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Study on Embryo Transfer System for Production of Transgenic Pigs

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ABSTRACT

In the last 10 years, porcine somatic cell nuclear transfer to generate transgenic pig has been performed tremendous development with introduction and knockout of many genes. However, efficiency of porcine somatic cell nuclear transfer is still low and embryo transfer (ET) is one of important step for production efficiency. In porcine ET for production of transgenic cloned pig, we can consider many of points to increase production rates. In respect of seasonality and weather, porcine ET usually is not performed in summer and winter. Cloned transgenic embryos must be transferred into reproductive tracts of recipients where embryos are located after natural fertilization with similar estrous cycle. If cloned embryos with 2～4 cell stage are transferred, they must be transferred into oviducts in periovulatory stage. Number and deposition sites of transferred cloned embryos are important. And we must compare the methods of ET between surgical and non-surgical ones in respect of production efficiency. Sow recipients after natural estrus is most preferred recipients however its cost is must be considered. Here we will review many of current studies about porcine embryo transfer to increase production efficiency of transgenic pigs and strategies for further studies.

Key words: embryo transfer, porcine, recipient, somatic cell nuclear transfer

INTRODUCTION

Genetic modified transgenic pigs play an important role in biomedical and biological research (Vajta and Callesen 2012). Among many different techniques to make better transgenic pigs, embryo transfer is considered to be one of the most important techniques. However, the efficiency of transferred piglets is still less than 2% when it was measured as development to term as against the proportion of used oocytes (Koo *et al.,* 2010). To improve efficiency of porcine embryo transfer, this review organized many possible factors that can affect the success of embryo transfer in terms of porcine recipients.

Many studied showed that seasonality and temperature influence the result of pig cloning (Peltoniemi *et al.,* 2000). Although pigs are not considered as seasonal breeders, these studies show differences between different seasons. For example, spring and autumn have higher farrowing rate and litter size. In addition, winter has the lowest fertility among all seasons (Peltoniemi *et al.,* 1999). There is no significant difference between summer and autumn.

Also induced estrus of porcine recipients can affect the fertility of transgenic piglets. When transfer was performed on recipients that was in estrus 24 h later than the donors, fertility was the highest (Angel *et al.,* 2014; Wilde *et al.,* 1988). In addition, fertility gets lower when transfers are performed with more than 2 days of asynchrony (Geisert *et al.,* 1991). Accordingly, estrus synchronization and the timing of embryo transfer are important.

Total number of transferred embryos and site of embryo transfer may also affect the efficiency of embryo transfer. Accordingly, transfer of 60～120 embryos had better pregnancies and deliveries (62%) than the one (24%) of 40～60 embryos. Moreover, both site deposition transfer had a significant higher delivery rate (74% vs. 44%) and mean litter size (6.1 vs. 4.1) than the single site deposition transfer (Schmidt *et al.,* 2010). Therefore, site of embryos and the proper amount of embryos

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should be analyzed as well.

Embryo transfer has been applied in the pork industry and mainly surgical technique has been using since embryo transfer had invented (Martinez *et al.,* 2014). However, surgical technique has been limited, because they need the surgical procedures. Recently, many studied have been working on non-surgical technique and improving the efficiency of non-surgical ET (Yoshioka *et al.,* 2012). Accordingly, it may decide the results of non-surgical embryo transfer to deposit the embryos into deeper anterior location in the uterine horns (Li *et al.,* 1996, Martinez *et al.,* 2004), and volume of transfer medium can affect the result as well (Galvin *et al.,* 1994).

In this study, we will review many of current studies about porcine embryo transfer to increase the production efficiency for generation of transgenic pigs in terms of seasonality and weather when ET is performed, estrous cycles of recipients, number of transferred embryos and deposition sites in oviducts. Additionally, we will review that comparison of production efficiency between surgical vs. non-surgical ET methods and natural vs. induced estrus.

MATERIALS AND METHODS

1. Season and Weather

Seasonal infertility in a pig industry is generally considered as a potential financial loss to the pig industry. Not only the farrowing rate, but also the age of puberty in gilts and the litter size of piglets are influenced by season (Peltoniemi *et al.,* 1999). Accordingly, full-term pregnancies (FTP), which represents the number of surrogate sows giving birth to piglets, was the highest in May and the lowest in November (Love *et al.,* 1993). Furthermore, May, June, August and September are the most suitable months for embryo transfer (Huang *et al.,* 2013). Summer groups and autumn groups had typically no differences but the results were significantly different from the winter groups in terms of pregnancy rate (Koo *et al.,* 2010). This is because embryo development is vulnerable to the cold ambient temperatures and the loss of embryos during gestation was observed to be of a considerable percentage. Moreover, in a parthenogenesis experiment, blastocysts are made from oocytes more in spring than those in winter, though the rate of production of transgenic pigs were not different between spring and winter (Ma *et al.,* 2009). In addition, winter could influence the pregnancy rate and delivery rate during the surgery due to the cold temperature. Although litter size is still controversial, present studies indicated that litter size is indeed affected by seasons (Xue *et al.,* 1994). Litter size in spring months was the highest, while the smallest size was observed in late summer and early autumn. This is due to the fact that high temperatures can reduce milk production. Although there is a minimal variation, the seasons might also influence litter size as well. From the studies, it can be seen that weather and seasonal changes may influence milk production and the gestation period.

2. Ovulation Status

As mentioned above, the degree of synchrony between embryos in a donor and estrous cycle of recipient significantly influences pregnancy and birth outcome (production of piglets from *in vivo*). Therefore in order to ameliorate the efficiency of embryo transfer, proper ovulation status must be analyzed.

According to the research, 500 surrogates were analyzed to be divided into two groups. 267 recipients were in the pre-ovulation group and 233 were in the post-ovulation (AO). PO group had a higher pregnancy rate (36.3% vs 22.7%) and also delivery rate (9.4% vs 2.1%) than the AO group. Although mean litter sizes were similar, other rates were significantly higher (Koo *et al.,* 2010).

Generally, ovulation occurs within 38 hrs after estrus in pigs. Most of the recipients one day after estrus are in the PO stage, and most surrogates two days after estrus are in the AO stage, while ovulated oocytes become mature at the metaphase 2 stage. Oocytes *in vitro* at the metaphase 2 were used in this experiment. It means AO should be more suitable for being transferred than at the PO stage. Ironically, some studies in the present showed that the PO stage had better results than in the AO stage.

Martinez's research explained that in the estrous stage of uterine, it is not compatible with the needs of the embryos in the earlier stages, and embryos grow slower than their *in vivo* produced (Martinez *et al.,* 2007; Youngs 2001). Thus PO stage yielded better results. As explained, synchronization one day early seems to be suitable in embryo transfer. In other words, PO stage is more suitable for recipients to conduct embryo transfer.

3. Number of Embryos and Site of Deposition

To improve the results, we should focus on the embryo

transfer as well as the small surgical methods. This is why either one-side deposition or both-side deposition should be analyzed and it is necessarily considered why both-side deposition is applied in a surgical method.

Schmidt's experiment showed production of pigs with embryo transfer is improved by the total numbers of embryo transferred and site of the deposition. In experiment 1 of Schmidt, 46 surrogates received 40～60 embryos, and 39 recipients received 60～120 embryos. Transfer of 60～120 embryos had better pregnancy and delivery (62%) than the one (24%) of 40～60 embryos. Moreover the mean litter size was much higher (5.1) in transfer of 60～120 embryos than the one (6.0) in transfer 0f 40～60 embryos. In experiment 2 of Schmidt, the site of transferred embryos was in two groups, as 59 of recipients embryos were transferred to one of the uterine horns and 26 recipients embryos were transferred to both of uterine horns. The both-side deposition transfer had higher delivery rates (74% vs. 44%) and mean litter size (6.1±0.7 vs. 4.2±0.6) than the other one (Schmidt *et al.,* 2010). This study explained that the pregnancy rate and the mean litter size are correlated to the number of transferred embryos as well as the site of the deposition. It is necessarily performed with a larger amount of transferred embryos in the both-side of the deposition.

4. Surgical vs. Non-Surgical Methods

In the pork industry, embryo transfer has been applied and has been the main surgical technique used since its invention (Martinez *et al.,* 2014). However, surgical techniques have always been limited, because of the need to learn surgical procedures. Due to the complicated nature of surgeries, there are a lot of studies currently been done on using non-surgical techniques and have showed success of piglet production. Despite this, the efficiency of the techniques has been low compared to those of surgical techniques.

In order to improve the efficiency of non-surgical techniques, there are factors we should consider. Actually the previous study indicated that it is effective to use the flexible double-walled polythene catheter to induce more favorable results (pregnancy following non-surgical egg transfer in pigs). The factors that need to be organized are (1) the embryo deposition site and (2) the volume of transfer medium.

According to the studies, the non-surgical embryo transfer was to deposit the embryos into a deeper anterior location in the uterine horns (Li *et al.,* 1996; Martinez *et al.,* 2004). This is the main factor in the success of non-surgical embryo transfer. Flexible catheter, which is devised for the non-surgical embryo transfer, makes deposition a few inches deeper.

Galvin's study showed how adjusting the volume of transfer medium can be suitable to improve efficiency. First up, they divided into 3 groups with a different medium volume (1.6 ml, 3.2 ml, 10 ml). The results interestingly showed that a smaller volume (1.6 ml) had the most efficient result. First group (1.6 ml) had a higher pregnancy rate (80%) than the second (75%) and the third (25%). The abortion rates were 20%, 75% and 25%, respectively. These showed that a large volume does not to be physiological and it can makes the flow back from the vagina (Galvin *et al.,* 1994).

5. Natural vs. Induced Estrus

Reproductive management in the pig industry optimizes convenience and profits. Also batch-farrowing management of pigs creates a more convenient process in management and for pigs to match and farrow. Batch-farrowing management research demonstrated that approximately 90% of pigs come into estrus within 8 days after weaning and was the most effective way to make estrous synchronization (Blitek *et al.,* 2010). However, it is difficult for farms to synchronize pigs with the estrus period, especially for small farms that have fewer pigs. As we mentioned, natural selection is the most effective way to select the pigs on estrus. However, this is hard to apply on smaller farms. Therefore a lot of studied have conducted on drug-induced estrous synchronization (Ziecik *et al.,* 2005).

Estienne's study conducted an experiment to compare the drug-induced estrus with natural selection (Estienne *et al.,* 2001). In the experiment, All cycling gilts received the progestin with 15 mg/d for 18 d. After 24 hrs, they received an injection of 400 I.U. PMSG and 200 I.U hCG. Interestingly, ovulation rate was greater in the pigs with P.G. The resulting rate was 28.8% in pigs with P.G, equivalent to the natural selection rate.

CONCLUSION

This review study supports previous reports on the efficiency of embryo transfer recipients affecting the results of embryo transfer in its entirety.

As previous research showed, seasonality and weather influences the effectiveness of embryo transfer in terms of recipients. Between spring, summer and autumn, there is no visible dra-

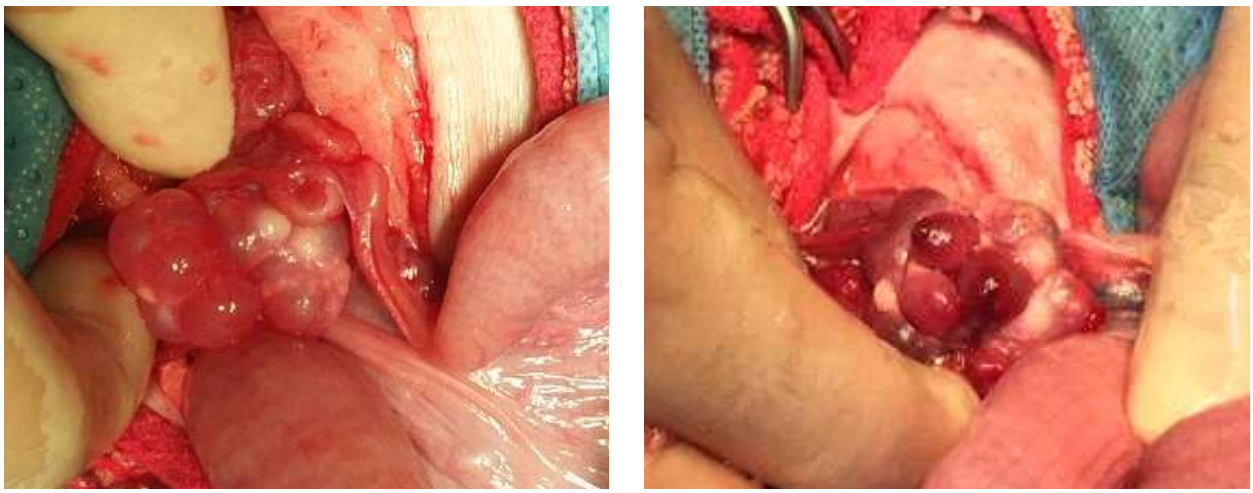

Fig. 1. Ovary at post-ovulation stage in A and at after-ovulation stage in B. Both were received different days after progesterone injection. There are a lot of full follicles in A. It is obviously seen at the stage of post ovutation under estrous hormones. In the picture of B, a number of corpus luteum is seen. It means the ovary in B is under progesterone hormones, which is stimulated by a corpus luteum.

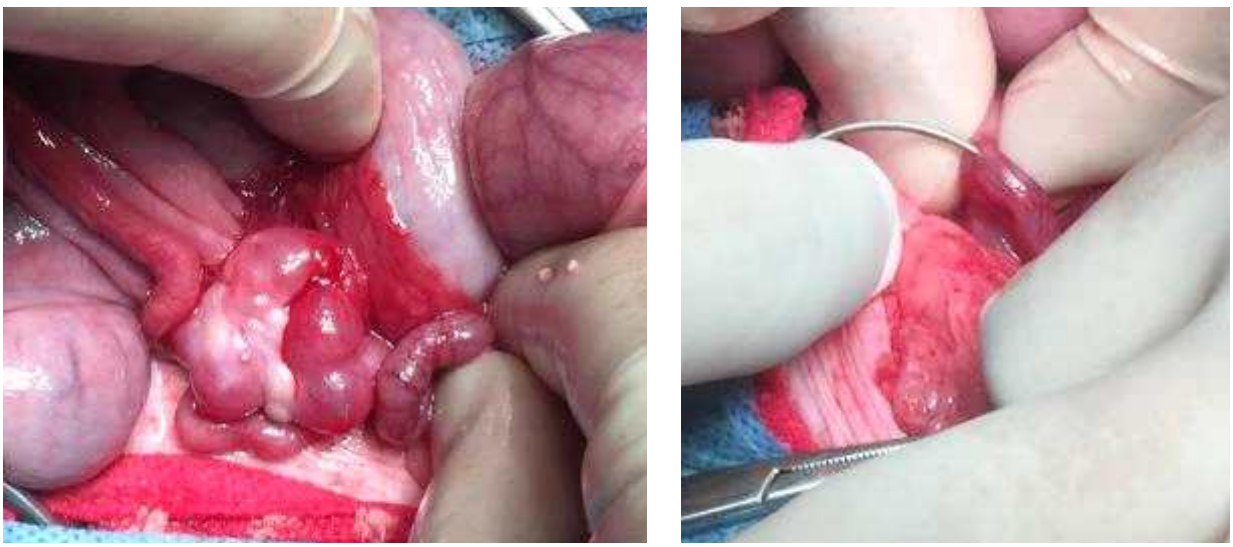

Fig. 2. Ampulla of oviduct with ovary in A. Surgical hole in ampulla of oviduct in B. In the picture of A, There is ampulla of oviduct where the embryos are injected. In B, the process is making a hole and the embryos will be injected.

matic difference. However, winter noticeably has less pregnancy rates and delivery rates when compared to other seasons. It is considered that there should be a cold shock applied on recipients and transferred embryos. Weather may also affect the litter mean size. Specifically, late summer and early autumn had the lightest size of piglets. High temperatures may influence the reduction of milk and result in the small size of piglets. To proceed, following studies should discuss ways to decrease the damage embryos sustain and improve the effectiveness of the embryo transfer during late autumn and winter. This study also indicated that recipients in the PO stage have better developmental results. Results showed *in vitro* environment is significantly different from the recipients. This results in different data in the embryos environment, and PO stage has better results. As many studies have supported this fact, it is recommended to transfer embryos for recipients during the PO stage. One research showed that both-side deposition transfer has better results than the one-side transfer. The data showed

that both-side deposition transfer had significantly higher results (74% vs, 44%). This study also showed as embryos transfer increased, delivery rates rose. To clarify, 60～120 embryos resulted in higher delivery rates than 40～60 embryos (62% vs 24%). To improve the efficiency, these factors carefully analyzed. For almost a decade, many researchers have worked on non-surgical techniques to improve shortcomings within the field. In one instance, flexible catheters are now used to put the semen into the deep uterine horns. Also suitable volume of transfer medium was discussed. This study showed that a smaller volume (1.6 ml) had the most efficient results. This suggests that a high volume of semen can make it flow back to the vagina. Therefore, smaller volumes are suitable for embryo transfer.

In conclusion, there are a lot of factors that can improve the results of embryo transfer and the whole pig industry, including the topics discussed. Although currently there are many studies being conducted on the subject, continued research on embryo transfer is needed for further improvement.

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