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Characterization of follicle and CL development in beef heifers using high resolution three-dimensional ultrasonography

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A B S T R A C T

The aim was to characterize dominant follicle (DF) and CL development through the estrous cycle of cattle using three-dimensional (3D) ultrasonography while making a comparison with conventional two-dimensional (2D) B-mode ultrasound (US) and to relate the measures taken to systemic concentrations of steroid hormones and gonadotropins. After synchronization of estrus, the ovaries of crossbred beef heifers (N = 5) were assessed using daily US with a GE Voluson *i* US scanner until the end of the first follicle wave, then every other day until emergence of the final (ovulatory) wave, when daily US resumed until ovulation. Follicle and CL growth were recorded and mapped. Measures of diameter (2D) and volume (3D) of the DF from the first and ovulatory waves of the cycles; and CL development were captured and stored for further analysis. Blood flow to the DF and CL were assessed using 3D power Doppler US measuring vascularization index (VI; %), vascularization flow index (0/100) and flow index (0/100). Jugular blood samples were collected every 24 hours for progesterone from the first estrus until the second ovulation. Concentrations of estradiol (E2) and follicle stimulating hormone (FSH) were measured every 8 hours from estrus to second follicle wave emergence; then, E2 only was measured from final follicle wave emergence until ovulation. Data were analyzed using PROC MIXED and PROC REG in SAS. Dominant follicle blood flow tended to decrease during follicle wave emergence and DF VI increased ($P < 0.05$) 24 hours before ovulation after peak E2. Measures of the DF and CL volume (3D) were highly predictive of 2D diameter measures throughout the cycle ($P < 0.0001$). Predictive values (r^2) for day of wave emergence and day from ovulation were similar for 2D and 3D measures; however, 2D measures had higher repeatability when compared with 3D measures. There was no relationship between CL VI and progesterone early in the cycle ($r^2 = 0.12$; $P = 0.1$); however, there was a strong positive relationship approaching ovulation ($r^2 = 0.77$; $P < 0.0001$). In conclusion, 3D power Doppler measures of blood flow appears to be representative of vascular changes in the DF and CL throughout the estrous cycle. However, the extra time required to acquire and analyze a 3D image and the relatively little additional information obtained over that achievable with 2D imaging in terms of follicle and CL development might preclude its widespread use other than for detailed research purposes.

1. Introduction

The use of ultrasound imaging for the noninvasive examination of reproductive organs in cattle has contributed to

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substantial advances in biology over the past two decades [1–3]. Conventional B-mode imaging was first used in cattle in the 1980s [4]. It has since played an integral role in reproductive management of cattle. The characterization of bovine follicular waves and pregnancy diagnosis using ultrasound has been particularly important [5,6], giving rise to the development of improved synchronization protocols and a new understanding of follicular dynamics during the estrous cycle [7]. Before the availability of ultrasound examination it was only possible to observe the ovaries after surgery or slaughter and so the proposed existence of waves of follicular growth could not be verified [8].

It is now well known that in cattle, two or three follicle waves occur during each estrous cycle, identifiable by the emergence of a cohort of follicles (>5 mm), that is stimulated by a transient increase in follicle stimulating hormone (FSH) concentrations [9,10]. The growing cohort secrete inhibins and estradiol (E2) that decrease FSH concentrations to basal levels and during this period, selection of a dominant follicle (DF) occurs [10]. The follicle undergoing selection preferentially develops LH receptors in its granulosa cell layer and benefits from enhanced insulin-like growth factor-I bioavailability [11]. Ovulation of the DF is dependent on LH pulse frequency and amplitude which is typically not sufficient for ovulation early in the cycle during the luteal phase [12]. However, during the follicular phase, LH pulse frequency increases, allowing for final maturation and ovulation of the DF [10,13]. An adequate supply of blood to the DF is vital for its survival and development and an increase in blood flow to the follicle is known to be associated with ovulation [14]. Survival of the CL is dependent on successful angiogenesis after ovulation and then maintenance of the vascular network to allow for sufficient progesterone (P4) secretion [15], therefore blood flow should be identifiable in corpora lutea during the luteal phase.

Two-dimensional (2D) B-mode ultrasound is only capable of allowing visualization of external features of an organ whereas, three-dimensional (3D)/four-dimensional (4D) imaging can reveal internal features, and possibly provide new information about the organ of interest [16]. Three-dimensional imaging was first developed in the 1990s and is now a standard tool in obstetrics and gynecology used routinely for the diagnosis of various clinical problems such as fetal abnormalities [17] and tumor growth [18] in humans. A 3D ultrasound examination produces a series of still volumes that can be displayed in any plane after the examination. Real-time 3D imaging, also referred to as 4D imaging, incorporates the fourth dimension of time meaning that the volume data set of a region of interest can be stored and later visualized as one single dynamic image [16]. Recent animal studies have adopted the use of 3D/4D imaging in their research, successfully characterizing events such as equine and elephant fetal development [19,20] and pregnancy diagnosis in cats and dogs [16]. Image acquisition using 3D/4D technology has improved with the introduction of high frequency ultrasound transducers, allowing volume data sets to be produced much faster which might help to overcome commonly reported problems of breathing artifacts and animal movement.

The aim of this study was to characterize DF and CL development in beef heifers using 3D/4D imaging technology and to establish the relationship between 3D and 2D measurements to assess the potential advantages of 3D imaging compared with the standard B-mode techniques in bovine research.

2. Materials and methods

2.1. Animals and treatments

All experimental procedures involving animals were licensed by the Department of Health and Children, Ireland. Protocols were in accord with the Cruelty to Animals Act (Ireland 1876) and the European Community Directive 86/609/EC and were sanctioned by the Institutional Animal Research Ethics Committee in University College Dublin.

All animals were housed indoors on straw bedding for the duration of the experiment and were offered ad libitum access to a diet consisting of 50:50 maize silage (Dry matter (DM); 344 g/kg, Crude Protein (CP); 76 g/kg, and Metabolisable Energy (ME); 11.8 MJ/kg DM) and grass forage (DM 239 g/kg, CP 101 g/kg, and ME 11.1 MJ/kg DM) in addition to 8-kg concentrates (DM 883 g/kg, CP 281 g/kg, and ME 12.9 MJ/kg DM) per day.

The estrous cycles of crossbred beef heifers (N = 8), predominantly Charolais and Limousin breeds aged between 18 and 24 months, were synchronized using an 8-day Controlled Internal Drug release (CIDR 1.9 g; Pfizer) intravaginal device with administration of a $PGF_{2\alpha}$ analogue (2 mL; Estrumate, Schering Plough Animal Health, Hertfordshire, UK) equivalent to 0.5 mg cloprostenol 1 day before CIDR removal. Heifers were observed for signs of estrus four times per day commencing 30 hours after CIDR removal and only those recorded in standing estrus within the next 24 hours (Day = 0; N = 5) were used.

2.2. Ultrasound examinations

Transrectal ultrasound scanning began on Day 0 (i.e., day of estrus) and was carried out daily throughout the first follicular wave then every other day until emergence of the ovulatory wave when daily scanning was resumed until ovulation. Follicle wave emergence was determined as the day when a cohort of between five and 10 follicles larger than 5 mm emerged on the ovaries. Three-dimensional power Doppler and 2D images of the DFs and CL during the first follicular wave and approaching ovulation were captured and stored for further analysis. Examinations were carried out using a Voluson *i* portable ultrasound system (GE Healthcare, Vienna, Austria) equipped with a convex RNA 5-9 probe which has 144° × 90° field of view. Identical preinstalled instrument settings (color gain, pulse repetition frequency, color-power, wall motion filter, frame rate) were used during all examinations. Examinations were carried out by the same operator at the same time (between 9 AM and 11 AM) each day, taking on average 30 minutes per animal. Heifers were sedated with a low dose of xylazine hydrochloride (0.2 mL per animal; Chanelle Pharmaceuticals Manufacturing Ltd., Galway, Ireland) which was diluted in 5 mL of Norocaine (Norbrook Laboratories Ltd., Newry, Northern Ireland) and administered as