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Effect of exogenous estradiol Benzoate on uterine blood flow in postpartum dairy cows

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ABSTRACT

The objective of this study was to assess the uterine blood flow following estradiol benzoate administration in Holstein-Friesian dairy cows by trans-rectal color Doppler ultrasonography. Six healthy lactating Holstein–Friesian cows were examined daily for 10 days starting at 4 weeks postpartum. All the cows, which were clinically healthy based on vaginal mucus scoring and endometrial cytology, were examined by trans-rectal Doppler ultrasonography to measure pulsatility index (PI), resistance index (RI), time average maximum velocity (TAMAX), blood flow volume (BFV) and diameter in the uterine arteries ipsilateral and contralateral to the previously pregnant uterine horn. On the third day of the experiment, the six cows were administered 10 mg intramuscular injection of estradiol-17β (E_2).Blood samples were collected at the time of daily examination for the assessment ofE_2concentrations.The PI and RI values decreased while TAMAX, BFV and diameter of uterine arteries increased in response toE_2 administration ($P < 0.05$).There was a high correlation between both the ipsilateral and contralateral uterine arteries for all variables that were studied($r = 0.860$, $P < 0.0001$, $r = 0.922$, $P < 0.0001$, $r = 0.651$, $P < 0.0001$, $r = 0.879$, $P < 0.0001$, r = 0.861, $P < 0.0001$ for the PI, RI, TAMAX, BFV and uterine arteries diameter, respectively).In conclusion, the greater blood concentrations ofE_2may be responsible for the greater TAMAX, BFV, increased diameters and decreased PI and RI of the uterine arteries during the puerperium in dairy cows.

1. Introduction

The physiological changes and control of the blood flow of the domestic animals reproductive tract have received great interest in the last several decades. One of the reasons is the introduction of Doppler ultrasonography to be used in animal reproduction. The blood flow of the uterine arteries has been studied during the estrous cycle by electromagnetic probes implanted surgically in cows (Ford et al., 1979; Ford and Chenault, 1981), sheep (Roman-Ponce et al.,1983; Reynolds et al., 1984)and sows (Ford and Christenson, 1979; Ford et al.,1982a; Ford et al.,1982b). These studies mainly demonstrated that the rhythmic changes in blood flow were correlated with the circulating concentrations of steroid hormones.

Recently, Ginther (2007) reported that color Doppler ultrasonography has been used on a wide range in assessing vasculature in the reproductive tract of large animals. The introduction of this technology in current research has allowed re-evaluating beliefs that

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were previously considered definitive regarding the physiology of reproduction. Color Doppler ultrasonography has increased the diagnostic and predictive abilities of theriogenology in farm animals.

The effect of estrogen in the physiological control of the uterine circulation has been an area of interest in reproductive physiology for long period. There is clear evidence that estrogen isa potent vasodilator compound. Thus, uterine hyperemia caused by estrogen injection has been estimated in sheep by direct collection of uterine venous blood (Huckabee et al., 1970), by flowmeters (Greiss and Anderson, 1970; Rosenfeld et al., 1973) and by microspheres (Rosenfeld et al., 1973). The endogenous estrogen produced during the estrous cycle appears to have a similar effect on uterine blood flow.

Trans-rectal color Doppler sonography of the uterine arteries in cows has been conducted only during the estrous cycle (Bollwein et al., 2000), pregnancy (Bollwein et al., 2002a; Panarace et al., 2006; Honnens et al.,2008a) and the normal puerperium (Heppelmann et al., 2012; Krueger et al., 2009). To the best of our knowledge, however, no published study has ever assessed the blood flow in the uterine arteries after exogenous estrogen injection. In addition to, exposure of the reproductive tract of postpartum cows to estrogen, there is a beneficial effect of preventing the premature secretion of PGF2α which could be the reason for the occurrence of short luteal phases. The aim of the present study, therefore, was to investigate the effect of exogenous estrogen administration on blood flow in the uterine arteries during the postpartum period in healthy cows.

2. Materials and methods

2.1. Experimental design

This study was conducted on a commercial dairy herd in Miyazaki City, Miyazaki prefecture, Japan. All experimental procedures were approved by the Animal Care and Use Committee of the University of Miyazaki (2013-08-01-Z4). Cows were housed in free stalls, fed a diet formulated according to the standard guidelines and milked twice a day. Cows with a history of normal parturition and puerperium were used. Six reproductively sound Holstein–Friesian cows were randomly selected for this study. All cows were examined daily for 10 days starting from the 4th week (32 ± 1 days: mean ± SD) postpartum. In addition to the ultrasonographic examination, the gynecological examination included vaginal mucous scoring and endometrial cytology (EC) on the first day to exclude cows with subclinical or clinical endometritis. All cows with vaginal mucous scoring more than 0 (mucus containing flakes of purulent material) or cows with polymorphonuclear leukocyte percentage (PMN%) greater than 5%, were excluded from this experiment.

2.2. Endometrial cytology samples

Endometrial cytology (EC) was conducted as described previously (Kasimanickam et al., 2004) with some modifications. In brief, EC samples were collected using a small brush (31 mm core length and 1 mm diameter, the brush part made from nylon with 25 mm length and 4 mm diameter). The brush was threaded onto a stainless steel rod (510 mm length and 2.4 mm diameter) by using a connector. Then the brush was placed in a stainless steel tube(500 mm in length and 4 mm in diameter) to facilitate its passage through the cervix. The prepared instrument was placed in a disposable plastic sheath (Fujihira Industry Co., LTD, Tokyo, Japan) then it was placed in a sanitary plastic sleeve (IMV Technologies, France). All the samples were taken while the animals were restrained in the stanchion and the tail tied by a rope and fixed to the neck of the cow. After washing and disinfection of the vulva, the prepared and covered instrument was inserted into the vagina forward toward the external opening of the cervix, where the sanitary sleeve was punctured, and the instrument was passed through the cervix into the uterine body where the stainless steel tube was retracted to expose the brush. The EC samples were collected by gentle rotation of the brush while being in contact with the uterine endometrium. After sample collection, the brush was retracted into the stainless steel tube beforefinal removal from the uterus.

Immediately after sample collection, slides for EC were prepared by rolling the brush part on two clean glass slides for each sample and fixed immediately with absolute methanol Cyto-fixative. On arrival to the laboratory, the slides were immersed in a Diff-Quik(SysmexCo., Ltd., Japan) Solution 1, for 20 s, and counterstained with Solution 2, for 20 s, then washed with distilled water and left to dry. The EC assessment was used to calculate the PMN %by counting a minimum of 400 cells of PMN and endometrial cells at ×400 magnification for quantitative assessment of endometrial inflammation. The stainless steel instrument was disinfected by antiseptic solution 0.05% benzalkonium chloride: (Alkyldimethylbenzylammonium chloride, surface acting quaternary ammonium disinfectant) between successive examinations. The brushes with the connector were sterilized by formaldehyde gas sterilization and neutralization equipment (HollSteri 130, Asukamedical Co., LTD, Osaka, Japan) before each farm visit.

2.3. Vaginal mucus collection

Vaginal mucus was collected as described by McDougall et al.(2007) with some modifications using vaginal mucus collection device (Metricheck, Simcro Tech, New Zealand). The mucous material within the inner surface of the Metricheck and/or adherent to its outer surface was scored (Sheldon et al., 2006) on a 0–3 scale (0 = clear translucent mucus, 1 = mucus with flakes of white discharge, 2 = discharges containing < 50% purulent material and 3 = discharges containing > 50% purulent material). The Metricheck device was disinfected with antiseptic solution before each use.