RESEARCH ARTICLE

Extraction process of livestock animal sex control semen

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Abstract: Sex control technology can speed up the breeding speed of good breeding animals, effectively release the reproductive potential of female animals, and provide female animals for dairy animals and female animals for meat. Animals provide the male livestock. Isolating male animal semen can not only improve the offspring quality but also the reproductive efficiency. Animal sex control semen separation, as an important part of animal genetic breeding, has an important role in the development of animal husbandry. This paper reviews the progress of flow cytometry semen separation and semen purity evaluation methods to provide a reference for the exploration of new methods.

Keywords: Flow cytometry; Sex control; Sperm separation; Purity and evaluation method

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1 Introduction

Animal sex control technology is called sexual control technology, which is a kind of artificial control of animal technology. The reproductive process of animals is mainly the efficient separation of X and Y sperm. Isolated sperm were used to fertilize the female animal and produce the desired sex.later generations. Not only does it help reduce the incidence of human sexual genetic diseases and we can save rare animals that are endangered, but more importantly, it allows dairy animals to produce more females and meat animals to produce more males to meet human needs. To meet the daily needs of the masses and promote the development of animal husbandry production. How to achieve this quickly and effectively in semen separation has become a hot topic of sex enhancement management technology. For semen isolation involves the development of separation methods or the optimization of methods. As a technical support, a rapid and effective semen evaluation system is indispensable. This review summarizes the progress of flow cytometry semen isolation and semen purity assessment. Methods to provide a reference for exploring new methods.

In 1925, Rush according on the differences in rabbit semen concentrations. In particular, centrifugation was used to study the sex of the rabbit offspring. After that, many studies were conducted based on the physical differences between X sperm and Y sperm. After some experiment and exploration, the expected result was not obtained, but it can be reproduced. Sex is bad. Experts identified specific proteins in cattle sperm to separate cow semen to obtain a percentage of male calves greater than 90%. In 1970, Barlow et al. [1] first reported the discovery of F bodies in Y sperm and concluded that the existence of F bodies was later confirmed because they exist in human and livestock sperm. Found only in the Y sperm. Li Xin et al. [2] cited the rabbit cardon electrophoresis method when semen and anodized semen was collected and insemination, most of the rabbits born were females. The female rabbits with cathodal sperm insemination were 74.3% and 15.1%, respectively. Expert 1:32 antibody serum injection into the vagina of estrous mother rabbit for 10-15 minutes. By natural mating with the

male rabbit, the two rabbits born will be female. Following with the continuous exploration and improvement of the researchers, the flow cytometry separation method was adopted. The production of sexual semen has entered into commercial production.

Flow cytometry Flow cytometry is possible to screen sperm by using differences in the DNA content of Y sperm. To perform an efficient separation. Studies have shown that the DNA of mammalian Y sperm contains less DNA than the X sperm and X sperm chromosome of cattle, horses, sheep, pigs and other animals. The DNA content was 3.8%, 4.1%, 4.2% and 3.6% [3]. First, the collected animal semen was filtered and diluted. Costained with a fluorescent dye. This color smoothly reaches the sperm lipid membrane and interacts with the double-stranded minor groove of the DNA. The AT-rich area. Because the amount of DNA varies between X sperm and Y sperm, X sperm and Y sperm. After semen separation, the X and Y sperm are stained with a fluorescent dye. When the device is illuminated by a UV laser, a blue excitation light will appear, that is, once the sperm is located by the device, the computer and the device will use the fluorescence from the semen to locate the sperm. Analysis to determine the difference in light intensity. If the droplet containing semen is illuminated by the laser, X semen (positive) and Y sperm (negative) are given different charges, the charged sperm uses the bias gradient plate (electric field) through a continuous current with a variety of electric charges. Sperm are deflected, and X sperm and Y sperm remain, making it difficult to distinguish between them.

In the 1960s, flow cytometry was a technique used to detect small differences in cellular DNA.open up before one's eyes. Experts began studying mice in 1982 and found that the difference in DNA content between mouse X sperm and Y sperm was now 3.2 percent. Initially initially initially until the cell counter is used to separate the sperm head. Improvements in modern flow cytometry go beyond isolating sperm. The head also improves the separation efficiency, which can effectively separate live sperm. Significant improvement. In 1991, the specialists achieved a successful separation. Sexual sperm of pigs produce 74% female piglets. Since then, semen from various animals has been isolated by flow cytometry to produce semen. The mother bird successfully produced the offspring. With the development of scientific research technology, researchers began to use spray instruments. Improve the nozzle, electric field and other systems, combined with voltage regulation, improve the separation effect rate will be significantly improved. Currently, novel improved flow cytometry isolation

The accuracy was significantly improved, and the accuracy of semen separation was kept at about 90%. Yes, more than 28 million different types of sperm can be separated per hour. XY has independently manufactured and improved the SX-MoFlo sperm separation device. The modified flow cytometer separates from 240,000 to 300,000 effective cells per minute.sperm. In 1997 to 1999,11 bulls by Seidel et al. Semen was separated by flow cytometry and the isolated semen was cryopreserved before use. Artificial insemination of cows showed that 83% of cows fertilized with X sperm gave birth to baby cows and 83% of cows fertilized with Y sperm. Rates of 90%, and higher conception rates in cows using frozen semen than in control cows. The 20% reduction in calf weight (calf) and calf health) was not significantly different from the control group. Study staff used flow cytometry to isolate Iberian red deer semen. Isoland isolated Y sperm for artificial insemination and bucks had 93% accuracy, while unsorted sperm had 55% accuracy for male calves. Some scholars are using microfluidic sperm separator (microfluidic sperm sorter, MFSS) to control sperm separation, sperm motility and line speed, allowing us to provide high quality products for in vitro fertilization. sperm. In the early 2000s, UK Cogent used flow cytometry. Separation technology for the production of animal semen and gender management after separation semen have been promoted to the commercial market. The development of semen separation technology in China started late, but it has developed rapidly. In recent years, some fields have been

unremitting and achieved remarkable results. Has reached the international top level. In 2002, Guangxi University conducted the first overseas experiment, flow cytometry and related equipment were introduced and established under the guidance of Professor Lu Huan. A research group was formed and studies on semen of buffalo sex was initiated. Specialists used flow cytometry to isolate the buffalo semen and use the isolated semen.

Buffalo semen was used for insemination, and the world's first external sex-controlled buffalo was established in Guangxi University. Because China is a big country of animal husbandry, the demand for sex semen is relatively high.big. From 2003 to 2005, overseas acquired related enterprises in the same industry. Several flow cytometers were installed, initially only to separate cattle semen, but later gradually expanded.

Nationwide commercial push for sexual semen in sheep and other animals has achieved significant success. Different grades. Zhao Xianlin et al. [4] studied the production process of sex semen in dairy cows. After improvement, sperm motility, acrosome integrity rate and cow conception rate were all improved. Improved, significantly increased effective sperm content of sex-specific semen. Sexual semen has great potential in the development of future varieties in China. The development of gender semen studies has led to large scale in farms where sexually control semen is used for reproduction. Despite some progress in animal semen separation, few studies target pigs. Pig semen differs from cattle or sheep semen because DNA varies between X and Y sperm. They are small and difficult to separate. In 2006, experts separated pig semen by flow cytometry and then by the fallopian tube method. Luchuan sows were inseminated, and one sow injected with Y sperm produced six piglets. Pigs, all piglets were male, with 100% sex accuracy, and X number of sperm were infused

There were three sows with litter sizes of 5,4, and 3. The accuracy was: 91.67%, and the sex of the piglets was consistent with the sperm separation results. In 2018, Fortunately, Hong Chao selected X and Y sperm from Saneng dairy goats and found that the most abundant sperm existed. The best staining solution was 34 μ L, percentage of effective staining area and X sperm. The ratios were 70.93% and 29.75%, respectively, which were superior to the staining solution dose 32. 36μL. Sun Fengjun et al. [5] found that bull sex control was practiced in Montberia.

2 How to evaluate the purity of gender semen

2.1 Evaluation method of birth sex determination

This is a method of determining the birth animal sex ratio for the following areas: It has been widely used in early clinical trials. Seidel et al used flow cytometry using instruments to separate bovine semen, and the isolated male and female semen were used to test experimental females. Cows are artificially inseminated, and the accuracy of X sperm and Y sperm fertilization varies. And 83% and 90%. Ding Weiliang [6] selected X sex semen from 597 dairy cows. After mating, a total of 579 were successful calving, including 46 male calves and 579 female calves. With 533 calves, the X sex semen accuracy rate was 92.06%. Although the assessment method is very reliable, the cycle is too long for cattle and takes 280 days.114 days for pigs, about 30 days for rabbits, and 148 days for sheep. The assessment method for sex fixation at birth is not useful for optimizing semen purity assessment methods.insemination also leads to semen damage or death, which can affect the accuracy of the test. To be precise, the sex of the upcoming child cannot be determined.

2.2 Embryo Sex identification and Evaluation Methods

The main methods of embryo sex identification and evaluation are as follows:

The sex of the embryo was determined by medicine, genetics, and biology. This identification method is considered one of the most accurate evaluation methods. More commonly used are methods for determining the sex of the embryo.

The embryonic molecular biology evaluation method is to detect the Y chromosome of embryonic cells, test whether there is a sex-specific gene SRY of the Y chromosome, thus affecting the accuracy of semen separation, if there

is a specific signal in the detection process, otherwise it is male. For women. The embryo molecular biology evaluation method has high sensitivity and high accuracy. The embryonic molecular biology evaluation method was used for embryonic cell fluorescence in situ hybridization and sex identification of PCR embryos. Fluorescent in situ hybridization of embryonic cells requires searching for specific gene DNA on the sex chromosome, labeling with a probe, and then in situ hybridization using specific DNA interacting with embryonic cells to calculate the sex of the embryo based on the hybridization results. Other ratios were used to calculate the accuracy of the semen separation. The PCR embryo sex assessment method requires designing primers for specific genes such as SRY based on the specific gene sequence, collecting embryo samples for PCR amplification, and analyzing PCR products. Gel electrophoresis was performed to determine embryo sex based on electrophoresis results and experts performed embryo studies of cows based on bovine SRY specific genes. Sex identification, a total of 17 embryos participated in the identification, identification accuracy reached up to 100%. PCR embryo sex evaluation method identification time compared its shorter length and relatively high accuracy. This method allows for the accurate isolation and purification of the semen. It has a great promotion value.

Embryonic Immunological evaluation Method The embryonic immunological evaluation method is to apply the distinction of H-Y monoclonal antibody or H-Y antiserum and embryonic cell immune response to determine the sex of the embryo, and to obtain sex control by counting the sex ratio of the embryo. Precision of semen. The embryonic immunological evaluation methods were divided into indirect fluorescence immunoassay and blastocyst formation inhibition methods. White et al. The accuracy of X and Y sperm was 89%. The accuracy rate of child insemination was 79%. Ramalho et al. For the inhibition of blastocyst formation using this production method, the accuracy of X sperm insemination was 81.82% and Y sperm insemination was 80%. Indirect fluorescence prevalence method is also defective in the identification of embryonic sex. Factors that may influence the identification accuracy; methods to inhibit blastocyst formation are more effective for slowly developing women. The embryos can easily lead to misjudgment and affect the experimental results. The experiments and the species differences of immunogenicity and H-Y antigen have a great effect on the results.

Some cells need to be removed from the embryonic trophoblast and treated with colcemids, which become fixed once mitosis reaches metaphase. Final staining, depending on the presence of the Y chromosome, using microscopic observation of the karyotype of the cell regardless of whether you estimate the accuracy of the semen, this method is at most 100% accurate. Monk et al. used the embryonic cytological detection and assessment method when we verified the sex control rate, the correct answer rate in male mice was 100%, the accuracy of the female rats was 91%. However, karyotyping is analytical and microdissection requires high operational skills. This will lead to an experimental failure.

2.3 Flow cytometry reanalysis and evaluation method, flow cytometry reanalysis method description

Flow cytometry was used to again separate the isolated semen and measure the semen. Fluid separation accuracy. Ultrasication of sperm must be performed before re-analysis. To improve sperm orientation efficiency, sperm tail and fluorescence. Sperm that needed to be reisolated were restained with a bright dye and reisolated. To check the accuracy of the sperm separation. Some researchers have used this method for sex semen from Gram boars by flow cytometry for sex semen X sperm purity 88%, motility 35%, Y sperm purity 83%, and motility 21%. However, this value is high if the difference in DNA content between species X and species Y sperm is less than 3%.

In this case, the separation becomes very difficult. Bovine current FACS is more difficult to separate semen in animals with relatively small differences in DNA content due to more usage. The larger the size, the more affected the separation accuracy is, and the more expensive the

flow cytometer is. Is much more expensive and difficult to construct in a typical laboratory.

2.4 Fluorescent in situ hybridization evaluation method is also known as fluorescence in situ hybridization evaluation method

The FISH evaluation method based on the principle of base complementation has the following requirements: the corresponding chromosome arrangement in the nucleus and the staining of non-radioactive fluorescent material use the specific nucleic acids of the fluorescent labeled chromosome to detect the chromosome sequence. Needle hybridization was used to effectively distinguish between X and Y sperm under a fluorescence microscope. Hamano et al. broke the tail of bovine semen and determined the purity of the liquid by FISH evaluation and determined the purity of Y sperm.

In vitro mature bovine oocytes were microinjected up to 82% and 80% of the progeny were males. Not only is the evaluation method accurate, but also the probes are stable and the reagents used are excellent. Although toxic, but the identification process is time-consuming, and the reagent requirements are relatively strict.

2.5 PCR evaluation method

The PCR evaluation method is highly enriched with target DNA as fragments, and weak sex-specific signals are amplified,

The amplified fragments were examined by gel electrophoresis to identify the sperm. Sex and the purity of the final sperm separation were assessed. Brien et al. [44] used flow cytometry using cell counting method and using single sperm PCR, X sperm purity was 89.7% and 79%. The researchers are using quantitative real-time PCR (quantitative real-time PCR) to verify the purity of bovine X and Y sperm as follows:

A yield curve for PCR amplification products can be generated by adding corresponding probes in the PCR reaction system, PCR reactions using fluorescent probes for PCR amplification and detection PCR computer processing according to each amplification cycle. The ratio of X sperm to Y sperm also can be inferred from the curve, as described. The purity of bovine semen was determined by real-time PCR by Expert et al. Furthermore, neither evaluation was significant compared to the flow cytometry reanalysis results.difference. Real-time quantitative PCR assessment methods have also proved to be highly accurate. However, this method of assessment requires special equipment. And the semen cannot be collected directly.

3 Looking forward

The application of flow cytometry separation method in the separation of animal X and Y sperm improves the semen separation efficiency and separation accuracy. Many companies use flow cytometry for the commercial production of animal sex control.semen. The gender separation of semen collected from excellent breeding animals has promoted the development of animal husbandry around the world. Despite its importance, flow cytometry is expensive and semen separation is difficult. The required techniques are also relatively complex, making the isolated seminal semen relatively expensive. Continuous improvement of flow cytometry, separation efficiency and cost gradually increase. It will be lower and lower, which is conducive to the large-scale promotion and application of sexual semen. At the same time, the gender semen purity evaluation method also has many defects, serious impact. Embryo identification method, flow cytometry reanalysis method and other popularization speed operation process is complex and high cost. With the progress of science and technology, with the development of subbiology, medicine and other related fields, flow cytometry separation of sperm has also been carried out. Improve semen accuracy, improve sexual semen vitality and pregnancy rate after separation. Improve the success rate and accuracy of various semen purity assessment methods.

Lay a good foundation and make contributions to promoting the development of animal husbandry.

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