Endometrial echotexture parameters in Turkish Saanen Goats (Akkeci) during oestrus and early pregnancy

M. Cengiz a,*, H. Kanca b, S. Salar b, A. Bastan b, I. Kucukaslan c, H. Alkan b, K. Karakas b, O. Yuksel b, A. Hayirli d

a Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Ataturk University, 25240 Erzurum, Turkey
b Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Ankara University, 06110 Ankara, Turkey
c Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Dicle University, 21300 Diyarbakir, Turkey
d Department of Animal Nutrition and Nutritional Disorders, Faculty of Veterinary Medicine, Ataturk University, 25240 Erzurum, Turkey

A R T I C L E   I N F O

Article history:
Received 21 May 2013
Received in revised form 7 January 2014
Accepted 6 February 2014
Available online 18 February 2014

Keywords:
Goat
Echotexture
Endometrium
Oestrus
Early pregnancy
Computer assisted image analysis

A B S T R A C T

This experiment was conducted to evaluate endometrial echotexture in oestrus and early pregnancy and its association with ovarian hormones and foetal count in goats. Akkeci goats (Saanen × Kilis crossbreed, n = 40) were randomly divided into two groups. Ten does (NAT) were mated on natural oestrus and 30 does (SYN) were subjected to synchronisation-prior to mating. The uterus was scanned on the days of sponge insertion (d −14), sponge removal (d −2) and mating (d 0) as well as 17 (d 17) and 30 (d 30) days after mating. Mean gray level (MGL), homogeneity (HOM) and contrast (CON) values were calculated. Blood samples were collected on days ultrasonography was performed. Data were analyzed by Chi-square, ANOVA, regression tests. HOM value reached the highest level on the mating day and then continuously decreased (P < 0.0001). Overall, HOM values were greater for SYN does than for NAT does after mating. CON values were virtually stable during the experimental period. MGL value fluctuated during the breeding period (P < 0.03) at a similar fashion in NAT and SYN does. Foetal count was not correlated with plasma hormones and echotexture parameters. Plasma progesterone concentration was correlated with echotexture parameters (r = −0.28 for HOM; r = 0.29 for CON; r = 0.25 for MGL; P < 0.05 for all) during post-mating. In conclusion, echotexture parameters changed during the breeding period, in association with plasma progesterone concentration. Future studies should test if the echotextural changes during embryonic fixation days can be used as a marker for early detection of pregnancy in does.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

In the past 3 decades, B-mode real time ultrasonography has become an important tool for reproductive management and research in small ruminants (Erdogan, 2012). Ultrasonographic imaging is based on the ability of tissues to reflect high frequency sound waves and reflection varies with tissue density (Pierson and Adams, 1995; Singh et al., 1997). A portion of the waves is reflected by tissue interfaces, while other parts of the waves are propagated. Images are displayed as two-dimensional maps of grayscale based upon location and strength of the echoes returning from the tissue interfaces (Zagzebski, 1996).

A two-dimensional ultrasonographic image is a matrix of square picture elements (pixels), which vary in grayscale values ranging from 0 (absolute black) to 255 (absolute
White) (Griffin and Ginther, 1992; Pierson and Adams, 1995; Singh et al., 1997). The ultrasonographic appearance or image pattern of a tissue is termed echotexture that varies by the tissue histological structure. Echotexture of the sonographic images is usually determined by subjective visual evaluation of the examiner. However, the density and gross features of the tissue can not be quantified by the human eye that can only distinguish 18–20 different shades of gray (Pierson and Adams, 1995). Computer algorithms (computer-assisted analysis) permits a quantitative assessment of the intensity of each pixel present on an image, allowing an objective evaluation of the image and thus, overcoming the subjectivity of the visual analyses (Singh et al., 2003).

In recent years, computer-assisted ultrasonographic image analysis of B-mode ultrasonography has been used for evaluation of reproductive tissues, including testes (Gabor et al., 1998), ovarian follicles and CL’s (Vassena et al., 2003; Davies et al., 2006; Liu et al., 2007) and uterus in farm animals (Bertmann et al., 2004). Cyclical endometrial echotexture changes in relation to peripheral concentrations of ovarian hormones have been reported in cows (Schmauder et al., 2008) and mares (Ginther, 1998). Furthermore, a recent study by Kauffold et al. (2010) showed that pregnant and cyclic pigs differed in the uterine echogenicity during the early pregnancy. However, only a few studies (Simões et al., 2007; Arashiro et al., 2010) have been performed in goats, in which computer-assisted analysis technique was employed for reproductive tissues and luteal function. It was hypothesized that peripheral progesterone and oestradiol 17-β concentrations as well as embryo/foetus number would affect uterine echotexture in early pregnant goat endometrium. The objectives of the present study were to investigate endometrial echotexture in oestrus and early pregnancy and its association with ovariian hormones and foetal count in goats.

2. Materials and methods

2.1. Animals and experimental protocol

The experiment was conducted on a dairy goat farm located in the vicinity of Ankara, Turkey (39° 53′ E; 32° 45′N; altitude of 938 m) during the natural breeding season (October and November 2011). Forty Akkéci goats (Saanen × Kilis crossbreed) at the age of 2–4 years old and weighing of 30–35 kg with body condition score of 3–4 (Suiter, 1994) were used in the trial. All animals were kept in an outdoor paddock under conditions of natural light and temperature. The animals were fed alfalfa hay and pellets. Fresh water and mineralized salt licks were available ad libitum.

Does were randomly divided into two groups. Ten does (NAT) were mated on natural oestrus, serving control group. Thirty does (SYN) were subjected to synchronised oestrous cycle by inserting 20 mg flugestone acetate impregnated sponges (Chronogest CR®, MSD, Istanbul, Turkey) for 12 days. SYN does were then injected with 500 IU eCG (i.m., Chronogest/PMSG®, MSD, Istanbul, Turkey) at sponge withdrawal. Forty-eight hours later, three fertile Akkéci bucks (2–5 years old) fitted with marking harnesses were introduced to the does.

2.2. Ultrasonography

Real-time ultrasound scanning of uterus was facilitated via the transrectal route on the days of sponge insertion (d –14), sponge removal (d –2) and mating (d 0) as well as 17 (d 17) and 30 (d 30) days after mating. Transrectal ultrasonography of the uterus was done using a 7.5 MHz linear-array transducer connected to a portable B-mode ultrasound scanner (Wed 3000®, Shenzhen WELLD Medical Electronics co., Ltd., Shenzhen, China) as described previously (Kähn, 2004). Standardized machine settings (i.e., depth, echo-amplification, persistence and pre- and post-processing) were used throughout the entire study. All ultrasonographic examinations were performed by the same observer. At least three sagittal uterine images of each animal were recorded in each examination (Fig. 1A).

An experienced operator performed foetal counting 60 days after mating as described by Vinoles-Gil et al. (2010).

2.3. Computer-assisted image analyses

Digital echotexture analysis of the stored uterine ultrasonographic images was performed on a personal computer. Recorded images were saved in the non-compressed bitmap file format, with a resolution of 720 x 480 pixels. Computer analysis was performed using a custom-developed software (BS200 Pro® Image Processing and Analysis Software, BAB, Ankara, Turkey) as described by Kucukaslan (2010). Briefly, four “Regions of Interest” (ROI) of 20 x 20 pixels were identified in each digitised images and analysed for echotexture parameters, which included mean gray level (MGL), homogeneity (HOM) and contrast (CON). The ROI encompassed only endometrial tissue, avoiding myometrial tissue and intrauterine fluid if presented.

2.4. Blood samples and plasma hormone concentrations

Blood samples from all does were collected from jugular vein into vactainers containing sodium EDTA, following transrectal ultrasonographic examinations. Blood samples were centrifuged at 894 x g for 20 min at 5 °C and aliquots were stored in a freezer at −20 °C.

Plasma oestradiol and progesterone concentrations were determined using commercial kits (ESTR-CTRIA and PROG-CTRIA, respectively, Cis-bio International, Gif-sur-Yvette, France) by radio-immunossay in a gamma counter (Mini-Assay type 6-20; Mini Instruments Ltd., London, England) (Leonhardt et al., 2003). The intra- and inter-assay CV were, respectively, 3.5 and 4.9% for oestradiol-17β and 3.0 and 4.5% for progesterone. The assay sensitivity was 7 pg/ml for oestradiol-17β and 0.05 ng/ml for progesterone.

2.5. Statistical analysis

A commercial software was used in data analyses (Statistical Analysis System, SAS, Version 9, Cary, NC). Group
association with foetal count was determined by Chi-square analysis using the PROC FREQ procedure. The effect of groups (NAT vs. SYN) on plasma and ultrasonographic texture parameters were tested by one-way ANOVA with the repeated measures option using the PROC MIXED procedure. Pearson’s correlation coefficients among response variables were generated using the PROC CORR and PROC REG procedures. Statistical significance was declared at $P<0.05$.

3. Results

All SYN does showed oestrus sign and were mated on the same day. Both NAT and SYN does were confirmed pregnant by ultrasonography. Although SYN does received eCG injection, their foetal count was not different from the NAT does (1.77 ± 0.11 vs. 1.50 ± 0.22; $P=0.26$, Table 1).

Plasma progesterone profile of NAT and SYN does was similar before and after mating periods ($P<0.26$; Fig. 2A).

![Fig. 1. A sagittal B-Mode image of a goat uterus. (A) Ultrasonographic texture characteristics of the uterine horn at the time of sponge removal with a mean gray level value of 112, a homogeneity value of 0.038 and a contrast value of 222. (B) Identification of “Regions of Interest” in the point of larger curvature (arrows). U: Urinary bladder.](image)

![Fig. 2. Plasma progesterone (A) and oestrogen (B) concentrations of Akkeci goats (Saanen × Kilis crossbreed) subjected to natural mating (NAT; dark bar) and synchronisation before mating (SYN; white bar). “d 0” indicates the mating time. Different letters among bars indicate differences by day. Asterisk (*) indicates group difference within the day.](image)
The mean plasma progesterone concentration continuously decreased after sponge application and reached the lowest level at time of mating, and then it started to increase more than the levels before mating ($P<0.0001$; Fig. 2A).

Plasma oestradiol 17-$\beta$ profile during the experimental period did not differ by the group ($P>0.18$; Fig. 2B). The mean plasma oestradiol 17-$\beta$ concentration remained constant until the mating time and peaked at the mating time, and then sharply decreased to pre-mating period level ($P<0.001$; Fig. 2B). Plasma oestradiol 17-$\beta$ concentration at time of mating was greater for SYN does than for NAT does ($P<0.05$).

Ultrasonographic texture characteristics of the uterine horn changed during breeding (Fig. 3). HOM value reached the highest level at the mating time and the lowest level 30 days after mating ($P<0.0001$; Fig. 3A). Although HOM value was greater for NAT does than for SYN does at the mating time, SYN does had greater HOM value than NAT does during the post-mating period ($P<0.01$; Fig. 3A).

Although CON values were virtually stable on $d-14$, $d-2$, $d0$ and $d17$, maximum average value was measured on $d30$ ($P<0.0001$; Fig. 3B). Changes in CON value during breeding did not differ by the group (Fig. 3B). MGL value fluctuated over time ($P<0.03$) at a similar fashion in NAT and SYN does ($P<0.0.48$; Fig. 3C).

Foetal count was correlated with neither plasma hormones nor echotexture parameters. Plasma oestradiol concentration was not correlated with echotexture parameters during post-mating. However, plasma progesterone

---

**Fig. 3.** Ultrasonographic texture characteristics (A) Homogeneity, (B) Contrast and (C) Mean Gray Level of the uterine horn in Akkeci goats (Saanen × Kilis crossbreed) subjected to natural mating (NAT) and synchronisation before mating (SYN). “d 0” indicates the mating time. Different letters among bars indicate differences by day. Asterisk (*) indicates group difference within the day.

**Effect:**
- Group, $P>0.33$
- Time, $P<0.0001$
- Group x Time, $P<0.01$

**Effect:**
- Group, $P>0.32$
- Time, $P<0.0001$
- Group x Time, $P>0.79$

**Effect:**
- Group, $P>0.60$
- Time, $P<0.03$
- Group x Time, $P>0.48$
concentration was positively correlated with CON \((r=0.29; \text{Fig. } 4\text{B})\) and MGL \((r=0.25; \text{Fig. } 4\text{C})\) values and negatively correlated with HOM value \((r=−0.28; \text{Fig. } 4\text{A})\) during post-mating.

4. Discussion

All SYN does showed oestrus signs and mated. However, synchronization did not alter foetal count (Table 1). This result is compatible with the previous study by Amarantidis et al. (2004) who reported no increases in twin and triplet rate in goats in response to administration of sponge plus PMSG. The result might have been associated with embryonic loss due to inability of the uterus to carry overabundant number of embryos (Ahmed et al., 1998; Robinson, 1989) and/or underfeeding (Mani et al., 1992).

The hormonal statuses were almost similar in groups, except plasma oestradiol 17-\(\beta\) concentration being higher in the SYN does than the NAT does on d 0 (Fig. 2B). This could be related to probable excessive number of follicles, resulting from releasing higher oestrogen (Błaszczyk et al., 2004). The decrease in plasma progesterone concentration after insertion of the sponge was remarkable (Fig. 2A), suggesting that medroxy progesterone acetate and flugestone acetate sponges might suppress the ovarian activity, ovulation and endogenous progesterone concentration...
Table 1  
The foetal count in Akkeci goats (Saanen × Kilis crossbreed) subjected to different breeding programme.\(^a\)

<table>
<thead>
<tr>
<th>Foetal count</th>
<th>Groups</th>
<th>Synchronized before mating (n = 30)</th>
<th>Naturally mated (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6 (80.0)</td>
<td>10 (33.3)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3 (30.0)</td>
<td>17 (56.7)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1 (10.0)</td>
<td>3 (10.0)</td>
<td></td>
</tr>
<tr>
<td><em>X^2</em> = 2.40; <em>P</em> &gt; 0.30</td>
<td>1.50 ± 0.22</td>
<td>1.77 ± 0.11</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Counting was done 60 days after mating.

(Al-Merestani et al., 2003; Menegatos et al., 1995; Zarkawi et al., 1999).

The reproductive hormones, oestrogen and progesterone, were reported as effective factors on the uterine echotexture that depends on endometrial oedema in various animal species. The uterine echotexture was reported to increase in heifers (Bonafos et al., 1995) and decrease in mares (Griffin and Ginther, 1991) during the oestrus and peri-ovulation days. In cows exhibiting oestrus, while Pierson and Ginther (1987) reported increased heterogeneity using real-time ultrasonography, Schmauder et al. (2008) reported increased HOM using computer-assisted analyses of the uterine sonographic images. In the presented study, HOM reached the highest value in oestrus as shown in cows (Schmauder et al., 2008) and started to decrease after gestation (Fig. 3A). Interestingly, plasma oestradiol concentration was not correlated with the any of echotexture parameters in goats as in a previous study by Schmauder et al. (2008). However, plasma progesterone concentration was correlated with HOM value. That is, increase in HOM might be associated with increase in the uterine tone rather than oedema occurring in oestrus or changes in plasma oestriadiol concentration that occurs more rapidly than oedema absorption in endometrium (Ginther, 1998; Schmauder et al., 2008). On the other hand, significant increase of plasma progesterone concentration, vascularisation and blood flow (especially around conception sites) during pregnancy might lead to the uterine relaxation, which in turn causes HOM to decrease (Fig. 3B) and CON to increase (Fig. 3B). The similar influence of progesterone on endometrial echotexture was also reported in met- and dioestra in cyclic cows (Schmauder et al., 2008).

Embryonic fixation process might also be effective on the echotexture parameters during pregnancy in heifers (Bonafos et al., 1995), mares (Griffin and Ginther, 1991) and pigs (Kauffold et al., 2010). These studies demonstrated that notable increases in echotexture were seen on d 21 in cows, on d 15–16 in mares and on d 16 in pigs after mating. In the current study, MGL value peaked on d 17 post-mating (Fig. 3C). Increase in MGL value on d 17 is shown to be associated with (1) phenomena related with maternal recognition and embryonic fixation such as trophoblast activity (Spencer and Bazer, 2004), (2) binucleate cell migrations and formation of syncytial plaques (Wooding et al., 1993), (3) release of secretory substances from endometrial glands such as interferon tau, placental lactogen and growth hormone (Moffatt et al., 2006; Spencer et al., 1999; Spencer et al., 2006) and (4) increase in microvascular volume density and microvascular plexuses which results in blood flow increase (Honnens et al., 2008; Reynolds et al., 1984; Reynolds and Redmer, 1992) in the pregnant uterus. The reciprocal communication between conceptus and endometrium, which begins on d 15–16 and continues until the 50–60th d of pregnancy in sheep (Spencer et al., 1999), might lead to MGL increase (especially on d 17) during pregnancy in does (Fig. 3C).

Despite changes in echotexture parameters over time, there were no correlations between foetal count and echotexture parameters. As hypothesised, this may suggest that endometrium might give equally reactions to the embryonic fixation regardless of the number of conceptus. Correlations of post-mating plasma progesterone concentration with echotexture parameters (Fig. 4) could be used for early detection of pregnancy.

5. Conclusion

The echotexture parameters and their correlations with reproductive hormones and average foetal count were evaluated during breeding in Turkish Saanen goats (Akkeci). The echotexture parameters changed during the pregnancy period, in association with plasma progesterone concentration. The echotexture changes in mated does during embryonic fixation days (especially 15–20 days after mating) can be evaluated as a marker for early detection of pregnancy. Future studies are need for defining echotexture parameters of cyclic and non-cyclic (pregnancy suspected) does.

Acknowledgments

We express our gratifications to Lodumlu Dairy Goat Farm staff and its owner Aslan Saracoğlu for allowing us to perform this experiment.

References


