学位論文内容の要旨

博士の専攻分野名称：博士（農学）　　氏　名：Weihong Fan

学位論文題名

Study on the role of zona pellucida in pre- and post-implantation development of mouse embryos

(マウス胚の着床前後の発生における透明帯の役割に関する研究)

The zona pellucida (ZP) plays various roles in embryonic development both *in vivo* and *in vitro*. The use of assisted zona hatching in *in vitro* production is widely used. Recently, the zona pellucida removal (ZPR) procedure is also applied at the early embryonic cleavage stage and is mostly used for blastomere separation for producing identical multiplets such as twins and quadruplets combined with genomic analysis, somatic cell nuclear transfer clones, chimeric animals, and improving the cytoplasmic fragmentation embryos. Despite the need for ZPR for reproductive research, little is known about the role of ZP on pre-implantation development, differentiation as well as post-implantation development. In this study, I investigated the role of ZP in the pre- and post-implantation development of mouse embryos.

1. Establishment of the optimized *in vitro* culture system for zona free mouse embryos

In this chapter, I investigated the optimized *in vitro* culture system for zona free mouse embryos by comparing the ordinary flat culture, commercially available Well of Well (WOW) individual culture well system used for human and bovine embryos, and customized WOW with deep and small well suitable for mouse embryos with small size (cWOW). The cWOW system significantly improved the compaction process and blastocyst rate compared with the microdroplets system and commercial WOW *in vitro*. These results suggest the suitable well size for embryo size could be an important factor to keep the 3-dimensional embryo structure for further development and differentiation.

2. Effect of ZPR on the pre-implantation development and gene expression of mouse embryos

In this chapter, I investigated the effect of ZPR on pre-implantation development and gene expression. ZF embryos showed a faster compaction speed than control embryos. Expression analysis of inner cell mass (ICM)- and trophectoderm (TE)- specific genes has revealed that the expression of ICM-related genes was increased both in ZF-morula and blastocyst, whereas expression of TE-related genes was decreased by ZPR. An increase in ICM- specific protein

(OCT4) and decrease in TE-specific protein (CDX2) were consistent with gene expression in ZF embryos.

3. Effect of ZPR on the post-implantation development

In this chapter, I investigated the effect of ZPR on post-implantation development. After embryo transfer, a lower rate of implantation and a lower number of live fetuses were observed in ZF embryos than in ZI embryos. There was no difference in fetal weight at birth. However, the placental weight of ZF embryos was significantly increased than ZI embryos. RNA-seq analysis of the placenta showed a total of 473 differentially expressed genes that are associated with biological processes. Overall results suggest that ZPR affects post-implantation development.

4. Effect of ZPR on the blastomere allocation at the early stage and subsequent development and gene expression

In this chapter, I investigated the effect of blastomere structure by the number of contact points at the early stage (4-cell) embryos on the subsequent development and gene expression of the differentiation regulatory factor gene *Carm1* after ZPR. Blastocyst rate and total cell number were significantly decreased in ZF 4-cell embryos with low blastomere contact points. Both gene and protein expression of Carm1 was rapidly and significantly increased by ZF. Besides, the expression of DNA methylation-related genes was also significantly decreased in the ZF embryos than in ZI embryos. Overall results suggest ZPR affects the morphological changes of early 4-cell and development blastocyst as well as potential epigenetic modification.

5. Establishment and evaluation of a new ZPR method for the development of ZF embryos

Although acid Tyrode's solution (ATS) and proteinase K (PK) are widely used for ZPR, there is a concern about the possible toxicity for embryos. In this chapter, I developed a less toxic protocol for ZPR by combining two treatments (two-step ZPR protocol) and evaluated the subsequent development and embryo quality. This two-step method significantly reduced ZPR time and improved blastocyst rate by increasing the total cell number and reducing the apoptotic cells compared to ATS and PK alone. Overall, this two-step ZPR protocol is beneficial for assisted reproductive technology in mammalian embryos.

In conclusion, I elucidated the role of zona pellucida not only keeps the blastomere structure for development, but also affects the gene expression and differentiation of pre- and post-implantation development. Besides, new methods for quick and less harmful zona removal and culture systems suitable for mouse ZF embryos will contribute to the development of assisted reproductive technology.