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Article

Polymorphism of Selected Regions of *Ovar-MHC* and the Health Status of the Ovine Mammary Gland

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Simple Summary: The common cause of economic losses in sheep breeding are udder diseases (*mastitis*). The main cause of disease is pathogenic microbes. Their infection adversely affects the quality and quantity of milk and the effects of lamb rearing. Methods of treating this disease so far haven't brought satisfactory results. Therefore, in this study, an attempt was made to search for natural resistance to udder diseases. The research was carried out on sheep of the Polish Heath and Polish Lowland breeds. Genetic variability of the major histocompatibility complex (MHC) genes was analyzed. MHC molecules enable recognition of the pathogen and activate other cells of the immune system (T and B lymphocytes) to organize the defence. The content of somatic cells in milk (as an indicator of udder health) and the percentage of lymphocyte subpopulation were also assessed in the examined sheep. Among the many alleles of the MHC genes (*OLADRB1, OLADRB2, OMHC1*), two of them, 488 bp (*DRB1*) and 284 bp (*DRB2*) in length, were more frequently reported in sheep with healthy udders, while carriers of the 508 bp (*DRB1*) and 272 bp alleles (*DRB2*) were more prone to subclinical mastitis. The obtained results justify the need for further research aimed at searching for molecular markers of sheep' innate immunity to mastitis.

Abstract: Udder diseases (*mastitis*) are a serious cause of economic losses in sheep breeding as they have a negative impact on lamb rearing and the quality of dairy products. So far the progress in treatment and prevention of these diseases has been insufficient, giving ground for searching possibilities of using natural immunity to combat mastitis. The aim of the study was to assess the relationship between the microsatellite polymorphism of selected *Ovar-MHC* genes and the health status of the mammary gland of sheep. The research was carried out on sheep of the Polish Heath and Polish Lowland breeds. In ovine milk the number of somatic cells (SCC) and the percentage of the lymphocyte subpopulation were assessed. On the basis of genomic DNA, molecular analysis of the *Ovar-MHC* gene fragments (*OLADRB1, OLADRB2, OMHC1*) polymorphism was performed. Significant differences were found in SCC and the percentage of lymphocytes (CD4, CD8, CD19) in the milk depending on the alleles of the *Ovar-MHC* genes. Alleles of 488 bp (*DRB1*) and 284 bp (*DRB2*) were found more frequently in sheep with healthy udders, while carriers of the 508 bp (*DRB1*) and 272 bp (*DRB2*) alleles were more prone to subclinical mastitis. The obtained results justify the need for further research in order to better understanding the genetic basis of mastistis and to search for effective molecular markers that can be used in breeding practice.

Keywords: sheep; microsatellite polymorphism; Ovar - MHC; mastitis; lymphocytes

1. Introduction

Inflammation of the udder (mastitis) is a major cause of economic losses in sheep farming. During udder infection, the content of certain milk components (casein, lactose, fat) and

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microelements in milk (calcium, phosphorus, potassium, magnesium) decreases, while the number of somatic cells (SCC) and neutrophils increases. Losses in milk production and changes in its physicochemical properties caused by mastitis adversely affect primarily lamb rearing (growth and development, diseases, falls), as well as the quality of dairy products [1-5]. The main cause of udder diseases are pathogenic microorganisms, mainly staphylococci and streptococci, much less often bacilli (Gram+, Gram-), bacilli and mycoplasmas [6-16]. In addition to pathogenic microorganisms, development and course of mastitis are also influenced by genetic factors (individual, breed and species specific), biological factors (number of lactations and their phase, general health condition of the organism) and environmental factors (nutrition, care, type of premises, zoohygienic conditions) [17-19]. Immunity is a complex physiological process in which the major histocompatibility system plays a fundamental role. This system includes many polymorphic genes coding for the surface structures of the body's cells, known as MHC molecules. Class I MHC molecules present antigens to cytotoxic T lymphocytes ($CD8^+$), while class II molecules initiate an immune response by presenting antigens to helper T cells ($CD4^+$). One of the consequences of this presentation is stimulation of B lymphocytes ($CD19^+$) to proliferate and produce antibodies specific for given antigens. Many studies clearly show that during bacterial infections of the mammary gland in ruminants, due to the increased permeability of the udder epithelium, there is an increased flow of blood serum components and leukocytes from the blood into the milk, of which the most numerous are neutrophils [20]. At the same time, there is a significant decrease in the percentage of T lymphocytes (total $CD2^+$ and the $CD4^+$ subpopulation, $CD8^+$) and B lymphocytes ($CD19^+$) as well as cells with the MHCII receptor [21-24]. Several studies found an increase in the percentage of lymphocyte subpopulations during bacterial infections in cattle [25-27]. Many authors used microsatellite sequences to assess variability of the MHC genes in ruminants. Molecular studies conducted in sheep have shown significant STR polymorphism within the *Ovar-DRB1* and *OMHC1* genes and the *Ovar-DRB2ps* pseudogene [28- 37]. Further studies showed that some of the microsatellite alleles of MHC genes may be associated with sheep resistance to diseases, including such as: sprays [38], leukemia [39] or parasitic invasions [40-44]. Correlation between the polymorphism of the selected *Ovar-MHC* genes and the yield and quality of sheep milk as well as weight gain of lambs was also noted [30,34,45,46].

The aim of this study was to evaluate the relationship between the polymorphism of selected *Ovar-MHC* genes and the health status of the mammary gland of sheep expressed in the number of somatic cells and the percentage of lymphocyte subpopulation in milk.

2. Materials and Methods

This research was carried out on 150 sheep of the Wrzosówka breed (Polish Heath Sheep- PHS) and on 154 lowland sheep of the Żelaźnieńska breed (Polish Lowland Sheep - PLS). Both breeds are included in the national conservation breeding program. PHS sheep are characterized by a delicate body structure, easy adaptation to even very difficult environmental conditions, good viability, fertility and resistance to diseases. PLS sheep were produced by crossbreeding, enhancing the regional Łowicz sheep. As a result of many years of breeding work, quite massive sheep were obtained, characterized by good meat and wool performance, and high fertility. Research material was collected once from mothers of both breeds, in the 4th week of lactation (first or second), always at the same time of the day (morning), 2 hours after their lambs were weaned. Milk samples (20 ml) were taken from each half of the udder separately into two test tubes. At the same time, 8 ml of blood (tubes with K_2EDTA) were collected from the same mothers from the external jugular vein for molecular testing. Health of the sheep udder was assessed on the basis of the number of somatic cells in 1 ml of milk (Somacount 150, Bantley). The level of 200×10^3 was adopted as the physiological norm [47]. Identification of immune system cells (ISCs) in sheep milk was made by flow cytometry using monoclonal antibodies against CD2, CD4, CD8 (T cells), CD19 (B cells), and MHC II (B cells, APC) (VMDR Inc. Pullman), labelled with fluorescein (FITC) or phycoerythrin (PE) (Medac) [48,49]. Results were read using a FACScalibur flow cytometer (Becton-Dickinson) and the CellQuest program. Genomic DNA was isolated from sheep blood with the phenol-chloroform method.

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Concentration and purity of the isolated DNA was determined with a Gene Quanty spectrophotometer (Pharmacia). The tested fragments of the *Ovar - DRB1* gene (exon 2, intron 2, MHC class II), *OMHC1* (MHC class I), *Ovar - DRB2ps* (intron 5, MHC class II) were amplified using the classic PCR technique [45,49,50]. Identification of microsatellite alleles was performed using a capillary POP sequencer. 4. (Perkin Elmer) and Gene Scan 2.1 (Perkin Elmer). Based on the results of molecular analysis for each breed of sheep, the parameters describing genetic variation were estimated using the Cervus 3.0.7 program [51]: frequencies of alleles and genotypes (ALFreq program - [52]) of microsatellite sequences (fragments of *Ovar-DRB1* genes, *OMHC1*, *Ovar-DRB2ps*), coefficients: observed heterozygosity (H_O) and expected heterozygosity (H_E), Polymorphic Information Content (PIC), compliance of genotype distribution with the Hardy-Weinberg law (chi^2 test). The Fis and Fis parameters for multilocus (10000 - permutation) were estimated according to the formulas of Weir and Cockerham [53] and Robertson and Hill [54]. Also, with the help of the GENETIX 4.05.2 program [55], the genetic distance [56] between the studied breeds was estimated and, on the basis of the Factorial Correspondence Analysis - CA [57], graphs were made showing the relationships between genotypes individual multilocus. The Bayesian clustering method implemented in the program STRUCTURE 2.3.4 [58] was used with an admixture model and correlated allele frequencies to detect substructure in the data, assign individuals to cluster and identify potentially admixed genotypes. The optimal number of clusters (K) was set by running program from K = 1 to K = 5, with 10 repetition of 1000000 MCMC chain steps after a burn - in period of 1000000 steps for each K. STRUCTURE results were visualized in STRUCTURAL HARVESTER [59] implementing the method of Evanno et al. [60]. Graphical output was performed in DISTRUCT 1.1. [61]. As part of the statistical analysis of the results, the median value of somatic cells and lymphocyte subpopulations in sheep's milk was also calculated for all Ovar - MHC alleles and genotypes. The significance of differences between the compared groups was assessed using non-parametric Mann-Whitney or Kruskal-Wallis tests (depending on the number of levels). All calculations were made using the Statistica 13.3 program.

3. Results

Detailed results of the molecular analysis of selected Ovar - MHC regions: *Ovar*-DRB1, OMHC1, *Ovar*-DRB2ps are summarized in Table 1. Different frequency of individual alleles of *Ovar* MHC genes both within and between races was noted. The 488bp allele (*Ovar*-DRB1), which was most common in sheep, was distinguished by over seven times lower frequency in PLS. In turn, the 566bp and 508bp alleles in PLS were noted 2-3 times more often than in PHS. In both breeds of sheep, six out of the seven identified OMHC1 alleles were of identical length (from 188bp to 202bp), the allele of 208bp was found only in PHS sheep. In the case of the 192bp and 196bp allele frequencies, large interracial differences were observed. High frequency of a given allele in one breed of sheep corresponded to low frequency in the other breed (allele 192bp in PHS - 32.3% in PLS - 6.8%, allele 196bp in PHS - 4.0% in PLS - 25.6%). The difference in frequency between the remaining alleles in PHS and PLS was much smaller. Analysis of the Ovar-DRB2 pseudogene polymorphism revealed the absence of 272bp and 276bp alleles in PHS sheep, while the 284bp allele in PLS.

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Table 1. Frequency of the *Ovar* – MHC alleles in the studied sheep breed.

Allele	Allele frequency (%)								
***Ovar - DRB1* (bp)**	**488**	**508**	**516**	**520**	**526**	**530**	**540**	**566**	**590**
PHS	46.1	11.0	10.2	1.6	7.5	1.6	5.1	13.4	3.5
PLS	6.1	32.0	15.0	3.7	4.4	1.4	8.8	28.6	-
***OMHC1* (bp)**	**188**	**190**	**192**	**194**	**196**	**202**	**208**		
PHS	8.3	8.7	32.3	29.0	4.0	10.0	7.7		
PLS	4.9	12.7	6.8	35.7	25.6	14.3	-		
***Ovar - DRB2* (bp)**	**262**	**268**	**272**	**274**	**276**	**284**	**290**		
PHS	17.2	32.8	-	20.9	-	22.0	7.1		
PLS	7.1	38.8	25.9	12.9	12.9	-	2.4		

PLS - Polish Lowland Sheep; PHS - Polish Heath Sheep.

The results of the microsatellite polymorphism analysis of the three *Ovar*-DRB1, OMHC1, and *Ovar*-DRB2ps loci were also used to estimate the parameters of genetic variation (Table 2). Compared to PHS, PLS showed lower Ho coefficients for the *Ovar*-DRB1 and *Ovar*-DRB2ps loci, and a higher value for OMHC1. However, the values of H_E coefficients for all analysed loci in both breeds of sheep were at a similar level (0.74 - 0.78). The PIC values were slightly lower than the H values. The presented parameters indicate that the PHS breed was characterized by greater heterozygosity than the PLS breed, however the differences in the values of these parameters between the tested breeds (except for *Ovar-DRB1*) were small. This is also confirmed by statistics. For the *Ovar-DRB1* and *OMHC1 loci* in the PHS and *OMHC1* in the PLS, negative values were obtained for the F_{IS} statistics, which may indicate that more heterozygotes were identified for the studied microsatellite *loci* than expected. In addition, no significant deviations within HWE were found in most loci (except *Ovar*-DRB1 and *OMHC1* in PLS). Estimated on the basis of the polymorphism of three selected Ovar - MHC regions, the genetic distance between the PLS and PHS breed was 0.444. Comparison of the genotype distribution between the studied breeds, made with the use of factorial correspondence analysis, clearly separated the two breeds (Figure 1). The best - supported number of clusters in STRUCTURE was K = 2 separating PLS from PHS Figure 2. The existing differences between the studied breeds are also presented in Figure 3.

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Table 2. Analysis of genetic variability of the *Ovar* - MHC microsatellite *loci* in the Polish Heath Sheep (PHS) and the Polish Lowland Sheep Żelazna variety (PLS).

Breed			**PHS**						**PLS**					
Locus	**N**	**No. of alleles**	**H_O**	**H_E**	**PIC**	**HW[b]**	**F_{is}**	**N**	**No. of alleles**	**H_O**	**H_E**	**PIC**	**HW[b]**	**F_{is}**
Ovar-DRB1	126	9	0.75	0.74	0.71	NS	-0.01060	147	8	0.58	0.78	0.75	***	0.25184
OMHC1	149	7	0.80	0.78	0.75	NS	-0.02306	154	6	0.84	0.77	0.73	**	-0.10286
Ovar-DRB2	133	5	0.74	0.77	0.73	NS	0.03869	147	6	0.70	0.75	0.71	NS	0.70440
Mean		7	0.76	0.76	0.73		0.00169[a]		6.67	0.71	0.77	0.73		0.07445[a]
±SD		1.63	0.03	0.02	0.02				0.94	0.11	0.01	0.02		

N - number of analysed individuals, H_O - observed heterosigosity, H_E - expected heterosigosity, PIC- Polymorphism Information Content,.

F_{IS} - inbreeding coefficient, [a)] multilocus F_{IS}, [b)] Hardy-Weinberg equilibrium significance level: *** significant at p≤0.001, ** significant at p≤0.01, NS - insignificant.

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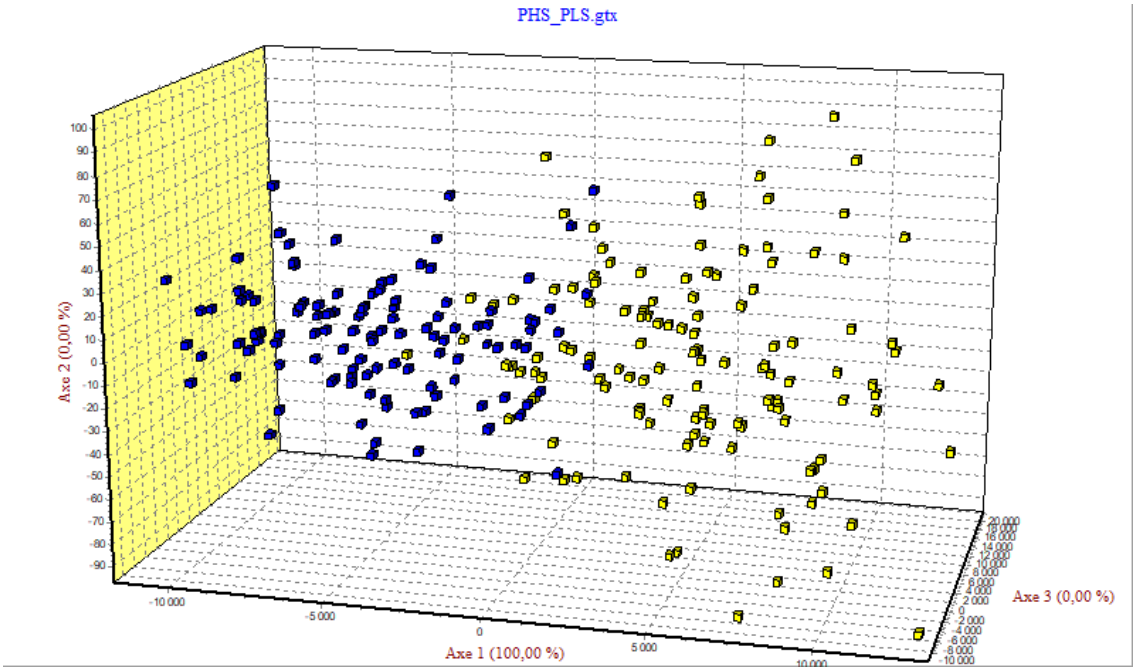

Figure 1. The factorial correspondence analysis performed in Genetix on the base of 3 *Ovar* – MHC *loci*. Genotype distribution between the two breeds: PLS - Polish Lowland Sheep (blue); PHS - Polish Heath Sheep (yellow).

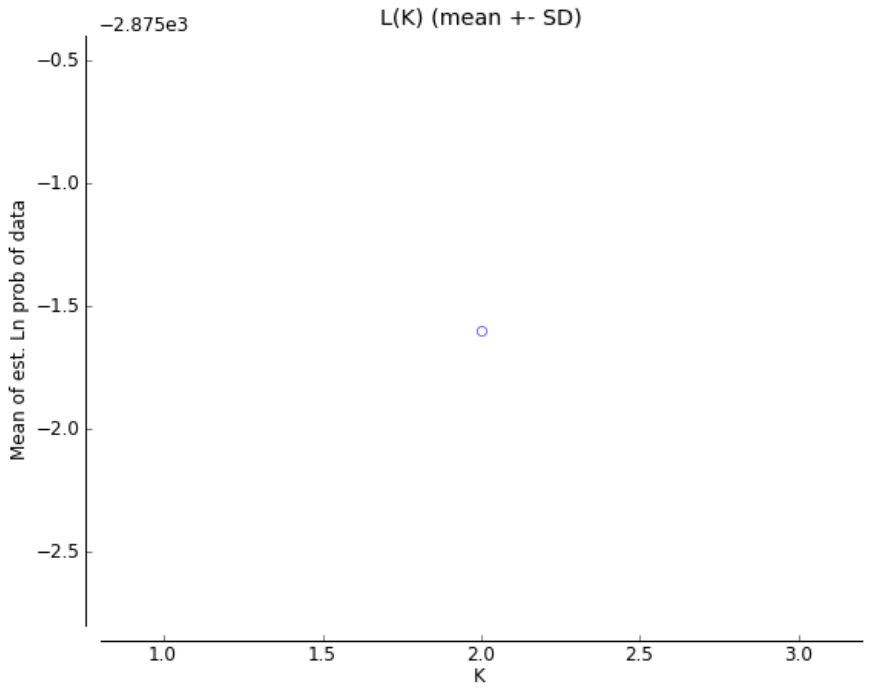

Figure 2. The posterior probability Ln (K) of the data.

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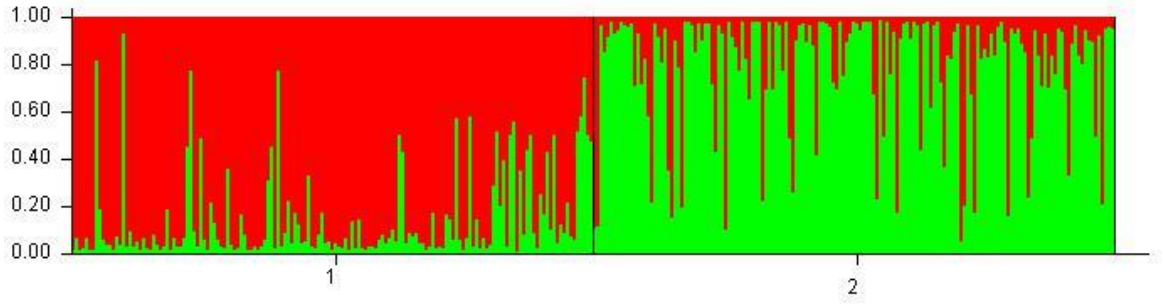

Figure 3. STRUCTURE analyses performed of Bayesian clustering analysis of the two breeds PHS (1) and PLS (2) analysed on the basis of three *Ovar* – MHC *loci*. Each individual is represented by one vertical bar that is divided into segments representing the proportion of memberships to the respective breeds. The results are displayed for two (K=2) suggested clusters.

Research showed significant differences between the breeds in the content of the analysed milk indicators (Table 3). PLS showed a significantly higher level of somatic cells (305.5 × 10^3) than PHS (142 × 10^3). In PLS a significantly lower percentage of lymphocytes ($CD2^+$, $CD8^+$, $CD19^+$) and cells with the MHC II surface receptor was associated with a higher SCC level. The reverse relationship was observed in PHS. Results of the cytometric analysis of sheep milk presented in Table 4 indicate a reverse relationship between somatic cells and cells of the immune system (ISC). In the samples of milk with SCC content> 200 × 10^3, a lower percentage of lymphocyte subpopulation was observed (except for $MHCII^+$ cells) compared to the level <200 × 10^3. For $CD2^+$, $CD4^+$, $CD19^+$ lymphocytes those differences were statistically significant. Table 5 presents the assessment of the relationship between the polymorphism of the Ovar-MHC regions under study and the level of SCC and ISC in the milk of both sheep breeds. Among the *DRB1* alleles (with the highest frequency), the 488 bp allele deserves attention (Table 1). The lowest number of somatic cells (M = 129 × 10^3) and a significantly higher percentage of $CD2^+$, $CD4^+$, $CD19^+$, $MHCII^+$ cells were found in the milk of sheep carrying this allele. On the other hand, the lowest (except for $CD8^+$) percentage of immune system cells was observed in sheep carrying the 508 bp allele. In the case of OMHC1, there was no significant relationship between the identified alleles and the analyzed SCC and ISC cells. Among the DRB2ps alleles, sheep carrying the 272bp allele were characterized by the lowest percentage of immune system ells, which also had the highest level of SCC (M = 277.5 5 × 10^3). It should be noted that this allele was present only in the PLS breed. A similar trend was observed in carriers of the 268bp allele. The highest (statistically significant) percentage of the $CD2^+$, $CD4^+$, $CD8^+$ lymphocyte subpopulation was recorded in sheep carrying the 284bp allele (this allele was present only in the PHS breed). Molecular analysis of selected *Ovar* - MHC regions in two breeds of sheep revealed the presence of 31 *DRB1*, 24 *OMHC1* and 24 *DRB2ps* genotypes. Table 6 shows the variability of the SCC and ISC against the genotypes with the highest frequency. The presented data indicate a large influence of a given allele (Table 5) on the phenotypic value of the genotype. The 488bp / 488bp homozygotes (as well as the alleles - Table 5) had a lower SCC value (M = 124 × 10^3) and a higher percentage of ISC compared to the other genotypes (508bp / 508bp, 566bp / 566bp). A similar relationship was found between the DRB2ps genotypes (284bp / 284bp and 268bp / 268bp, 268bp / 272bp).

Table 3. Median of somatic cells number and proportions of immune cells in the milk of the Polish Heath Sheep (PHS) and the Polish Lowland Sheep Żelazna variety (PLS).

Breed	Median	Median of immune cells (%)				
	SCC × 10^3	$CD2^+$	$CD4^+$	$CD8^+$	$CD19^+$	$MHCII^+$
PHS	142.0^A	55.0^A	22.8	31.0^A	18.0^a	58.5^A
PLS	305.5^B	43.5^B	21.0	17.3^B	14.0^b	40.0^B

Significant values – different letters (ab - $p \leq 0.05$, AB - $p \leq 0.01$).

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Table 4. Relation between the number of somatic cells and proportion of the lymphocyte subpopulation and MHCII cells in the milk of studied sheep.

SCC × 10^3	Median of immune cells (%)				
	CD2+	CD4+	CD8+	CD19+	MHCII+
<200	53.5^{A}	24.0^{A}	25.0	18.0^{A}	45.0
>200	47.0^{B}	19.3^{B}	22.0	14.0^{B}	53.0

Significant values- different letters (AB - $p \leq 0.01$)

Table 5. Median of somatic cells number and proportions of immune cells in the milk of the Polish Heath Sheep (PHS) and the Polish Lowland Sheep Żelazna variety (PLS) in relation to the analysed *Ovar* – MHC regions.

Allele (bp)		n	Median	Median of the immune cells (%)				
			SCC × 10^3	CD2+	CD4+	CD8+	CD19+	MHCII+
Ovar-DRB1	**488**	133	129.0	54.5^{aA}	25.5^{aA}	30.0^{aA}	17.6	50.0^{a}
	508	122	232.5	44.5^{B}	17.0^{B}	18.8^{B}	11.0	37.0^{b}
	516	70	256.5	53.0^{a}	21.3^{a}	21.8^{a}	19.0	38.3
	566	118	254.0	46.5^{B}	20.0^{a}	16.3^{B}	18.0	46.0
OMHC1	192	118	171.0	52.0	21.5	26.0	15.0	47.0
	194	195	211.0	53.5	22.8	25.0	17.0	52.0
	196	88	329.0	46.8	22.8	19.0	15.0	41.3
	202	74	193.0	49.3	19.8	21.8	14.0	48.0
Ovar-DRB2ps	268	202	206.5	47.0^{a}	21.5^{a}	21.0^{ab}	16.5	47.0^{a}
	272	72	277.5	37.0^{bA}	15.0^{A}	15.3^{b}	13.5	30.0^{bB}
	274	92	265.0	50.0^{a}	19.0^{a}	25.8^{a}	18.0	59.5^{aA}
	284	59	156.0	56.5^{aB}	27.0^{aB}	31.0^{a}	16.5	45.0^{a}

Significant values – different letters(ab - $p \leq 0.05$, AB - $p \leq 0.01$).

Table 6. Median of somatic cells number and proportions of immune cells in the milk of the Polish Heath Sheep (PHS) and the Polish Lowland Sheep Żelazna variety (PLS) in relation to the selected O*var* – MHC genotypes.

Genotype (bp/bp)		n	Median	Median of immune cells (%)				
			SCC × 10^3	CD2+	CD4+	CD8+	CD19+	MHCII+
Ovar-DRB1	**488/488**	19	124.0	55.5	31.0	26.0	21.0	60.0
	508/508	24	219.0	37.5	17.0	17.5	10.0	32.5
	566/566	22	231.5	42.0	21.5	8.0	19.0	59.0
OMHC1	192/194	31	159.0	46.3	20.0	16.0	21.5	47.0
	194/196	36	282.0	54.8	29.5	20.0	15.3	44.3
	194/202	23	226.0	50.0	20.0	25.0	14.0	49.0
Ovar-DRB2ps	268/268	34	221.0	43.0	28.5	22.0	18.0	63.5^{A}
	268/272	17	1176.0	37.5^{a}	15.0	8.0	13.0	23.5^{B}
	284/284	10	132.0	68.0^{b}	35.2	26.2	17.0	42.5

Significant values – different letters (ab - $p \leq 0.05$, AB - $p \leq 0.01$).

4. Discussion

The usefulness of microsatellite sequences for the assessment of *Ovar*-MHC gene polymorphism in sheep has been reported in many scientific papers [62]. Paterson [31], analysing microsatellite polymorphism in exon 2 of the Ovar-DRB1 gene, found, in the Scottish primitive Soay

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sheep, the presence of 8 alleles ranging in length from 205bp to 287bp. Similar polymorphism was demonstrated in our own studies (Table 1, Table 2), even though a different and longer fragment (exon 2 / intron 2) of this gene was analysed. Much greater genetic diversity of the *Ovar-DRB1* gene (14 alleles - from 148 bp to 238 bp) was reported by Worley et al. [33] in sheep of various breeds in Norway, Canada and Alaska, Geldermann et al. [34] in German merino (16 alleles) as well as Gowane et al. 63] in Malpura (23 alleles). The *OMHC1* gene polymorphism demonstrated in this study (Table 1, Table 2) is also confirmed in papers by other authors. Groth and Wetherall [65] identified 8 *OMHC1* alleles in Australian merino, Paterson [31] in Soay sheep 5 alleles (184bp-206bp), Worley et al. [33] in sheep of various breeds 11 alleles (178bp-200bp) and Santucci et al. [35] also have 14 alleles (168bp-212bp) in different breeds of sheep. Blattman and Beh [28], analysing microsatellite polymorphism of the *Ovar-DRB2* pseudogene, found 11 alleles (265bp - 295bp) in the studied sheep, while Worley et al. [33] found more than twice as many - 23 alleles (238bp-286bp). Those results differ significantly from both the results of our own research and those obtained by Paterson [31], who identified only 6 alleles in the Soay sheep (265bp - 283bp). Summing up, it should be stated that high values of heterozygosity coefficients (H_E> 0.7) in both breeds of sheep (PHS and PLS) for all analysed *Ovar*-MHC genes indicate that these loci can be used as genetic markers. The analysis of the variability of the lymphocyte subpopulation in the milk of ruminants depending on the udder health has been researched by many authors. Chaffer et al. [66] showed that milk obtained from bacteria infected cow udder quarters contained a significantly lower percentage of T lymphocytes ($CD4^+$ and $CD8^+$) compared to milk obtained from healthy quarters. Winter and Colditz [21], when infecting the udder of sheep with *S. epidermidis*, observed in the milk of these animals 24 and 48 hours after injection, a significantly lower percentage of $CD4^+$ and $CD8^+$ lymphocytes than in the milk of the control group. In other studies, Persson-Waller and Colditz [23], as early as 4 hours after stimulation of the udder of sheep, *S. aureus* and *E. coli* noted a significantly lower percentage of $CD8^+$ and $CD4^+$ lymphocytes in the secretion of the dried udder of the experimental group compared to the control group. Similar results were obtained in the own research (Table 4). The lowest percentage of $CD2^+$, $CD4^+$, $CD8^+$ lymphocytes recorded in milk> 200 × 10^3 / ml is probably the consequence of the early phase of the body's immune response to an infectious agent, characterized by a significant decrease in the share of lymphocytes and an increase in the percentage of neutrophils. Different results were obtained by Taylor et al. [25] and Riollet et al. [27], who found in the milk of sheep and cows during bacterial infections of the mammary gland a greater percentage of $CD4^+$, $CD8^+$ lymphocytes compared to milk from the infection-free udder. Those differences in the above-cited studies and in our own research on the participation of $CD4^+$ and $CD8^+$ in milk may depend not only on the heterogeneous conditions of the research (breed, physiological state, lactation period, time after infection) but also on the type of microorganisms causing udder diseases. This is confirmed in the research by Soltys and Quinn [67], which shows that T lymphocytes ($CD4^+$, $CD8^+$) are selectively recruited in udder infection depending on the type of bacteria present in the mammary gland. B lymphocytes ($CD19^+$) and cells with the MHCII receptor (mainly antigen presenting cells) are involved in the complex process of the humoral response, i.e. the next phase after inflammation in which neutrophils play an essential role. An increase in the proportion of neutrophils in the initial phase of response to an infectious agent reduces the proportion of cells involved in the subsequent humoral response process. This conclusion is based on many scientific studies [21-24] in which animals were experimentally infected. Results of our own research (Table 4), especially regarding changes in the proportion of B ($CD19^+$) lymphocytes in sheep milk, are consistent with other results, presented earlier. Different result was only obtained for $MHCII^+$ cells. The variability in health status of sheep udder (SCC) depending on the identified allele of the microsatellite sequence of the *DRB1* gene fragment (MHC class II) noted in our own research (Table 5) was reflected in the variability of the percentage composition of the analysed immune cells in milk. Sheep, which carry the 508 bp allele (*Ovar*-DRB1), with higher SCC levels, had a significantly lower percentage of T lymphocytes ($CD2^+$, $CD4^+$, $CD8^+$) and $MHCII^+$ cells compared to these, which carry the 488 bp allele. It should be emphasized that the 488bp allele in the PHS breed was more than 7.5 times more frequent than in the PLS breed (Table 1), which is more prone to mastitis. As shown by many studies [21-24], the decrease in the share of immune cells involved in

proper antigen recognition ($CD4^+$, $CD19^+$, MHC II^+) is a symptom characteristic of the initial phase of an ongoing inflammatory process, when mainly the number of neutrophils increases.

Considering the above, significant differences in the percentage composition of lymphocytes in milk (Table 5), which reflect the indicators of the mammary gland health status, observed between carriers of the 508bp and 488bp alleles may indicate a different susceptibility of sheep bearing these alleles to udder diseases. The obtained results may suggest that the 488 bp allele of the *Ovar*-DRB1 gene may be associated with resistance to mastitis in sheep, while the 508 bp allele with susceptibility to this disease. Among the analysed alleles of the *DRB2* pseudogene, the presence of the 272bp allele in the sheep genotype may favour inflammation of the mammary gland, in contrast to the 284bp allele, which may be associated with resistance to udder diseases (Table 5). It should also be emphasized that the 272bp allele was present only in PLS, which are more susceptible to mastitis, while the 284bp allele was identified only in PHS, more resistant to this disease. Such suggestions regarding the link of certain alleles with susceptibility or resistance to udder diseases are confirmed by the identified genotypes presented in Table 6.

5. Conclusions

Studies showed statistically significant differences in the health condition of the mammary gland of the Heath sheep (PHS) and lowland sheep of the Żelazna variety (LS). The varied health status of the udders of these sheep breeds was reflected in the variability of the percentage of cells in the immune system. It was found that in PHS the percentage of lymphocyte subpopulation in milk was significantly higher than in PLS. A higher percentage of lymphocytes in milk was associated with a lower level of somatic cells - meaning healthy udders. Molecular analyses allowed to identify significant microsatellite polymorphism of the three studied *Ovar* - MHC regions in the two sheep breeds. This polymorphism and the estimated high values of expected heterozygosity coefficients for the majority of the analysed fragments of Ovar - MHC genes indicate the possibility of searching for genetic markers of sheep resistance to diseases among alleles of these genes. Based on the analysis of the values of Ho, H_E and PIC parameters, it can be concluded that PHS sheep were characterized by greater heterozygosity than PLS sheep. Significant differences in the health status of sheep udder and the share of immune system cells depending on the identified alleles of the *Ovar* - MHC genes were also shown. The 488bp (*DRB1*) and 284bp (*DRB2*) alleles were more frequently found in sheep with healthy udders, while the carriers of the 508bp (*DRB1*) and 272bp (*DRB2*) alleles were more prone to mastitis. For the *OMHC1* alleles, no clear relationship was observed between them and condition of the sheep udder and activity of immune cells. Previous studies have shown that resistance to different diseases is influenced by many factors, but one of the most important is genetic variation. Despite many scientific achievements, there are still difficulties in precisely determining the role that genetic variation plays in the individual shaping of the immune response process induced by pathogens. The results of many studies, however, indicate that further improvement and standardization of the research methods should enable both better understanding of the complex defence processes, and the determination of such genetic markers that can be successfully used in breeding practice.

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