SECTION 9.
BIOLOGY AND BIOTECHNOLOGY

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CELLULAR AND GENETIC TECHNOLOGIES IN REPRODUCTIVE BIOTECHNOLOGY

Cellular and genetic methods provide fundamentally new opportunities for managing the reproductive potential of animals: from the selection and modification of genes that control fertility to the creation of clones and transgenic lines. These technologies allow preserving and distributing valuable genotypes, investigating the mechanisms of embryo development, and optimizing breeding programs [4, 5].

Cloning by somatic nuclear transfer (SCNT). SCNT involves transferring the nucleus of a somatic cell to a degraded or removed oocyte nucleus, stimulating the development and implantation of the embryo into the recipient. The technology allows the genetic identity of the donor nucleus to be restored, which is useful for preserving rare or valuable genotypes [6, 9].

The main obstacles to cloning by somatic nuclear transfer are the low rate of development before birth, epigenetic alterations (incomplete epigenome rewriting), placentation abnormalities and high costs. In swine, the percentage of successful pregnancies after SCNT is traditionally low compared to other species, which limits its widespread use [4, 7].

Ways to improve the efficiency of SCNT include optimizing the state of donor cells (phases of the cell cycle), using epigenetic modifiers (HDAC inhibitors, DNMT inhibitors) to correct mitotic/epigenetic defects, and improving oocyte and embryo culture media. Omnix analysis and high-throughput sequencing help identify markers associated with reconstitution success [1, 8].

Induced pluripotent stem cells (iPSCs) and embryonic stem cells. iPSCs and embryonic stem cells (ESCs) open up the possibility of creating cell lines for studying early embryonic development, toxicity testing and as a potential source of gametes in the future. In animal husbandry, iPSCs can serve as a "platform" for gene modification and subsequent generation of clones or hybrid embryos [5, 6].

In practical animal husbandry, the use of iPSCs/ESCs is limited: differences between lines by species, risks of teratogenicity, difficulty in differentiating into functional gametes. The need for standardized protocols and safe control of their use is key [1].

In vitro technologies: IVM, IVF, ICSI. Oocyte- and embryo-oriented procedures. IVM (in vitro maturation), IVF (in vitro fertilization) and ICSI (intracytoplasmic sperm injection) are the central methods for obtaining embryos outside the body. There are successes in pig breeding, but the rates are lower than in some other species, due to the characteristics of oocytes and sperm [2, 7].

In vitro oocyte manipulations are modern approaches that allow studying the mechanisms of fertilization and creating new animal lines. In vitro maturation (IVM), in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) methods are successfully used in experimental pig breeding to improve fertilization results and optimize breeding programs [2].

Technical factors for success are oocyte quality (metabolic state, lipid level), cultivation environment (osmotic conditions, antioxidants), sperm-oocyte contact time, immunological and epigenetic effects. ICSI allows you to bypass problems with low sperm motility, but requires specialized equipment and skills [6].

Genome editing (CRISPR/Cas, TALENs, ZFNs). CRISPR/Cas allows point-specific gene modifications, creation of knockouts or introduction of specified alleles. In reproductive biotechnology, it is used to correct mutations, increase disease resistance, improve reproductive characteristics and create transgenic lines [8, 9].

Molecular genetic control of reproductive qualities - the use of fertility gene markers (ESR1, FSHB, RBP4, PRLR, LEP) allows for the early identification of animals with high genetic potential. Genotyping using PCR, SNP analysis and next-generation sequencing (NGS) has become the basis of molecular breeding [4].

Problems that arise - off-target effects, mosaicism in embryos, epigenetic consequences and regulatory/ethical restrictions. Careful population and long-term phyto- and zoo-experiments are necessary to assess the safety and stability of phenotypes [7].

Transgenic technologies and biopharmaceutical lines. Transgenic animals are used for research purposes and the production of recombinant proteins (pharmaceuticals). Transgenicity is less commonly used in pig production, but the technology has the potential to improve reproductive and productive characteristics [6].

Epigenetics and omics approaches. Epigenetic changes (DNA methylation, histone modifications) determine the success of SCNT transcription and embryo