Establishment of an *in vitro* bovine model for exploring factors involved in embryo attachment to the mother endometrium

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Abstract

Fertilization rate in dairy cows after insemination is high. However, pregnancy losses percentages in the early stages, even before it can be detected, is a numerous and cause considerable economic damage. It is estimated that early pregnancy loss may occur due to abnormal processes at the physiological, endocrine, paracrine, cellular or molecular levels durung fetal development, embryo attachment, endometrial receptivity and selectivity, embryo-uterine connection, or placental development and function. The processes of embryo attachment and implantation to the endometrium are complex and include well-timed extracellular and molecular procedures. Therefore, conducting research to reveal the molecular events and mechanisms of those processes in vivo since, although the implantation process is an autonomous conserved process - there are many differences in the mechanism of implantation between different species. There are several models for in-vitro implantation research in humans and mice, and there is also an in vivo model for studying embryo implantation in mice, yet, since the process of embryo implantation in ruminants is completely diverse from that in mice and humans, those models cannot be used to explore embryo implantation in livestock. The aim of the study was to develop an in vitro model for embryo attachment and implantation to the endometrial cells in farm animals. For this purpose cows uteri were collected from Slaughterhouse. We have successfully established protocols for isolating, freezing and thawing as well as generating primary endometrial cells culture from those uteri. Using anti-vimentin and anti- cytokeratin specific antibodies we have verified that the cells we have isolated and grown are endometrial cells. Moreover, by using lanti-viruses

encoding GFP (green fluorescent protein) gene, we have also shown that it is possible to genetically modify these endometrial cells, which may be most useful in examining their function in embryonic implantation in future studies. Attempts were also made to grow spheroids from the CT-1 cell line, cells formed from the trophoblast cells of bovine blastocysts from day 11th of pregnancy. Unfortunately, we were unable to thaw those cells and grow spheroids to examine their adhesion to the endometrial cells. Next, we have tried to wash embryos from heifers underwent superovulation treatment. Although the ovaries responded well to the hormonal treatment, we could not collect any embryo. Thus, we could not examine bovine embryos ability to attach to endometrial cells in vitro. Hopefully, the data and experience gained in this study will contribute in the future to develop an efficient in vitro model for embryo attachment using either spheroids or real embryos.