INFLUENCE OF SOME MOLECULAR MARKERS OF OOCYTE COMPETENCE ON REGULATION OF EARLY EMBRYONIC DEVELOPMENT IN BOVINE

Presented by

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Abstract

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Abstract

Influence of Some Molecular Markers of Oocyte Competence on Regulation of Early Embryonic Development in Bovine. Mohamed Ashry, Cairo University, Faculty of Veterinary Medicine. Ph.D. Thesis in Theriogenology, 2015.

Brilliant cresyl blue (BCB) is a super vital stain that has been used to select competent oocytes in different species. The objectives of the first part of the present studies were to determine mRNA abundance for select TGFβ superfamily components, SMAD2/3 and SMAD1/5 phosphorylation levels and transcript abundance for other oocyte (JY1) and cumulus cell (CTSB, CTSK, CTSS and CTSZ) markers of oocyte quality in bovine oocytes and or adjacent cumulus cells classified based on developmental potential using BCB staining. The ability of exogenous FST, JY1, or cathepsin inhibitor treatment to rescue development of embryos derived from poor quality oocytes selected based on BCB staining was also determined. Cumulus oocyte complexes (COCs) from abattoir derived ovaries were subjected to BCB staining and GV stage oocytes and cumulus cells harvested from control, BCB+ and BCB- (poor oocyte quality) groups for real time PCR or Western blot analysis. Remaining COCs underwent in vitro maturation, in vitro fertilization and embryo culture in presence or absence of above described treatments. Levels of FST, JY1, BMP15 and SMAD1, 2, 3 and 5 transcripts were higher in BCB+ oocytes whereas abundance of CTSB, CTSK, CTSS and CTSZ mRNAs was higher in cumulus cells surrounding poor quality BCB- oocytes. Western blot analysis revealed SMAD1/5 and SMAD2/3 phosphorylation were higher in BCB+ than BCB- oocytes. Embryo culture studies demonstrated that follistatin and cathepsin inhibitor treatment but not JY-1 treatment can rescue developmental competence of BCB- oocytes. Results provide further understanding of molecular indices of oocyte competence. The focus of the second part of the present studies was d to elucidate the regulatory role of protein kinase B "AKT" in oocytes and embryo competence and potential link to the embryotrophic actions of FST. The objectives of these studies were to determine the relationship between *AKT* transcript abundance/signaling activity and oocyte competence determined by BCB staining, and characterize the temporal changes in *AKT* mRNA during oocyte maturation and early embryogenesis *in vitro*. Effects of AKT inhibition on early embryonic progression and effects of follistatin supplementation on developmental capacity of AKT inhibitor treated embryos and signaling activity of AKT and its downstream targets were also analyzed. *In vitro* embryo production model was utilized to study the effect of AKT inhibition and FST supplementation on early embryos, qRT-PCR and Western blot were used for analysis of mRNA transcript abundance and signaling activity of investigated pathways respectively. Both *AKT* mRNA and phosphorylation level were higher in BCB+ than BCB- oocytes. Abundance of mRNA for *AKT* was increased in pronuclear through 8-cell stage embryos relative to GV stage oocytes, then decreased at 16-cell stage and further decreased in morula and blastocyst stage embryos. AKT inhibition during the initial 72 h of embryo culture blocks early cleavage, reduces total cleavage, 8-16 cell stage and blastocyst formation rate. FST supplementation partly rescues the effects of AKT inhibition but didn't affect the phosphorylation level of AKT despite the significant increase in p-AKT at 1 and 10 h after FST supplementation. Results suggest a positive relationship between *AKT* transcript abundance/ signaling activity and oocyte competence determined by BCB staining. Results also demonstrate a temporal regulation of *AKT* mRNA abundance during early embryogenesis and indicate that embryotrophic actions of exogenous FST may be mediated, at least in part, by modulation of AKT signaling pathway. Further studies are required to elucidate the functional role of AKT and mechanism of action of FST in regulation of early embryonic development in bovine.

Key words: bovine, oocyte competence, Brilliant cresyl blue, gene expression, Western blotting.

Publications derived from the dissertation

1. **Ashry, M., Rajput, S. K., Folger, J. K., Knott, J. G., Hemeida, N. A. and Smith, G. W. (2014).** Regulation and potential regulatory role of AKT in bovine oocyte competence. In: *47th Annual Meeting of Society for the Study of Reproduction*. Michigan, USA, 19–23 July, 2014.

2. **Ashry, M. and Smith, G. W. (2015).** Application of embryo transfer using in vitro produced embryos: Intrinsic factors affecting efficiency. *Cattle Practice*, 23(1):1-8

3. **Ashry, M., Lee, K. B., Mondal, M., Datta, T., Folger, J. K., Rajput, S. K., Zhang, K., Hemeida, N. A. and Smith, G. W. (2015).** Expression of TGF β superfamily components and other markers of oocyte quality in oocytes selected by brilliant cresyl blue staining: Relevance to early embryonic development. *Molecular Reproduction and Development*. published online 20 Feb 2015

4. **Ashry, M., Rajput, S. K., Folger, J. K., Knott, J. G., Hemeida, N. A., Kandil, O. M. T., Ragab, R. S. A. and Smith, G. W. (2015).** Evidence support a potential role for the AKT signaling pathway in mediating embryotrophic actions of follistatin on bovine early embryonic development. In: *48th Annual Meeting of Society for the Study of Reproduction*. San Juan, Puerto Rico, USA, 18–22 June 2015

Dedication

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Mohamed Ashry

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