



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

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Abstract

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Thesis Title: Influence of Some Molecular Markers of Oocyte Competence on Regulation of Early Embryonic Development in Bovine

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 TGF β 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 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

I would like to dedicate this humble dissertation with lots of love and respect to my father, my mother, my wife and my children Ahmed and Marwan. Without their support, love and care, I would not have realized my dreams in life.

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CONTENTS

Topics	Pages
Abstract	I
Publications	II
Dedication	III
Acknowledgements	IV
Table of Contents	VI
List of Tables	X
List of Figures	XI
Introduction	1
Literature Review	
1. Application of Embryo Transfer Using <i>in Vitro</i> Produced Embryos: Intrinsic Factors Affecting Efficiency	5
1.1.Applications of embryo transfer using <i>in vitro</i> produced embryos	8
1.2.Factors that limit efficiency of <i>in vitro</i> embryo production	12
2. Oocyte developmental competence	21
2.1.Morphological indicators of oocyte competence	22
2.2.Molecular indicators of oocyte competence	24
2.3.Biological indicators of oocyte competence	34
2.4.Biochemical indicator of oocyte competence	35
2.5.Factors affecting oocyte developmental competence	41
Materials and General Methods	
1. <i>In vitro</i> embryo production	49
2. Brilliant cresyl blue (BCB) staining	52

3. Gene expression	53
4. Western blot analysis	55
5. Statistical analysis	58
6. Software	59
Part I: Analysis of Putative Bovine Oocyte Quality Markers Using BCB Staining Model	
Specific objectives	61
Methods	62
I.1. Quantification of <i>FST</i> , <i>JY-1</i> , <i>CTSB</i> , <i>CTSS</i> , <i>CTSZ</i> , <i>GDF9</i> , <i>BMP15</i> , and <i>SMAD1</i> , 2, 3 and 5 in BCB screened GV stage oocytes.	62
I.2. Quantification of <i>FST</i> , <i>CTSB</i> , <i>CTSK</i> , <i>CTSS</i> and <i>CTSZ</i> in the cumulus cells harvested from GV stage oocytes screened with BCB.	62
I.3. Expression and phosphorylation level of SMAD proteins in BCB screened oocytes	62
I.4. Effects of exogenous follistatin and JY-1 supplementation on indices of embryo developmental progression observed for embryos derived from good and poor quality oocytes based on BCB staining.	63
I.5. Effects of cathepsin inhibitor treatment during meiotic maturation on developmental capacity of <i>in vitro</i> fertilized embryos derived from control oocytes and oocytes classified based on BCB staining.	63
Results	64
I.1. Specific oocyte and cumulus expressed transcripts are associated with higher developmental competence of bovine oocyte	64
I.2. Cumulus cell expression of <i>FST</i> and oocyte expression of <i>CTSB</i> , <i>CTSS</i> , and <i>CTSZ</i> : relationship with BCB staining and oocyte quality.	67
I.3. A possible link between SMAD signaling pathway and oocyte competence	69

I.4. Effects of exogenous follistatin supplementation on indices of embryo developmental progression observed for embryos derived from good and poor quality oocytes based on BCB staining.	73
I.5. Effects of exogenous JY-1 treatment during initial 72 h of <i>in vitro</i> embryo culture on developmental capacity of bovine embryos derived from BCB screened and control oocytes.	77
I.6. Effects of cathepsin inhibitor treatment during meiotic maturation on developmental capacity of <i>in vitro</i> fertilized embryos derived from control oocytes and oocytes classified based on BCB staining	80
Discussion	83
Part II: Regulation and Potential Regulatory Role of AKT in Bovine Oocyte Competence	
Specific objectives	91
Methods	92
II.1. Characterization of <i>AKT</i> transcript abundance and signaling activity in oocytes with different developmental potential determined by Brilliant Cresyl Blue staining.	92
II.2. Temporal regulation of <i>AKT</i> mRNA during oocyte maturation and early embryonic development <i>in vitro</i> .	92
II.3. Influence of the phosphatidylinositol analogue SH-6 on AKT phosphorylation	93
II.4. Effect of AKT inhibitor supplementation during the initial 72 h of <i>in vitro</i> culture on indices of embryo developmental progression	93
II.5. Influence of follistatin treatment on developmental capacity of AKT treated embryos.	94
II.6. Effect of exogenous follistatin supplementation on activation of AKT signaling pathway in early bovine zygotes	94
Results	96
II.1. A possible link for <i>AKT</i> transcript abundance/signaling activity with oocyte developmental competence determined by BCB staining.	96

II.2. Temporal regulation of <i>AKT</i> mRNA abundance during oocyte maturation and early embryogenesis.	98
II.3. Influence of the phosphatidylinositol analogue (AKT inhibitor) SH-6 on AKT phosphorylation	100
II.4. Effect of AKT inhibitor supplementation during the initial 72 h of <i>in vitro</i> culture on indices of oocyte developmental progression	102
II.5. Influence of follistatin treatment on developmental capacity of AKT inhibitor treated embryos.	104
II.6. Effect of exogenous follistatin supplementation on activation of AKT signaling pathway in early bovine zygotes	108
Discussion	111
Summary and Conclusions	119
References	123
Abbreviations	161
Arabic Abstract	ا
Arabic Summary	ب

List of Tables

No.	Title	Pages
1	Sequence of primers used for real-time PCR.	56
2	Sources of the antibodies used in the study	59
3	Software used in the study	59
4	Effects of exogenous follistatin supplementation on indices of embryo developmental progression for embryos derived from good and poor quality oocytes based on BCB staining	75
5	Effects of exogenous JY-1 treatment during initial 72 h of in vitro embryo culture on developmental capacity of bovine embryos derived from BCB screened and control oocytes	78
6	Effects of cathepsin inhibitor (E-64) treatment during meiotic maturation on developmental capacity of in vitro fertilized embryos derived from control oocytes and oocytes classified based on BCB staining	81
7	Influence of Follistatin treatment on developmental capacity of AKT inhibitor treated embryos	106

List of Figures

No.	Caption	Pages
1	Expression of follistatin, JY-1, BMP15, and GDF9 in BCB screened GV stage oocytes.	65
2	Cumulus cell cathepsins expression in BCB screened GV stage oocytes	66
3	Cumulus cell expression of follistatin and oocyte expression of Cathepsin B, Cathepsin S, and Cathepsin Z.	68
4	Expression of SMAD1, SMAD2, SMAD3 and SMAD5 in BCB screened GV stage oocytes.	70
5	Activity of SMAD2/3 signaling pathway in GV oocytes selected based on BCB staining.	71
6	SMAD 1/5 signaling pathway activity in GV oocytes selected based on BCB staining.	72
7	Effects of exogenous follistatin supplementation on indices of embryo developmental progression for embryos derived from oocytes selected by BCB staining	75
8	Effects of exogenous JY-1 treatment during initial 72 h of in vitro embryo culture on developmental capacity of bovine embryos derived from BCB screened and control oocytes	78
9	Effects of cathepsin inhibitor (E-64) treatment during meiotic maturation on developmental capacity of in vitro fertilized embryos derived from control oocytes and oocytes classified based on BCB staining.	81
10	Association of <i>AKT</i> transcript abundance/signaling activity with oocyte developmental competence determined by BCB staining.	97