Reproductive Biology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Reproductive Biology

journal homepage: www.elsevier.com/locate/repbio

Uterine artery flow velocity waveform, arterial flow indices, follicular dynamics, and sex hormones during preovulatory period in synchronized ovulatory cycle of *Bos indicus* beef cows

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ARTICLE INFO

Keywords:
Doppler velocimetry
Northern Thai native cattle
Preovulatory period

ABSTRACT

A greater understanding of the uterine artery's (UtA) biology is essential to the increase in female reproductive abilities. The UtA flow velocity waveform, blood flow volume (BFV), pulsatility and resistance indices (PI and RI), blood flow velocities, dynamics of the dominant follicle (DF), and estradiol (E2) and progesterone (P4) levels in an induced ovulatory cycle were evaluated in Thai native cattle. Twenty cows were induced with synchronized ovulation through a P4-releasing device, from Day −9 to Day −4, concurrent with the administration of two doses of a gonadotropin-releasing hormone on Day −9 and Day −1, and two doses of prostaglandin $F_{2\alpha}$ on Day −4 and 8 h later. Day 0 was designated as the day of ovulation. The cows underwent Doppler sonographic determination and blood collection from Day −4 to Day 0. The cows were classified in the non-ovulating (n = 5) and ovulating groups (n = 15). The ovulating cows presented higher BFV values, blood flow velocities, DF growth rates, and E2 levels; yet lower PI values and P4 concentrations, than those of the non-ovulating cows. The BFV values and the blood flow velocities were greater, but the RI and PI values were lower in the ovulatory side UtA than in the contraovulatory side UtA. The BFV values were positively correlated with blood flow velocities, DF growth rates and E2 concentrations in the ovulating cows; confirming the importance of UtA blood flow, follicular growth, and E2-vasodilation during preovulatory phase in the induced ovulatory cycle of *Bos indicus* beef cows.

1. Introduction

The blood flow and perfusion directed to the uterus and ovary are critical to the support of the nutrient, hormone, and growth factors in the development of uterine and ovarian cells [1]. Doppler ultrasonography is considered to be an effective, non-invasive method for the quantitative determination and clinical application of artery blood flow in humans and animals [2]. Uterine volumetric blood flow is measured through semi-quantitative examination, using the so-called Doppler indices; namely, the pulsatility index (PI) and resistance index (RI) [3]. The Doppler ultrasonographic indices were calculated automatically from the flow velocity waveform (FVM) [4]. In domestic animals, Doppler sonography is extensively applied to evaluate uterine artery (UtA) blood flow in sows [5], ewes and goats [6], *Bos taurus* cows [7], *Bos indicus* cows [8], as well as buffalo cows [9]. In *Bos taurus* dairy cattle (Holstein Friesian cows), changes in the UtA blood flow during the estrous cycle were dependent on reproductive patterns, regulated by sex-steroid hormones, such as 17β-estradiol (E2) and progesterone (P4) [7]. Moreover, the blood flow impedance (RI value) was the lowest on the day of ovulation during the spontaneous estrous cycle in the *Bos indicus* dairy cattle (Sahiwal cows) [8]; however, characterization of uterine artery flow velocity waveform (UtAFVW), blood flow volume (BFV), UtA indices, and blood flow velocities during the preovulatory phase in a synchronized ovulatory cycle had not yet been investigated in *Bos indicus* beef cattle. Bovine breeds are not only important livestock species, but their follicular and luteal developments make them ideal models for humans [10,11]. The application of the bovine model has resulted in a greater expansion of reproductive knowledge regarding follicular dynamics and luteal development in mammalian animals, as well as humans. Similar to women, cattle are monoovulatory species, and exhibit a wave-like pattern of antral follicular development [10,12,13]. To obviate the practical difficulties that limit experimental

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https://doi.org/10.1016/j.repbio.2018.01.008
Received 23 September 2017; Received in revised form 17 January 2018; Accepted 20 January 2018

design in humans, the *in vivo* bovine model is required to study the relationship among UtA blood flow, follicular dynamics, and sex hormones during the preovulatory period. Thus, the aim of this study was to determine the UtAFVW, BFV, Doppler indices, blood flow velocities, follicular growth, and the sex-steroid hormones obtained during the preovulatory periods in an induced ovulatory cycle of ovulating and non-ovulating White Lumphun beef cows (northern Thai native cattle).

2. Materials and methods

2.1. Animal assurance

Twenty multiparous White Lamphun cows of average age (6.3 ± 1.8 years) were studied. Prior to the experiment, all cows were examined to ensure absence of reproductive problems, using a transrectal ultrasound machine, and were vaccinated against foot and mouth disease (FMD). All cows were managed under optimal management practices, as per the standard criteria fixed for maintenance of breeding beef cows in the Beef and Dairy Cattle Research Unit, Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, Thailand (Latitude: 18°45′ N/Longitude: 98°55′ E). The beef cows were housed in individual pens, providing sunshade and rain cover. Each individual pen comprised an area of approximately 8.0 m^2 (2.0 m × 4.0 m), and had rubber sheet flooring in the cattle stall. The animals were exposed to the natural photoperiod and regional weather. The beef cows received sweet corn stover silage as roughage *ad libitum,* containing a commercial concentrate (14% crude protein, 13% moisture, 12% crude fiber, and 3% crude fat) supplementation. Clean water and mineralized salt licks were also available *ad libitum*.

2.2. Short-term five-day synchronization regime

Twenty non-pregnant *Bos indicus* beef cows were induced with synchronized ovulation, through the five-day CO-Synch + controlled internal drug release (CIDR) device-based protocol (Fig. 1), as previously described by Bridges et al. [14]; and Kasimanickam et al. [15]. On the first day of the synchronization treatment (Day −9), the first 10 μg dose of gonadotropin-releasing hormone (GnRH; Receptal, MSD Animal Health, New Zealand) was applied to induce ovulation and reset follicular growth. At the same time (Day −9), a CIDR (1.9 g of synthetic P4, Eazi-Breed CIDR, Zoetis Inc., New Zealand) was inserted intravaginally into the beef cows for five days, from Day −9 to Day −4 (short-term five-day synchronization regime). To induce luteal regression (luteolysis), the beef cows received two 500 μg doses of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; Estrumate, MSD Animal Health, New Zealand), with the first administration given on Day −4, followed by a second administration, 8 h later. On Day −1, the second dose of GnRH (10 μg) was administered to induce ovulation of the new dominant follicle (DF).

2.3. UtA visualization and location

Transrectal color Doppler ultrasonography was performed with a 5.0 MHz linear-array transducer for B mode (gray scale) and color imaging (SonoTouch 30VET; Chison Medical Imaging Co., Ltd., China) in order to visualize both UtAs (left and right) of the 20 beef cows. The Doppler sonographic determinations, as well as arterial evaluations, were scanned via an electromagnetic blood-flow transducer once daily, from Day −4 until ovulation. All UtA evaluations were performed by the same examiner, and lasted approximately 15 min for each beef cow. The Doppler blood flow evaluated the main UtAs [7,16]. The Doppler probe was oriented transversely and faced dorsally, such that the aorta was observed and followed caudally until its branching, through which the main UtAs were basically identified as movable arterial vessels in the mesometrium [7,16,17]. The UtAs, the main arterial vessels supporting the uterus, were found to have diameters > 5.0 mm in the non-pregnant cows [3,16]. The relative position and dimension of the main UtAs were sketched and recorded on arterial charts in order to calculate the cross-sectional area. To eradicate the signals from slowly moving tissues and arterial wall movements in the route of the Doppler machine pulse, a linear-array probe and a high-pass filter were provided at 5.0 MHz and 50 Hz, respectively [16,18]. A Doppler blood-flow probe was deposited across the arterial vessel, with the aim that the angle of insonation would fall within the range of 20°–60° between the color Doppler beam and the arterial vessel [16,19]. Each Doppler recording was digitalized and videotaped for later analysis.

2.4. Follicular dynamics and ovulatory capacity

Follicular evaluation through scanning with transrectal ultrasonography was conducted once daily, from Day −4 to Day 0, in order to confirm ovulation (Fig. 1). During the preovulatory period (Day −4 to Day 0), ovulation was considered to have occurred given the disappearance of the DF ≥7.0 mm in diameter [20,21]. The mean rates of follicular growth were calculated using the diameter of the DF at its detection, as well as on the day of evaluation, divided by the time of the growth period [22]. On Day 0, the 20 beef cows were classified by monoovulatory appearance, as they “did not exhibit ovulation” (non-

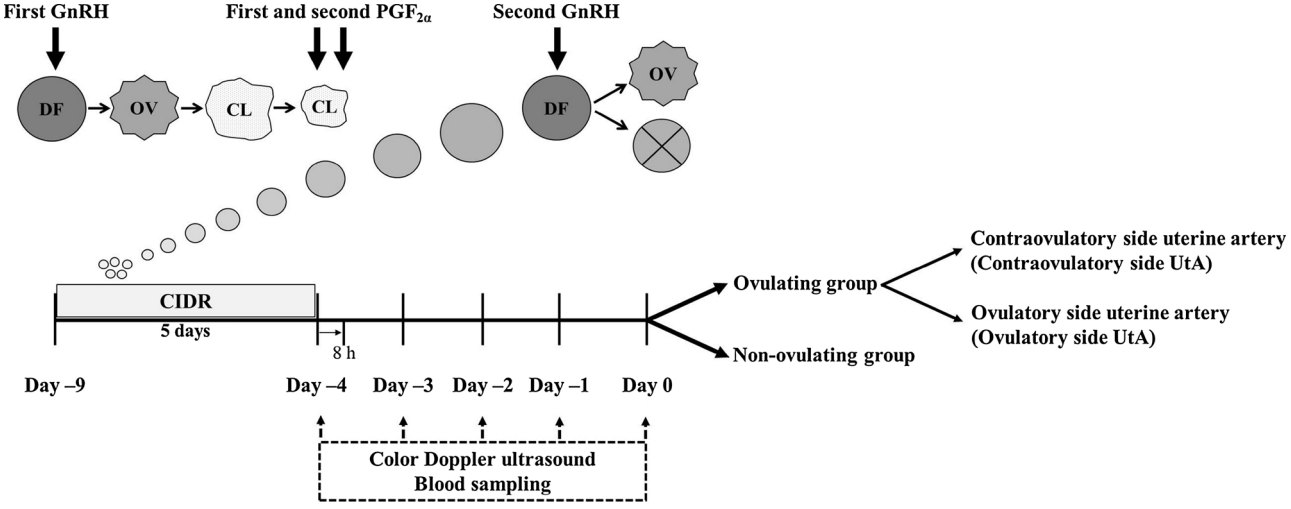

Fig. 1. The experimental design and description of the controlled follicle and corpus luteum. Abbreviations: CIDR, controlled internal drug release device; CL, corpus luteum; DF, dominant follicle; GnRH, gonadotropin-releasing hormone; OV, ovulation; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; UtA, uterine artery.