7.3 Phenotypic evaluation and comparison of production performance

7.3.1 Cattle (mammary expression of pharmaceutical proteins / functional improvement of dairy products)

In long-term breeding, growth, reproduction, and health indicators were comparable to controls; no significant differences were found in the nutritional composition of milk and meat, indicating agricultural application potential and food safety (Yum et al., 2024). The target proteins (such as lactoferrin or recombinant human proteins) were highly and stably expressed in milk, and metabolomic/proteomic and functional assays showed enhanced antibacterial and bioactive properties, consistent with the evidence chain presented in Section 7.2.

7.3.2 Pigs (rhPC bioreactor / disease resistance / xenotransplantation)

The milk expression level of rhPC in transgenic pigs ranged from 100-1800 μg/ml among different constructs or lines and remained stable within the same line. Reproductive and lactation performance were normal, making them suitable for large-scale biopharmaceutical production (Van Cott et al., 1997). For xenotransplantation purposes, multi-gene-modified miniature pigs exhibited stable health and physiological performance while successfully expressing multiple human complement-regulating and anticoagulant proteins, significantly improving organ compatibility indicators. In addition, the breed of recipient sows and embryo transfer conditions significantly affected pregnancy and delivery rates, providing a basis for optimizing breeding procedures.

7.4 Research outcomes and risk assessment conclusions

The genetic stability of exogenous genes is the core prerequisite for ensuring phenotypic consistency and production predictability. Defined insertion sites, single-copy integration, and stable expression patterns constitute the foundation of a reliable genetic system. The use of nonviral-mediated gene transfer and targeted integration technologies can significantly reduce the risks of structural variations and epigenetic silencing, thereby enhancing genetic safety and long-term expression consistency (Van Cott et al., 1997; Yum et al., 2024).

In terms of risks and countermeasures, early random integration strategies were often associated with potential issues such as repetitive sequence insertions and expression drift, leading to genetic instability or functional loss. To prevent such risks, comprehensive molecular characterization analyses should be performed, including breakpoint sequencing or whole-genome sequencing (WGS), copy number quantification, and methylation profiling, to accurately assess integration features. Meanwhile, combining insulators and safe harbor site strategies can effectively isolate adverse chromatin effects and reduce the probability of transgenerational silencing. Long-term population tracking can further verify the stable transmission of both genetic and phenotypic traits. Additionally, optimizing recipient breed selection and embryo manipulation procedures in breeding and transplantation stages helps improve reproductive success rates and maintain trait consistency.

At the application and translational level, research and industrial practice in cattle and pigs have fully demonstrated that transgenic livestock hold sustainable potential for agricultural production and biomedicine. The establishment of standardized phenotypic evaluation and continuous safety monitoring systems not only ensures product safety and functional reliability but also provides scientific and transparent evidence for regulatory review and public communication, thereby promoting the social acceptance and regulated development of transgenic technology (Yum et al., 2024).

8 Challenges and Future Directions

8.1 Technical aspects: precision of gene editing and controllability of insertion sites

Although transgenic livestock technologies have made remarkable progress, many challenges remain at the technical level. The precision of gene editing is a primary concern. Current mainstream systems such as CRISPR/Cas9, TALEN, and ZFN possess high editing efficiency but still may cause off-target effects, leading to unintended gene mutations or chromosomal rearrangements. These molecular events may disrupt key genes or regulatory elements, resulting in physiological abnormalities or phenotypic drift, which could affect the reliability of research conclusions and industrial safety. For instance, in some pig-editing experiments, off-target mutations disrupted immune gene expression, leading to reduced disease resistance. Therefore, improving editing system specificity and developing controllable gene repair mechanisms will be central to future technical optimization.