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Application of Different Methods in Animal Sex Control Technology

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Abstract: Animal sex control technology enables targeted offspring gender selection in livestock through artificial reproductive intervention, offering significant economic value. It leverages biological differences in X/Y sperm DNA content, surface proteins, and metabolic activity. This research will discuss the application performance of different methods in animal sex control technology. Flow cytometry (85%-95% accuracy via fluorescent sorting) is high cost, low efficiency, and damage to fragile sperm (e.g., 40% survival loss in pigs), thus limiting the industrial use. Immunological methods (TLR7/8 pathway modulation) achieve 90% female offspring in ruminants but lack validation in pigs. CRISPR-based gene editing ensures near-100% accuracy but faces commercialization hurdles due to off-target effects (0.5%-5%) and ethical concerns. Emerging technologies like microfluidic chips (10,000 sperm/minute via laminar flow) and nanomagnetic beads (89% purity and 92% survival in pigs using H-Y antigen markers) show high-throughput, low-cost potential. Future integration with AI algorithms (98% accuracy) and cross-species molecular marker research could drive broader adoption.

Keywords: animal sex, control technology, gene editing, CRISPR

1. Introduction

Animal gender control technology is a key technique that involves artificially intervening in the reproductive process of animals to obtain offspring of a specific gender. The core of this technology lies in utilizing the differences between X and Y sperm in terms of DNA content, surface protein expression, and metabolic activity to achieve efficient sperm separation or early identification of embryo gender. Gender control technology has significant economic value in livestock production. For example, the dairy industry requires female calves to enhance milk production efficiency, while the beef industry relies on male calves to accelerate fattening. Through gender control, breeding costs can be significantly reduced, and resource allocation can be optimized [1-3]. Advances in sperm separation techniques for livestock sex control highlight the need for cost-effective solutions [4, 5].

In terms of biological basis, the gender of mammals is determined by sex chromosomes. The combination of X sperm and an ovum results in a female (XX), while Y sperm results in a male (XY). There is a significant difference in DNA content between X and Y sperm. For example, the DNA content of X sperm in cattle is 3.8% higher than that of Y sperm. This characteristic provides a theoretical basis for separation using flow cytometry. Additionally, the specific expression of TLR7/8 receptor proteins in X sperm is closely related to the development of immunological separation techniques, revealing regulatory mechanisms at the molecular level [3].

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As the only commercialized technology, flow cytometry is based on the DNA content difference between X and Y sperm (e.g., the DNA content of X sperm in cattle is 3.8% higher than that of Y sperm). It achieves gender control through fluorescence staining and electric field separation. Although the accuracy rate can reach 85%-95%, it has limitations such as expensive equipment, low separation efficiency, and differences in species applicability. For example, pig sperm have fragile membrane structures, and their viability significantly decreases after separation, making it difficult to apply industrially [2, 3].

Immunological methods achieve separation by identifying the differences in surface proteins between X and Y sperm. For example, the TLR7/8 signaling pathway activator R848 can selectively inhibit the mitochondrial activity and glycolytic capacity of X sperm, reducing its motility by more than 80%. This technology has been validated in mice, dairy goats, and Qinchuancattle and does not affect sperm fertilization ability, making it a potential alternative to flow cytometry [3]. For embryo screening technology, such as PCR and DNA probes require in vitro fertilization and embryo transfer, these methods are complex and costly, and are only applicable to high-value species [1, 2]. For gene editing technology, although it has achieved a nearly 100% female calf rate in mice, it is still limited by off-target effects and ethical controversies, restricting its large-scale application [2].

This research systematically compares the principles, efficiency and applicability of existing and emerging animal control technologies, including flow cytometers, immunology methods, CRISPR-based gene editing, microfluidic chips and nanomagnetic bead classification. It emphasizes the limitations of commercial technology (for example, the high cost and inefficiency of flow cytometers), and explores the potential of emerging technologies to meet these challenges. It also analyzes the industrial application progress of cattle, goats, pigs and emphasizes China's leading role in optimizing dairy flow cytometers. It also discusses the ethical controversy surrounding gene editing and the need to establish a global regulatory framework. Finally, the future direction of multi-technology integration (such as artificial intelligence-driven platforms) and cross-species molecular marker research is proposed to achieve sustainable and inclusive progress in this field.

2. Emerging animal control technologies

2.1. Application performance

Animal sex control technology can realize the screening of offspring of specific sex through various methods [6]. There are significant differences in the principle, effect, cost and scope of application of different technologies [7, 8]. As the only commercial technology at present, flow cytometer can achieve 85-95% gender control accuracy through fluorescent staining and electric field separation based on differences in X/Y sperm DNA content, which is especially suitable for dairy farming and can achieve 100% female offspring [3]. However, due to the high cost of this technical equipment (more than $500,000), low separation efficiency (about 3,000 sperm/minute) and poor applicability to species with fragile membrane structure, the survival rate of sperm after treatment can be reduced by more than 40% [2], limiting its wide application in industrial production.

Immunological methods achieve separation by identifying specific proteins on the surface of X sperm. For example, the activator R848 can selectively inhibit the mitochondrial activity and hemolysis ability of X sperm, reducing its athleticity by 80% [3]. Among ruminants such as Qinchuan cattle, the proportion of female calves can reach 90% [3], and the cost of equipment is lower than that of flow cytometer [1]. However, at present, this technology is mainly limited to the separation effect of cows, goats and other species on single-gastric animals such as pigs, and has not been fully verified [2]. Further research on cross-species molecular labeling and regulatory mechanisms is still needed.

Embryo screening technology relies on in vitro fertilization and embryonic sex detection (such as PCR or DNA probe). The accuracy rate is high, but the operation process is very complicated,

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requiring up to 72 hours, and the success rate is significantly affected by the operator's experience [1]. It is difficult for this technology to be popularized in general animal husbandry. Gene editing technology (such as CRISPR/Cas9) can directly modify the gender-determined gene and achieve nearly 100% of the female ratio in mouse experiments [2]. Theoretically, it has cross-species universality, but in the face of extra-target effects and ethical disputes [3].

Using the laminar flow effect and inertial focusing principle, the microfluidic chip can increase the separation speed of bovine sperm by 10,000 times per minute, which is more than 3 times more efficient than that of flow cytometer, and reduce the cost of equipment by 80% [3], while reducing the damage to fragile sperm (such as pigs). By labeling specific proteins (such as H-Y antigens) on the surface of X sperm, nanomagnetic bead technology achieved a purity of 89% in pig sperm separation experiments and maintained a survival rate of 92% [3]. Although these technologies are still in the laboratory stage, their high efficiency and low cost provide the possibility for future large-scale applications.

2.2. Comparison between different methods

China is at the forefront of gender control technology internationally. For example, the application of flow cytometry in dairy cows has achieved a 100% female calf rate, significantly improving the efficiency of dairy farms [3]. Additionally, sperm separation technologies in dairy goats and Qinchuan cattle have validated the universality of the TLR7/8 pathway, providing a basis for the promotion of this technology across multiple species [3].

In the research and application of animal gender control technology, the existing technology and the latest progress show a multi-dimensional contrast and complementarity. As the only commercial technology at present, flow cytometry is based on the difference in X/Y sperm DNA content (for example, the DNA content of X sperm in cows is 3.8% higher than that of Y), and achieves an accuracy of 85%-95% through fluorescent staining and electric field sorting. However, its sorting efficiency is only about 3,000 sperm/minute, and the equipment cost is more than 50 million US dollars, and the survival rate after pig sperm sorting has decreased by more than 40%, and industrial applications are limited. In contrast, microfluidic chip technology uses laminar flow effect and inertial focusing principle to achieve mark-free sorting. The efficiency can reach 10,000 sperm/minute, the equipment cost is reduced by 80%, and the survival rate of pig sperm with fragile membrane structure is maintained at more than 90%, showing significant low damage advantages. At present, microfluic control technology has entered the pilot stage and verified its universality in cattle and goats. In the future, it may improve the efficiency to 20,000 sperm/minute in combination with nanomagnetic bead sorting, which has become the core direction of industrialization [6].

In the field of surface protein identification, immunological methods achieve 90% of female offspring in cattle and goats by inhibiting X sperm mitochondrial activity (such as TLR7/8 pathway regulation), but the effect on pig sperm separation has not been verified; while nanomagnetic bead technology achieves physical selection by coupling H-Y antigen and other markers, pigs The purity of sperm sorting reaches 89%, the survival rate is 92%, and the equipment cost is 80% lower than that of flow cytometry, and the sorting speed can reach 20,000 sperm/minute [8]. Its flexible adaptation to the needs of multiple species provides the possibility of filling the gap in pig sex control technology.

At the embryo level, embryo screening technology relies on *in vitro* fertilization and PCR detection, such as CRISPR-based gene editing method (Figure 1). Although the accuracy rate is high, it takes more than 72 hours and is only applicable to high-value species. CRISPR gene editing can achieve nearly 100% female offspring within 24 hours by directly modifying the SRY gene, but the off-target effect (0.5% -5%) and ethical disputes (such as the EU ban) restrict its animal husbandry application [5]. In comparison, although the traditional centrifugation method (Percoll) is simple to operate, high

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reactive oxygen damage causes sperm survival rate to decrease by more than 30%, and the proportion of females to be only 60%. The new inertial focusing sorting technology combines microfluidic control and nanomagnetic beads to synchronously improve the sorting speed (20,000 sperm/min) and purity (More than 90%), the survival rate of pig sperm is 50% higher than that of the traditional method, which has become a key technology to replace the centrifugation method.

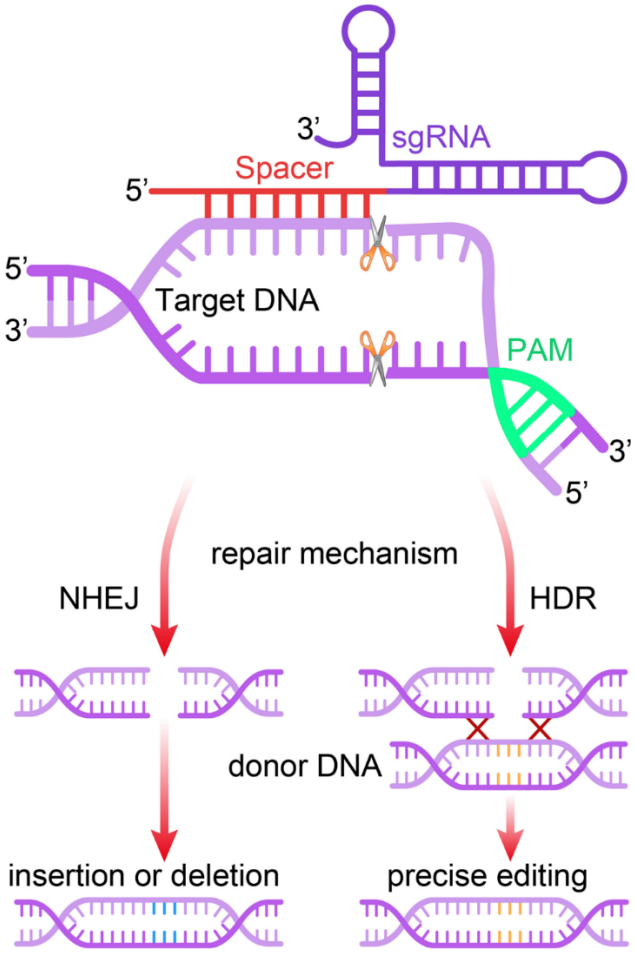

Figure 1: Mechanism of the CRISPR-based gene editing methods [5]

In terms of technology iteration, the new generation of genesis flow cell system improves the sorting speed to 8,000 sperm per second through automated optimization. The conception rate of cows is close to the conventional semen level, but the equipment cost exceeds 1 million US dollars. Although CRISPR is efficient in rats, the complex structure of pig sperm membrane and ethical issues are difficult to commercialize. In the future, multi-technology integration (such as AI-driven microfluidic control-magnetic bead integration platform) may become a breakthrough direction. The Chinese team has increased the sorting accuracy from 95% to 98% through AI algorithms and explored the universality of cross-species molecular markers. In general, the gender control technology of ruminants is becoming mature, while single-gamach animals such as pigs still need to rely on the efficiency optimization and cost breakthrough of emerging technologies such as microfluiology control and nanomagnetic beads. At the same time, the ethical compliance of gene editing urgently needs to be standardized by the global framework to achieve a balance between technical benefits and ecological safety.

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2.3. Future directions

For technological mechanisms, further clarification of the molecular marker differences between X and Y sperm is needed. For example, the regulatory mechanisms of the TRAF6/PI3K/GSK3α/β phosphorylation pathway in the TLR7/8 pathway on sperm energy metabolism should be explored, and separation parameters for different species (such as cattle, pigs, and sheep) should be optimized [3]. For efficiency improvement, it can develop microfluidic and nanoseparation technologies to increase separation speed to more than three times the current level and reduce equipment costs [3]. For ethical standards, it can establish a safety assessment system for gene editing technology to balance technological benefits with ecological risks [3, 7].

3. Conclusion

The current animal sex control technologies exhibit a coexistence of precision and limitations. Although flow cytometry shows mature and highly accurate (85%-95%), it is constrained by high costs and low efficiency, hindering its industrial application. Immunological methods have shown significant effects in ruminants, but the sorting mechanism for monogastric animals such as pigs still needs verification. Embryo screening relies on *in vitro* fertilization and PCR detection, which is complex and time-consuming. Gene editing technology, though efficient, is limited by ethical controversies and off-target risks. Among emerging technologies, microfluidic chips have increased sorting efficiency to 20,000 sperm per minute through label-free sorting, while nanomagnetic bead technology offers both high purity (89%) and low damage (survival rate of 92%), providing a new path for sex control in fragile sperm of pigs and other species. Future research should focus on three areas: deepening the study of molecular marker differences between X and Y sperm (such as the regulatory mechanism of the TRAF6/PI3K pathway) and optimizing sorting parameters for multiple species, promoting the integrated application of microfluidics, nanomagnetic beads, and AI technology to achieve breakthroughs in sorting efficiency and reduction in equipment costs, establishing a global ethical framework for gene editing and standardizing the assessment criteria for off-target effects to balance technological innovation and ecological safety. Through the synergy of multiple technologies and standardization, animal sex control technologies are expected to enhance the economic benefits of animal husbandry while moving towards a new stage of high efficiency, inclusiveness, and sustainability.

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