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Original article

USE OF BIOTECHNOLOGICAL METHODS FOR ACCELERATED IMPROVEMENT OF KALMYK CATTLE

N.V. Chimidova, A.V. Ubushieva, Z.V. Bochkaeva, A.I. Khakhlinov, V.S. Ubushieva

Abstract

Background. The reduction in the number of some domestic breeds raises concerns among livestock experts. One of the reasons for the reduction in the number of livestock is the lack of purebred bulls, which are able to preserve the breed. Kalmyk breed of cattle is the only ancient aboriginal domestic breed of meat direction. It is kept on year-round pasture keeping, thus on productivity indicators it is not inferior to foreign breeds of meat direction. In this connection preservation and improvement of the gene pool of this breed is important. Kalmyk cattle breed is one of the few indigenous Russian breeds that still possesses unique genetics.

Currently, the breed needs to be restored and protected. In order to increase the number and improve the breed qualities of Kalmyk animals, selection is necessary, which should be based on reliable information about the origin of animals and identification of candidate genes marking productive and reproductive qualities. Restoration and improvement of breed qualities of Kalmyk cattle is possible only under the condition of accelerated reproduction of purebred genotyped stock with the use of biotechnological methods in reproduction. The analysis of complex evaluation of animals showed the variability of live weight of bulls-producers and cows in different periods of growth, from 20, 22 kg at birth to 320, 380 kg at 15 months. Evaluation of the genetic structure of the Kalmyk population for meat productivity genes CAPN1, TG5, GH, revealed a high level of homozygous individuals with desirable genotypes, more than 40%. The average level of heterozygous individuals is 30%. The results obtained on the genetic structure of Kalmyk cattle in the Republic of Kalmykia demonstrate a moderate level of genetic diversity and suggest the possibility of restoring a

"pure" gene pool. The selected schemes of hormonal polyovulation for the arid breed revealed a high percentage of fertilization (81%) and 92.3% of donor cows responding with superovulation. On average, 8.8% of embryos were obtained per donor, and 5.5% of them were suitable. Thus, the results of research work on the use of biotechnological methods in reproduction of indigenous breeds confirm the fact of successful approbation and further utilization of genetic potential of highly valuable animals.

Purpose. The aim of the research is to study the way of using biotechnological methods for accelerated improvement of Kalmyk cattle breed.

Materials and methods. As part of the research work conducted at Kalmyk University on the use of biotechnological methods in indigenous animal husbandry, the Kalmyk breed of cattle bred in breeding farms of the Republic of Kalmykia served as an object of research. Animals were annually evaluated for purebredity in a complex way. We have processed and analyzed the data on animals: primary accounting on productivity indicators, genealogical affiliation (data of 33,135 heads, year of birth 2014-2023 were studied). When analyzing the complex evaluation, we relied on the methodology of evaluating the breeding value of beef cattle, approved by the Eurasian Economic Commission, for further implementation of the evaluation of the breeding value of the Kalmyk breed.

Results. Identification of candidate genes associated with qualitative and quantitative indicators of meat were investigated in breeding farms of the Republic of Kalmykia. Population analysis was carried out in steers of Kalmykian breed at the age of 8 months. 1626 heads were studied and live weight of young animals was evaluated. The data on genotypes associated with live weight are shown in the diagram.

Conclusion. Experimental studies conducted on the experimental herd on the use of biotechnological methods in the reproduction of indigenous breeds have shown the fundamental possibility of using reproductive biotechnology in the reproduction of Kalmyk meat breed. During the research work more than 150 pieced with Kalmyk cattle embryos were received and frozen.

Keywords: indigenous breed; variants of selective breeding; domestic livestock breeding; biotechnology in purebred breeding

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Научная статья

ИСПОЛЬЗОВАНИЕ БИОТЕХНОЛОГИЧЕСКИХ МЕТОДОВ ДЛЯ УСКОРЕННОГО СОВЕРШЕНСТВОВАНИЯ КРУПНОГО РОГАТОГО СКОТА КАЛМЫЦКОЙ ПОРОДЫ

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Аннотация

Обоснование. Сокращение численности некоторых отечественных пород вызывает опасения у экспертов-животноводов. Одной из причин сокращения численности поголовья является нехватка чистокровных быков-производителей, которые способны сохранять породу. Калмыцкая порода крупного рогатого скота – это единственная древнейшая аборигенная отечественная порода мясного направления. Содержится на круглогодичном пастбищном содержании, при этом по показателям продуктивности не уступает зарубежным породам мясного направления. В связи с этим сохранение и улучшение генофонда данной породы важно. Калмыцкая порода КРС одна из немногих коренных российских пород, до сих пор обладающая уникальной генетикой. В настоящее время порода нуждается в восстановлении и защите. Для увеличения численности и совершенствования породных качеств калмыцких животных необходима селекция, которая должна опираться на достоверную информацию о происхождении животных и выявлении кандидатных генов, маркирующих продуктивные и воспроизводственные качества. Восстановление и улучшение породных качеств калмыцкого скота возможно лишь при условии ускоренного размножения чистопородного генотипированного поголовья с использованием биотехнологических методов в воспроизводстве. Анализ комплексной оценки животных показал вариабельность живой массы быков-производителей и коров в разные периоды роста, от 20, 22 кг при рождении до 320, 380 кг в 15 месяцев. Оценка генетической структуры калмыцкой популяции по генам мясной продуктивности CAPN1, TG5, GH, выявила высокий уровень гомозиготных особей с желательными генотипами, более 40%. Средний уровень имеют гетерозиготные особи - 30%. Полученные результаты о генетической структуре калмыцкого скота в Республике Калмыкия демонстрируют умеренный уровень генетического

разнообразия и предлагают возможность восстановления «чистого» генофонда. Подобранные схемы гормональной полиовуляции для аридной породы выявили высокий процент оплодотворяемости (81%) и получения 92,3% среагировавших суперовуляцией коров-доноров. В среднем на одного донора получено 8,8% зародышей, пригодные из них оказалось 5,5%. Таким образом, результаты НИР об использовании биотехнологических методов в воспроизводстве аборигенной породы подтверждают факт успешной апробации и дальнейшего использования генетического потенциала высокоценных животных.

Цель. Цель исследования изучить способ использования биотехнологических методов для ускоренного совершенствования крупного рогатого скота калмыцкой породы.

Материалы и методы. В рамках научно-исследовательской работы, проведенной в Калмыцком университете, по использованию биотехнологических методов в аборигенном животноводстве, объектом исследований служила калмыцкая порода КРС, разводимая в племенных хозяйствах Республики Калмыкия. Животные ежегодно комплексно оценивались на чистопородность. Нами были обработаны и проанализированы данные о животных: первичный учёт по показателям продуктивности, генеалогическая принадлежность (исследовано данных 33135 голов, год рождения 2014-2023 гг.). При анализе комплексной оценки опирались на методику оценивания племенной ценности мясного скота, утвержденной Евразийской Экономической Комиссией, для дальнейшего внедрения оценки племенной ценности калмыцкой породы.

Результаты. Определение кандидатных генов, связанные с качественными и количественными показателями мяса, были исследованы в племенных хозяйствах Республики Калмыкия. Популяционный анализ проводился у бычков калмыцкой породы в возрасте 8 месяцев. Было исследовано 1626 голов, а также проведена оценка живой массы молодняка. Данные о генотипах, ассоциированных с живой массой, отображены в диаграмме.

Заключение. Экспериментальные исследования, проведенные на опытном стаде по использованию биотехнологических методов в воспроизводстве аборигенной породы, показали принципиальную возможность применения репродуктивных биотехнологий в воспроизводстве калмыцкой мясной породы. За время проведения научно-исследовательской работы было получено и заморожено более 150 пайет с эмбрионами калмыцкого скота.

Ключевые слова: аборигенная порода; варианты селекционного разведения; отечественное животноводство; биотехнология в чистопородном разведении

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методов для ускоренного совершенствования крупного рогатого скота калмыцкой породы. *Siberian Journal of Life Sciences and Agriculture*, *17*(6-2), 475-493. https://doi.org/10.12731/2658-6649-2025-17-6-2-1570

Introduction

The development of specialized beef cattle breeding in the Russian Federation is necessary for the stability and independence of the national market, while it is important to stimulate the production of high-quality and competitive products [1; 2].

Candidate genes of meat productivity in cattle (cattle) play an important role – they determine the potential of animals to fat deposition and meat quality [3; 4]. Research in this area is actively continuing on indigenous breeds, including domestic breeds [5; 6; 7].

Research in the genetics of meat productivity continues to this day, the search for new candidate genes can be revealed as genomic technologies like genomic sequencing and genome association studies are developed [8; 9; 10].

One of the modern reproductive technologies in beef cattle breeding today is embryonic transplantation, which can significantly improve the genetic characteristics of the herd [11; 12; 13].

Specialized beef cattle breeding requires breeds to comply with the following parameters: 1) maternal instinct and pasture instinct, 2) energy cost savings achieved by eliminating and reducing many energy-intensive technological processes, 3) use of cheaper voluminous fodder [14]. Kalmyk breed meets these requirements [14; 15]. Thus, the development of specialized meat cattle breeding based on the Kalmyk breed allows to solve the problem of high-quality beef production and gives the opportunity to provide the meat processing industry with domestic raw materials, thereby increasing the food security of the country [16; 17]. However, despite the unique biological and economically useful qualities of the Kalmyk breed, the current state causes concerns [18]. According to Federal State Budgetary Scientific Institution All-Russian Research Institute of Breeding Business (2024), a significant decrease in the number of animals is observed in the cattle of the oldest domestic Kalmyk breed. Thus, in 2024 the specific weight of this population in the total balance of all meat breeds of the country stopped at the boundary of 33.5% with the number of cattle of 86990 heads, while in the initial year 2010 this indicator was – 49.3% with the number of animals in the amount of 131153 heads [19]. As the data of the International Union for Conservation of Nature show, almost one third of native breeds in Russia have disappeared in recent decades, and about 20% have

critical status [20]. Aboriginal domestic breed of meat direction of productivity has truly unique qualities, genetics, and therefore needs to be restored and protected [21; 22; 23]. In connection with the above, the scientists of Kalmyk State University conducted research on accelerated reproduction of the gene pool Kalmyk breed using embryo transplantation biotechnology. In our research we developed methods for superstimulation of polyovulation in the indigenous Kalmyk breed. Eggs of donor cows were fertilized with sperm of highly productive bulls, using artificial insemination, which is also considered as a kind of breakthrough in reproduction of indigenous breeds, which are characterized by their "wildness". These studies serve as an example in increasing the genetic progress of the Kalmyk population. The possibilities of using genetically valuable bulls that may not be available for natural insemination due to pronounced breed characteristics are shown.

Materials and methods

As part of the research work conducted at Kalmyk University on the use of biotechnological methods in indigenous animal husbandry, the Kalmyk breed of cattle bred in breeding farms of the Republic of Kalmykia served as an object of research. Animals were annually evaluated for purebredity in a complex way. We have processed and analyzed the data on animals: primary accounting on productivity indicators, genealogical affiliation (data of 33,135 heads, year of birth 2014-2023 were studied). When analyzing the complex evaluation, we relied on the methodology of evaluating the breeding value of beef cattle, approved by the Eurasian Economic Commission, for further implementation of the evaluation of the breeding value of the Kalmyk breed.

Polymorphism of beef productivity genes CAPN1, TG5, GH was determined by molecular genetic analysis in the genomics laboratory of Kalmyk University according to the defined Scheme.

To determine polymorphisms of candidate genes CAPN1, TG5, and GH, polymerase chain reaction was performed using oligonucleotide primers. Detection was performed in agarose gel.

Commercial reagent kits were used for DNA isolation from biological material of animals (blood): MagnoPrime VET in the amount of 100µl (Fig. 1).

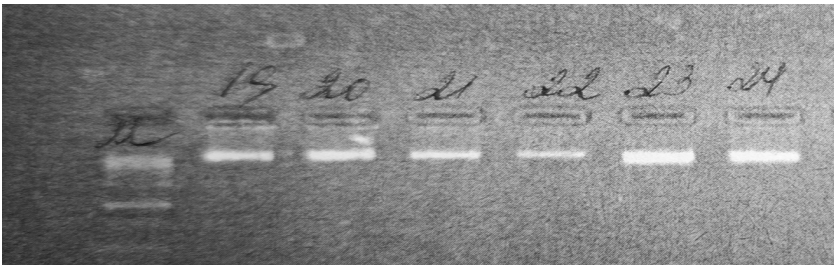

Fig. 1. Example of determination of DNA isolation from blood of Kalmyk animals using MagnoPrime VET reagent kit

The nucleotide sequences were determined using the Sanger method. Before sequencing, PCR products were pre-purified from the reaction mixture using a commercial kit by spin-column method.

Sequencing was performed using capillary electrophoresis of a Nanofor 05 genetic analyzer. Mutation Surveyor software (USA) was used to decipher capillary electrophoresis data.

The methodological basis of the study is based on generally accepted methods of systematization and analysis.

Official materials of yearbooks on breeding work in beef cattle breeding (editions of All-Russian Research Institute of Breeding Business), Federal State Statistics Service of the Russian Federation and analytical reviews of NRA (National Rating Agency) served as the information base.

During experimental researches scientists constantly increased the welfare of experimental animals, observed the norms of their protection in accordance with modern scientifically – substantiated requirements. Maintenance, feeding and veterinary care of animals, scientific purposes were carried out in accordance with the Recommendations of the Board of the Eurasian Economic Commission from November 14, 2023 N 33.

Experiment scheme

Studies on practical application of modern biotechnological methods in Kalmyk cattle were conducted in the period 2022-2024. The animals belonged to breeding farms of the republic: "Budda", "Plodovitoe", "Khoshud", "Kirovsky", "Agrofirma Uralan". Ten bulls and 50 breeding stock from each farm were selected by sampling. Kalmyk animals were selected by year of birth, belonging to the same breed and phenotype expression (best meat forms). After that, a donor herd was formed on the basis of the Regional Research and Production Center for Reproduction of Farm Animals of the Federal State Budgetary Edu-

cational Institution of Higher Education "Kalmyk State University named after B.B. Gorodovikov".

Results

The composite score on the average live weight of breeding bulls at different periods of growth and development (at birth, 205 days, 12 and 15 months) was 22.1, (SD – 5.3; Cv – 24.3), 205,3, (SD – 16.3; Cv – 6.7); 320,0 (SD – 24.2; Cv – 6.2); and 379.0 kg (SD – 26.0; Cv – 5.3) (Fig. 2).

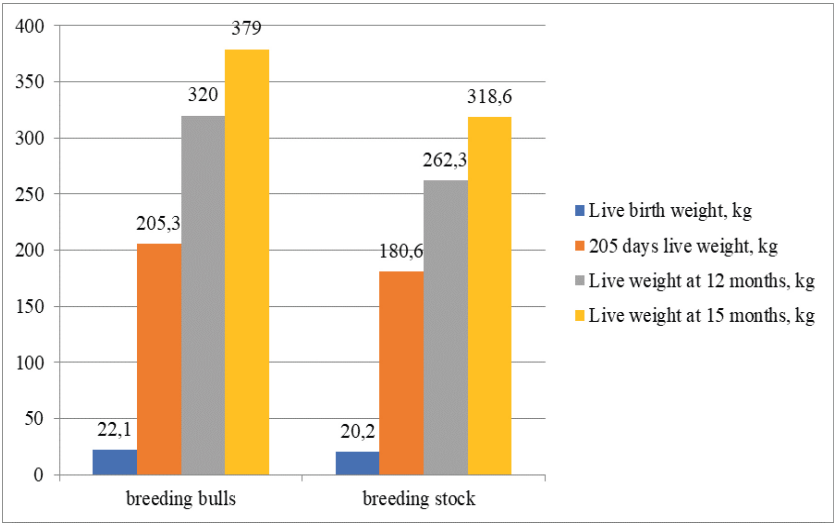

Fig. 2. Monitoring of animal live weight, kg

Results of tracking live weight of cows at different periods of growth and development – 20.2, (SD – 1.6; Cv – 8.0), 180.6, (SD – 14.0; Cv – 7.7), 262.3, (SD – 16.0; Cv – 6.0), 318.6 kg (SD – 15.4; Cv – 4.8) accordinglyThe classiness of the producers is rated as elite and elite-record.

For the first time the population features of the breed bred on the territory of the republic were studied. Identification of candidate genes associated with qualitative and quantitative indicators of meat were investigated in breeding farms of the Republic of Kalmykia. Population analysis was carried out in steers of Kalmykian breed at the age of 8 months. 1626 heads were studied and live weight of young animals was evaluated. The data on genotypes associated with live weight are shown in the diagram (Fig. 3).

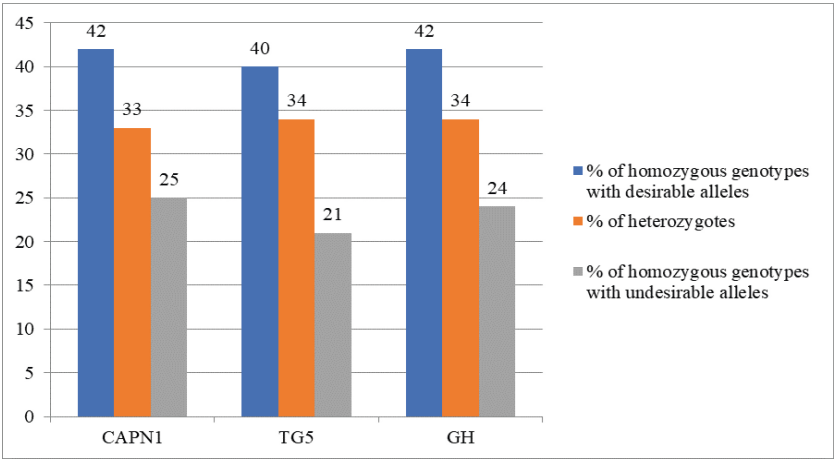

Fig. 3. Monitoring of steer genotypes, %

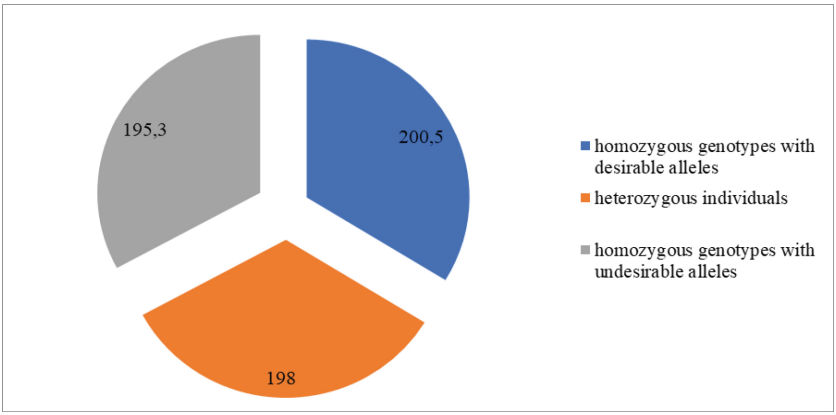

Fig. 4. Differences in live weight of young animals of different genotypes, kg

Evaluation of the genetic structure of the Kalmyk population for the candidate genes of meat productivity CAPN1, TG5, GH, revealed a high level of homozygous individuals with desirable genotypes, more than 40% of them. The average level has heterozygous individuals – more than 30%. The results obtained on the genetic structure of the Kalmyk cattle population in the Republic of Kalmykia demonstrate a moderate level of genetic diversity and suggest the possibility of restoring a “pure” gene pool.

A comparative analysis of the live weight of young Kalmyk cattle by different genotypes was carried out. The data is shown in the diagram (Fig. 4).

At 8 months of age, steers with homozygous genotype carrying desirable alleles outperformed their peers - heterozygotes and homozygotes with undesirable alleles in their genotypes – by 2.5 kg and 5.2 kg, respectively. This study can serve as a confirmation of the influence of the studied genes on the meat productivity of Kalmyk cattle.

The analysis of the obtained results on genotypes and their association with live weight indicates the possibility of improving the breed based on the use of genetic markers in the selection of animals. Kalmyk breed, being the only domestic aboriginal breed of meat direction of productivity, is of great value for breeders. Animals with genotypes CC, TT and VV have high live weight. Consequently, animals with specified genetic complexes should be used to a greater extent in selection and targeted breeding.

Within the framework of research work conducted by scientists from Kalmykia, a scientific experiment on the use of biotechnological methods in the reproduction of indigenous livestock and its approbation was conducted. The Kalmyk breed served as an example in the experiments. From the number of progenotyped animals 10 pedigree purebred cows and 4 bulls – producers of Kalmyk breed were selected as embryo donors according to the indicators of meat productivity taking into account the origin. The animals formed the core of the donor “elite” herd on the basis of the University.

As it is known, in the technology of embryo production the problem of obtaining stable results of polyovulation is especially acute. The existing generally accepted scheme of polyovulation stimulation for indigenous breeds is labor-intensive and often impossible. This problem was solved by the developed scheme of prolongation of follicle-stimulating hormone (FSH) action with the help of polyvinyl alcohol. According to the stimulation scheme, the number of injections was reduced to 3 instead of the generally accepted 8.

In the experiment on implementation of embryo transplantation biotechnology methods in application to Kalmyk breed, 3 donor cows with established sexual cycle were used. Injections were administered subcutaneously in the area of the scapula on days 9, 10 and 11 of the sexual cycle, observing an interval of 24 hours. Total FSH was injected in a total dose of 50 units. On the 11th day of the cycle, prostaglandin “estrophan” was injected intramuscularly in a dose of 500 mcg.

Artificial insemination of donor cows was performed with freshly obtained chilled semen, 3 cows were inseminated twice, the interval was 60 hours and 72 hours after estrophan administration.

Polyovulation was established by rectal examination in all 3 donors, which allowed for embryo retrieval.

The procedure was performed in a standardized manner – a non-surgical method was used. A rubber Folley catheter with an air bubble-fixer was used in uterine horn washing. Dulbecco's medium consisted of gentamicin (300 ml volume) and bovine serum albumin (300 ml volume).

A total of 23 embryos and oocytes were obtained from donor cows. By evaluating the quality, 10 excellent and good embryos were identified (Fig. 5). Thus, the yield of quality embryos of Kalmykian breed was 3.3 on average per donor, summarizing, a positive result was obtained, which indicates the possibility of using biotechnological methods of reproduction of Kalmykian breed of cattle.

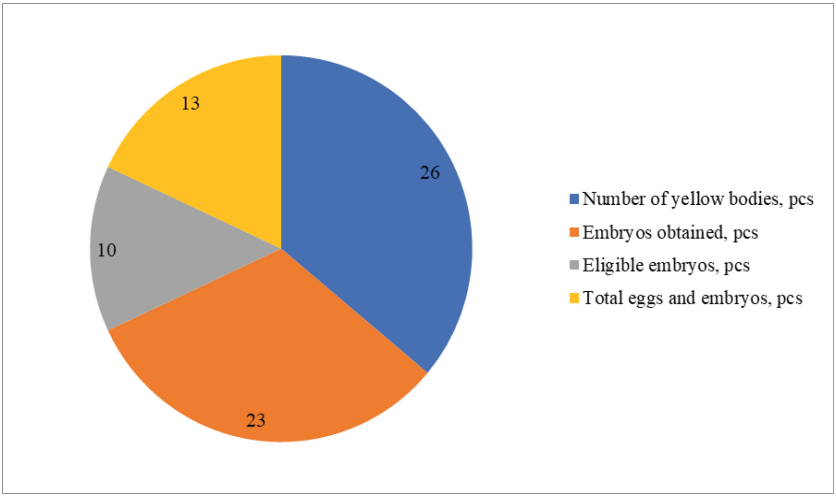

Fig. 5. Results on the use of biotechnological methods 1 stage

In this experiment, excellent and good embryos in the morula-blastocyst stage of development were cryopreserved for later transplantation.

The second stage of research work on the introduction of biotechnological methods of reproduction in the Kalmyk breed was the development of polyovulation induction. The improved scheme of hormonal treatment consisted in a single injection of a prolonged form of FSH-p in a dose of 50 units of Armorov standard with an additional injection of 500 IU of follimag.

According to the developed scheme, hormonal polyovulation was carried out, which was induced 13 times in 10 donor cows of the donor herd. Donor cows

were stimulated with prostaglandin F2α "Estrophan" and then inseminated with fresh chilled semen of breeding bulls – producers carrying desirable alleles in their genotypes. The results of stimulation of donor cows are presented in Fig. 6.

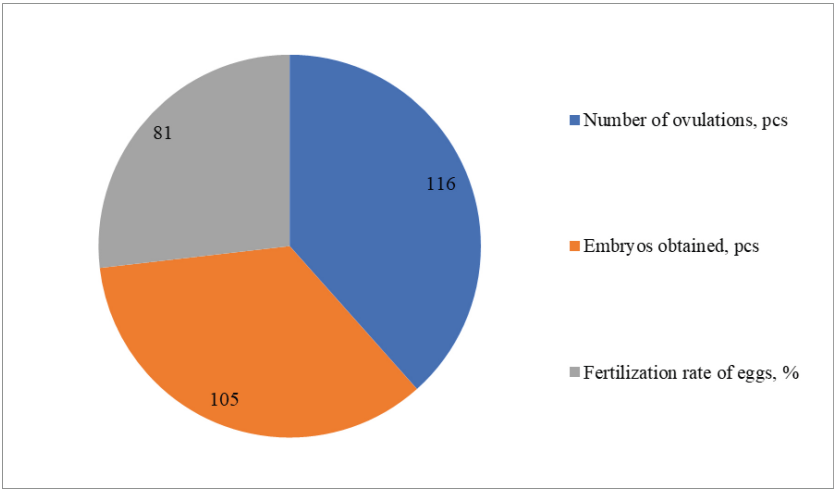

Fig. 6. Results on the use of biotechnological methods Stage 2

The experiment on approbation of the new prolonged FSH action scheme proved to be effective and the yield of quality embryos was significantly increased.

Conclusion

The search for marker genes associated with qualitative and quantitative production indicators in Kalmyk cattle revealed different frequencies of genotypes. The frequency of animals with desirable alleles is relatively high, more than 40% in the study population. There is a high rate of heterozygous individuals, their frequency was equal to 30%. These results can speak about the probability of obtaining desirable genotypes under the condition of selection of animals with given complexes. Monitoring of live weight of steers of different genotypes, determined differences in the results obtained, the difference was 2.5 kg and 5.2 kg. These studies confirm that CAPN1, TG5, GH genes are associated with meat productivity in Kalmyk cattle breed.

Experimental studies conducted on the experimental herd on the use of biotechnological methods in the reproduction of indigenous breeds have shown the

fundamental possibility of using reproductive biotechnology in the reproduction of Kalmyk meat breed. During the research work more than 150 pieced with Kalmyk cattle embryos were received and frozen.

Thus, the results of research work on introduction of biotechnological methods in reproduction of indigenous breed, confirm the fact of successful utilization of genetic potential of highly valuable animals. Two variants of selective breeding of the domestic meat breed – Kalmyk cattle, considered and tested by us, proved to be effective in increasing the productivity of cattle and accelerating its reproduction.

Conflict of interest information. The authors declare that they have no conflict of interest.

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