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β-casein gene polymorphism use in terms of brown dairy cattle preservation

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SUMMARY

Biologically important components of milk are fat, protein, lactose and minerals. Thanks to them, it is a significant and essential part of the human diet. In recent years, among the protein's components the beta-casein has gained great importance and popularity among people who care about their health. Therefore, the beta-casein composition of milk protein and dairy products has become an important breeding feature of dairy animals. This study aims at assessing the prospects for the use of polymorphism of the beta-casein locus in the selection of brown cattle to determine the possibility of creating gene pool herds with unique characteristics of milk – exclusively with beta-casein A2. The identified genetic structure of cows and bulls of the Lebedyn breed by beta-casein, the involvement of bulls of the original Brown Swiss (BS) breed according to the proposed reciprocal model, allows forming homozygous populations of A2A2 genotypes in subsequent generations. An individual approach to the issues of matching and selection, control, with the help of DNA diagnostic methods, by the processes of transmission of genes from parents to offspring, will allow replenishing herds with carriers of the A2 gene. The proposed breeding model in combination with the use of sexed sperm and genotyping of heifers, has the highest selection acceleration of the creation of a homozygous population for beta-casein A2A2.

Uso del polimorfismo del gen de la β-caseína en términos de preservación del ganado lechero marrón

RESUMEN

Los componentes biológicamente importantes de la leche son la grasa, la proteína, la lactosa y los minerales. Gracias a ellos, es una parte significativa y esencial de la dieta humana. En los últimos años, entre los componentes de la proteína, la beta-caseína ha ganado gran importancia y popularidad entre las personas que se preocupan por su salud. Por lo tanto, la composición beta-caseína de proteínas lácteas y productos lácteos se ha convertido en una característica de cría importante de los animales lácteos. Este estudio tiene como objetivo evaluar las perspectivas para el uso del polimorfismo del locus de beta-caseína en la selección de ganado marrón para determinar la posibilidad de crear rebaños de piscina genética con características únicas de la leche – exclusivamente con beta-caseína A2. La estructura genética identificada de las vacas y toros de la raza Lebedyn por beta-caseína, la participación de los toros de la raza original de la raza suiza marrón (BS) según el modelo recíproco propuesto, permite formar poblaciones homocigotas de genotipos A2A2 en generaciones posteriores. Un enfoque individual para las cuestiones de coincidencia y selección, control, con la ayuda de métodos de diagnóstico de ADN, por los procesos de transmisión de genes de padres a descendientes, permitirá reponer rebaños con portadores del gen A2. El modelo de cría propuesto en combinación con el uso de espermatozoides sexados y el genotipado de novillas, tiene la mayor aceleración de selección de la creación de una población homocigota para beta-caseína A2A2.

PALABRAS CLAVE
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INTRODUCTION

There is global problem of biodiversity preserving of animals genetic resources is in the world. FAO states that aboriginal breeds should be considered as a valuable genetic heritage. In Ukraine, brown cattle of the North-Eastern region are local and represented by Lebedyn (L) and Ukrainian brown (UB) dairy breeds. In order to further preserve such herds, scientists must look for unique features of the productive qualities of livestock. Since these are dairy breeds, the main area of research was the analysis of productive qualities of livestock. Many scientists (Drozdov et al., 2009; Kopylov et al., 2016; Kovalyuk et al., 2019) consider native breeds of farm animals to be carriers of unique genetic information that cannot be reproduced by modern breeding methods. The study of their gene pool is conducted in terms of finding new useful genes of quantitative or other traits (productivity, disease resistance, stress) for use in further selection.

The Ukrainian Lebedyn dairy cattle bread (L) with its high adaptability to specific environmental conditions, in our opinion, deserves to be preserved and further used in the breeding process. Of course, it is difficult for this breed to compete for the highest yield with such world leaders as the Holstein and Brown Swiss (BS) breed, but it is still possible. Then the quality and uniqueness of the products received should come to the fore. Revival of this breed can be carried out at the expense of both natural and artificial methods of reproduction (Ladyka et al., 2019; Sklyarenko et al., 2017).

The limited genealogical diversity of the Lebedyn breed sires, the sperm of which is stored in breeding centers, does not allow to avoid completely inbreeding in gene pools, which can lead to undesirable consequences. Therefore, we are introducing a fundamentally new scheme of breeding work by the method of population reciprocal crossbreading (Mukhamadieva et al., 2016). In addition to preventing the negative effects of inbreeding, it is important to maintain a livestock population that is distinguished by its predominant quality milk composition. This, in turn, increases revenue, stimulating the conservation and increase of unique livestock.

MATERIAL AND METHODS

Biochemical composition of milk and casein. Studies have shown that the biochemical composition of milk is highly dependent on genetic factors such as breed and cow origin (Bobe et al., 2007; Marchi et al., 2007; Bonfatti et al., 2010). Currently, the study of the relationship between hereditary factors that determine the types of proteins and enzymes, and the economic-beneficial features of animals is of scientific interest (Ilyina, 2014; Khlestkina, 2013; Islam et al., 2014; Drozdov et al., 2009; Molee et al., 2015).

Casein is a major component of milk proteins. It is represented by three fractions – alpha, beta and kappa. β-casein accounts for 24-28% of total milk protein (Parashar and Saini, 2015). It has 209 amino acids in at least 12 variants, which differ in their structures. The most common of these are A1 and A2 (Kovalyuk et al., 2018; Kovalyuk et al., 2019; Kononova et al., 2016; Kopylov et al., 2016). According to (Kučerova et al., 2006), four alleles (A1, A2, A3 and B) of the casein locus were detected in the genotyped animals. Scientists (Ng-kwai-hang et al., 1986) testify to the presence of nine different types (A1A1, A1A2, A1A3, A1B, A2A2, A2A3, A2B, A3A3 and A3B) of beta-casein.

Many scientists have determined the frequency of occurrence of A1 and A2 genotypes in different dairy cattle populations (Kovalyuk et al., 2018; Kovalyuk et al., 2019; Parashar and Saini, 2015; Kononova et al.,2016; Barany et al., 1993; Ng-kwai-hang et al., 1986; Demirel and Çak, 2018; Kyselová et al., 2019; Kovalyuk et al., 2019). In particular, (Boro et al., 2016) reports that the frequency of the A1 allele in the Guernsey breed is in the range of 4-2%, in the Brown Swedish - 34-30%, in the Jersey - 50-37%, in the Holstein - 56-47%, in the Ayrshire - 60- 51%, in the Red Danish - 77%. Demirel and Çak (2018) has systematized the results of many studies and concludes that milk from Guernsey, Jersey and Asian cattle, human milk, and milk from other animals (sheep, goats, donkeys, yaks, camels, buffalo, etc.) contains mainly β-casein A2. The milk of Holstein cows mainly contains β-casein A1.

Some scientists have linked beta-casein polymorphism with the ability to preserve local breeds. In the Romanian animal population, the A2 allele frequency was found to be 0.563 for gray cattle; 0.400 – for Pinzgauer, Romanian Red; 0.750 – for Black Romanian (Gradinaru et al., 2018). In Poland, the proportion of β-casein A2 in Polish red sires and cows was 0.58 and 0.37, respectively (Cieslinska et al., 2019).

Beta-casein polymorphism and human health. It is well known that milk is a source of high quality protein and trace elements – calcium, magnesium and phosphorus for humans. However, in 2012, the Food Agriculture Organization (FAO) reported an increase in the number of many chronic diseases caused by milk consumption (Boro et al., 2016).

It is now scientifically proven that when digested with enzymes of the gastrointestinal tract of milk, which contains a fraction of beta-casein A1, peptide β-casomorphin 7 (BCM 7) is formed in a much larger amount than with the corresponding splitting of milk having a fraction of beta-casein A2 (Kovalyuk et al., 2019; Molee et al., 2015). It should be noted that this substance has opioid properties (Kononova et al., 2016). It is known that BCM 7 can be formed not only from milk but also from yoghurt, cheese and other dairy products (Kuzmenko and Kuzina 2016).

Researchers (Kononova et al., 2016) claim that milk obtained from cows with the A1A1 genotype adversely affects human health. In particular, there is a significant relationship between milk protein and the incidence of type 1 diabetes. Scientists say a higher level of BCM 7 is associated with higher death rates from coronary heart disease. In addition, neurological disorders such as schizophrenia, autism and sudden infant death syndrome can also be caused by the consumption of cow's milk (Kovalyuk et al., 2018; Kaminski et al., 2017; Elliott et al., 1999; Parashar and Saini, 2015; Boro et al., 2016; Truswell, 2005). Kuzmenko and Kuzina (2016) states that the exclusion from the diet fraction A1 β-casein can be a prevention of both intestinal motility disorders, lactose digestion, and problems related to the local immune response. In addition, Chinese studies suggest that gastrointestinal symptoms in some subjects with lactose intolerance may be related to type A1 β-casein and not to lactose itself (He et al., 2017). This is confirmed by other researchers (Jianqin et al., 2016; Clarke and Trivedi, 2014).

The evidence that β-casein A2 consumption is appropriate is due to the fact that, according to studies, there were virtually no heart disease in some populations, such as Masai (East African) and Samburu (Northern Kenya), despite the consumption of a diet rich in animal milk. At the same time, the milk they consumed was obtained from cattle that are exclusively carriers of the A2 allele (Boro et al., 2016). Milk containing allelic variant A2 of β-casein is considered low allergenic compared to variant A1 (Kononova et al., 2016). Summing up, it can be stated that A2 milk has a positive effect on human health and avoids the risk of many diseases.

Marketing of milk A2. Commercial companies have been making money since the early 2000s, selling A2 milk in niche markets. In particular, New Zealand's A2 Milk Company licenses and markets all products of this milk in Australia, China and the US. According to the consumer surveys, the decisive factor in buying such a product is the actual selling price, which is based on the fact that A2 milk is taken at the price of organic milk (Kovalyuk et al., 2018; Kovalyuk et al., 2019; Mukhamadieva et al., 2016; Herbstreit, 2018).

At the same time, farmers are interested in switching to producing such milk, given its high purchase price (Mukhamadieva et al., 2016).

Thus, the high frequency of occurrence of animals with the A2A2 genotype by β-casein in populations of local cattle breeds, combined with the beneficial properties of this product and its positive effects on the human body and, given the high purchase cost of raw materials, creates prerequisites for preservation of valuable livestock in the world.

The purpose of the research was to evaluate the prospects for the use of β-casein locus polymorphism in brown cattle breeding in order to determine the possibility of creating gene pools with unique dairy productivity characteristics - A2 milk production.

Research methods. The studies were conducted at the laboratory of the Institute of Animal Production of the National Academy of Agrarian Sciences of Ukraine (Kharkiv). DNA samples, obtained from the hair bulbs of cows and heifers of the Lebedyn breed (n=50), belonging to the breeding reproducer of private agricultural enterprise "Komyshanske", Okhtyrka district, Sumy region, and sperm of sires of the Lebedyn breed (n=5), the European Brown Cattle (BV, n=5), the crossbreed of Lebedyn and European Brown Cattle (BV, n=7) and the Original Brown Cattle (OBV, n=3), were studied. Genotypes were determined using the allele-specific PCR method (AS-PCR). The resulting amplification fragments of size 244 bp were visualized by horizontal electrophoresis in agarose gels stained with ethidium bromide under the action of ultraviolet.

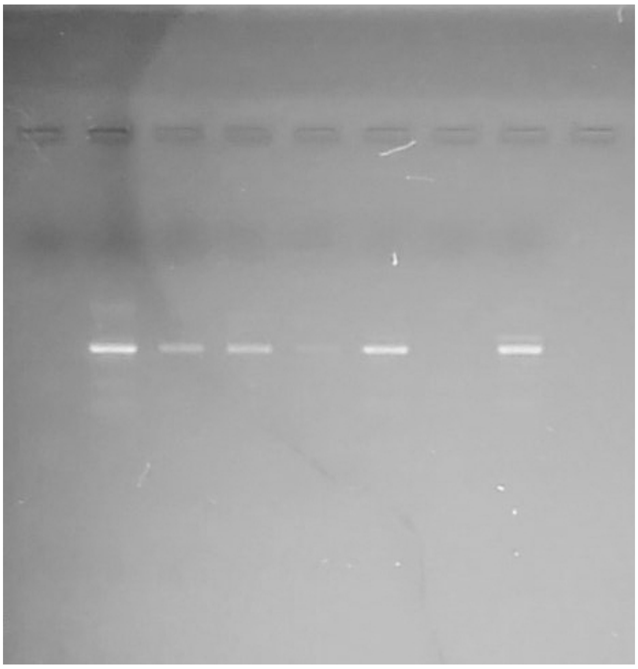
Breeding enterprise catalog data were used to determine the genotype of the sires by β-casein gene used in the selection schemes. (https://www.ggi.de/ru/glavnaja/; http://ct.wwsires.com/; Semex.com; altagenetics.ru)

RESULTS

The conducted study of sperm production of sires on β-casein gene was aimed at studying the genetic characteristics of each of the sires in order to indirectly determine the genetic structure of the studied population for β-casein gene and to determine the possibility of using their sperm production in custom pairs.

Polymorphism analysis of the genes analyzed is shown in **Figure 1**.

As a result of the studies, it was found that among nine sires tested by β-casein genes of the desirable homozygous A2A2 genotype, three sires were homozygous by the genotype A1A1, eight sires were heterozygous by the genotype A1A2 (**Table I**).



Fiure 1. Polymorphism analysis of the genes analyzed (Análisis de polimorfismo de los genes analizados).

According to the results of molecular genetic analysis, the frequency of distribution of brown breeds sires genotypes and alleles by β-casein gene was determined. In particular, it was found that the frequency of the desired genotype A2A2 among the tested animals was 0.20 in the Lebedyn breed; the European Brown Cattle (BV) - 0.80; their cross-breed - 0,14; Original Brown Cattle (OBV) - 1.00. The proportion of A1A1 genotype carriers is 0.20; 0; 0.29; 0 respectively. The frequency of the heterozygous A1A2 genotype carriers was 0.60; 0.20; 0.57; 0 respectively. The frequency of A1 allele carriers in the animals tested is 0.50; 0.10; 0.80; 0; and of the desired A2 is 0.50; 0.90; 0.20; 1.00 respectively (**Table II**).

The results obtained almost coincide with those obtained by Kaminski et al. (2017) and Kučerova et al. (2006). According to their results, the frequency of the A1 allele in the sires of Swiss breed is in the range of 0.09-0.15. The frequency of the desired allele A2 in the sires ranges from 0.72 to 0.77.

Thus, in order to preserve Lebedyn breed and for further formation of the herd A2, it is scientifically sound to choose for the selection purposes the following sires: Zorkyj 9902 of Lebedyn breed; Gotor 8011946865, Abel 593920645, Foniks 536673172, Harison 666623864 of European brown cattle (BV); Final 1008, a cross-breed of mentioned above; Nimrod 814720783, Dzhuleks 814660509, Urano 110027139002 of Original Brown Cattle. In the work with the Original Brown Cattle, it is desirable to use the scheme developed by us (Ladyka et al., 2019).

Table I. **Characteristics of sires by beta-casein genotype** (Características de los sementales por genotipo de beta-caseína).

Nickname and sire's number	Breed	Genotype
Bujnyj 102	L	A1A2
Dykyj 7933	L	A1A1
Karyj 12273	L	A1A2
Zorkyj 9902	L	A2A2
Kachur 5296	L	A1A2
Rogiz 5002	L*BV	A1A2
Final 1008	L*BV	A2A2
Zalp 17505	L*BV	A1A1
Parom 2075	L*BV	A1A1
Zajchyk 17000	L*BV	A1A2
Murat 79	L*BV	A1A1
Chystyj1 7035	L*BV	A1A2
Gotor 8011946865	BV	A2A2
Drago 758976242	BV	A1A2
Abel 593920645	BV	A2A2
Foniks 536673172	BV	A2A2
Harison 666623864	BV	A2A2
Nimrod 814720783	OBV	A2A2
Dzhuleks 814660509	OBV	A2A2
Urano 110027139002	OBV	A2A2

Note: L – Lebedyn breed; BV – European Brown Cattle; OBV – Original Brown Cattle.

Table II. **Frequency of distribution of genotypes and alleles of brown breeds bull-sires by β-casein gene** (Frecuencia de distribución de genotipos y alelos de razas marrones de machos de toro por β-caseína por el gen de la caseína β).

Genotype / allele	Frequency of distribution of β-casein genotypes and alleles in an animal group			
	L	BV	L*BV	OBV
A_1A_1	0,20	-	0,29	-
A_2A_2	0,20	0,80	0,14	1,00
A_1A_2	0,60	0,20	0,57	-
A_1	0,50	0,10	0,80	-
A_2	0,50	0,90	0,20	1,00

Note: L – Lebedyn breed; BV – European Brown Cattle; OBV – Original Brown Cattle.

The next stage of our research was the analysis of animals of the basic breeding herd in relation to β-casein polymorphism (**Table III**).

It was determined that the desired A2A2 genotypes by beta-casein gene in cows and heifers of Lebedyn breed were 63%, and A1A2 heterozygotes - 37%. The frequency of distribution of alleles in the sample is respectively A1– 0.184 and A2 - 0.816

Of the 49 females evaluated, 41 had a male parent evaluated by β-casein genotype. According to the results of our studies, it was found that the parental identity of the animals determines the relationship between the allelic variant of β-casein in the milk of the offspring (**Table IV**).

Table III. **Frequency of distribution of genotypes and alleles of cows and heifers of Lebedyn breed by β-casein gene** (Frecuencia de distribución de genotipos y alelos de vacas y novillas de raza Lebedyn por β-caseína).

Number of animals tested	Genotype						Alleles	
	A_1A_1		A_1A_2		A_2A_2		A_1	A_2
	n	%	n	%	n	%		
49	-	-	18	37	31	63	0,184	0,816

Accordingly, in homozygous sires A2A2, 72% of daughters had a similar genotype and in heterozygous sires A1A2 – 67% (**Table V**).

Based on the presence of daughters of the studied bulls, the frequency of distribution of genotypes by β-casein and possible combinations when crossing animals of different genotypes (**Table VI**), it can be concluded that the breeding livestock of the basic farm is represented by 75% of animals with genotypes A1A2 and A2A2 by β-casein.

The identification of dairy cattle productivity by the locus of β-casein gene should be performed to address a number of breeding issues and, above all, the creation of A2 milk herds. The number of animals with the desired homozygous A2A2 and heterozygous A1A2 genotype can be increased by selecting parent pairs. Full penetrance, i.e. 100% frequency and probability of phenotypic gene expression can be achieved if:

♂ ♀

A2A2 x A2A2 = 100% penetrance;

A2A2 x A1A2 = 50-70% penetrance;

A1A2x A2A2 = 50-70% penetrance;

A2A2 x A1A1 = 50-70% penetrance;

A1A2 x A1A2 = 25-50% penetrance;

A1A1 x A1A2= 25-50% penetrance;

A1A2 x A1A1= 25-50% penetrance.

An individual approach to the issues of matching and selection, control of the processes of transfer of genes from parents to posterity, by means of DNA-diagnostic methods, will allow to replenish the herds with A2 gene carriers in a relatively short period of time.

To obtain A2 milk, cows must have the A2A2 genotype. Assessment of the genotype of animals is currently available and is carried out by PCR method in appropriate laboratories (for example, in the Laboratory of Sumy National Agrarian University).

Crossing of two animals in both of which the A2A2 genotype yields 100% offspring of A2A2, as in the case of two A1A1 carriers: 100% of their offspring will have the A1A1 genotype. Since one or another variant of β-casein depends solely on genetics, there is no quick fix. Breeding cattle that gives only A2 milk takes time. With an active approach, only offspring from cows identified as A2A2 according to the results of the research could be allowed to reproduce. The passive approach could only mean the selection of bulls-sires with the A2A2 genotype. In the second approach, the frequency of β-casein A1 in milk would decline twice in each generation, i.e. every 5 years (Kovalyuk et al., 2019).

Thus, on the basis of the scheme of distribution of the desired alleles A2 (**Table VI**), for each herd it is necessary to develop its own scheme of selection of parental pairs to achieve the desired homozygous combination of alleles by β-casein gene among the offspring.

We have proposed a method of creating a population of dairy cattle with the desired genotype by the gene β-casein, which is represented by the scheme of crossing:

A1A1 (female parent) + A2A2 (male parent). In the first generation, according to the genetic scheme, we will receive 100% of individuals with heterozygous genotype AB. In the second generation, the next crossing pattern should be applied.

A1A2 + A2A2 = 2 A1A2 (50%) + 2 A2A2 (50%). That is, already half of the offspring will have the desired A2A2 genotype. The remaining heterozygous offspring with the A1A2 genotype should also follow the crossing pattern A1A2 + A2A2 = 2 A1A2 (50%) + 2 A2A2 (50%).

Already in the second generation, there will be significant dominance of individuals with the desired A2A2 genotype by β-casein gene - 87.5% of the total population. In subsequent generations, the proportion of the desired genotypes will increase (**Table VII**).

Although updating the herd is a rather slow process, we recommend that farms who wish to obtain as many heifers with the desired genotype by the test trait as possible introduce continuous insemination of heifers with sexed sperm of A2A2 β-casein genotype. Through these measures, farms can predict with high accuracy the production of heifers with the desired genotype, thereby increasing the proportion of A2A2 animals. At the same time, the use of sexed sperm of the bulls-sires of desirable genotypes creates an opportunity to increase livestock independently by repairing the herd without importing cows and heifers.

A powerful means of accomplishing the task of creating A2A2 herds is the embryo transplantation method, which will ensure the rapid increase in the number of animals of the required genetic characteristics.

Table IV. The dependence of the genotype of cows and heifers of Lebedyn breed on the inheritance of the father on β-casein gene (La dependencia del genotipo de vacas y novillas de Lebedyn se reproducen en la herencia del padre en el gen de la β-caseína).

Nickname and sire's number	Sire's genotype	Number of daughters evaluated	Daughters genotypes		
			A1A1	A1A2	A2A2
Goldmin 198015	A2A2	4	-	2	2
Protezhe 68159838	A2A2	5	-	-	5
Gotor 8011946865	A2A2	5	-	1	4
Harison 666623864	A2A2	4	-	2	2
Altasidi 198358	A1A2	5	-	1	4
Bush 68129315	A1A2	5	-	2	3
Sesdeblum 68144448	A1A2	2	-	1	1
Lester 9695540	A2B	1	-	1	-

Table V. The ratio of the number of different genotypes bulls and their daughters (La proporción del número de toros genotipos diferentes y sus hijas).

Number of bulls/genotypes	total	Daughters					
		A1A1		A1A2		A2A2	
		n	%	n	%	n	%
4/A2A2	18	-	-	5	28	13	72
3/A1A2	12	-	-	4	33	8	67
1/ A2B	1	-	-	1	100	-	-

CONCLUSIONS

1. The genetic structure of (L) Lebedyn breed bulls (80% of heterozygotes on A2), which is available, allows to form homozygous by the given trait populations in the following generations.

2. Bringing the bulls of the Original Brown Cattle (OBV) to the reciprocal crossing of 100% A2A2 gives a chance to accelerate the growth of homozygous bulls and heifers for breeding.

3. The breeding stock of Lebedyn breed (62% of homozygotes and 38% of heterozygotes) predicts a significant increase in A2A2 beta-casein homozygosity in the next generation, especially when using homozygous bulls.

4. Breeding model (Tables 6, 7), in combination with the use of sexed sperm and heifer genotyping, has the highest breeding acceleration of the A2A2 beta-casein homozygous population.

Table VI. **Possible combinations when crossing animals of different genotypes by β-casein** (Posibles combinaciones al cruzar animales de diferentes genotipos por β-caseína).

		Parent 1				Parent 1				Parent 1				Parent 1	
		A1	A2			A1	A2			A1	A2			A2	A2
Parent 2	A1	A1A1	A1A2	Parent 2	A2	A1A2	A2A2	Parent 2	A1	A1A1	A1A2	Parent 2	A1	A1A2	A1A2
	A1	A1A1	A1A2		A2	A1A2	A2A2		A2	A1A2	A2A2		A1	A1A2	A1A2
		50% A1A1 50% A1A2				50% A1A2 50% A2A2				25% A1A1 50% A1A2 25% A2A2				100% A1A2	

Table VII. **Methods of creating a population of dairy cattle with the desired genotype by β-casein gene** (Métodos para crear una población de ganado lechero con el genotipo deseado por β gen caseína).

Initial parental forms	Offspring genotype	Generation
A_1A_2 + A_2A_2	2 A_1A_2 (50%) + 2 A_2A_2 (50%)	I
A_1A_2 + A_2A_2	2 A_1A_2 (12,5%) + 2 A_2A_2 (87,5%)	II
A_1A_2 + A_2A_2	2 A_1A_2 (6,25%) + 2 A_2A_2 (93,75%)	III
A_1A_2 + A_2A_2	2 A_1A_2 (3,125%) + 2 A_2A_2 (96,175%)	IV

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