the postpartum period in cows and thus negatively affect reproductive performance (Opsomer et al., 2000; Beagley et al., 2010). Many studies have been performed to examine the effect of oxytocin on the incidence of RFM and subsequent reproductive performance (Hickey et al., 1984; Mollo et al., 1997; Stevens and Dinsmore, 1997; Jeremejeva et al., 2010; Palomares et al., 2010). However, there is no systematic information on uterine contractions in response to oxytocin administration in cows with RFM, in which oxytocin may elicit decreased or little response.

The objectives of the present study were to investigate (1) the suitability of transrectal colour Doppler sonography of the uterine arteries as a non-invasive tool for evaluating the uterine response to exogenous oxytocin; (2) the differences in uterine response depending on time after parturition, and (3) the uterine response to oxytocin in cows with RFM.

## Materials and methods

The present study was approved by the Care and Use of Agricultural Animals of Obihiro University (No. 23-92) and performed according to its guidelines.

*Animals*

*Study 1: Time-dependent changes in the response of uterine blood flow to oxytocin in healthy cows*

Study 1 was conducted on all cows that calved between June to September 2011 in the Field Center of Animal Science and Agriculture, Obihiro University of Agriculture and Veterinary Medicine. Clinical examinations, i.e. rectal temperature (every day), systemic signs (every day), vaginal lochia (twice a week) and haematological examination (once a week) were performed until 3 weeks postpartum. Cows with an abnormal peripartum period including dystocia and twin birth, hypocalcaemia, mastitis and ketosis were excluded from the study, which left 12 Holstein cows (mean ± SD lactation number: 3.5 ± 1.2) that were clinically healthy without any signs of systemic illness and so were used for the study.

The experiments were conducted on days 2 and 5 pp, and the cows were divided into two groups as follows: (1) Oxytocin group ($n = 7$); 50 IU oxytocin (Oxytocin; Nagase) injected IM; (2) control group ($n = 5$); 5 mL saline was injected IM.

*Study 2: Response of uterine blood flow to oxytocin in healthy cows and cows with RFM*

Six Holstein cows with mean (±SD) lactation number of 3.8 (±1.2) were used. The animals belonged to the same herd as the cows of study 1. All six had retained their fetal membranes for longer than 12 h after expulsion of the fetus. Fifty IU oxytocin (Oxytocin; Nagase Co.) were injected IM. Results were compared to the oxytocin group from study 1.

*Uterine blood flow assessment*

Transrectal Doppler ultrasound examinations were performed on healthy cows on days 2 and 5 pp in study 1. On the basis of the results from study 1 in which the uterus responded to oxytocin on day 2 pp but not on day 5 pp, the same OCT was applied in study 2 for cows with RFM ($n = 6$) on day 2 pp. Uterine blood flow was investigated immediately before (-5 min) and 5, 30, 60, and 120 min after the administration of oxytocin (OT group) or saline (control group). Prior to each examination, the cows received epidural analgesia (3.0 mL lidocaine 2%, Mylan) to reduce intestinal peristalsis which makes it difficult to keep the angle between the Doppler beam and the uterine artery consistent.

Uterine blood circulation was investigated in both uterine arteries, ipsilateral (UA-ipsi) and contralateral (UA-cont) to the former pregnant horn. All the sonographic examinations were performed by the same operator (FM) at the same time of day (between 19.00 and 22.00 h), using an SSD-5500 ultrasound machine (Aloka) equipped with a 7.5 MHz linear transducer (UST-900L2-7.5, Aloka). Uterine blood flow was assessed using the blood flow volume (BFV, mL/min) and pulsatility index (PI).

Using a computer-assisted image analysis program (PixelFlux, Version 1.0), the time-averaged maximum velocity (TAMV, cm/s) over 1 cardiac cycle was calculated using the following formula:

$$\mathrm{TAMV} = \mathrm{TAMF} \times c/(2F \times \cos\alpha)$$

where TAMF is the time-averaged maximum frequency shift (Hz), $c$ is the ultrasound propagation speed in the body (cm/s), $F$ the transmitted wave frequency (Hz), and $\alpha$ the angle between the ultrasound beam and the direction of blood flow.

BFV was calculated as follows:

$$\mathrm{BFV} = \mathrm{TAMV} \times \pi \times (D \times 0.5)^2 \times 60$$

where $D$ is the diameter of the uterine artery (cm).

PI was calculated as the ratio of the difference between the peak systolic frequency shift (PSF) and minimum-diastolic frequency shift (MDF) to the TAMF over the cardiac cycle, i.e.:

$$\mathrm{PI} = (\mathrm{PSF} - \mathrm{MDF})/\mathrm{TAMF}$$

The PI increases if the vascular bed distal to the site of measurement constricts (Krueger et al., 2009). The BFV and PI values of the two analysed consecutive pulse waves within three wave pairs were averaged. Because there were large variations in the uterine BFV and PI between individual cows, relative changes (%) of BFV and PI were calculated to evaluate the changes after oxytocin/saline injection.

*Statistical analysis*

Statistical analysis was performed using Stat View 5.0 (SAS Institute) and JMP 6 (SAS Institute). The Shapiro–Wilk test was used to test for normal distribution of the data, and the parameters PI and BFV showed normal distribution. For these variables, repeated measures analysis of variance (ANOVA) were used to determine the influence of injection (oxytocin and saline) or cows (healthy and RFM). One-way ANOVA was performed to determine effects of the time after injection. Significant results were further analysed with Dunnett's test, and the values after injection were compared with those before injection (control).

## Results

*Study 1: Time-dependent changes in the response of uterine blood flow to oxytocin in healthy cows*

The mean diameter ± SD of the uterine artery was 12.4 ± 3.1 mm (ipsilateral) and 6.7 ± 1.1 mm (contralateral). The absolute values of BFV and PI in both the uterine arteries, ipsi- and contralateral to the former pregnant horn, did not significantly differ between groups prior to oxytocin/saline injection (Table 1). The absolute values of BFV were higher ($P = 0.0002$), and PI were lower ($P < 0.0001$), in the UA-ipsi compared to those of the UA-cont. Blood flow volume decreased ($P = 0.0304$) and PI increased ($P = 0.0001$) from day 2 to day 5 pp in both uterine arteries.

On day 2 pp, BFV decreased rapidly 5 min after oxytocin administration in both uterine arteries (UA-ipsi: $P = 0.0001$, UA-cont: $P = 0.048$) and remained at a lower level for 60 min (contralateral) and 120 min (ipsilateral), respectively (Fig. 1). In contrast, there were no significant changes in BFV after oxytocin administration in either uterine artery on day 5 pp (UA-ipsi: $P = 0.09$, UA-cont: $P = 0.756$). On day 2 pp, PI increased ($P = 0.042$) at 5 min after the injection, remained at a higher level for 60 min, and returned to the basal level ($P = 0.949$) after 120 min in the UA-cont (Fig. 2). PI values did not change after oxytocin injection in the UA-ipsi ($P = 0.3976$). There was no significant change in diameter of either artery after oxytocin injection (UA-ipsi: $P = 0.9997$, UA-cont: $P = 0.9872$).

**Table 1**
Absolute values (mean ± SD) of blood flow volume (BFV) and pulsatility index (PI) in the uterine arteries ipsi- and contralateral to the former pregnant horn prior to oxytocin/saline injection (-5 min) on days 2 and 5 pp.

| Group | $n$ | Uterine artery | Day 2 | Day 5 |
|---|---|---|---|---|
| *BFV (mL/min)* | | | | |
| Oxytocin | 7 | Ipsi | 4928 ± 2870[a] | 2532 ± 1572 |
| | | Contra | 1018 ± 393[b] | 454 ± 99 |
| Saline | 5 | Ipsi | 4181 ± 2414[a] | 1926 ± 791 |
| | | Contra | 778 ± 331[b] | 504 ± 252 |
| *PI* | | | | |
| Oxytocin | 7 | Ipsi | 1.31 ± 0.24[a] | 2.24 ± 0.45[a,*] |
| | | Contra | 1.99 ± 0.38[b] | 3.07 ± 0.50[b,*] |
| Saline | 5 | Ipsi | 1.21 ± 0.19 | 1.72 ± 0.59 |
| | | Contra | 1.99 ± 0.52 | 2.44 ± 0.73 |

[a,b] Values with different letters differ within the same time ($P < 0.05$).
[*] Significant difference between day 2 and day 5 pp ($P < 0.05$).