Animal Reproduction Science 127 (2011) 7–15

Contents lists available at ScienceDirect

Animal Reproduction Science

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

Ultrasound image analysis offers the opportunity to predict plasma progesterone concentrations in the estrous cycle in cows: A feasibility study

Johan M. Thijssen[a,*,1], Kathrin Herzog[b,1], Gert Weijers[a], Maren Brockhan-Luedemann[b], Alexander Starke[b], Heiner Niemann[c], Heinrich Bollwein[b], Chris L. de Korte[a]

[a] Clinical Physics Laboratory, Radboud University Medical Centre, P.O. Box 9101, 6500HB Nijmegen, The Netherlands
[b] Clinic for Cattle, University of Veterinary Medicine Hannover, Bischofsholer Damm 15, D-30173 Hannover, Germany
[c] Institute of Farm Animal Genetics, Friedrich Loeffler Institute (FLI), Hoeltystrasse 10, Mariensee, D-31535 Neustadt, Germany

ARTICLE INFO

Article history:
Received 13 December 2010
Received in revised form 22 June 2011
Accepted 13 July 2011
Available online 23 July 2011

Keywords:
Corpus luteum
Estrous cycle
Luteal phases
Cow
Progesterone level
Computer Assisted Ultrasound (CAUS)
Ultrasound imaging
Image analysis
Ultrasound parameters

ABSTRACT

In recent years, several attempts have been made to evaluate the activity of a corpus luteum by determining its sonographic echo texture. In all of these studies the values of the echo texture parameters depended on the type and settings of the ultrasound machine. Therefore, the aim of the study was to investigate if a quantitative analysis of ultrasound (US) images of the *corpus luteum* (CL) after calibration of the ultrasound machine enables the assessment of the peripheral plasma progesterone (P4) level. Ten Holstein Friesian cows were examined daily at Days 4 to 8, 10 to 16, and −5 to −1 (Day 1 = ovulation) of the estrous cycle. B-mode sonography of the corpora lutea was performed and blood samples were taken for plasma P4 analysis. US images were calibrated and analyzed using a software package (CAUS) developed by the authors. In addition to the area of the CL (Total Area, TotA; Tissue Area interactive, TisAi; Tissue Area Automatic, TisAa), the following US parameters were calculated from the gray level histogram and from the size of the speckles: Mean, Standard Deviation (SD) and Signal-to-Noise Ratio (SNR = Mean/SD) of echo levels, Residual Attenuation (ResAtt), Axial and Lateral speckle size (Ax and Lat, respectively). The inter-individual variability of the P4 level was expressed by the coefficient of variability (CV), averaged over all days. It appeared that the CV of the absolute P4 was high (0.65) and the P4 relative to that at Day 4 and at Day 16 was of comparable magnitude. Correlations of US parameters with P4 were highest for the P4 relative to Day 16 (P4_rel_D16). This relative P4 measure was then used for further analysis. The correlations of P4_rel_D16 with TotA, TisAa (CL area after automatic segmentation of tissue) and ResAtt were found the highest ($R = 0.68$, 0.74, and −0.42, respectively). Multiple linear regression analysis, incorporating all US parameters revealed the formula: $P4\_rel\_D16_{pred} = -0.315 + 0.225TisAa - 0.023ResAtt$, and a goodness of fit: $R^2 = 0.59$ ($p < 0.001$). This formula was then used to "predict" for each image the P4_rel_D16 from the estimated US parameters. A high correlation of the predicted with the measured P4_rel_D16 was found: $R = 0.77$. Classification of images using the predicted P4_rel_D16 to be in the range >0.80 (corresponding to 0.95 times the average_P4_rel_D16 measured during the "static" phase of the luteal cycle) by ROC analysis was correctly made in 88% of cases. In conclusion the quantitative analysis of calibrated ultrasound images may yield a good prediction of cyclic changes of P4 levels and has potential for predicting the phase in the estrous cycle of a cow.

* Corresponding author. Tel.: +31 24 3619061.
E-mail address: j.thijssen@cukz.umcn.nl (J.M. Thijssen).
[1] These authors equally contributed to this work.

0378-4320/$ – see front matter © 2011 Published by Elsevier B.V.
doi:10.1016/j.anireprosci.2011.07.006

1. Introduction

The determination of peripheral plasma progesterone (P4) concentration is of great importance in high-yielding dairy cows during the estrous cycle and during early pregnancy (Mann and Lamming, 2001). Low plasma P4 concentrations play a pivotal role in the diagnosis of subfertility (Mann and Lamming, 2001). The analysis of P4 concentration in plasma during the daily routine is characterized by relatively high costs and a time interval between blood withdrawal and availability of the diagnostic result. For this reason non-invasive means to obtain information about the P4 productivity of the corpus luteum (CL) have been developed. One of the most promising techniques was the use of ultrasound (US) imaging of the CL. In a few studies a correlation could be shown between the peripheral P4 concentration and luteal size during the early phase (Kastelic et al., 1990; Ribadu et al., 1994), as well as during the late phase of the estrous cycle (Assey et al., 1993; Son et al., 1995). These results are indicating a possible relation between luteal size and P4 concentration.

A second ultrasound technique being used is color Doppler imaging, where the number of colored image pixels is considered to be indicative of luteal blood flow (LBF) and perfusion. Although several authors have indicated that a strong correlation is present between luteal perfusion and peripheral P4 concentration (e.g., Bollwein et al., 2002; Ginther et al., 2007; Herzog et al., 2010), it has been concluded that the evidence is not strong enough to use color Doppler imaging to predict the P4 concentration.

Quantitative B-mode ultrasound was used by Siqueira et al. (2009). These authors investigated the estrous cycle related behavior of the following US parameters: CL area, Mean pixel value and pixel heterogeneity (Standard Deviation of echo levels), as well as the correlation of these parameters with P4 level. However, these authors did not calibrate their equipment, nor did they consider the depth dependence of the texture characteristics of the images. They kept the main settings of the US equipment fixed during the experiment (overall gain, depth gain and transmit focus). No information was provided about the contrast and post processing settings of the equipment. Estrous cycle related behavior was observed for CL area and heterogeneity, not for Mean pixel value. Significant correlations of all three parameters with P4 level were found.

Herzog et al. (2008) also reported a quantitative ultrasound study and they found a significant cyclic behavior of CL area, Mean gray level and several second order statistical image texture parameters. They kept the equipment settings stable throughout the study but again the system was not calibrated, nor the effects of depth dependence of the texture taken into account. In the present study, quantitative analysis was applied to US images of the CL using the (pre)processing scheme and the software package for Computer Aided Ultrasound (CAUS) (Thijssen et al., 2008; Starke et al., 2010). In addition to the CAUS parameters, the luteal area was estimated over the whole estrous cycle. The hypothesis that the estimated US parameters could be used for predicting the plasma P4 concentration by using the multiple regression formula of P4 level vs. optimal US parameter set was tested.

2. Materials and methods

2.1. Subjects

In this study, healthy Holstein Friesian cows ($n = 10$) were selected and examined between October 2007 and May 2008 at the Research farm of the Institute of Farm Animal Genetics, Friedrich-Loeffler Institute, Mariensee, Germany. The study was approved and conducted in accordance with the German legislation on animal welfare (Lower Saxony Federal State Office for Consumer Protection and Food Safety, 33.9-42502-04-07/1370). Animals were between 2.8 and 8.0 years old, had a Mean lactation number of 1.9, and were examined 114 ± 12 (Mean $\pm$ SD) days after calving. The cows were housed in tie-stalls from December 2007 till April 2008, fed a total mixed ration ad libitum (6.6 MJ NEL/kg dry matter, 14.9% crude protein), and had free access to water. They were pastured from May till November 2008 and, in addition, individually fed concentrates commensurate with milk production (approximately 1 kg concentrate for each 2 kg of milk produced).

2.2. Study design

All cows were subjected to the OvSynch protocol, which consists of subcutaneous administration of GnRH analogue (10 μg Buserelin i.m., Receptal®, Intervet Inc., Unterschleissheim, Germany). After seven days an injection of $PGF_{2\alpha}$ analogue (657.5 μg Cloprostenol-sodium salt i.m., Estrumate®, Essex Inc., Munich, Germany) was carried out and followed by an injection of GnRH two days later. The cows were monitored for ovulation at 12, 24, and 36 h after the final injection. The day that the dominant follicle could no longer be visualized by US examination was taken as Day 1 of the cycle Cows were examined daily at Days 4 to 8, 10 to 16, and −5 to −1 (Day 1 = ovulation) of the estrous cycle and blood samples were taken for plasma P4 analysis after each US examination of the ovaries, which was performed between 08.00 and 12.00 am.

To compare parameters between estrous cycles of different lengths, we considered Days 4 to 16 and Days −5 to −1. The estrous cycle was subdivided into three different periods (Tom et al., 1998): *growing phase* (Days 4 to 8), *static phase* (Days 10 to 16) and *regression phase* (Days −5 and −1).

2.3. Plasma P4 concentration

Plasma was separated by centrifugation within 30 min after a blood sample was taken. Samples were stored at −20 °C until analysis was performed. Plasma P4 was assessed by using a commercially available chemiluminescence immunoassay (Immulite®, Siemens Healthcare Diagnostics, Deerfield, IL 60015-0778, USA). The intra- and inter-assay coefficients of variation were <10%. The lower detection limit was 0.5 ng/ml. The intra- and inter-assay coefficients of variation (CVs) were <10%.

Because it appeared that the P4 concentrations were different in various animals by a factor of four, the hypothesis was tested that the P4 level relative to the one at Days