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EMBRYO SURVIVAL TO ACCELERATE GENETIC PROGRESS IN DAIRY HERDS

(review)

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Abstract

Continuity of genetic progress and the use of advanced technologies in the breeding of highly productive livestock are the distinctive features of modern dairy cattle breeding (G.R. Wiggans et al., 2017; B.V. Sanches et al., 2019). An example of Holstein cows of North American selection indicates the achievement of genetic changes (more than 56,0 %) in animals over 50 years (1963-2013), when milk yield doubled from 6619 kg to 12662 kg (A. Garcia-Ruiz et al., 2016). Along with this, genetic improvements aimed at higher milk yields have decreased the reproductive capacity and impaired health of cows (J. Kropp et al., 2014; L. Hyun-Joo et al., 2015, B. Fessenden et al., 2020) that is a global problem (E.S. Ribeiro et al., 2012; K.J. Perkel et al., 2015). High-yielding cows are 30-50 % susceptible to mastitis, metritis, lameness and other diseases (I. Cruz et al., 2021), and the average calving rate is about 40-50 % with 90-95 % fertilization (M.G. Diskin et al., 1980; P. Humblot, 2001). The embryonic period of cows which is up to 42-45 days of gestation (J. Peippo et al., 2011) is characterized by high (up to 40 %) embryonic mortality (D.C. Wathes, 1992; K.J. Perkel et al., 2015; P. Rani et al., 2018), the multifactorial etiology of which has not yet been elucidated. Loss of genetic potential (unborn bull sires, replacement heifers, mothers of bull sires, and embryo donor cows) slows down selection process in dairy herds (M. Ptaszynska, 2009). This review focuses on the genetic predisposition of the embryo to survival as one of the important factors determining the onset and development of pregnancy of dairy cows. Blastocysts retain the ability to survive in stressful conditions of in vivo or in vitro production after cryopreservation-thawing (J.L.M. Vasconcelos et al., 2011; C. Galli, 2017; H. Erdem et al., 2020) and bisection (microsurgical division of the embryo in half for two demi-embryos) (Y. Hashiyada, 2017). The information on embryo survivability becomes more genetically founded as candidate genes associated with high embryo competence to development are found (M.C. Summers and J.D. Biggers, 2003; A. El-Sayed et al., 2006). Molecular genetic technologies make it possible to study the entire set of genes that endow the blastocyst with the ability to develop sustainably (A.M. Zolini et al., 2020), as well as epigenetic changes of gene expression patterns before and after embryo implantation (A. Gad et al., 2012; P. Humblot, 2018). It will help to develop methods for marker-assessed diagnostics of embryonic disorders, to regulate embryonic genes expression, to elevate the pregnancy rate in cows possessing economically valuable traits and, finally, to accelerate genetic progress in dairy cattle populations.

Keywords: genomic selection, transcriptomes, high-yielding cows, embryonic mortality, genetic progress, molecular genetic markers

Holstein cattle are common in herds in many parts of the world [1]. However, the increase in milk productivity caused numerous health problems in cows (in 30.0-50.0% of cases of all registered diseases, these are mastitis, metritis, lameness, milk fever, ketosis) [2], and also led to a decrease in reproductive ability [3-7], which was the result of one-sided selection and genetic improvements and has a negative impact on modern dairy cattle breeding [8-10].

The average calving rate in high-yielding cows is about 40-50% with a fertilization of 90-95% [11, 12]. Frequent events include high (up to 40.0%) embryonic mortality [13-15] in the period from fertilization to 42-45 days of pregnancy [16]. Fetal death between 40 and 80 days of pregnancy occurs in 2.0-6.0% of cases, in the remaining period in 4.0% of cases [17]. In addition, industrial housing conditions lead to injury, stress, hyperthermia, pyrexia [18] and, consequently, to a longer period between calving, infertility, a significant percentage of early culling [19]. This slows down the rate of genetic progress. i.e., the continuous improvement in productivity rates provided by the continuity of the breeding process, the effectiveness of which depends on the rapid reproduction of animals with economically significant genotypes.

The etiology of the high embryonic mortality that is recorded in herds is still not understood [20]. Among the factors influencing embryonic death in pregnant high-yielding cows are the oviduct environment which regulates the development of the embryo up to the blastocyst stage [21-23], and the uterine environment before embryo implantation [24, 25]. In addition, attention is focused on the natural mechanism of adaptation of the blastocyst to environmental conditions, due to the genetic predisposition of the embryo to survival [17, 26, 27] which is determined by hereditary factors [28], i.e., the genetic information transmitted to the embryo from the egg [29] and from the sperm [30, 31].

Molecular studies of the relationship between gene expression and early embryonic development or its delay can provide insight into the genetic and epigenetic mechanisms that ensure the viability of the embryo.

The purpose of this review is to summarize data on the ability of bovine embryos to survive when obtained in vivo or in vitro, after cryopreservation and thawing, after microsurgical division, and during transplantation and pregnancy.

Embryo transplantation technology (ETT), widely practiced in the breeding of high-producing animals, makes it possible to obtain a large number of embryos from genetically valuable donor cows fertilized by the semen of outstanding sires in a short period [32, 33]. Among the methods that form the basis of TTE, MOET (multiple ovulation and embryo transfer method) through which embryos are obtained in vivo [34] and IVP (in vitro production method) designed to obtain embryos in vitro [35] are important.

The transfer of in vivo or in vitro derived embryos to less valuable recipient heifers allows faster reproduction of more offspring than natural reproduction [36, 37]. According to the International Embryo Transfer Society (IETS), more than 20 million dairy and beef cattle embryos were received worldwide between 2000 and 2019, and in 2019 in 39 countries, which accounted for approximately half of the world livestock (Russia, USA, Canada, Brazil, France, Italy, etc.), and 1,419,336 commercial embryos suitable for transplantation have been produced [38]. With TTE, a significant part of embryos in vivo degenerates and dies before reaching the blastocyst stage. On days 6-7, in superovulated dairy donor cows with a productivity of 85-95%, approximately 50% of viable embryos were retrieved [39].

Embryo survival with the MOET method. The essence of the MOET method is that in a genetically valuable cow (an embryo donor), the growth and maturation of many egg-producing follicles (superovulation induction) is artificially activated by the administration of follicle-stimulating hormone (FSH) preparations. On day 7 after insemination of a donor cow, in vivo embryos are removed from its reproductive organs and transplanted (freshly obtained or frozen-thawed) to less valuable recipients [40]. The technological process and the means used in this manipulation imply stresses and traumatization of both the resulting embryos and donor cows. Donor cows get into a stressful situation already in

preparation for the superovulation induction procedure, when the animal is caught and fixed. The FSH preparation is used in strict accordance with the scheme (8-10-fold injection every 12 hours for 4-5 days). In response to multifactorial influences, physiological and metabolic processes in the body change. The number of in vivo embryos produced by donor cows varies over a wide range [41-43]. According to international practice, 30% of donors have no ovarian response to exogenous gonadotropins [44], 30% of donors show an extremely low ovarian response with a number of ovulations of 1-3, which corresponds to the natural process of ovulation. And only in one third of donors there is a superovulatory response with the number of ovulations from 5 to 12 [45-47].

The oviduct of the female cattle serves as a place for the fertilization of the egg and the location of the embryo during the first 4 days. At the 16-cell stage of development (early morula), the embryo moves into the uterine cavity, where it develops to the morula stage and on day 7 to the blastocyst stage (pre-implantation stage of development) [48, 49]. On days 8-9, the blastocyst cavity significantly increases in size, its zona pellucida stretches, becomes thin, and breaks (hatching process), removing the embryo from the zona pellucida [39, 50]. Further, the blastocyst attaches to the endometrium of the uterus, the low receptivity of which causes failures in the implantation of the embryo, including during IVF programs. The processes that determine the readiness of the endometrium to accept an embryo in cattle are not well understood [51)], while in humans, the genes of the HOX family (Homeobox) and the proteins encoded by them, for example, HOX10 and HOX11, are known, which are involved in the regulation of implantation and serve as key regulators of receptivity processes. endometrium [52, 53].

In MOET programs, 7-day-old embryos are removed from the reproductive organs of a donor cow in a non-surgical way using specialized equipment. The technical removal of an embryo from its natural environment increases stress [54]. In addition, unavoidable losses of embryos occur during retrieval, ranging from 60-80 to 20-30% of the counted number of corpora lutea [55].

After retrieval, the embryos are placed in an artificial environment and labeled based on the International Embryo Transfer Society (IETS) guidelines [56, 57]. The stages of embryo development are determined (stage codes from 1 to 8) and their quality is assessed for suitability for transplantation (quality codes from 1 to 4). Embryos with a quality code of 1 (excellent or good), which are at the stages of development from compact morula (stage code 4) to blastocyst (stage codes 5 or 6), provide the highest pregnancy rates, including after cryopreservation. Embryos with quality codes 2 (satisfactory) and 3 (poor) after cryopreservation show low pregnancy rates in recipients, so they are used for transplantation only in a fresh form. In embryo collections, in addition to embryos suitable for transplantation, as a rule, there are oocytes (unfertilized eggs), unicellular or degenerated embryos that are not viable (quality code 4) and must be disposed of. According to many years of world practice, an average of 58.0% of embryos suitable for transplantation are detected in embryo collections, and the rest are degenerated embryos (11.0%) and unfertilized eggs (31.0%) [58]. After one session of MOET, on average, 6.2 in vivo embryos suitable for transplantation are obtained from one donor cow [59], and over 40 embryos in vivo for 1 year when using this method every 45 days [60]. Cases have been registered when up to 50 in vivo embryos suitable for transplantation were obtained from one donor cow during one session of superovulation stimulation [45]. The ability of freshly obtained in vivo embryos to survive when transplanted to recipients is evidenced by the pregnancy rate of 45.0-55.0%, after transplantation of frozen-thawed embryos, this figure is 30.0-45.0% [61-65]. Therefore, a significant part of the embryos

repeatedly subjected to technological stresses demonstrates the ability to survive which is confirmed by the birth of calves.

Embryo survival in the IVP system. The IVP method is even more aggressive than MOET, but both methods serve as an important tool in cattle breeding to increase the number of offspring from animals of high genetic value, which maximizes the reproductive capacity of cows over a shorter period of time [66].

In the production of IVP embryos, eggs are obtained in vivo or post mortem (after the slaughter of the animal). A transvaginal aspiration method is used, which is commonly known as the OPU (ovum pick-up) method. i.e., the collection of immature oocytes from the ovaries of donor cows under ultrasound control [33, 67]. The essence of IVP is that the resulting oocytes are cultivated in the laboratory under in vitro conditions for maturation (in vitro maturation, IVM), artificially matured oocytes are subjected to in vitro fertilization (IVF), after which the fertilized oocytes (zygotes) are cultured in a growth medium (in vitro culture, IVC) to develop the embryo to the blastocyst stage [68]. Oocytes are able to resume meiosis during IVM, split after fertilization (IVF), develop to the blastocyst stage in IVC, and induce pregnancy leading to the birth of healthy offspring, which is generally interpreted as developmental competence of oocytes [69].

In addition to hormonal stimulation, follicular wave phase, follicle diameter, feeding conditions, and donor age, the developmental competence of oocytes is affected by the in vitro culture process [70]. The transfer of oocytes from one culture medium to another, as well as the composition of the medium and culture conditions during IVC, can cause physico-chemical (temperature, osmolality and pH), oxidative (pro-oxidant and antioxidant balance) and energy (use and accumulation of nutrients, synthesis) in the embryo. ATP) stresses leading to misregulation of homeostasis at an early stage of development [71].

With the use of molecular technologies, it became possible to study various indicators of embryo development at all stages of IVP. It has been shown that the development of embryos under certain culture conditions leads not only to a change in the expression of genes associated with metabolism and growth, but also to a change in the concept and development of the fetus after transfer to recipients [72]. Embryo stress responses during in vitro culture correlate with transcriptomic changes associated with energy metabolism, signaling pathways, and extracellular matrix remodeling [71, 72]. It is assumed that the transcriptomic changes that occur during the blastulation period are the result of the adaptation of the embryo to environmental factors, and such adaptation, under suboptimal cultivation conditions, can cause epigenetic changes leading to metabolic imbalance that negatively affects the process of implantation, development of the embryo, and its health in the postnatal period [71, 73].

Embryos derived from in vitro matured oocytes are less viable than embryos derived from naturally ovulating oocytes [74-78]. As practice shows, 90% of oocytes extracted from the follicles of a donor cow are capable of meiosis and maturation, 80% of fertilized oocytes (zygotes) develop to the 2-cell stage, but only 30-40% of them can develop to the blastocyst stage [39, 79-81]. On one donor cow, the IVP method is used every 15 days, receiving more than 72 in vitro embryos within one year, when three in vitro embryos are produced on average in one technological cycle [60]. After transfer of embryos in vitro, the pregnancy rate in recipients is 10-40% lower compared to embryos in vivo, in addition, 60.0% of pregnancies are terminated during the first 6 weeks, and live calves are born in 27% of cases [82]. Compared to in vivo embryos, in vitro embryos are characterized by lower cryotolerance during cryopreservation [83-85], and the engraftment rates of vitrified embryos in vitro, recorded by Sanches et al. [86], on day 30 after

transplantation, were 35.89±3.87% (84/234) vs. 51.35±1.87% (133/259) after transplantation of freshly obtained embryos. The observed (albeit small) percentage of calves that developed from oocytes subjected to numerous manipulations outside their natural environment indicates the presence of an adaptation mechanism, the understanding of which will become possible with the accumulation of experimental data.

Microsurgical division of the embryo in half and the survival of demi-embryos. Cattle are singletons that give birth to one calf per year. With natural reproduction, the appearance of twin calves (mono- and dizygotic twins) occurs extremely rarely, in 3-5% of cases in dairy cattle and in no more than 1% of cases in beef cattle, the percentage of birth of monozygotic twins is even lower [87]. In dairy cattle, the probability of having monozygotic twins occurs in no more than 0.001% of calvings [88].

The developed method of microsurgical division of the embryo in vivo in half (bisection) [89-92] offered a simple way to increase 2-fold the number of in vivo embryos. During bisection, the embryo (at the morula or blastocyst stage) is placed on a laboratory watch glass or in a Petri dish with an artificial nutrient medium. After fixation, the embryo is divided under a microscope into two halves [93, 94], which should be of the same size, and blastomeres and trophoblast cells should be evenly distributed [95]. The bisection method is based on the unique property of totipotency that mammalian gametes (egg and sperm) acquire immediately after fertilization: the zygote begins to split, forming blastomeres, while each blastomere is able to generate a full-fledged organism, but this ability is lost in the course of embryo development with the onset of cell differentiation [96]. After microsurgical division of the embryo, each of the halves within several hours (from 1 to 3 hours) in a nutrient medium at room temperature restores the spherical shape typical of the embryo (demi-embryo) [89, 97]. Immediately after recovery, demi-embryos can be transferred to recipients.

Embryonic death in demi-embryos is recorded much more often in comparison with intact (intact) embryos [98], but after the demi-embryo engraftment, it develops similarly to the intact one [95]. According to Hashiyada [99], the pregnancy rate in recipients after transplantation of demi-embryos in vivo is 36.4-53.2%, according to Lopatarova et al. [100] 48.8-56.5%. The absence of pregnancy after demi-embryo transfer is mainly due to damage and loss of blastomeres during the bisection procedure, as well as with insufficiently effective methods of culturing halves of a divided embryo [101]. Despite the damage caused to the embryo during bisection, for several decades, thousands of twin calves without signs of developmental anomalies were obtained from demi-embryos around the world [102]. However, the bisection method has not been widely used in practice, since it is difficult to perform such manipulations in a farm environment [33].

The development of molecular technologies has expanded the scope of the bisection method, which makes it possible to conduct scientific research on monozygotic genetically homologous demi-embryos [99]. Thus, the expression patterns of genes associated with the genetically determined ability of the embryo to survive in the mother-embryo system were studied. In the studies of Zolini et al. [17, 27], to identify marker genes that correlate with embryo survival, one part of a demi-embryo was transplanted into the recipient, and other part was used for RNA-seq analysis. The bisection method is also used in breeding farms in testing sires for offspring. This reduces the interval between generations, which allows the use of such bulls at a younger age [99].

The genetically determined ability of the embryo to survive. Gene polymorphism has been recognized as the most effective mechanism that ensures both the homeostasis of the organism and the dynamic constancy of

the population [103]. The regulation of gene activity and activation of regulatory genes play an important role [104]. Due to gene polymorphism, the embryo is programmed to be resistant to damage, and its genotype has an individual potential for variability depending on environmental conditions [103, 105].

Before the advent of modern molecular genetic technologies, the study of genes involved in early embryonic development was difficult, but whole genome studies are now possible using advanced microarrays that allow profiling of gene expression based on quantitative measurements [106]. Zolini et al. [17, 27] studied gene activity in transferred bovine embryo survivors and non-survivors. In embryos obtained in vivo, among the genes differentially expressed in viable and nonviable embryos, the most transcribed cluster was associated with membrane proteins, especially those involved in the development and functioning of the nervous system, in particular in the formation of the olfactory function [17]. Interestingly, in the survivors derived from in vivo embryo transplantation, there were the genes for oxidative phosphorylation the activity of which was suppressed [17]. In case of engraftment of embryos obtained in vitro [27], many differentially expressed genes involved in survival were associated with cellular responses to stress. The authors suggested that this is a consequence of disturbances caused by embryo culture. It also turned out that the set of genes associated with the survival of embryos, and the biological functions associated with these genes, are significantly different in embryos obtained in vivo and in vitro.

In bioptates of 7-day-old bovine blastocysts, Salehi et al. [106] revealed 6765 genes associated with numerous biological processes, such as regulation of the metaphase-anaphase transition of the cell cycle, regulation of chromosome segregation, mitochondrial translation, ubiquitination associated with the K48 protein, and mitotic nuclear fission. El-Sayed et al. [72], who studied gene expression in in vitro blastocyst bioptates transplanted into recipients, showed that the regulation of gene activity was different in the absence of pregnancy and in the case of calf birth. For a number of genes, such as *TNF* (pro-inflammatory cytokine), *EEF1A1* (enzymatic delivery of aminoacyl-tRNA to the ribosome), *PTTG1* (oncogene), *AKR1B1* (glucose metabolism), and *CD9* (implantation inhibitor gene), increased expression was found, which correlated with the inability to induce pregnancy. The implantation-associated genes (*COX2* and *CDX2*), genes for carbohydrate metabolism (*ALOX15*), growth factor (*BMP15*), signal transduction (*PLAU*), and placental development (*PLAC8*) were involved in calf birth. In bovine blastocysts cultured in vitro, Suwik et al. [78], when profiling transcripts of the *IGF1R*, *IGF2R*, *OCT4*, *SOX2*, and *PLAC8* genes, showed a change in their expression depending on the stage of blastocyst development and quality. In a transcriptomic analysis of in vitro blastocysts obtained from oocytes exposed to elevated concentrations of non-esterified fatty acids (NEFA), Van Hoeck et al. [107] found physiological changes in developing embryos and a decrease in their survival compared to controls.

At present, the entire set of genes and transcriptomes associated with the characteristics of the development and survival of the bovine embryo is not fully understood. It is expected that the study of transcriptome abnormalities will lead to methodological progress in assessing embryonic competence [71, 108, 109] which is understood as its development from the zygote stage (single-cell embryo) to the blastocyst (multi-cell embryo, pre-implantation stage), capable of causing pregnancy, culminating in the birth of a calf [109].

Epigenetic aspect of embryo survival. In living organisms, epigenetic regulation of gene activity is widespread, which is not associated with a change in the primary structure of DNA, but modifies the functioning of the genome depending on internal and external factors [110]. It has been shown that

epigenetic regulation is carried out through chemical modification of the DNA structure (DNA methylation, histone modifications, non-coding RNAs) or chromatin [111]. DNA methylation in blastocysts is a reversible and dynamic epigenetic mechanism involved in the remodeling of the chromatin structure, including in critical regulatory regions of the genome, and thereby affecting gene expression [112]. To date, the complex relationship between epigenetic modifications, chromatin state, and transcriptional activity in bovine embryos has not been sufficiently studied [111]. A comparative analysis of the degree of modification of certain parts of the genome in normal and pathological conditions can reveal epigenetic predictors associated with disturbances in the regulation of gene expression, which are associated with the survival of the embryo.

While still in the oviduct, the embryo undergoes epigenetic changes that affect its subsequent development, implantation and postnatal phenotype [113, 114] which is important for ensuring the correct set of genes transcribed during embryonic genome activation (zygotic genome activation, ZGA) [115)]. After fertilization, the first zygotic divisions occur in the mode of transcriptomic silence, which persists until the activation of the embryonic genome is completed. In this regard, early embryos in vitro show increased sensitivity to culture-related stress compared to later stages of pre-implantation development [71]. A study by Dobbs et al. [116] showed dynamic changes in DNA methylation in bovine embryos, that is, a decrease in methylation from the 2-cell to 6-8-cell stage during ZGA followed by an increase during further development to the blastocyst stage. This indicates that embryonic cells after ZGA acquire transcriptomic variability, providing sensitivity to external environmental conditions [117, 118].

With the expansion of experimental data, epigenetic studies of gene expression patterns in response to changes in environmental conditions before and after embryo implantation will become a source of important information on the regulation of embryonic development in MS [119, 120].

The role of MOET, IVP and embryo bisection in molecular genetic studies of embryonic development. Molecular technologies make it possible to obtain a large amount of genomic information on many biological processes in the animal body. Significant progress has been made through genomic selection (particularly in dairy farming) [80, 121].

Prior to the introduction of TTE in livestock farming practices in the 1980s, genetic progress in dairy herds was slow due to the long breeding cycle and one calf per cow [122]. The advent of MOET and IVP accelerated it by shortening the generation interval and using the best females. The combination of these methods with genomic selection for milk production traits further reduced the generation gap and increased the genetic effect due to the high selection accuracy [34]. An example of Holstein cows of North American breeding indicates the achievement of genetic changes (more than 56.0%) in the body of animals over 50 years (1963-2013), when the annual milk yield doubled from 6619 kg to 12662 kg [1]. Also, thanks to the use of TTE in combination with genomic selection for 7 years (from 2008 to 2015), in the USA, when producing genetically valuable cows and sires, the interval between generations was sharply reduced from about 7 years to < 2.5 years, and when obtaining bull-producing cows from 4 to 2.5 years [1]. There is evidence that more than 90% of Scandinavian dairy cows in Denmark, Sweden and Finland in 2018 were born from bulls that were only 3.1 years old [123]. Therefore, despite the fact that genomic selection has been used for a relatively short time, the results achieved confirm its positive impact on the efficiency of dairy cattle breeding [1].

Currently, research is ongoing on a set of genes and transcriptomic data associated with the embryonic development of cattle. It is expected that the

identification of molecular genetic markers specific for the development of a certain pathological process in early embryos will contribute to the development of methods for assessing pathogenic factors leading to early embryonic death. The CattleQTLdb database (https://www.animalgenome.org/cgi-bin/QTLdb/BT/index) integrates ever-growing volumes of data on quantitative trait loci (QTL) obtained in different countries of the world during the study of the bovine genome , as well as providing tools to study the genetic mechanisms that control traits of interest in this farm animal species [124]. In CattleQTLdb, you can quickly find relevant genotype-phenotype information for trait analysis [125], including those associated with various aspects of fertility and successful pregnancy.

The results of experiments on the study of transcriptomes of bovine embryos (from oocytes to late blastocysts), including those using next-generation sequencing (NGS) technologies, are summarized at http://emb-bioinfo.fsaa.ulaval.ca/ IMAGE/. However, the assessment of the relationship between the transcriptome profile of the preimplantation blastocyst and the onset of pregnancy in cows is still difficult, since there are no unified algorithms and approaches to interpreting data from different sources.

The decisive factors affecting the reliability of genomic estimates and predictions are the increase in the number of individuals in the reference population, which determines the relationship between phenotypes and markers, as well as the increase in the size of the reference population and the accuracy of the phenotypes of interest [127]. The MOET and IVP methods are becoming important for maintaining the genetic potential [120], making it possible to obtain tens of hundreds of embryos from genetically valuable animals in a short period of time [128]. In addition, in vitro embryos serve as a model object for molecular studies of biological functions from the unicellular stage to the blastocyst [37] and the study of oocyte maturation, fertilization, early development and implantation [78].

The use of bisection is common in the study of transcriptomes in embryos in connection with the onset of pregnancy [17, 27].

Thus, the methods of multiple ovulation, in vitro maturation, microsurgical division of embryos in half (bisections) and transplantation are quite well developed and are applicable to obtain viable embryos from genetically valuable donor cows, which, after transplantation to recipients and engraftment, can develop up to the birth of offspring. Combined with genomic research, these methods form the basis of modern reproductive biotechnologies used to accelerate genetic progress in herds. Significant factors determining the onset and development of pregnancy include the genetically determined ability of the embryo to survive in different environmental conditions, so the search for candidate genes associated with embryonic development is an important area of research aimed at increasing pregnancy rate in high-yielding cows. Modulation of the expression of embryonic genes may become a promising direction in reproduction. To implement this approach, genetic and epigenetic markers are needed to detect both violations of embryonic development and the high competence of the embryo.

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