Concentrations of progesterone and 17β-estradiol in blood and milk and those of natural inhibitors in milk of goats in various physiological stages

Abstract

The aim of the study was to investigate how the physiological condition of goats affects the contents of progesterone and 17β-estradiol in the blood and milk and the presence of natural inhibitors in milk. The experimental material consisted of the milk and blood from 13 two- and three-year old goats, in which heat and ovulation were synchronized. Blood and milk samples were collected in the consecutive days, counting from the moment of ovulation: -47, -19 (the day of sponge administration), -2, 0, 2, 6, 18, 21, 40, 66 and 104. The presence of natural inhibitors in milk was determined using BRT®. The concentrations of progesterone and 17β-estradiol in the blood and milk were radioimmunoassayed using tagged hormones [125I].

The highest concentration of estradiol in blood (76.26 pmol/l) was found 2 days before ovulation. Progesterone content reached a very high level starting from the 6th day of pregnancy, assuming the value of ca 62.5 nmol/l on day 66 and 104 of pregnancy. The levels of both progesterone and estradiol in milk were strongly correlated with the content of a given hormone in blood (r>0.9; p=0.0001). BRT showed the presence of inhibitors in the milk of all the investigated goats on the 6th day after ovulation, which may not be explained either by the action of progesterone, or estradiol per se.

Key Words: goat, progesterone, 17β-estradiol, natural inhibitors, blood, milk

Zusammenfassung

Titel der Arbeit: Progesteron und 17β-Estradiol Konzentration im Blut und in der Milch sowie der Gehalt an natürlichen Hemmstoffen in der Milch bei verschiedenen physiologischen Zuständen von Ziegen

Das Ziel der Untersuchungen war zu prüfen, wie sich der physiologische Zustand der Ziegen auf die Konzentration von Progesteron und 17β-Estradiol im Blut und in der Milch sowie auf den Gehalt von natürlichen Hemmstoffen in der Milch auswirkt. Das Untersuchungsmaterial, Milch und Blut, wurde zwei- and dreijährigen Ziegen entnommen, bei denen die Brunst und Ovulation synchronisiert wurde. Die Blut- und Milchprobenentnahme erfolgte, ab Ovulationstag, am -47, -19 (Deponierung der Vaginalschwämmchen), -2, 0, 2, 6, 18, 21, 40, 66 und 104 Tag. Der Hemmstoffgehalt in der Milch wurde mittels BR-Test® überprüft. Die Progesteron- und 17β-Estradiolkonzentration im Blut und in der Milch wurde durch einen spezifischen Radioimmunoassay bestimmt [125I].

Die höchste Estradiolkonzentration im Blutplasma (76,26 pmol/l) wurde zwei Tage vor der Ovulation festgestellt. Die Progesteronkonzentration war ab 6. Tag der Trächtigkeit sehr hoch und am 66. und 104. Trächtigkeitstag betrug sie ca. 62,5 pmol/l. Sowohl die Progesteron- als auch die Estradiolkonzentration in der Milch waren mit dem Gehalt beider Hormone im Blut hoch korreliert (r>0,9; p=0,0001). Der BR-Test ergab bei dem Hemmstoffgehalt in der Milch bis zum 6. Tag nach der Ovulation eine Übereinstimmung hinsichtlich der Hormongehalte, während danach ungerichtete Effekte beobachtet wurden.

Schlüsselwörter: Ziege, Progesteron, 17β-Estradiol, natürliche Hemmstoffe, Blut, Milch

Introduction

The content of hormones in blood may be reflected in their contents in the secretions and excretions of the animal’s organism, but it is not always the case. The level of progesterone in the saliva of the Hawaiian monk seal (Monachus schauinslandi) is correlated with the blood progesterone level (PIETRASZEK and
ATKINSON, 1994), whereas in the false killer whale (*Pseudorca crassidens*) no such correlation was found (ATKINSON et al. 1999).

The processability of goat milk depends – among other things – on the physiological stage of the animal (WSZOŁEK, 1997). The Provincial Department of Veterinary Medicine in Poznań repeatedly informed that frequently inhibitors were found in the milk obtained from goats in the state of sexual activity, in spite of the assurances by the owners that foreign inhibitors did not get to the milk. Thus, a hypothesis was formulated that the milk of sexually active goats contains natural inhibitors (DANKÓW et al., 1997).

The aim of the conducted investigations was to examine how the physiological status of goats affects the contents of progesterone and 17β-estradiol in their blood and milk and the presence of natural inhibitors in the milk.

### Material and methods

The experimental material consisted of the milk and blood of 13 two- and three-year old goats of the White Improved breed and crosses of this breed with the Boer breed, in which heat and ovulation were synchronized using the Chrono-Gest® method (INTERVET 1997). Vaginal sponges containing 45 mg Cronolone preparation (Flugestone acetate) were inserted into the goats in the middle of August for the period of 17 days. Forty eight hours before the sponges were removed goats were given an intramuscular injection of 500 IU PMSG. Thirty six hours after the sponges were removed goats were serviced. The service was repeated after 12 hours. All the goats were fertilized and kidded at the turn of January and February (the length of gestation 148 – 152 days).

Blood samples (*Vena jugularis externa*) were collected from the goats 11 times (from the middle of July to November) during the morning milking to determine progesterone and 17β-estradiol contents. At the same time milk samples were collected to determine the contents of the above mentioned hormones and detect the possible presence of inhibitors.

Samples were collected on the consecutive days, counting from the moment of ovulation: -47, -19 (the day of sponge administration), -2, 0, 2, 6, 18, 21, 40, 66 and 104, respectively.

The presence of natural inhibitors in milk was determined using BRT® (Brillant Reduction Test), in the three-hour incubation at the temperature of 64-65°C, using *Bacillus stearothermophilus var. calidolactis* C-953 as the testing organism, at the Laboratory of Milk Hygienic Quality of the Regional Dairy Cooperative, Poznań-Dębiec (PN-91/A-86033).

The levels of progesterone and 17β-estradiol in blood serum and milk were assayed by radioimmunoassay using Progesteron Tests [¹²⁵I] SPECTRIA and Estradiol [¹²⁵I] SPECTRIA (OBRI Polatom, Otwock-Świerk, Poland). Cross-reactivity of the progesterone antiserum was 3.9% for pregnenolone and less than 1% for other steroids, whereas estradiol antiserum demonstrated 1.4% cross-reactivity with ethinyloestradiol and less than 1% with other steroids. Intra- and interassay variations were 3.2% and 2.3% for estradiol and 4.2% and 5.1% for progesterone, respectively. Measurements were taken on the LKB Wizard gamma counter. The method of direct determination of hormone levels was applied, and milk samples were subjected to the action of ultrasounds prior to the determination in order to obtain uniform material. To
confirm the obtained results random testing was applied, using the method of high performance liquid chromatography. The levels of the above mentioned hormones were determined at the Isotopic Laboratory, Department of Animal Physiology and Biochemistry, the Agricultural University of Poznań.

Pearson’s correlations were calculated between hormone contents and a univariate analysis of variance was performed using the least square method in order to assess changes in the hormone levels between recordings (SAS®, 1996).

Results

The mean level of progesterone in the blood of goats up to the day of ovulation did not exceed 2 nmol/l (0.629 ng/ml), Fig. 1. During the consecutive recordings a considerable increase in the blood content of this hormone was observed; it remained at the level of 43-53 nmol/l (13.5-16.7 ng/ml) between day 6 and 40 of gestation, and reached approx. 62 nmol/l (19.5 ng/ml) on day 66 and 104 of gestation.

The mean level of progesterone in milk was as a rule lower than that in blood and strongly correlated with it \( (r=0.92; p=0.0001) \). Differences in progesterone levels in blood and milk are distinct since the 6th day of gestation (Fig. 1). Between day 6 and 66 of pregnancy the progesterone level in milk was stable (38.23 –42.17 nmol/l) and did not show changes found in case of the blood progesterone content. On day 104 of gestation the level of progesterone in milk dropped considerably to the value of 25.28 nmol/l, whereas the blood progesterone level was at that time 62.02 nmol/l.

The mean estradiol level in the blood of goats during the first two recordings did not exceed 8 pmol/l (2.18 pg/ml), Fig. 2. Two days before ovulation it reached the highest value of 76.26 pm/l (20.77 pg/ml), followed by a rapid drop to 14.58 pmol/l (3.97 pg/ml) on the day of ovulation. Next it was growing until day 40 of gestation, it fell on
day 66 of gestation and again rose reaching the value of 41.27 pmol/l (11.24 pg/ml) on day 104 of gestation.

The mean level of estradiol in milk was strongly correlated with the blood level of this hormone \( r=0.95; p=0.0001 \). Changes in milk estradiol contents differed from those in blood; the increase between the day of ovulation and the 40\(^{th}\) day of pregnancy was less pronounced, followed by a decrease (Fig. 2). Similarly as in case of progesterone, on day 104 of gestation the highest difference was observed in the contents of estradiol in blood (41.27 pmol/l) and milk (13.34 pmol/l).

The correlation between progesterone and estradiol contents was not statistically significant \( p>0.05 \).

Except for three recordings natural inhibitors were not detected in milk. On day 2 after ovulation ambiguous results (±) were obtained for the milk of all the investigated goats. On day 6 after ovulation all the results were positive (+), whereas on day 18 after ovulation the ± result was found in the milk of 4 animals, with the negative result for all the other animals.

**Discussion**

Similar levels to those in the investigations conducted by the authors of this study in case of progesterone contents in the blood of goats before and after ovulation (0.42 ng/ml in the autumn and 0.27 ng/ml in the spring) were also observed by BŁASZCZYK et al. (2004).

Also ENGELAND et al. (1997) found a similar progesterone level (1.9 nmol/l) in the blood of non-pregnant goats 20 days after ovulation. However, blood progesterone levels found in the investigations by the authors of this study during later recordings were higher than those reported by other researchers.
DE CASTRO et al. (1999), while investigating the ovarian activity in non-pregnant goats, found the level of progesterone of approx. 5 ng/ml on day 6 after ovulation (14.8 ng/ml in the investigations conducted by the authors of this study).

SOUSA et al. (1999), who investigated blood progesterone levels in pregnant goats, found that it differed significantly between the 1st and 3rd week of gestation. Later changes within the two-week intervals were not that distinct, although in goats of the caninde breed the blood progesterone level was also increasing between the 7th and 11th week of gestation, similarly to the results of this study. However, SOUSA et al. (1999) during the whole pregnancy did not observe progesterone levels exceeding 10 ng/ml.

Also ENGELAND et al. (1996) found in healthy pregnant goats changes in the progesterone levels similar to those in this study, although the progesterone level during the 110 days of gestation did not exceed in the cited study the value of 35 nmol/l.

ENGELAND et al. (1997) observed progesterone contents in the milk of pregnant goats starting from day 20 at the level of 20.4 nmol/l (in this study – 41.84 nmol/l on day 21 of gestation, respectively).

Milk progesterone level is correlated with the level of this hormone in blood, which makes it suitable for pregnancy diagnostics e.g.: in domestic cattle (HEAP et al. 1976), the domestic buffalo (SINGH and PUTHIYANDYM 1980) and the bottle-nosed dolphin (WEST et al. 2000). Also in case of domestic goats methods using milk progesterone levels (ELISA and latex agglutination) are considered to be good pregnancy diagnostic methods, although they are inferior in comparison to those using blood progesterone (RIA) or the observation of behavior indicating heat (ENGELAND et al. 1997). Progesterone in milk (RABIEE et al. 2001, MCCOY et al. 2002, REKSEN et al. 2002) and in butterfat (WALDMANN et al. 2001) is used in the studies on domestic cattle because “Milk progesterone monitoring offers an accurate and objective measurement of factors associated with postpartum ovarian activity which will assist in investigating the genetic and environmental factors affecting fertility” (LAMMING and DARWASH 1998).

BŁASZCZYK et al. (2004) obtained results concerning the estradiol levels in the blood of goats in heat similar to those in this study. The highest blood estradiol content did not exceed 25 pg/ml in the autumn and 20 pg/ml in the spring. DE CASTRO et al. (1999) found changes similar to those in these investigations in the blood estradiol contents in goats during heat and the luteal stage of the sexual cycle, although estradiol content was lower. It did not exceed 20 pg/ml; on the day of ovulation it was 2.7 pg/ml, whereas before oestrus it was < 3 pg/ml. DE CASTRO et al. (1999) observed the occurrence of a second, lower peak in the blood estradiol content in goats on the second day after ovulation (7.6 pg/ml), which in the investigations conducted by the authors of this study was rather slight. Reports on the second peak in the blood estradiol content in case of goats at the luteal stage are contradictory (ABAYAWARDENE and POPE 1990, LEYVA-OCARITZ et al. 1995).

In this study a strong correlation was observed between estradiol contents in the blood and milk of goats. PERHEENTUPA et al. (2002) found that the administration of estradiol in the amounts of 50-100 µg/24 h to nursing women affects the level of this hormone in their blood, but not their milk (at the application of a test with the quantity sensitivity of 5 pmol/l).
Neither of the investigated hormones explains the occurrence of inhibitors in milk. The peak of inhibitor contents in milk occurs on the 6th day after ovulation, i.e. 8 days after the peak of estradiol content in blood and milk, thus it is unjustified to conclude that estradiol per se is the inhibitor detected using BRT. The peak of inhibitor contents coincides with the increase in progesterone contents in blood and milk; however, later inhibitors are not detected, while the progesterone level does not decrease, thus also progesterone does not explain the presence of inhibitors. It may be concluded that inhibitors detected in goat milk are other substances active during the oestrus cycle or their metabolites.

References


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