Part 3. Biotechnology and genetics

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EFFECTS OF MTHFR GENE ON REPRODUCTIVE HEALTH AND PRODUCTIVE TRAITS OF DAIRY COWS

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Summary. One-carbon metabolism of mammals is one of the key points of metabolism and the pleiotropic effects of human MTHFR gene determining reproductive health are known. The aim of this study was an evaluation the role of MTHFR gene in lactating dairy cows. Cows were genotyped by sequencing. The plasma homocysteine level, bone mineral density, traditional production and reproduction traits were analyzed. Statistical methods included Pearson's chi-squared and *t* criteria, Pearson's and Spearmen correlation coefficient *r* and ANOVA. Allele frequencies of SNP 8137C/T of MTHFR gene were: 0.943 (C) and 0.057 (T). Distribution of genotypes was 88.6% (CC) : 11.4% (CT) : 0% (TT). Investigated group of animals was in Hardy-Weinberg equilibrium. We had founded that calving interval were shorter in cows with the CC genotype than in CT animals, 378.6 vs 405.9 days, and than in the herd as a whole — 378.6 vs 388.5 days. Lactation period of CC cows shorter by 10 days than in CT cows, 321.7 vs 331.5 days. Analysis of traits of CC cows and animals in the herd had demonstrated that the age of first insemination and the age of first calving are significantly lower in CC cows than in the herd as a whole, 525.8 ± 17.8 vs 642.9 ± 7.5 days, and 808.6 ± 18.5 vs 936.6 ± 8.0 days. Higher bone mineral density values as an indicator of body health are observed in CC cows compared with CT animals, 3,580.3 vs 3,359.0 mg/mm^3. The study of MTHFR gene associated with reproductive traits in cows is relevant as a basis for breeding and biochemical correction of gene effects causing the reproductive disorders of animals.

Keywords: dairy cows, MTHFR gene, homocysteine, bone mineral density, milk production, reproduction of cows

Introduction. One-carbon metabolism of mammals is one of the key points of metabolism, and its research is a perspective direction for the development of pharmacological correction of failures. Methylenetetrahydrofolate reductase (MTHFR) is one of the main regulatory enzymes in the metabolism of homocysteine that catalyses the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Single nucleotide polymorphisms (SNPs) in MTHFR gene lead to decreased activity of enzyme, efficiency of the homocysteine-methionine cycle and hyperhomocysteinemia. The C677T transition in the exon 4 in human MTHFR gene leading to a thermolabile protein, with decreased enzymatic activity and it has been investigated for the past 20 years in different countries and ethnic groups. Numerous investigations have been performed on the associations of MTHFR gene SNPs with low fertility of women. Results of these studies have demonstrated a relationship between genotypes of MTHFR gene and pregnancy complications (placental insufficiency, premature detachment of normally situated placenta, late gestosis), fetal malformations (cleft neural tube, anencephaly, deformities of the facial skeleton), children born with a chromosomal abnormality.

So this MTHFR enzymatic activity process is lowered in subjects with MTHFR 677TT and 677CT genotypes and these individuals might require an increased intake of folate or another dietary factors to maintain or control blood levels of plasma folate or homocysteine (Soligo, Barini and Annichino-Bizzacchi, 2017; Hwang et al., 2017; Al-Achkar et al., 2016; Asim et al., 2015; Vanilla et al., 2015; Luo et al., 2015; Poursadegh Zonouzi et al., 2012; Altomare, Adler and Aledort, 2007; Stonek et al., 2007; Ananth et al., 2007; Doolin et al., 2002; Zetterberg et al., 2002).

It was presented by Zimin et al. (2009), that cows and humans have sufficient DNA sequence similarity to enable to map the human genome almost entirely onto cow. Authors were able to demonstrate a large majority, approximately 91%, of the genome has been placed onto the 30 *Bos taurus* chromosomes. Researchers identified 25,710 RefSeq proteins representing 18,019 distinct human genes, and aligned these to the cow genome. Of the 18,019 human genes, 17,253 (95.7%) mapped to cow (Zimin et al., 2009). In this connection, we believe that cows could be a suitable model for studying the effects of genes associated with mammalian reproduction.

The results obtained Yapan et al. (2011) after research of cows reproductive genetics and MTHFR gene were demonstrated that 8137C/T SNP was identified in exon 7 of MTHFR. Association analysis revealed that the 8137C/T was associated with cow abortion. According to the conclusion of researchers MTHFR may be a beneficial candidate gene to control cow abortion.

At the same time it could be noted a number of factors that affect one carbon metabolism such as age, gender, nutrition, genetics, medication, physical activity, climatic conditions and management of animals. That is why it is actual to conduct research on the Ukrainian selection breeds in the technological and fodder conditions of Ukrainian farms.

The aim of the study was the evaluation of role of MTHFR gene in lactating dairy cows.

Material and methods. The study was carried out with the analysis of materials and biological samples of lactating dairy cows from the State Enterprise Research Farm 'Nyva' of Institute of Animal Breeding and Genetics named after M. V. Zubets of the National Academy of Agrarian Sciencies of Ukraine during 2016–2017.

The type of production in SE RF 'Nyva' is organic. In this farm cows had milk production (6,514 L), milk fat (3.65%) and milk protein (3.20%) concentration during 2016–2017. The base total mixed ration (TMR) was alfalfa/haylage based with corn silage, corn and other silage. Part of soybean meal is 3–5% of the grain mixture. The system of keeping cows is traditional. All animals had the same light/dark schedule, humidity and temperature. Part of time animals spent in the walking areas. They do not receive hormonal and other medications, even to stimulate ovulation. The animals are excluded from infectious diseases.

For the study 35 cows at the age of 2nd–7th lactation after calving were selected randomly. All cows have the same pedigree — on average, every animal has 79.5 ± 1.9% of Holstein breed, 16.3 ± 1.92% of Simmental, 2.89 ± 0.82 of Montbeliarde. Traditional production and reproduction parameters were analyzed.

Cows were milked between 6.00 and 8.00, and from 17.00 to 19.00 daily. Samples of milk were individually stored and analyzed by Ecomilk-Standart (Bulteh 2000 Ltd, Bulgaria) for fat, protein. Bone mineral density (BMD) of cows was estimated by ultrasonic densitometry of the middle third of 12 pair rib-bone by Sunlight Omni 7000 (Sunlight Medical Ltd, Israel).

The plasma homocysteine levels were analyzed using commercial ECLIA test kits. The PCR reaction was carried out according Yapan et al. (2011). PCR products were analyzed with ethidium bromide stained 1.0% agarose gels electrophoresis, purified DNA fragments were isolated from gel using GeneJET Gel Extraction Kit (Life Technologies, USA) and then they were sequenced. Analysis of the gel images was carried out by ChemiDoc™ XRS+ System (Bio-Rad, USA). Sequencing was performed on an Applied Biosystems 3130 Genetic Analyzer (Life Technologies, USA). The results were analyzed with Chromas 2.1 Sequencing Software (Technelysium Pty Ltd, Australia) and BLASTN (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Statistical analysis was performed with the Shapiro-Wilk and Kolmogorov-Smirnov tests for normality and hypotheses — criteria *t* and χ^2. The deviation of allele frequencies from Hardy-Weinberg equilibrium was tested using Pearson's chi-squared test. The relationship between traits was estimated by the Spearman and Pearson correlation analysis. Means for two groups were compared by ANOVA (Atramentova and Utevskaya, 2008).

Results and discussion. The analysis of the results obtained in the present study indicated cows allele and genotype frequencies of 8137C/T in exon 7 of MTHFR gene (Table 1). Investigated group of animals was in Hardy-Weinberg equilibrium. C allele frequency was 0.943, T allele frequency — 0,057.

Table 1 — Allele and genotype frequencies of MTHFR gene in cows group, $\bar{x} \pm s_x$

Parameter	SNP 8137C/T of MTHFR gene		
Allele	**C**	**T**	
Frequency	0,943	0,057	
Genotype	**CC**	**CT**	**TT**
n factual	31	4	0
%	88.6	11.4	0
n theoretical	31	4	0
%	88.9	10.7	0.4
BW, kg	528.7 ± 7.3	524.5 ± 8.0	—
BMD, mg/mm^3	3,580.3 ± 61.4*	3,359.0 ± 89.5*	—
Hcy, µmol/L	5.85 ± 0.82	4.70 ± 0.54	—
CI, days	378.8 ± 5.7**	405.9 ± 10.7**	—
First lactation			
MY, kg/year	5,973.5 ± 92.5	5,868.0 ± 118.8	—
F, %	3.64 ± 0.03	3.67 ± 0.05	—
P, %	3.24 ± 0.02	3.27 ± 0.02	—
LP, days	321.7 ± 5.3	331.5 ± 13.7	—

Notes: $\bar{x} \pm s_x$ — mean ± standard error; BW — body weight, BMD — bone mineral density, Hcy — homocysteine level, CI — calving interval, LP — lactation period, MY — milk yield, F — milk fat, P — milk protein, * — differences are significant at $p < 0.05$, ** — $p < 0.01$.

Distribution of genotypes in cows group is 88.6% : 11.4% : 0%, CC : CT : TT, respectively. Our findings are consistent with the results published before by Yapan et al. (2011) — no homozygote of TT were found. In the work of Chinese authors the genotypic frequencies for CC, CT, and TT were 76.3% : 32,7% : 0%, and the deviation from the Hardy-Weinberg equilibrium was observed. Probably, analysis of a large group of cows (n = 569) allowed to show the negative effects of the T allele on the fertility of animals: cows with CT genotype had significantly greater relative risk of abortion (OR = 2.05, $p = 0.0002$) than those with CC genotype (Yapan et al., 2011).

The our analysis had demonstrated that calving interval was shorter in cows with the CC genotype — 378.6 vs 405.9 days in CT animals ($p = 0.01$). Evidently, on the one hand, more inseminations were required for CT animals, since it is known that subjects, human and cows, with CT genotype are characterized by lower MTHFR enzymatic activity, DNA and RNA synthesis, protein methylation and a higher homocysteine level in the blood. We have already shown a significant direct correlation between homocysteine level in the blood and the number of inseminations carried out before successful fertilization and development of pregnancy of cows — $r = 0.36$ ($p = 0.05$).

On the other hand, longer days-open in CT cows may be associated with and more active lactation and prolactin's effects include inhibition of ovulatory cycle, follicle-stimulating hormone and gonadotropin-releasing hormone secretion, prolongation of luteal phase and prevents pregnancy during the lactation. In our research — lactation period of CC cows shorter by 10 days than in CT animals, 321.7 vs 331.5 days.

The analysis showed that the homocysteine level in blood of animals was in the range of 2.96 to 27.9 μmol/L, reaching an average of 5.85 ± 0.82 μmol/L in CC cows group and 4.70 ± 0.54 μmol/L in CT group. At the same time, other authors found differences in homocysteine levels in cows with CC and CT genotypes in a large herd (Yapan et al., 2011). According them, the blood homocysteine levels of animals with CT genotype were significantly higher than those with CC genotype during the first 6 months of pregnancy and in non-pregnant cows.

It is well known that a higher level of homocysteine in the blood plasma associated with the CT genotype was also shown in humans (Doolin et al., 2002) and could be associated with various reproductive problems. For example, it had been demonstrated that in women the lower level of homocysteine in the follicular fluid was associated with a better chance of clinical pregnancy (Ocal et al., 2012). A meta-analysis of human studies had documented that high homocysteine level increased the abortion risk by 40% (Ren and Wang, 2006).

Bone mineral density is an indicator of body health and its effect contributed to successful inseminations, good fertility of cows and health pregnancy. Higher BMD values were observed in CC cows compared with CT animals, 3,580.3 vs 3,359.0 ($p = 0.05$). It is important to note that previously we found a negative correlation between bone mineral density and duration of days-open of cows — $r = -0.50$ ($p = 0.05$), and, respectively, the number of inseminations carried out before successful fertilization and development of pregnancy of cows — $r = -0.46$ ($p = 0.08$). More insemination was required for animals with a lower level of bone mineral density.

Analysis of traits of CC cows and all animals in the herd had demonstrated that the age of first insemination and the age of first calving are statistically significantly lower in CC cows than in the herd as a whole, 525.8 ± 17.8 vs 642.9 ± 7.5 days ($p = 0.000007$), and 808.6 ± 18.5 vs 936.6 ± 8.0 days ($p = 0.000003$), respectively. Calving interval were shorter in cows with the CC genotype than in the herd — 378.6 ± 5.7 vs 388.5 ± 4.6 (F = 5.65, $p = 0.01$).

Our study found no association between cows with different genotypes on productivity traits, although this position requires further study.

Conclusions. We analyzed association of 8137C/T MTHFR gene with reproductive traits of dairy cows.

The relationship between genotypes and reproduction traits of dairy cows — calving interval, age of first insemination, age of first calving, efficiency of insemination, homocysteine level in the blood plasma and bone mineral density of cows was shown.

Polymorphism 8137C/T of MTHFR gene might play an important role in development and fertility of cows and could be useful in breeding programs.

The study of genes associated with reproductive traits in cows is relevant as a basis for selection and biochemical correction of gene effects causing the reproductive disorders of animals.

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