Application of modern trends and models in rabbit breeding: A review

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Abstract

The domestic rabbit is a valuable species because of the qualities of its meat. It is also used for the production of wool and fur. In Bulgaria, the rabbit is mainly raised for the production of rabbit meat. Breeding of rabbits in intensive and semi-intensive farms is carried out mainly through hormonal stimulation and artificial insemination. In recent years, in rabbit breeding has been used modern molecular methods including various analyzes at the DNA level – microsatellite markers, RFLP, QTL etc. In Bulgaria, there are very few studies associated with the genome of rabbits and the phenotype expression of genes. The experimental work at the molecule level and the application of new techniques contributes to the development and fast progress in this direction, and this has a significant advantage in creating different ways of working in this field.

Key words: rabbit breeding, reproduction, rabbit genome, modern trends

Приложение на съвременни тенденции и модели в зайцевъдството: Обзор

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Резюме

Домашният заек е ценен вид, за което допринасят качествата на неговото месо. Използва се също за производство на кожа и козина. В България заекът се отглежда главно за производство на заешко месо. Развъждането на зайци в интензивни и полуинтензивни ферми се извършва главно чрез хормонална стимулация и изкуствено осеменяване. През последните години в развъждането на зайци се използват съвременни молекулярни методи, включващи различни анализи на ниво ДНК – микросателитни маркери, RFLP, QTL и др. В България има много малко изследвания, свързани с генома на зайците и фенотипната експресия на гените. Експерименталната работа на молекулно ниво и прилагането на нови техники допринася за развитието и бързия напредък в тази посока и това има значително предимство при изследванията в тази област.

Ключови думи: зайцевъдство, размножаване, геном на зайците, съвременни тенденции

Introduction

The domestic rabbit (*Oryctolagus cuniculus*) is known for its short gestational period (29–31 days), high fertility rate (a large number of offspring), and the ability to recover quickly after birth which are the most important economic traits for animal breeding in general. For example, a rabbit doe can be inseminated several times per year and have several generations from different bucks. Sexual maturity begins early – between the fourth and eighth month from birth. In one year, a sexually mature rabbit doe can deliver between 4 to 7 times (Esteves et al., 2018).

The rabbit is an animal that is bred for food industry due to the high quality of its diet meat, which is considered a delicacy, as also for rabbit wool and fur. It is also an experimental animal bred (*in vivo* and *in vitro*), as there are contributions to the medical field – in immunology, the development of vaccines, genetics, in the study of development and prevention from infectious diseases. The rabbit suffers from some inherited human diseases (aortic arteriosclerosis, cataracts, hypertension, hypertrophic cardiomyopathy, epilepsy, spina bifida, osteoporosis), therefore it makes it a useful object for research related to medicine and other scientific tendencies (Carneiro et al., 2011; Peng et al., 2015).

In Bulgaria, the rabbit is bred mainly for its meat. Although the sector is not well-developed, rabbit meat production is essential due to its diet properties, such as low fat and high protein content (Sotirov et al., 2009).

Artificial insemination is used in the breeding of rabbits, which is widespread in Italy, France and Germany. It saves time by drastically improving the breeding process (Dimitrova et al., 2008).

According to data from the Ministry of Food and Agriculture in Bulgaria till 2019 the number of rabbits raised in the country was about 115 thousand individuals. The sector is not strongly represented, but is growing fast, establishing new markets for rabbit production and farm development and also maintaining the current ones. (https://www.mzh.government.bg/media/filer_public/2020/12/03/agd_2020_web.pdf).

Characteristics of rabbit breeding

In rabbit breeding, studies have been made on frequency of ovulation and uterine volume, with techniques such as laparoscopy, CT and X-ray findings. With the selection of reproductive traits in rabbits, three main directions were created: fertility and lactation improvement, growth rate and qualities of carcass and meat (by paternal line), amelioration and general improvement of growth qualities and number of the litter size (multifunctional lines) (Khalil et al., 2008). The capacity of the uterus is genetically linked with a number of the newborn offspring. The most common criteria used in maternal selection programs are related to litter size at birth or at weaning (Khalil et al., 2008). Breeding for the number of kits in rabbits is associated with an increase in the frequency of ovulation with minor changes in prenatal survival. Average daily gain is the most important trait in the period after weaning, which implies the need of individual breeding. Statistical models such as BLUP (best linear unbiased prediction) are also used (Sánchez et al., 2008; Youssef et al., 2008).

There is lots of research focused on the morphology and physiology of the rabbit, as well as its genetics. For example, the study of the *KIT* gene responsible for the so-called English spotting coloration in Checkered Giant Rabbits, which is also associated with a condition known as Mega cecum and Congenital Megacolon, is associated with the same locus – The English spotting locus (Fontanesi, L. et al., 2014). Mutations in tyrosinase (TYR), melanocortin 1 receptor (MC1R), agouti signal protein (ASIP), melanophilin (MLPH) and tyrosinase-linked protein 1 (TYRP1) genes are associated with different coat color in rabbits (Fontanesi, L. et al., 2014).

In such studies, it is important to use a control population, together with the selected population, in which selection is excluded. Also, by applying statistical methods, the development of a genetic line in the offspring can be tracked. The use of statistical methodologies to estimate the weight gain of selected individuals is also an important part of the experimental work. For example, the Gompretz curve for weight-age correlation can be applied (Maj et al., 2009). The formula used is:

$$y(t) = Ae^{-Be^{-Kt}}$$

where: *y* is the weight in grams, (t) is the age in days, A is the average weight of the rabbit in adulthood, K is the growth rate, and B is the scaling parameter (integration constant, related to the initial weight), e – this is a natural logarithm with value (e = 2.71828...) Maj et al., therefore examine the standard deviation, taking into account parameters such as daily gain, fresh and cold carcass weight, carcass percentage for fat, bone and pure meat content. (Metzger et al., 2006).

Reproduction by hormonal stimulation. To increase ovulation rate in rabbit does, some breeders use hormones to speed up the reproduction process. The rabbit has no fixed ovulation period during estrus, due to the fact that ovulation occurs after mating (Yusuff, 2015). Those hormones are naturally secreted by the pituitary and the hypothalamus in vertebrates. Ganadotropin-releasing factor also known as luteinizing hormone-releasing factor (LHRH) or luliberin is one of the most important ovulation-stimulating hormones and it belongs to the group of gonadotropic-releasing hormones. Buserelin is also one of them. It is an analogue of the gonadotropic hormone, which is most often used in the practice of artificial insemination by muscle injection of the desired rabbit, after coitus (Yusuff, 2015). GnRH is a hypothalamic multipeptid molecule, which plays an essential role in the regulation of reproductive processes (Conn and Crowley, 1994).

Artificial insemination in rabbit breeding. The artificial insemination includes the use of hormones or techniques of “bio-stimulation”. It is widly used in rabbit farms, as it significantly facilitates the reproduction process. The susceptibility of the rabbit doe has a great effect on this type of insemination. Ovulation in rabbits occurs after copulation with the male rabbit between 10 and 13 hours after mating. Therefore, to stimulate ovulation, hormonal treatment with maternal serum from pregnant mare gonadotropin (PMSG) is applied to increase susceptibility (Nassif et al., 2020). It is generally accepted that a dose of 20–25 IU of PMSG, 48 hours before insemination of lactating rabbits, 11 days after birth increases the susceptibility to successful fertilization (Velikov and Dimitrova, 2014 a; Velikov and Dimitrova, 2014 b; Nassif et al., 2020). Susceptible rabbit does at insemination produce three to four times more weaned rabbits than non-susceptible ones, especially when still breastfeeding.

The storage of male semen is also an important step in the process of artificial insemination. The process involves collecting semen from selected bucks, visually and microscopically evaluating the semen, diluting 10 to 20 times, pipetting, and injecting a gonadotropin-releasing hormone (GnRH) or analogue into the targeted animal in the time of insemination (Nassif et al., 2020).

Dimitrova et al. (2008) experimented with the New Zealand White and Californian rabbits. The results showed that the White New Zealand breed was more susceptible to stimulation (70% more successful inseminations with gonadotropic stimulating hormone-GnRH).

Application of modern technologies in rabbit breeding

Molecular markers. Modern techniques for establishing genetic diversity are increasingly used in animal breeding. With molecular markers very easy and accurate could be determined the population status of the different pedigree lines, breeding programs could be designed to select animals with desired traits, avoiding inbreeding.

Rabbit microsatellite research began at the end of the 20^{th} century and four sites – SOL03, SOL28, SOL08, and SOL30 were found using cloning techniques (Li et al., 2020).

In molecular genetic research, the main DNA markers are microsatellites (simple sequence repeats, SSR), restriction fragment length polymorphisms (RFLP), Amplified Fragment Length Polymorphism, Single Nucleotide Polymorphism, mitochondrial DNA and other technologies (Li et al., 2020).

Molecular markers are fragments in which differences in DNA sequences are observed, revealing polymorphism at the DNA molecule (Deb et al., 2012; Yadav et al., 2017). They slowly replace morphological and biochemical markers (protein) due to their high degree of informativeness (> 90%), while in blood groups it varies from 70% to 90% and in other biochemical markers – 40–60% (Ebegbulem and Ozung, 2013).

They are numerous and spread throughout the genome. These markers are inherited mainly codominantly and they are multiallelic, with mean heterozygosity for populations over 70% (Mitra et al., 1999; Vignal, 2002; Grover and Sharma, 2016).

In the study of Salma et al. (2018) the researchers applied 16 markers for genotyping new synthetic line known as Moshtohor rabbits (M-line) to identify polymorphism in the promoter region of the progesterone receptor gene (PGR). They observed a total of 108 alleles in the studied 4 populations. They recorded higher frequency with statistically significant difference of GG genotype of G/A2464 SNP located in the promoter region of PGR gene indicating its possible association with litter size.

Quantitative trait loci. The establishment of the quantitative trait loci (QTL), through the application of molecular markers is advanced and very accurate method, widespread in various sectors of the animal breeding. The domestic rabbit has 44 chromosomes, of which 70 loci are known in 6 binding groups: approximately 33% are associated with hair (color, structure), other 33% control blood groups and antibodies, adn the last 33% regulate hereditary diseases (Grassé and Dekeyser, 1995). According to the US National Center for Biotechnology Information, the rabbit has 29,098 genes distributed in 2,737.46 megabases, responsible for encoding about 19,000 proteins (Hristova et al., 2018).

The presence of polymorphisms in rabbit populations was studied in New Zealand and Californian breeds. This polymorphisms are essential in the research of the influence of the gene responsible for the formation of growth hormone GH, located on chromosome 19 in the rabbit genome (Gencheva et al., 2019). From its analysis by PCR-RFLP, they identified three single nucleotide polymorphisms (SNPs) – two nucleotide substitutions g.111 C > T g.156 A > G and a new g.255 G > A in the non-coding regions of rabbit Growth Hormone. The most common genotype was the homozygous genotype TT (0.93), with predominance of the T allele (0,97) above the C allele (0,03) (Gencheva et al., 2019).

In the study of Li et al. (2020) for evaluation of the New Zealand White rabbit they determine 43 SSR loci in 500 bp region in the range of 88 and 456 bp. They found 43 loci which were distributed throughout 18 pairs of chromosomes.

In conclusion, for the means of the effective evaluation of the rabbits from the New Zealand White, the appropriate number of loci should be 22 and the number of records 60. The acquired results provide a theoretical reference for future rabbit conservation (Li et al., 2020).

Future prospects of genetic progress in the industry

The rabbit is one of the earliest mammals used in research on the transfer of nuclear material. In 1988, the first cloned rabbit was created by embryonic blastomere nuclear transfer (Song et al., 2021). In 2002, the first cloned rabbit from somatic cells was successfully designed by Renard's group in France, by using freshly harvested cumulus cells such as nuclear donor instead of cultured somatic cells.

Gene targeting allowed precise manipulation of the genome avoiding the problems caused by random insertion. More important, it allowed scientists to study human genetic diseases by deletions and insertions of specific mutations in the animal genome in order to establish the roles of different genes in healthy and diseased animals.

Recently, with the discovery of gene editing nucleases, including, zinc finger nuclease (ZFN), transcription activator-like effector nuclease (TALEN), and clustered regularly interspaced short palindromic repeats/CRISPR associated protein-9 (CRISPR/Cas9), it is now possible to modify the rabbit genome precisely and efficiently (Song et al., 2021).

Different methods of transgenic animal production like sperm-mediated gene transfer, and viral-mediated gene transfer, have also been used, but these methods have considerable problems with reproducibility and transgene rearrangement as well as low efficiency.

In 1985, the first transgenic rabbit was produced by Robet E. Hammer and colleagues. They injected the pronuclear stage embryos with a fusion gene consisting of the human growth hormone stimulated by the mouse metallothionein-I promoter (MThGH) (Song et al., 2021).

ZFN, TALEN, and CRISPR/Cas9 have been proved as powerful programmable tools for genome editing. These engineered endonucleases are efficient in generating double-strand breaks (DSB) in the specific genomic loci that can be repaired either by error-prone, non-homologous end joining (NHEJ) leading to frameshifts which may result in a functional knock out of the target gene or by homology directed repair (HDR) to integrate a mutation or a DNA insert at a specific locus.

Cas9 is an endonuclease enzyme playing a protective role against foreign nucleic acids in the CRISPR adaptive immune system in bacteria. The characteristics of bacterial CRISPR immune system are that genetic materials taken up from previous invasive elements are expressed in crRNA, which could direct the Cas9 endonuclease to cut foreign DNA elements containing the same sequence (Song et al., 2021).

Conclusion

Innovative genetic methods and models allow the creation of modern breeding programs and at the same time the rapid improvement of the sector in our country. The experimental work at the molecule level and the application of new techniques contributes to the development and fast progress in this direction, and this has a significant advantage in creating different ways of working in this field.

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