CORRELATION OF PROGESTERONE CONCENTRATION IN LITHUANIAN RED COW MILK WITH MILK YIELD AND MILK COMPOSITION DURING OESTROUS

Aurimas Gavelis, Vytuolis Žilaitis, Arūnas Juozaitis, Vida Juozaitienė

Veterinary Academy, Lithuanian University of Health Sciences
Tilžės St. 18, LT–47181 Kaunas, Lithuania.

Corresponding author: Aurimas Gavelis
e–mail: aurimas.gavelis@lsmuni.lt

Abstract. Concentration of progesterone during oestrus is associated with cow fertility and success of the inseminate. The aim of the study was to evaluate milk yielding, milk composition and number of lactation relation with cow milk progesterone concentration during oestrus.

Clinically healthy Lithuanian Red (LR) breed cows without reproduction disorders, and 90–100 days after calving (n=30) during oestrus were divided by milk yield (20% less – first group, average – the second and the third 20% higher) and by lactation number (the first, the second and the third). Milk samples for progesterone and milk composition determining were taken during oestrus, after milking and stored in plastic containers (50 ml), not preserved. Milk was taken once, when oestrous was observed. Progesterone has been determined using the set HORMONOST MILK produced by BIOLAB GmbH company (Munich). Milk composition (fat, protein) was determined by device EKOMILK M, based on ultrasonic technology. Somatic cells were measured EKOMILK SCAN analyser.

Progesterone concentration in cows' milk Group II of cows was by 30.34% higher than in Group I, but by 17.65% lower than in group III ($P>0.05$). Difference of mean of progesterone compare III was by 40.79% higher than in Group I ($P<0.05$).

The highest concentration of progesterone was determined in the milk of cows of third and more lactation – (by 55.4% higher than of cows the second lactation and by 26.4% higher than cows of the first lactation. Progesterone concentration during oestrous correlated with milk yield $r=0.50$ ($P<0.01$), and milk protein concentration $r=–0.46$ ($P<0.01$).

Milk yield and lactation number of LR cows associated with inadequate progesterone concentration during oestrous and it may be the cause of decreased fertility of high yielding cows.

Keywords: milk yield, milk composition, fertility, progesterone

Introduction

It is noted that the increasing cow milk production in farms is related with poorer reproduction characteristics (Dobson et al., 2007; Walsh et al., 2011). In efficient cow reproductive function control practices, computerized herd management systems are used (Ostergaard and Grohn, 2001). In them, as wellness and reproductive function status markers, activity gauges, milk meters, electric milk conductivity meters, and body weight meters are used (Fricke et al., 2014). Progesterone concentration is a perspective cow reproductive wellness mark (Spencer et al., 2007).

Progesterone is a good fat–soluble steroid (Heap et. al., 1976), which during oestrous cycle is produced by a temporary endocrine gland – the corpus luteum (Petersson et al., 2007). By the end of the 17 days oestrous cycle, corpus luteum degenerates and on the 21st day a minimum concentration of progesterone (less than 2 ng/ml) is produced, and on the 19–23 day cow oestrous cycle begins again (Petersson, 2007). During oestrous cycle low level of progesterone associate with increase oestrogens. It determines the ovulation and oestrous behaviour. Furthermore, resulting incidences of ovulation were decreased at greater progesterone concentrations and presence of a corpus luteum, and increased at greater estradiol concentrations and presence of follicles >13.5 mm. (Stevenson and Pulley, 2016). G. E. Lamming and A. O. Darwash, (1998) state that the assessment of progesterone concentration in milk is an objective and accurate method, which allows assessing ovarian function and specify the time of insemination.

Progesterone concentrations in milk depends not only on the cow's physiological condition, but also on its breed. It is observed that aberrant lengthening of luteal phase is more common for the Swedish Holstein, rather than with the Swedish Red (Lindhé and Philipsson, 2001), nevertheless, it was established that the so called profile of progesterone heritability is significantly higher – from 0.16 to 0.30 (Royal et al., 2002; Petersson et al., 2007).

Positive correlation between fat content and progesterone concentrations is indicated (Nuti et al., 1975). In butterfat progesterone concentration is higher than that in blood serum (Petersson, 2007). Assessing the cow's reproductive condition by progesterone concentration is important when evaluating the factors that affect this indicator.

The objective of this experiment was to evaluate milk yielding, milk composition and lactation relation with oestrous cow milk progesterone concentration.

Materials and Methods

Clinically healthy Lithuanian Red breed cows without reproduction disorders, and 90–100 days after calving (n=30)

were selected for the research. The cows were monitored for 21 days oestrous on a daily basis, 0.5 hours after the morning and evening milking, and 15 minutes after the noon milking. The indications of oestrous cow were assessed according to the following features: an adequate oestrous behaviour, reduced milk production, liquid mucus – the first signs of oestrous. Decreased milk production, sticky mucus from the vagina, the reflex of standing were assessed as expressed signs of oestrous. The cows were divided into 3 groups by milk yield: group I – milk yield 20% less than the average in all groups of selected cows, (n=4), group II – average milk yield in groups (n=22), and III – 20% more than the average (n=4). The cows with similar yielding (n=30) were divided by age. Group I cows 1 lactation (n=12), group II – 2 lactation (n=12) and group III – 3 and more lactations (n=6).

Milk samples for progesterone and milk composition determining were taken from oestrous cows after milking and stored in the plastic containers (50 ml) not preserved. Milk was taken once, if oestrous was observed. Progesterone has been determined using the set HORMONOST MILK produced by BIOLAB GmbH company (Munich). The complete progesterone test kit is based on enzyme–linked immunosorbent method. For this purpose tubes with their surface coated with antibodies p against progesterone have been used. In the test tube is added the tested cow's milk and "milk diluter". Progesterone of the sample is reacting with antibodies. After 5 minutes, the progesterone–enzyme complex solution "enzyme" is added; the sample is mixed and incubated for 3 minutes. To remove any "unreacted substances", the samples are 6 times washed rinsing them in the test tubes. Then, the substrate is added. The quantity of separated substrate is proportional to the fixed amount of the enzyme with antibodies and inversely proportional to the concentration of the tested progesterone. The substrate concentration is measured using a Microlab colorimeter.

Milk composition was determined in the Lithuanian University of Health Sciences, Veterinary Academy, Department of Animal Breeding and Nutrition, Laboratory of Establishment of Animal Breeding Value and Selection. Milk composition (fat, protein) was determined by device EKOMILK M. Milk evaluation method based on ultrasonic technology. Somatic cells were measured EKOMILK SCAN analyser.

Descriptive statistic (mean – M, standard error – SE, standard deviation – SD) was calculated using R 2.1.0 package (http://www.r–project.org/). The results considered to be reliable $P \leq 0.05$.

Results

Having compared Group II and Group III respectively, statistically fat content and protein content ($P<0.05$) significantly decreased with increasing cow yielding. Progesterone concentration in cow milk of Group II cows was by 30.34% higher than in Group I, but by 17.65% lower than in group III ($P>0.05$). Difference of mean of progesterone compare III was by 40.79% higher than in Group I ($P<0.05$) (Table 1).

Table 1. **Milk composition indicators depend from yield**

Groups	Milk yield, kg	Fat [%]	Protein [%]	SLS $*10^3$/ml	Progesterone ng/ml
1	19.77±1.241	4.29±0.156	$3.70±0.058^a$	137.50±29.734	$2.25±0.929^d$
2	30.65±0.814	4.24±0.122	$3.60±0.058^b$	213.45±45.483	$3.23±0.445^e$
3	38.55±1.048	3.87±0.113	$3.11±0.124^c$	192.5±34.352	$3.80±0.187^f$
a:b; b:c; d:f; P<0.05					

Cow age has influence on milk progesterone level. The highest concentration of progesterone was determined in the milk of the third group cows – (by 55.4% higher than in the second and by 26.4% higher than in the first group cows). In lactation cows, the average progesterone concentration was 3.6±0.42 (Table 2).

Table 2. **Milk progesterone concentration ng/ml during individual lactation**

Groups	M	SE	SD
1	3.68	0.4482	1.553
2	2.23	0.7631	1.526
3	5.0	1.1564	2.830

Table 3. **Correlation of progesterone concentrations with milk yield and milk composition**

Features	Milk yield kg	Fat [%]	Protein [%]	SLS $*10^3$/ml
Fat %	–0.40**			
Protein %	–0.63**	0.01		
SLS $*10^3$/ml	–0.05	–0.002	0.15	
Progesterone ng/ml	0.50**	0.09	–0.46**	–0.21
*P<0.05; **P<0.01;				

The results of correlation analysis of the tested cow milk indicators. The analysis of the dependence of features has shown that the correlation factors of progesterone concentration with milk traits were statistically reliable. The tendency

of possible increase of progesterone concentration in milk with rising cow productivity and decrease with increasing milk protein content ($P<0.01$) was observed (Table 3).

Discussion

Currently, in cow breeding, the focus on the properties of cow reproduction has been increasingly growing. Intensive genetic improvement of cow productivity leads towards higher energy imbalance and morbidity (Pryce and Veerkamp, 2001) that tends to reduce fertility (Veerkamp et al., 2003). Oestrous duration is influenced by heredity, diet, milk, age, cow housing, and by the overall wellness (Žilaitis et al., 2013). One of the causes of cow infertility is the impaired metabolism of progesterone (Hansen, 2011). Progesterone concentration negatively correlate with oestrogens. This is indicated by high correlation between the visual symptoms of oestrous and estradiol concentrations (Lyimo et. al., 2000). High milk production decreases duration of oestrous probably due to decreased circulating concentrations of oestrogens. (Lopez et. al., 2004). Notice, that progesterone is inhibitory to oestrous (Allrich, 1994). By ours data, by increasing milk yield, progesterone concentration in milk during oestrous increased. That condition may explain the reduction of fertility of high yielding cows. Data coinside with others, that fertility depend from yield.

In lactating dairy cows, plasma and follicular fluid estradiol concentrations are negatively correlated with daily milk yield (Beam and Butler, 1998). Mounting evidence indicates that insufficient concentrations of progesterone during the development of the ovulatory follicle is a major impediment for fertility in high–producing dairy cows subjected to timed artificial insemination programs (Bisinotto et al., 2014; Wiltbank et al., 2014).

When analysing cow milk yield variation regularities, D. Hojman et. al., (2004) and M. Mellado et. al., (2011) established that daily milk yield increases with each lactation. When comparing the data of the first and the second lactation cows analysed by us, with those obtained by other researchers, similar results were observed. A study comparing cows of high or low genetic merit for milk production demonstrated a negative association between circulating progesterone concentration and genetic merit for milk production (Lucy and Crooker, 2001). A negative correlation between milk production level and peak concentration of progesterone (in the first ovarian cycle) was found for cows (Windig et. al., 2008). Studies have shown that during oestrous progesterone concentration in milk may increase with rising productivity of cows ($P<0.01$).

Increased progesterone concentration is related with atypical symptoms of oestrous. Historically, mounting behaviour and standing to be mounted have been considered the clearest indicator of anoestrous event, but its expression tends to decrease with greater milk production (Rivera et al., 2010). High–yielding dairy cows have an elevated metabolic rate, which increases liver blood flow and metabolism of steroid hormones (Sangsritavong et al., 2002). As a result, these cows experience decreased circulating estradiol concentrations that could be a possible cause of shorter and less intense expression of oestrous (Roelofs et. al., 2010; Rivera et al., 2010).

Cow milk protein concentration is correlative with progesterone concentration. On the other hand, high yield increases catabolism, which results in increased need for feed intake and metabolism of steroids changes (Sangsritavong et al., 2002; Wiltbank et al., 2014). Between progesterone concentration in milk and milk protein content a negative correlation ($P<0.01$) was defined. Royal et al., (2002) found a strong negative correlation between both milk yield and protein content and length cycle and progesterone mean. The connection between the fat content and progesterone concentration unproved.

Conclusion

Progesterone concentrations during oestrous depends on the cow milk yield. The difference of progesterone concentration of the cows with milk yield 20% higher than others, differs by 40.79%. Age has an impact on progesterone concentration in milk during oestrous: older cows has 55.4% more progesterone during oestrous than in the second and 26.4% higher than in the first group cows. The milk progesterone concentration depends on the milk protein. Progesterone concentration decrease by increase protein concentration in milk ($r=–0.46$, $P<0.01$).

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