MEIOTIC MATURATION AND IN VITRO MATURATION OF BOVINE OOCYTES[1]

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Contents: In vitro production of embryos, and, as part of this method, in vitro maturation of oocytes, have received great attention in last ten-fifteen years. It is well established in bovine. Here, in this review is presented importance of this method, usual meiotic division is described, as well, as importance of biochemical investigations of several protein factors and enzymes, which control these processes.

Key words: meiosis, maturation, IVM, bovine oocytes

Introduction

In vitro production of embryos (IVPE) in animal husbandry has received great attention and support in the last ten-fifteen years. The IVPE technology is well established in the bovine and it is increasingly used in practice.

IVPE includes in vitro oocyte maturation, in vitro fertilisation and the development of the fertilised oocyte to the blastocyste stage (in vitro embryo cultivation). Each of these developmental steps has to be completed successfully, if the embryos obtained are expected to establish a viable pregnancy that will deliver a normal offspring. The expected rate of transferable blastocysts after oocyte maturation, fertilisation and embryo culture in vitro reaches 30 to 40% . Generally, the main problem of the procedure of IVPE is the reduced viability of in vitro embryos compared with in vivo counterparts. To improve the efficiency, now the research is focussed on all factors influencing the parental gametes prior to their collection, and how gametes and embryos react to different culture systems. Hypothesis is that cultivation systems are suboptimal. In these investigations biochemical aspect of in vitro maturation is focused.

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[1] Review article- Revijalni rad

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Meiotic division

In cattle, the first potential primordial germ cells (PGCs) can be identified in 18-days-old embryos. They migrate to the forming gonad (ovary) and form clusters of dividing cells termed oogonia. After a period of mitotic proliferation, oogonia enter meiotic prophase and differentiate into primary oocytes, which begin their first meiotic division. In giving rise to oocytes, **oogonia undergo the two cell divisions of meiosis to reduce their diploid chromosome complement (2n) to the haploid state (n), and this is the main difference between meiosis and mitosis.** Entry in mitosis is influenced by the cyclin depended kinase cdc2/Cyc A**,** and entry into meiotic maturation is stimulated at least by kinases like the maturation promoting factor (MPF, also called M-phase promoting factor) which is a complex of cdc2 and cyclin B, mitogene activated kinases (MAPK1, 2) and the protein kinase Akt . In the contrast to mitosis number of chromosome where it remains diploid , in meiosis first meiotic division enables the reduction of chromosome number to haploid (the second meiotic division is very similar at least morphologically to the mitotic division). After the initiation of meiosis, the germ cells (primary oocytes) progress through the leptotene, zygotene and pachytene of the first meiotic prophase before arresting at the diplotene (dictyate) stage. As development proceeds towards the diplotene stage, the primary oocytes acquire an outer coat (zona pellucida) and cortical granules, accumulate ribosomes, mRNA, proteins and various nutrients required for further development.

It has been reported that in mouse oocytes, during the growing phase, only 15% of the mRNA is bound to ribosomes and only one third of ribosomes are involved in protein synthesis *(Paynton and Bachvarova, 1990, 1994).* That means, during this phase, a stockpile of macromolecules is formed which will be used in the further maturation process (e. g. during meiotic maturation), or which is necessary to promote early embryonic development.

In the follicle, the oocyte is surrounded by closely associated granulosa cells (cumulus cells), forming a compact cumulus cell-oocyte complex (COC). Gap junctions formed by different connexins allow a low molecular weight (up to 5000 Da) signal transfer between granulosa cells and the oocyte witch probably is involved in meiotic arrest. (*Trounson, 2003,DelÀquila et al, 2004*). COCs are embedded in follicular fluid which probably also provides substances which impair meiotic maturation. The preovulatory LH peak initiates final follicular and oocyte maturation and ovulation. Follicles that do not reach ovulation become atresic and are eliminated.

Table 1. Stages of meiotic maturation, summarized according to Karp, 2002
Tabela 1. Stadijumi mejotičkog sazrevanja prema Karp-u, 2002

STAGES	EVENTS
1. Prophase I	
Leptotene	Chromosomes condense
Zygotene	Synapsis-Homologous chromosomes align (**bivalents**); synaptonemal complex formation
Pachytene	Recombination nodules appear; crossing-over of genetic material occurs
Diplotene	Chiasmata formation, desynapsis; chromosomes decondense and engage in RNA synthesis
Diakinesis	Chromosomes condense, appear **tetravalent**, RNA synthesis ceases
GV stage	The nucleus of the oocyte is quite large in comparison to that of a somatic cell, called **germinal vesicle**
GVBD stage	**Germinal vesicle** breaks down(disassembly of nuclear lamina, and breakdown of the nuclear envelope)
2. Metaphase I	Bivalents line up on the metaphase plate of the spindle
3. Anaphase I	Chromosomes move apart
4. Telophase I	Formation of two daughter nuclei and extrusion of first polar body
5.Interphase	Very short, only G1 phase
6. Prophase II	Nuclear envelope breaks down and the new spindle forms,
7. Metaphase II	Remaining chromatides (half the initial number) in each daughter nuclei) promptly align again on the second meiotic spindle
8. Anaphase II	Chromatides start moving apart (subject to fertilization)
9. Telophase II	Formation of two daughter nuclei; extrusion of the second polar body

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Oocytes from ovarium can be collected and cultivated in vitro in a suitable cultivating medium, passing through *in vitro maturation*. Oocytes released from their follicle persist in the germinal vesicle stage (GV stage, diplotene of first meiotic division- 0 hours of maturation), and bovine oocytes in culture pass through germinal vesicle break down (GVBD) after 10 hours of maturation followed by the first meiotic division and finally reach the metaphase II at 20 to 24 hours of maturation.

Biochemistry of meiotic maturation and in vitro maturation

As described before, the relatively short period of meiotic *in vitro* maturation is characterised by morphological changes, among others, chromosome condensation, germinal vesicle breakdown (GVBD), and assembly of the meiotic spindle.

This phase is also accompanied by dramatic decrease of mRNA transcription and characteristic alterations in the protein synthesis (*Tomek et al., 2002a, b*).
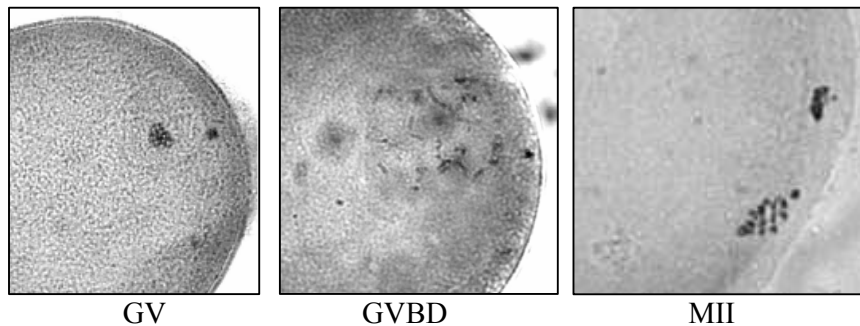

GV   GVBD   MII

Figure 1.Oocyte maturation (thanks to Dr. Fabiana Melo Sterza)
Slika 1. Sazrevanje oocita (zahvaljujući dr Fabiani Melo Sterza)

After the GVBD oocytes pass through short anaphase I and telophase I, very short interphase (only G1), prophase II and reaches MII stage (metaphase of second meiotic division) when   they should be competent for fertilisation. After fertilization the oocytes complete the second meiotic division and extrude a small polar body (”second oocyte” which consists of condensed DNA, and low amount of cytoplasm).

During in vitro maturation RNA synthesis strongly decrease because the condensed DNA present in oocytes from GVBD stage on, are practically disable for transcription. Translation increases from GV to GVBD, but than decreases up to metaphase II, where translation is almost impaired. Messenger RNA is accumulated, in the growing phase of oocytes and during meiotic division in the prophase until the diplotene. These mRNAs are believed to be translated after fertilisation to sustain the first mitotic divisions, in advance of the activation of the embryonic genome. In bovine, the embryonic genome is activated in the 8 cell stage but maternal RNA can be found even in blastocysts (Sirard et- al. 2002). One important question is how is translation regulated during meiotic maturation and which factors are involved in repressing translation in MII stage oocyte.

For analysing this issue we have investigated signalling cascades (MAPK, cdc2 and Akt pathways) which contribute to networks that affect the activity of regulators of initiation of translation. In detail, the translation initiation factor eIF4E, part of eIF4F complex, its repressor protein 4E-BP1, the poly-A-tail binding protein PABP1, and associated proteins Paip1 and Paip2, and also to eIF4F complex were investigated. In this way the cooperation between 3´ and 5´ mRNA binding factors were proofed to be involved in the modulation of translation rates during meiotic maturation of bovine oocytes. (*Smiljaković et al, 2003, Smiljaković et al, 2004, Tomek & Smiljaković, 2005, Miščević et al, 2005, Smiljaković, 2006*).

MEJOTIČKO I *IN VITRO* SAZREVANJE GOVEĐIH JAJNIH ĆELIJA

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Rezime

In vitro proizvodnja embriona i, kao deo ove metode, *in vitro* sazrevanje jajnih ćelija, privlače veliku pažnju poslednjih deset-petnaest godina. To je dosta korišćena metoda u uzgoju goveda. U ovom radu je opisana važnost ove metode, zatim, uobičajeni tok mejotičkog sazrevanja jajnih ćelija kod goveda, kao i važnost biohemijskih istraživanja različitih proteinskih faktora i enzima koji kontrolišu ovaj proces.

Literature

1. DELL`AQUILA M.E., CAILLAUD M., MARITATO F., MAR-TORIATI A., GERARD N., AIUDI G., MINOIA P., GOUDET G.(2004): Cumulus expansion, nuclear maturation and connexin 43, cyclooxygenase-2 and FSH receptor mRNA expression in equine cumulus-oocyte complexes cultured in vitro in the presence of FSH and precursors for hyaluronic acid synthesis. Reproductive biology and endocrinology; 2:44.
2. MIŠČEVIĆ B, ALEKSIĆ S, JOSIPOVIĆ S, PANTELIĆ V, SMILJAKOVIĆ T., OSTOJIĆ D. (2005): Importance of beef production in Serbia. 8th International Symposium ``Modern trends in Livestock production``, Belgrade, October 5th-8th, Biotechnology in Animal Husbandry, Vol 21 (5-6):9-15.
3. PAYNTON BV, BACHVAROVA R.(1990): Changes in maternal mRNAs during oocyte maturation. In B.Bavister , J. Cummins.,

ERS Roldan (eds). Fertilisation in Mammals. Norwell. Massachusetts: Serono Symposia,pp25-34.

4. PAYNTON BV, BACHVAROVA R.(1994): Polyadenylation and deadenylation of maternal mRNAs during oocyte growth and maturation in the mouse. Mol Reprod Dev.; 37(2):172-80.

5. SIRARD M.A, DUFORT I., COENEN K., TREMBLAY K., MASSICOTE L., ROBERT C.(2003): The use of genomics and proteomics to understand oocyte and early embryo functions in farm animals. Reprod. Suppl; 61: 117-29.

6. SMILJAKOVIĆ T., MELO STERZA F., KUBELKA M., VOHNIKOVA Z., TOMEK W. (2003): Aspects of cytoplasmatic maturation of bovine oocytes; Interplay between MAPK , mRNA-cap binding complex and cytoplasmatic complex and cytoplasmatic mRNA metabolism in regulation of translation. Biotechnology in Animal Husbandry, 19(3-4):1-8.

7. SMILJAKOVIĆ T., MAX M., TOMEK W. (2004): Abundance of poly (A) binding protein in bovine oocytes during in vitro maturation. Biotecnology in Animal Husbandry; 20(3-4), 5-14.

8. SMILJAKOVIĆ T. (2006): Cytoplasmic Maturation of Bovine Oocytes- Analysis of Signaling Cascades and Factors which Contribute to the Meiotic Maturation of Bovine Oocytes; doctoral thesis(Dr.rer.nat.) , accepted 5.12.2005., University in Rostock, Germany, mentor: Dr. Wolfgang Tomek.

9. TROUNSON A.O., GOSDEN R.G. (2003): Biology and Pathology of the Oocyte, Cambridge University Press.

10. TOMEK W., MELO STERZA F.A., KUBELKA M., WOLLENHAUPT K, TORNER H., ANGER M., KANITZ W. (2002a): Regulation of translation during in vitro maturation of bovine oocytes: The role of MAP kinase, eIF4E (Cap binding protein phosphorylation, and êIF4E-BP1. Biology of reproduction; 66:1274-1282.

11. TOMEK W., TORNER H., KANITZ W. (2002b) Comparative analysis of protein synthesis, transcription and polyadenilation of mRNA during maturation of bovine oocytes in vitro. Reprod. Domestic. Anim.; 37:86-91.

12. TOMEK W., SMILJAKOVIĆ T. (2005): Activation of Akt (Protein kinase B) stimulates metaphase I to metaphase II transition in bovine oocytes. Reproduction 130:423-430.