Temporal interplay between the genes that control interferon tau expression in early *in vitro* fertilization- and nuclear transfer-derived bovine embryos

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ABSTRACT

In the current work, the embryonic development and the temporal behavioral interaction of the genes involved in IFNτ gene expression and how they behave in an orchestrated manner to increase the developmental competence of IVF and NT produced embryos were investigated. Behavior of genes included ETS2, CDX2, GATA2, GATA3, OCT4 and NANOG was analyzed in early bovine IVF produced embryos, (from compact morulae to the blastocyst hatching stages), by semi- and relative quantitative PCR and compared between two *in vitro* culture (IVC) systems, two-step chemically defined medium and modified synthetic oviductal fluid (mSOF) containing 8 mg/mL, BSA.

Early embryonic development was found to be better in two-step chemically defined culture system than that of mSOF as indicated by the increment of blastocyst yield, 33.1% in two-step

culture system vs. 18.8% in mSOF medium, and the blastocyst hatching, 52.3% in two-step culture system vs. 33.5% in mSOF medium. Relative quantitative gene expressions showed harmonic behavior in the two-step culture system rather than the culture in mSOF, IFNτ showed even increase throughout the embryonic development in the two-step culture medium while it decreased with blastocyst hatching in mSOF culture condition.

Temporal dominance of OCT4 over all the transcription factors was found in regulation of IFNτ expression (the major factor of expression regulation but in inverse manner). However, ETS2, CDX2, GATA2 and GATA3 are potent IFNτ stimulator in cumulative manner but in case of OCT-4 decrement. CDX2 directly related with IFNτ, but still under OCT4 dominance and also regulated by the subservient of OCT4 which is NANOG.

These findings confirmed the usefulness of using the two-step chemically defined culture medium for increasing the developmental competence of IVF produced embryos and elucidated the dominance of OCT4 over the other genes implicated in regulation of IFNτ expression.

Moreover, bovine trophoblast cells (BTs) from IVM/IVF oocytes and *in vitro* produced blastocysts, were cultured, isolated and used them, for the first time, as donor cells for nuclear transfer and compared them with adult fibroblasts (AFs) as donor cells. BTs were reprogrammed in enucleated oocytes to blastocysts with similar efficiency to AFs (14.5% and 15.6% respectively, $P \leq 0.05$). The levels of IFNτ, CDX2 and OCT4 expression in IVF-, BT- and AF-derived blastocysts were analyzed using reverse transcription polymerase chain reaction and reverse transcription quantitative polymerase chain reaction (RT-PCR and RT-qPCR). IVF-

produced embryos were used as reference to analyze the linear progressive expression of IFNτ through mid, expanded and hatching blastocysts.

RT-PCR and RT-qPCR studies showed that IFNτ expression was higher in BT-derived blastocysts than IVF- and AF-derived blastocysts. Both IVF- and BT-derived blastocysts showed a progressive increase in IFNτ expression as blastocyst development advanced when it compared with AF-derived blastocysts. OCT4 was inversely related with IFNτ expression, while CDX2 was found to be directly related with IFNτ temporal expression. Persistence of high expression of IFNτ and CDX2 was found to be higher in BT-derived embryos than in IVF- or AF-derived embryos. These results could be a useful tool for understanding the IFNτ genetics and epigenetics.

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Key Words: Cow, somatic cell nuclear transfer, trophoblast cell, gene expression, implantation, Interferon-τ.

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GENERAL CONCLUSION

In the current thesis the temporal interaction among the genes that control IFNT was studied using different *in vitro* culture media and different donor cells for nuclear transfer (NT), including the IFNτ-secreting cell, the trophoblast.

The importance and the efficiency of using two-step chemically defined media was confirmed as a good tool for bovine embryos *in vitro* culture system because of; 1) Increased blastocyst yield, 2) Increased blastocyst cell number, 3) Increased blastocyst hatching rate, 4) harmonic and orchestrated gene interplay reflected by progressive increase in IFNτ expression which is a good sign for blastocyst developmental competence. 5) the dominance of OCT4 over all the other transcripts in regulation of IFNτ expression and subsequent IFNτ secretion.

The current study included the first attempt to isolate bovine trophoblast on a cheap and easily recovered feeder cells which are porcine granulosa cells instead of the conventional method which used mouse embryonic fibroblast. Morever, it showed the first attempt also to use bovine trophoblast as donor cells for nuclear transfer and produced *in vitro* competent embryos.

The temporal expression of IFNT showed a cumulative pattern in the embryos derived from triophoblast as donor cells, that can be a useful method for further studying IFNT epigenetics.

Using of trophoblast cells in NT could be one resource for further understanding of the interaction between OCT4 and CDX2 genes in the regulation of IFNT expression and trophoblast physiology.