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Interrelationships among morphology, echotexture, and function of the bovine corpus luteum during the estrous cycle

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ABSTRACT

It has been suggested that ultrasound image attributes are a potential indicator of physiological and functional status of the corpus luteum (CL) in several species, including cattle. The aims of this study were to evaluate CL morphological, functional and echotextural characteristics, and also to investigate the hypothesis that those attributes are correlated and change similarly throughout an estrous cycle. Ovaries of crossbred (*Bos taurus taurus* × *Bos taurus indicus*) heifers were evaluated using ultrasonography daily throughout an interestrus interval using a B-mode, real-time ultrasound machine equipped with a 5 MHz linear-array rectal transducer, during a natural estrous cycle (Experiment 1; $n = 12$) or during a shortened cycle, with luteolysis induction 10 d after estrus (Experiment 2; $n = 6$). Blood samples were collected for assay of plasma progesterone concentrations. Corpora lutea areas were measured and daily images of each CL were videotaped and digitized for computer-assisted analysis using custom-developed software. In Experiment 1, area of luteal tissue increased until a maximum value 10 d after estrus ($P < 0.001$), followed by a plateau phase, and then a decline beginning 14 d after estrus. Luteal tissue area was highly correlated to plasma progesterone concentrations ($r = 0.86$; $P < 0.001$). When luteolysis was induced in Experiment 2,

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loss of CL function (decrease in plasma progesterone concentrations to metestrous values) preceded tissue regression by 48 h (24 h compared with 72 h; $P < 0.001$). The mean pixel value of ultrasound images did not change in Experiment 1 ($P > 0.70$), but a day effect on this attribute was observed in Experiment 2 ($P = 0.052$). In contrast, mean pixel value was correlated to plasma progesterone concentrations in Experiment 1 ($r = -0.63$; $P < 0.05$), but not in Experiment 2 ($r = -0.28$; $P > 0.10$). In regard to CL heterogeneity, defined as the standard deviation of the mean pixel value of the luteal tissue, a time effect was observed following both natural (Experiment 1; $P < 0.009$) and luteolysis-induced (Experiment 2; $P < 0.05$) estrous cycles ($P < 0.05$). Moreover, this variable was correlated with plasma progesterone concentrations ($r = -0.71$ and -0.58 in Experiments 1 and 2, respectively; $P < 0.01$), indicating that CL images were more heterogeneous during metestrus and after luteolysis (functional regression). In summary, morphological and echotextural attributes were correlated with CL function and underwent similar changes during the estrous cycle. Luteal tissue heterogeneity, assessed by ultrasonography, is considered a potential indicator of CL functional status, because it is correlated to circulating progesterone concentrations.

1. Introduction

The corpus luteum (CL) is a transitory endocrine gland that secretes progesterone, and has a key role in establishment and maintenance of pregnancy in domestic mammals (Niswender et al., 1994; Fields and Fields, 1996; Schams and Berisha, 2004). In cattle and other domestic animals, lifespan of the CL is mainly controlled by the uterine luteolytic hormone, prostaglandin $F_{2\alpha}$, which affects estrous cycle length (Milvae, 2000).

Real-time B-mode ultrasonography has been extensively applied in animal reproduction research to study basic physiological events and morphological changes, such as ovarian follicular dynamics (Ginther et al., 1989; Fortune, 1993; Wiltbank et al., 2002), or as an intrinsic step in current biotechnology techniques, such as transvaginal follicular aspiration of cumulus–oocyte complexes (Pieterse et al., 1991; Kruip et al., 1994; Hashimoto et al., 1999; Galli et al., 2001). An increase in the use of ultrasonography during the last 10–15 years has allowed for the understanding of very important reproductive phenomena occurring during the estrous cycle (e.g. follicular recruitment, development and dominance; follicle wave patterns; CL development), and resulted in the establishment of more effective hormonal protocols for estrous/ovulation synchronization (Pursley et al., 1995) and superovulation (Bo et al., 2008).

Use of ultrasonographic examinations permits the sequential assessment of size (area), form, and consistency of the CL (Pierson and Ginther, 1987; Kastelic and Ginther, 1989). It is, therefore, possible to monitor the development of a CL throughout its lifespan. Use of ultrasonography to assess CL characteristics, however, has not permitted the differentiation between a developing or regressing CL (Pieterse et al., 1990; Hanzen et al., 2000).

Ultrasonography is based on the ability of tissues to differentially reflect or transmit high frequency sound waves, indicating variations in tissue density. Ultrasonographic images are composed of thousands of picture elements called pixels, and each of these pixels is represented numerically in a scale of 256 shades of gray (0 = black; 255 = white) according to their brightness intensity (Pierson and Adams, 1995; Singh et al., 1997, 1998; Tom et al., 1998). The technique of computer-assisted image analysis allows quantitative and objective assessment of brightness intensity of each pixel composing an image, overcoming the subjectivity of visual analyses. In the assessment of tissue echotexture, a mathematical matrix is generated using these numerical values, and two main variables are analysed: mean pixel value and pixel heterogeneity (Singh et al., 2003).