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Animal Biotechnology Roles in Livestock Production

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Abstract. Currently, meat and milk productions are significantly increasing especially in Asia. The supply of these products is vital to people's health and well-being, whereas the efficiency of beef production appears to be still lower than other meat productions. Improvements in the quality and functionality of their livestock products, as well as their production efficiency, are required for further production. Animal biotechnologies have contributed to genetic improvement, genetic diversity maintenance of domestic animals, etc. Basic animal biotechnologies, such as artificial insemination and embryo transfer, have been well established and applied as powerful tools for genetic improvement of livestock. In the applications of artificial insemination techniques, the use of sexed semen has been now widely spread, and also efforts are also made in the development of the technology using a small amount of sperm. For embryo transfer, several types of vitrification technologies have been applied to improve pregnancy rates and contributed to the international/domestic supply of livestock embryos. Conventional animal biotechnologies, such as in vitro fertilization and intracellular sperm injection, have been applied to not only livestock production and also human-assisted reproductive medicine. For in-vitro production of embryos in domestic animals, currently, oocytes have been collected from medium or large follicles (3-6 mm or larger in diameter) of ovaries. Although the oocytes derived from small follicles (less than 3 mm in diameter) exist more on the surface of ovaries, the developmental competence of the oocytes has been known to be significantly lower than those from medium follicles. If we could improve the competence of oocytes derived from small follicles significantly, we may be able to increase the number of female gamete resources for in vitro embryo production. Also, the development of techniques for producing transgenic and cloned animals has greatly contributed to the creation of pharmaceuticals and organs for xenotransplantation. Recently, furthermore, genome editing technologies, such as combined use of CRISPR/Cas9 and PiggyBac, have been developed and have made it possible to correct specific parts of the genome and introduce mutations by homologous recombination. In this review, I would like to discuss the application and progress of the above biotechnologies, including our recent research results.

1. Introduction

Animal production is the largest user of land in the world, through grazing or consumption of fodder and feed grains [1]. Currently, meat and milk productions, as well as livestock counts, are significantly increasing especially in Asia [2]. Since livestock production requires a significantly large amount of natural resources and is responsible for about 14.5% of total anthropogenic greenhouse gas emissions [3], improving the genetic ability and production efficiency is very



important for sustainable livestock production and should be friendly to the global environment. Although the supply of these products is vital to people's health and well-being, however, the efficiency of beef production appears to be still lower than other meat productions [2]. To produce 1 kg of meat, pork, poultry and milk productions require 6.4 kg, 3.3 kg and 0.7 kg of feed, respectively, while beef cattle require 25 kg [2]. Furthermore, improving fertility in dairy cows has been estimated to reduce methane emissions by 10-24% and nitrous oxide by 9-17% [3]. Improvements in the quality and functionality of their livestock products, as well as their production efficiency, are required for reducing anthropogenic greenhouse gas emissions and sustainable livestock production. Since animal biotechnologies have contributed to the genetic improvement, genetic diversity maintenance of domestic animals, etc., the application and progress of the above biotechnologies, including our recent research results are discussed in the current review.

2. Impact of basic animal technologies and the current innovation

Basic animal technologies, such as artificial insemination and embryo transfer, have contributed to improving livestock genetically as powerful tools. Especially, artificial insemination is the most powerful technique to provide the livestock industry for genetic improvement [4]. In Japan, the spread of artificial insemination and embryo transfer has boosted the milk yield of dairy cows by about 80% and 60%, respectively. Currently, timed artificial insemination, which is not required estrous detection, has already been very popular and its implementation rate is still gradually increasing [5]. The basic protocol of timed artificial insemination is the insertion of a progesterone or progestin-releasing device plus the intramuscular administration of estradiol at random days of the estrus cycle defined as day 0, device removal and intramuscular administration of prostaglandin, estradiol and equine chorionic gonadotropin on day 8 and timed artificial insemination 48 h later [5]. Various modifications have been challenged in the duration of treatment with progesterone or progestin devices and the timing of administration of prostaglandin, for example; two days before device removal. Furthermore, use of sexed semen sorted by flow cytometry for a small difference in DNA content between X-chromosome and Y-chromosome allows predetermination of calf's sex with close to 90% reliability and has benefits to both dairy and beef productions [6, 7], whereas usage of sexed semen for artificial insemination is much lower in the production of beef than dairy cattle. The conception rate appears to be still lower than the rate following artificial insemination with conventional semen [8-10] due to slower speed of sorting sperm as compared with the semen requirements of commercial dairy herds [11], although sexed semen has recently applied successfully not only to heifers but also lactating cows [12]. Therefore, the further breakthrough has been required in the preparation of sexed semen. In this year, one of Japanese group published an interesting scientific paper [13], which demonstrated that both Toll-like receptors 7 (localized in the tail) and 8 (localized to the midpiece), TLR7/8, coding the X chromosome were expressed in approximately 50% of the epididymal spermatozoa and that ligand activation of TLR7/8 selectively suppressed the mobility of the X chromosome-bearing sperm (X-sperm) but not the Y-sperm without altering sperm viability or acrosome formation. They reported that following in vitro fertilization using the ligand-selected high-mobility sperm, 90% of the embryos were XY male, whereas the TLR7/8-activated, slow mobility sperm produced embryos and pups that were 81% XX females [13].

Following artificial insemination, many spermatozoa are phagocytized [14] by polymorphonuclear leukocytes recruited into the cervix and uterus during proestrus and estrus [15]. In many species (including cattle and pigs), seminal plasma has been demonstrated to reduce phagocytotic ingestion of sperm in the female reproductive tract and in vitro [16-20]. Supplementation with seminal plasma at artificial insemination has been demonstrated to improve preimplantation embryo development [21]. Furthermore, supplementation of semen extender with caffeine and calcium chloride for artificial insemination of fresh and frozen-thawed spermatozoa has been demonstrated to reduce the recruitment of uterine polymorphonuclear leukocytes and the activity of phagocytosis [22-24]. Since hen's egg yolk protects sperm against

cold shock and preserves sperm functions and fertility following liquid or frozen preservation, the egg yolk has been supplemented in extenders for cryopreservation of boar spermatozoa [25] and directly deposited with sperm into the uterus [26]. We have demonstrated that the presence of egg yolk stimulates the phagocytotic activity of polymorphonuclear leukocytes in pigs but not in cattle [27]. Since sperm dosage of sexed semen for artificial insemination is usually relatively lower than that of conventional one, these treatments mentioned above may contribute to increasing the conception rate following timed artificial insemination with sexed semen.

Reproductive technologies for genetic improvements, such as multiple ovulation and embryo transfer (MOET) and juvenile in vitro fertilization and embryo transfer (JIVET) have contributed to accelerate genetic gain by increasing selection intensity and decreasing generation interval [28]. Especially in cattle, basic animal biotechnologies including embryo transfer, including superovulation, non-surgical embryo recovery, transfer, cryopreservation and in vitro production of embryos, have successfully developed, whereas improvement in cryopreservation of biopsied and in-vitro produced embryos will be required [29]. Techniques for embryo sexing based on detection Y chromosome-specific DNA sequence is currently the most reliable method [30]. As compared with PCR method, which has the risk of false positives due to DNA contamination during duplicate PCR procedures and/or electrophoresis, Loop-mediated isothermal amplification (LAMP) is a simple, rapid and novel DNA amplification method, which does not require special reagents or electrophoresis to detect the amplified DNA [30]. Recently, furthermore, non-surgical elongating conceptus transfer technique has also been developed and applied for sexing without specialized skills for biopsy [31].

3. Continuous development of conventional animal technologies

Conventional animal biotechnologies, such as in vitro fertilization and intracellular sperm injection, have been applied to not only livestock production and also human-assisted reproductive medicine. In cattle, in vivo oocyte collection by transvaginal ultrasound-guided follicle aspiration (ovum pick-up) and in vitro production of embryos are considered a reliable and cost-effective technique and have acquired a significant role in the breeding [32, 33]. In vitro maturation and fertilization technologies for embryo production in domestic animals have developed, the efficiency has been tried to improve by analyzing each condition during oocyte growth and maturation, fertilization and early development [32, 34-39]. In cattle, in vitro fertilization emerged as an alternative to superovulation and has become the technique of choice for embryo production, especially in Brazil [40]. In vitro embryo production overcame the main limitation of superovulation as a poor and inconsistent ovarian response to exogenous FSH stimulation commonly observed in most zebu breeds [41], the number of embryo production in vitro has been drastically increasing since 2002 in Brazil [40].

For in-vitro production of embryos, currently, oocytes have been collected from medium or large follicles (3-6 mm or larger in diameter) of ovaries in domestic animals [36, 42, 43]. Although the oocytes derived from small follicles (less than 3 mm in diameter) exist more on the surface of ovaries [44], both meiotic and developmental competences of the oocytes have been known to be significantly lower than those from medium follicles [45-47]. If we could improve the competence of oocytes derived from small follicles significantly, we may be able to increase the number of female gamete resources for in vitro embryo production. Levels of some cytoplasmic factors, such as transcript abundance of the MOS gene [47], cAMP and cGMP [48] or glutathione and metaphase-promoting factor [49] have also been demonstrated to differ between oocytes derived from small and medium follicles during culture for in vitro maturation. Recent research has demonstrated that the addition of factors secreted from not only oocytes, such as BMP15 and GDF9 [50-52], but also surrounding cumulus cells, such as vascular endothelial growth factor [53, 54], during in vitro maturation enhanced the meiotic and developmental competences of small follicle-derived oocytes.

Several types of vitrification technologies have recently been applied to improve pregnancy rates and contributed to the international/domestic supply of livestock embryos [55-58]. In birds, however, cryobanking of germplasm had been limited to the use of semen, due to the morphology of female gamete (very large egg), preventing conservation of the W chromosome and mitochondrial DNA. Recently manipulation techniques of primordial germ cells including purification, cryopreservation, depletion, long-term culture, and transplantation were developed in chickens, the concept of a poultry primordial germ cells-bank has been proposed [59]. These cut-in-edge animal biotechnologies will contribute to making a great breeding and sustainable new production system in the poultry industry.

4. Current breakthrough in animal technologies

Also, the development of techniques for producing transgenic and cloned animals has greatly contributed to the creation of pharmaceuticals and organs for xenotransplantation [60]. Recently, new efficient technologies to introduce foreign DNA or modify endogenous genes in oocytes, embryos and somatic cells, namely genome editing, such as use of CRISPR/Cas9 (to cut the target DNA at the region where we want) and PiggyBac transposon system (to transpose foreign DNA to the specific region), have been developed and has made it possible to correct specific parts of the genome and introduce mutations by homologous recombination [60, 61]. Since these technologies have been expected to reduce the risk in public health, such as preventing transmission of malaria and influenza [62] and have potential to functionally analyze new target molecules that could be used for therapeutic and vaccine purposes [63], genome editing technologies have been applied actively to studies on human disease, xenotransplantation of humanized organs, mainly in pigs [60, 64].

Furthermore, Japanese scientists greatly recently have made spermatozoa and oocytes from both embryonic stem cells and induced pluripotent stem cells, and they succeeded in producing pups [65, 66]. The *in vitro* generation of germ cells from embryonic stem cells in mice has also recently been succeeded [67]. In cattle, efficient derivation of embryonic stem cells derived from blastocyst has been reported [68]. In humans, oogonia have been produced from induced pluripotent stem cells [69]. These breakthroughs in reproductive technologies have proposed an idea to shift largely paradigm, namely “*in vitro* breeding”, that accelerates genetic improvement by shortening the generational interval [70].

5. Conclusion

Basic and conventional animal biotechnologies have developed to improve the efficiency and combined with those technologies to expand the application to not only animal production but also biomedical field. Furthermore, current new biotechnologies drastically make it possible to improve and create genetically domestic animals and quickly expand the utilization. To accelerate sustainable animal production, which is friendly with the current environment, further development and integration of cut-in-edge animal biotechnologies will be required.

6. Conflict of Interest

There is no conflict of interest with any financial organization regarding the materials discussed in this manuscript.

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