—Mini Review—

Current Status and the Future of Bovine Embryo Transfer using Sex Determined Embryos

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Abstract: *The embryo transfer technology represents a powerful tool for the acceleration of various breeding programs in cattle. In cattle breeding, it is important to know the sex of embryos in order to improve the genetic potential of the herd. Normally, only five to six calves can be obtained during the reproductive life-time of a cow. However, calving rates can be increased by superovulation and embryo transfer program. If embryo sexing techniques are applied for the program, embryos with the preferred sex can be obtained from cows with high genetic performance. This is a potent way to improve the genetic background and production of animals. However, the reliability, cost, practicality and time required for this approach must be considered as well. The present paper discusses the current status and the future of bovine embryo transfer using sex determined embryos.*

Key words: *Bovine, Embryo transfer, Sexing*

Introduction

The embryo transfer technology represents a powerful tool for the acceleration of various breeding programs in cattle. Recently more than 20,000 calves a year are produced by embryo transfer in Japan. In cattle breeding, it is important to know the sex of embryos in order to improve the genetic potential of the herd. Normally, only five to six calves can be obtained during the reproductive life-time of a cow. However, calving rates can be increased by using superovulation and embryo transfer (MOET) program. If embryo sexing techniques are applied for the program, embryos with the preferred sex can be obtained from cows with high genetic performance. The possibility of increasing the production of a desired sex has important economic implication for dairy and beef industries. For instance, dairy farmers can produce a large number of replacement heifers to offset poor fertility resulting in high culling rates for lactating herds, and they can sell sex determined embryos with a premium value. Likewise, for beef cattle farmers, an efficient increase in the proportion of male calves relative to cohort females is economically favorable. However, the reliability, cost, practicality and time required for sexing of embryos may limit the economical means of this approach. In this paper the current status and the future of bovine embryo transfer using sex determined embryos are discussed.

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Sex Determination of Bovine Embryos using Biopsy Procedures

Developments in embryo micromanipulation techniques, have led to the development of embryo biopsy procedures which, in commercial embryo transfer programs, have allowed the direct genetic analysis of preimplantation bovine embryos [1]. Several methods have been developed for early sex determination including karyotyping [2, 3], detection of sex-specific male antigens [4], assays based on sex-related metabolic differences [5], in situ hybridization [6], polymerase chain reaction (PCR) [1, 7–10], and loop-mediated isothermal amplification (LAMP) [11].

Embryo sexing based on detection of Y chromosome-specific sequences has been used to predict the sex of the offspring [2]. Polymerase chain reaction enabling the amplification of a target sequence from a small number of blastomeres has also been used for sexing of embryo [1, 8–10]. However, these methods require high levels of technical skills and are time consuming. Moreover, PCR has a risk of false positives as a result of DNA contami-

nation during the handling of the PCR product in duplicate PCR procedures and/or electrophoresis. Therefore, to make embryo sexing become widely used in the bovine embryo transfer industry, a simple rapid and precise sexing method had to be developed.

A rapid bovine embryo sexing procedure employing LAMP has been reported by Hirayama *et al.* [11]. LAMP is based on the specific DNA amplification under isothermal conditions with high specificity, efficiency, and speed [12]. The LAMP reaction is carried out under isothermal conditions (ranging between 60–65°C) using DNA polymerase with strand displacement activity. This reaction requires four specific primers (inner and outer primer sets) that recognize six independent sequences, and specifically synthesize a large amount of amplification products, which are a mixture of stem-loop DNAs with various size and cauliflower-like structures with multiple loops. When the target DNA is amplified by LAMP, a white precipitate derived from magnesium pyrophosphate (a byproduct of the LAMP reaction), is observed. LAMP does not require special reagents or electrophoresis to detect the amplified DNA [13]. The time needed for sexing by this way is less than 1 h. LAMP-based embryo sexing is suitable for field application [11].

Embryo sexing procedure by biopsy is simple and highly accurate (more than 90%) even under farm conditions [9, 14]. The pregnancy rates after the transfer of fresh, biopsied embryos were comparable to those obtained by the transfer of fresh and intact embryos [8]. However, determination of embryo sex using biopsy is not always economically optimal. When all recovered embryos are biopsied, approximately 50% of the collected embryos are of undesired sex and these embryos are usually discarded. Also, when combined with cryopreservation, the survival rate after freezing/thawing and pregnancy rate of biopsied embryos are compromised compared to that of intact embryos [14]. Recently, several vitrification methods have been developed for the low-temperature preservation of cow embryos; some embryo transfer groups have reported good survival and pregnancy rates after transfer of vitrified-warmed biopsied embryos [15, 16]. However, most of these protocols require several steps for removal of the cryoprotectant before embryo transfer [15]. Moreover, vitrification of sex-determined embryos requires the specific processes of warming, dilution and non-surgical transfer into recipients which must be applied under farm conditions very similarly to those used for artificial insemination.

Embryos Produced using Sex Sorted Sperm

Technologies for sorting bovine sperm into X- and Y-bearing fractions have a major impact on breeding programs in dairy cattle and other livestock species. At present, the only company that has a technology successful for the separation of X and Y bearing sperm is XY, Inc., Fort Collins, CO. Sex-sorting technology of sperm is based on about 4% difference in DNA content between X-and Y-bearing bovine sperm. The high-speed cell sorting machine employed can separate more than 10 million X- or Y-sorted sperm per hour with more than 90% purity [17]. Due to success in cryopreservation of flow-cytometrically sorted spermatozoa, X- or Y-sorted sperm are currently commercially available from selected bulls for artificial insemination (AI). If female embryos are produced with X-sorted spermatozoa, or male embryos are produced with Y-sorted spermatozoa, practically all embryos are of desired sex and disadvantages of embryo biopsy can be avoided. Sexing of embryos has demonstrated that the use of sex sorted sperm can result in a high female (using X-sorted sperms) or male (using Y-sorted sperms) embryo ratio, thereby eliminating the need to sex embryos [18]. Furthermore, the transfer of intact embryos is advantageous because it eliminates reduced viability caused by biopsy, and time/labor for embryo sexing as well as the added costs associated with the risk of discarding undesired embryos [19].

However, the use of sex-sorted sperm in superovulation programs results in less effective outcomes, especially regarding the number of transferable embryos, compared to the results obtained with non-sorted sperm [19–22]. Several factors, such as the reduced lifespan of sex sorted sperm in the female reproductive tract, fewer numbers of sorted sperm/straw for AI, possible pre-capacitation induced by the sorting procedure can affect fertility and embryo production following AI [18–21, 23, 24].

Currently, the retrieval of oocytes through the use of ultrasound guided aspiration of cumulus oocyte complexes, or ovum pick-up (OPU), is inescapably linked to the procedures for *in vitro* embryo production, as it can exploit the most elite males and females to combine for an accelerated genetic gain [25]. OPU has been shown to be a valuable technology greatly enhancing the potential of *in vitro* production systems in a variety of breeding conditions [26–28]. *In vitro* fertilization requires a much lower number of spermatozoa than does AI, to produce a reasonable number of embryos [23]. Moreover, a higher efficiency will ultimately be reached because producing

offspring of the desired sex will be cost effective; nearly half the cost needed to produce animals using unsexed sperm will be expected. Recently a large-scale ET with vitrified bovine blastocysts produced by *in vitro* fertilization with sex-sorted semen achieved a 41% pregnancy rate, which did not different from the pregnancy results for *in vivo* derived embryos (53%) [29].

The Future of bovine Embryo Transfer using the Sex Determined Embryos

In vitro production (IVP) of sex determined embryos by *in vitro* fertilization (IVF) of oocytes collected by OPU with sex sorted sperm is expected to be a powerful tool for the improvement of animal production in cattle. Currently, a large number of embryos are produce by IVF using sex sorted sperm in dairy *bos indicus* cattle in Brazil [30]. However, in Japan, the Livestock Improvement Association of Japan, Inc. and Genetics Hokkaido Association are allowed to use the sex sorted sperm only for artificial insemination but not for IVF destined for practical reproduction by XY, Inc. Sex-sorted sperm purchased from these companies can be used for IVP of bovine embryos for research purpose only. At this moment, we can not use sex sorted sperm for the OPU and IVF to produce embryos in the field work. We hope that these companies will allow us to use sex sorted sperm for IVP of bovine embryos in the near future. However, the demand of sex determined embryos from dairy farmers and the beef cattle farmers is growing; therefore, the number of embryos collected from superovulation treated cows inseminated with sex sorted sperm should be improved. More studies are needed to optimize artificial insemination protocols using sex sorted sperm in MOET programs to improve embryo production, especially in cows. On the other hand, supplies of sex sorted sperm dose not satisfy the demand of dairy farmers and beef meet cattle farmers, and the number of bulls used for the production of sex sorted sperm is limited. Some desired bulls are not yet available for sex sorted sperm. In such cases, sexing techniques using biopsy are still important for the production of sex determined embryos. However, the technical developments of cryopreservation methods need further breakthroughs to overcome the existing barriers of sensitivity of cryopreserved embryos that had been manipulated. For example, to use sexed- and -vitrified embryos extensively on fields, facilities for warming of cryopreserved embryos, the removal of cryoprotectant and non-surgical embryo transfer to recipients must be prepared on farms similarly to those for artificial insemination.

Conclusion

The embryo transfer technology represents a powerful tool for the acceleration of various breeding programs in cattle. Dairy and beef cattle farmers demand the technology that can control the sex of calves in the embryos supply system. Therefore, supply of higher number of sex determined embryos will be required. The development of a system which can produce high quantities of sex determined embryos at low costs with high pregnancy rates is indispensable.

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