ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA

VOL. LXI, 19 SECTIO DD 2006

[*]Katedra Biochemii i Fizjologii Zwierząt Akademii Rolniczej w Lublinie
[**]prywatna praktyka weterynaryjna

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The levels of triacylglycerols and cholesterol in blood plasma of mares in the periovulatory period

Stężenie triacylogliceroli i cholesterolu w osoczu krwi klaczy
w okresie okołoowulacyjnym

SUMMARY

Metabolism of lipids and their level in blood of animals and men is dependent on many factors, including the stages of estrus cycle. The aim of this study was to establish the level of triacylglycerols (TG) and cholesterol in blood plasma during the periovulatory period in mares. 12 Arabian mares were included in the investigation during the breading season. The number of days of estrus cycle was defined on the basis of results of transrectal ultrasonography conducted once a day. The animals were divided into two groups: 8 mares with recorded ovulation and 4 with anovulatory cycle. Blood samples were collected from jugular vein at rest in the morning, and plasma TG and cholesterol levels were determined by enzymatic methods. In blood plasma of ovulating mares the levels of TG and cholesterol decreased after ovulation. In the group of anovulatory mares the values of measured parameters were not statistically different.

Key words: triacylglycerols, ovulatory period, mares

INTRODUCTION

The triacylglycerols (TG) level in blood plasma of horses measured at rest is maintained in the range 0.1–0.7 mmol · l^{-1} [Winnicka 2004] and in appearance of hyperlipidemia it can achieve 5.5 mmol · l^{-1} [Mogg and Palmer 1995], reaching in extreme cases of hyperlipemia 75 mmol · l^{-1} [Watson and Love 1994]. In healthy horses the plasma TG level depends on the time of day [Yashiki *et al.* 1995], the level of fat in feed [Orme *et al.* 1997, Schmidt *et al*. 2001], the state of satiety [Frank *et al.* 2002], pregnancy or lactation [Ozpinar *et al*. 2004] and physical activity [Podolak *et al.* 2004]. In available literature there is a lack of information about the blood TG level during the estrus cycle of the mare. The studies on other animal species indicated that there exists the relation between TG blood concentration and period of sexual activity. An important increase of both cholesterol and TG plasma concentration was shown in bitches during metoestrus [Downs *et al*. 1994]. In flank organs of laboratory animals major lipid synthesis was observed under stimuli of progesterone (P) [Cabeza and Miranda 1997]. In oophorectomized women the

serum TG level were increased by estrogen (E) treatment, but not affected by the combination of estrogen plus testosterone (T) [Floter *et al*. 2004].

In mares the level of estrogens reach the top values two days before ovulation, then go down and two days after ovulation attain the basal level. In this time the progesterone synthesis is started and it increases concentration in blood from level below 1 to 10 ng/ml in 6–7th day of estrus cycle [Bielański and Tischner 1997].

The aim of this study was to establish the level of TG and cholesterol in plasma during the course of ovulatory cycle in mares.

MATERIAL AND METHODS

12 Arabian mares aged 3–17 years were included in the investigation during the breading season at the turn of March and April. The horses were fed a basal diet compared with oats and hay without fat supplementation. The number of days of estrus cycle was defined on the basis of result of transrectal ultrasonography realized once a day. The investigated animals were divided into two groups: 8 mares with recorded ovulation and 4 with anovulatory cycle, in which the greatness of follicles was alternately increasing and decreasing in the range 29 × 28 mm to 47 × 46 mm. Blood samples were collected from the jugular vein at rest in the morning to tubes with EDTA. The number of ovulatory cycle days was defined in compliance with Hughes' definition designating estrus cycle in mares as a period between ovulations [Hughes *et al.* 1980]. In the gathered blood plasma the TG and cholesterol levels were determined by enzymatic methods using diagnostic kits of Cormay. The data are shown as means ± standard errors. The changes in levels of the measured parameters between the sampling days were analysed using paired Student's t-test (ANOVA, Microsoft Excel NT).

RESULTS

Means ± SE of TG and cholesterol levels in blood plasma of ovulating mares on the following days of estrus cycle are shown in Fig. 1. The TG plasma level on the -2nd day and the 6th was significantly higher according to 0 and 2nd days ($p<0.05$). The concentration of cholesterol was on constant level on the -2nd and 0 days, than was decreased restrainedly on day 2nd ($p<0.08$)

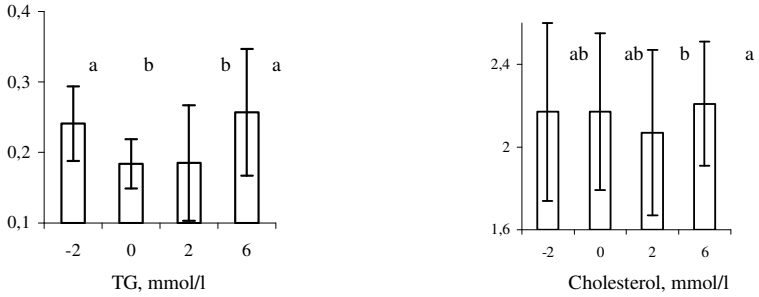

Fig. 1. Triacylglycerols (TG) and cholesterol levels in blood plasma of ovulating mares during periovulatory period (-2, 0, 2, 6 – the following days of ovulatory cycle; a, b – statistically different at $p \leq 0.05$)
Rys. 1. Stężenie triacylogliceroli (TG) i cholesterolu w osoczu krwi klaczy owulujących w okresie okołoowulacyjnym (-2, 0, 2, 6 – kolejne dni cyklu owulacyjnego; a, b – różnice istotne statystycznie przy $p \leq 0,05$)

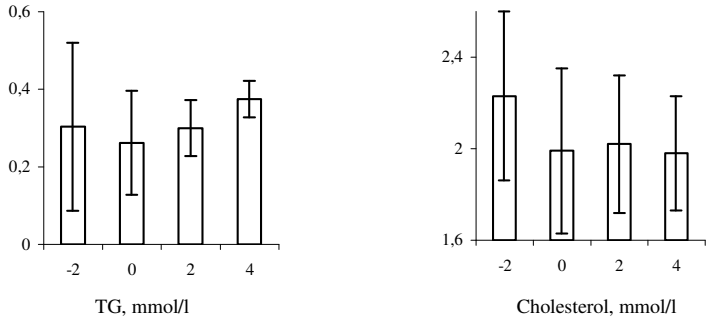

Fig. 2. TG and cholesterol levels in blood plasma of mares with anovulatory follicular cycle (details as in Fig. 1)

Rys. 2. Stężenie TG i cholesterolu w osoczu krwi klaczy z bezowulacyjnym cyklem jajnikowym (opis jak pod rys. 1)

and afterwards was statistically increased in 6th day ($p<0.05$). In the group of anovulatory mares the day of cycle was defined as 0 when the follicle had the greatest measure. The following data of TG and cholesterol levels in this group were not statistically different (Fig. 2).

DISCUSSION

Blood concentration of TG is the result of the difference between the rate of release of these lipids into the blood circulation and its removal from the same compartment via the activity of different organs and systems, mainly muscle, liver and reproductive organs.

Under the influence of glucagon, glucocorticoids and catecholamines, TG are mobilized from adipose tissue and free fatty acids are transported via blood to peripheral tissues, where they may be oxidized or used as steroids synthesis substrates. Excess of FFA is formed in horses' organism in the liver into TG again and then they come into very low density lipoproteins (VLDL) elevated in the blood. The hydrolysis of TG exists in VLDL lead to remove FFA again.

Increase of TG plasma level takes place when its production in the liver is not balanced by removal from the blood. Mobilization of TG from adipose tissue is stimulated by hormone-sensitive lipase activated by glucocorticoids, glukagon and catecholamines. Insulin inhibits the activity of this enzyme and promotes TG uptake into peripheral tissues by stimulating lipoprotein lipase [Naylor 1982]. The activity of these two enzymes is also dependent on the level of female reproduction steroids. Yamaguchi *et al.* [2002] observed periodical changes in the activity of these two enzymes among the stages of estrous cycle in female rats: the lipoprotein lipase activity reached a minimum at estrus, whereas hormone-sensitive lipase was the highest one. These changes in the activity of lipolytic enzymes lead to an increase in the blood TG concentration during this period, when the E level is the highest ones. In a study of oophorectomized women the TG serum level was also increased by E treatment [Floter *et al.* 2004]. The results of another work on flank organs of laboratory animals indicate that the lipid synthesis is also promoted by a high level of P [Cabeza and Miranda 1997].

Also, in the present work the TG level in mares' blood plasma was higher in follicular and luteal phases of estrus cycle, when the concentration of reproductive steroids reached the peaks. During ovulation TG level was lower, while as is known estrogens go down and P starts to increase. On the other hand, TG and cholesterol are used as substrates to synthesis of steroids into ovaries: the E in granulosa cells and P in corpus luteum. The results of investigations on cows and rats indicate that the high blood levels of TG and cholesterol lead to an increase of P production [Schuler *et al.* 1981, Carroll *et al.* 1990].

T, on the contrary, as endogenous as treated experimentally leads to a decrease of TG level in blood of women and men [Pietrzak *et al*. 2002, Gambineri *et al.* 2003, van Beek *et al.* 2004] and also it prevents the increase of blood lipids under the influence of estrogens [Floter *et al*. 2004]. A similar relation was not found during the investigation of castrated male rats, when TG level was not modified in spite of the radical changes in T concentration [Haug *et al*. 1984]. In the last two studies the level of cholesterol in blood went down under the influence of T. As was described previously in group of anovulating mares, the T level was significantly higher than in mares with denoted ovulation [Bobowiec *et al.* 2004]. The higher T concentration and lack of E fluctuations do not affected lipids metabolism, because TG and cholesterol level was not modified in anovulatory mares. The accurate mechanism of relation between reproductive steroids and lipids metabolism is still unknown.

To sum up, the levels of plasma TG and cholesterol in mares are dependent on the stages of periovulatory period. The fluctuations of levels of the investigated lipids in periovulatory period take place only in mares with denoted ovulation. In anovulatory mares the level of these lipids during the growing and atresis of follicle is stable.

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STRESZCZENIE

Metabolizm lipidów i ich poziom we krwi zwierząt i ludzi jest uzależniony od wielu czynników, między innymi od fazy cyklu rujowego. Celem pracy było określenie stężenia triacyloglicerol (TG) i cholesterolu w osoczu krwi klaczy w okresie okołoowulacyjnym. Badania prowadzono podczas sezonu rozrodczego w grupie 12 klaczy rasy arabskiej. Dzień cyklu owulacyjnego określano na podstawie badań jajników przeprowadzanych codziennie rano metodą ultrasonograficzną. Zwierzęta podzielono na dwie grupy: 8 klaczy, u których stwierdzono pęknięcie pęcherzyka jajnikowego i 4 klacze nieowulujące. Krew do badań pobierano w spoczynku z żyły szyjnej zewnętrznej. W uzyskanym osoczu stężenie TG i cholesterolu oznaczano metodami enzymatycznymi. W grupie klaczy owulujących poziom TG i cholesterolu w osoczu krwi obniżał się tuż po owulacji. U klaczy z cyklem bezowulacyjnym nie stwierdzono zmian stężenia badanych parametrów.

Słowa kluczowe: triacyloglicerole, okres okołoowulacyjny, klacze