Differential gene expression between in vivo and in vitro maturation: a comparative study with bovine oocytes derived from the same donor pool

Luiz Sergio Almeida Camargo1, Michele Munk2, Jose Nelio Sales3, Sabine Wohlrves-Viana2, Carolina Capobiano Romano Quintão1, João Henrique Moreira Viana4

1Brazilian Agricultural Research Corporation (Embrapa) - Dairy Cattle, Juiz de Fora, Brazil
2Federal University of Juiz de Fora, Juiz de Fora, MG, Brazil
3Federal University of Lavras, Lavras, MG, Brazil
4Embrapa - Cenargen, Brasilia, DF, Brazil

Ethical considerations: All applicable guidelines for the care and use of farm animals were followed. The Ethics Committee on Animal Use (CEUA) approved this study (protocol 32/2015).

ABSTRACT

Objective: In vitro maturation has been shown to influence gene expression in oocytes, but a common shortcoming in reports on the matter has been the use of different donors in each experimental group thus disregarding donor effects. This study aimed to investigate the abundance of mRNA in oocytes matured in vivo and in vitro obtained from the same group of donors.

Methods: A bovine model was used to assess the relative abundance of specific transcripts in in vitro-matured (IN VITRO-OPU) and in vivo-matured (IN VIVO-OPU) oocytes collected from the same donors by transvaginal ovum pick-up (OPU). Transcript abundance in oocytes from the IN VIVO-OPU group and oocytes matured in vitro but retrieved from different cows slaughtered at a commercial abattoir (IN VITRO-Abattoir group) was also compared. Total RNA was extracted from denuded oocytes and cDNA was produced via reverse transcription using an oligo(dT) primer for relative quantification of eight target transcripts by real-time PCR.

Results: Oocytes in the IN VITRO-OPU group had lower (p<0.05) abundance of peroxiredoxin 1 (Prdx1), heat shock protein 70.1 (Hsp70.1), growth and differentiation factor 9 (Gdf9), and maternal antigen that embryo requires (Mater) transcripts than the oocytes in the IN VIVO-OPU group, all obtained from the same pool of donor cows. Similar results were seen in the comparisons involving the IN VIVO-OPU and IN VITRO-Abattoir groups (p<0.05).

Conclusion: In vitro maturation affected the abundance of polyadenylated transcripts in the oocyte cytoplasm when compared to in vivo maturation induced by exogenous hormones in oocytes collected from the same donor pool.

Keywords: in vivo maturation, mRNA, gene expression, ovum pick-up

INTRODUCTION

In the roster of assisted reproductive technologies (ART), oocyte in vitro maturation (IVM) can be an alternative for patients with ovarian conditions prescribed treatments that may compromise their oocytes (Reinblatt & Buckett, 2008; Ata et al., 2010; Shalom-Paz et al., 2010) and individuals at risk of ovarian hyperstimulation syndrome (Sauerbrun-Cutler et al., 2015). IVM may also decrease the number of clinical consultations and the level to which patients may require drug therapy, thus reducing the cost of treatment (Picton, 2002). Despite its potential benefits, IVM is still marginally used in human ART, with applications mainly in fertility preservation (Kasum et al., 2015; Lamberti et al., 2015, Shirasawa & Terada, 2017). IVM is limited by oocyte developmental competence (Gilchrist & Thompson, 2007) and may result in lower fertilization and embryo production rates (Banwell & Thompson, 2008). IVM may also disturb the meiotic spindle, the morphology of human oocyte chromosomal alignment (Li et al., 2006), and expose gametes to reactive oxygen species (Combelles et al., 2009). Lower implantation and birth rates have been reported with IVM, when compared to conventional protocols in which maturation is induced in vivo (Suikkari, 2008; Smitz et al., 2011). Concerns over the long-term effects of IVM on offspring health have been reported (Suikkari & Soderstrom-Anttila, 2007). Unfortunately, little is known about oocyte maturation when compared to other developmental processes (Coticchio et al., 2015), therefore, a better understanding of the mechanisms involved in oocyte competence acquisition and the factors that might disturb it during IVM is crucial in the optimization of this technology.

During IVM, oocytes need to undergo nuclear and cytoplasmic maturation. Meiosis resumes and progresses up to metaphase II, while the cytoplasmic organelles undergo redistribution (Ferreira et al., 2009) required for cortical granule secretion and pronuclear formation. Nevertheless, before becoming fully competent oocytes also need to go through molecular maturation. Transcriptional activity is supposed to be low during maturation (Bettegowda & Smith, 2007) and much of the mRNA transcribed and stored in the cytoplasm during oocyte growth is degraded, whereas some of it is protected from degradation and conferred stability by the 3' untranslated regions (Brevini et al., 2007). Polyadenylated oocyte mRNAs are required not only for meiotic resumption, but also for early embryo development (Piccioni et al., 2005; Brevini et al., 2007; Evskov & Marin de Evskova, 2009). Studies have shown that during oocyte maturation some mRNA can undergo deamination (Brevini-Gandolfi et al., 1999; Lequarre et al., 2004), while other transcripts may accumulate in polyadenylated form (Tomek et al., 2002). A recent study using single oocytes and RNA-Seq showed that some polyadenylated transcripts increase while others decrease in abundance, showing the importance of cytoplasmic polyadenylation during oocyte maturation (Reyes et al., 2015). Different forms of mRNA and proteins are present in the ooplasm and may be required in early cleavage (Meirelles et al., 2004; Li et al., 2010), playing a role on embryonic genome activation (Schultz, 2002).

Previous studies reported that IVM might impact oocyte gene expression and alter the amount of mRNA stored in the ooplasm, which in turn would affect further embryo development. However, there is no consensus over such effect of IVM. A study showed that Rhesus monkey oocytes matured in vitro had different levels of expression of some maternal mRNAs when compared to oocytes matured in vivo.
in vivo (Zheng et al., 2005), while other authors described close similarities between oocytes matured in vitro and in vivo (Lee et al., 2008). The authors of the latter study performed cDNA-array analysis and found only 59 genes differentially expressed between oocytes matured in vitro and in vivo, which accounted for a mere 0.31% of the total probe set analyzed. In humans, global gene expression analysis revealed that more than 2,000 genes were differentially expressed between oocytes matured in vitro and in vivo (Jones et al., 2008). Despite a few differences, another study found that human oocytes matured in vivo and in vitro shared similar patterns of gene expression (Wells & Patrizio, 2008). In bovines, IVM impacted the amount of mRNA transcripts stored in the ooplasm when compared to in vivo maturation (Lonergan et al., 2003). Transcriptome analysis found distinct transcription patterns between bovine oocytes matured in vivo and in vitro (Katz-Jaffe et al., 2009). Therefore, the impact of IVM on oocyte transcriptome is unclear. A downside common to these studies is the use of different donors in each experimental group. Since oocytes accumulate transcripts as they grow, differences among donors in the amount of mRNA stored in the oocytes before maturation may affect the interpretation of the effects of IVM on the abundance of transcripts in the ooplasm.

This study aimed to investigate the abundance of polyadenylated mRNA in oocyte pools matured in vivo and in vitro harvested from the same group of cows in order to decrease the effects of individual variation among donors in the amount of transcripts. The study also compared the abundance of polyadenylated mRNA between oocytes matured in vivo and oocytes matured in vitro retrieved from different cows in order to evaluate whether the effects of IVM on mRNA abundance might also be seen in oocytes coming from different donors. A bovine model was used because of the practical and ethical limitations inherent to working with human oocytes, the similarities between human and bovine oocyte maturation (Ménézo & Héruel, 2002), and the well-established in vitro maturation protocols for bovine oocytes. The following genes were chosen according to the importance of their proteins for oocyte and early embryo development: maternal antigen that embryos requires (Mater), zygote arrest 1 (Zar1), growth and differentiation factor 9 (Gdf9), B-cell CLL/lymphoma 2 (Bcl-2), BCL-2-associated X protein (Bax), peroxiredoxin 1 (Prdx1), heat shock protein 70.1 (Hsp70.1) and high-mobility-group 1 (Hmgn1).

MATERIALS AND METHODS

Chemicals and animals

The chemicals used in this study were purchased from Sigma Chemical (St. Louis, MO, USA) unless otherwise indicated. The oocyte donors were mature crossbred cows kept in pasture with water ad libitum and under shadow.

Experimental design

This study compared the relative abundance of specific transcripts in oocytes obtained by transvaginal ovum pick-up (OPU) of the same donors matured either in vitro (IN VITRO-OPU group) or in vivo (IN VIVO-OPU group). Transcript abundance was also compared between oocytes matured in vivo (IN VIVO-OPU group) and oocytes matured in vitro (IN VITRO-Abattoir group), with the latter obtained from ovaries collected at a commercial abattoir from different cows. Five cows underwent OPU to collect oocytes for in vitro maturation (IN VITRO-OPU). Two weeks later, the same donors were submitted to hormone therapy to induce in vivo maturation and had their oocytes retrieved by OPU (IN VIVO-OPU group). In the IN VITRO-Abattoir group, the oocytes were retrieved post-mortem at a local commercial abattoir and transported to the laboratory. Maturation was deemed complete when the oocytes had fully expanded cumulus cells. Three pools with 10 matured denuded oocytes in each group were submitted to RNA extraction. Relative quantification of Prdx1, Hsp70.1, Gdf9, Mater, Zar1, Bax, BCL2, and Hmgn1 genes was performed with real-time PCR.

Ovum Pick-up

The oocytes in the IN VITRO-OPU and IN VIVO-OPU groups were harvested with the aid of a portable ultrasound device equipped with a sector scanner and a 7.5 MHz transvaginal transducer (Aquila Vet, Esaoote, Geneva, Italy). Ovum pick-up was performed with disposable 20 gauge needles (WTA Tecnologia, Cravinhos, SP, Brazil) at a vacuum pressure of 80 mmHg. The aspirated follicular fluid was collected in 50 mL tubes containing TALP-HEPES added with 125 IU/ml of heparin (Liquemine, Roche Lab, Brazil). In the IN VITRO-OPU group, the oocytes were harvested from crossbreds between Gir and Holstein not submitted to hormone therapy on a random day of the estrous cycle. In the IN VIVO-OPU group, OPU was performed two weeks later in the same cows. In both IN VIVO- and IN VITRO-OPU groups, all follicles measuring 3-8 mm in diameter were aspirated. The mean ± SEM number of oocytes collected by OPU/donor was 13.7±3.9 and 16.2±3.2 in the IN VIVO- and IN VITRO-OPU groups, respectively.

Oocytes collected from abattoir ovaries

The immature oocytes included in the IN VITRO-Abattoir group were aspirated from the follicles of ovaries picked randomly at a local abattoir. The ovaries were transported to the laboratory in 0.9% sodium chloride solution supplemented with 0.05 g/L of streptomycin at 33-37ºC within 3 h. In the laboratory, the ovaries were rinsed in sodium chloride solution at 35-37ºC and follicles measuring 3-8 mm in diameter were aspirated with a 21 G needle attached to a disposable syringe.

In vivo maturation

The oocytes in the IN VIVO-OPU group were harvested after hormonal stimulation of the pre-ovulatory LH surge to induce in vivo maturation. The cows were implanted a progesterone-releasing intravaginal device - a controlled internal drug release (CIDR) insert (Pfizer, São Paulo, Brazil) - and prescribed 2 mg of estradiol benzoate (Estrinov, Farmavet, São Paulo, Brazil) on Day 0. On Day 4, the cows were stimulated with 180 mg FSH (Folltropin, Bioniche, Canada) injected in six decreasing doses every 12 h. On Day 6, the cows were administered 0.53 mg of cloprostenol sodium (Ciosin, Cooper, São Paulo, Brazil). On Day 7, the CIDR insert was removed and the cows were injected 2.5 mg of gonadorelin (Gestran-Plus, Tecnocel, São Paulo, Brazil). OPU was performed 18 h after the gonadorelin injection. Only oocytes with expanded cumulus cells and homogeneous cytoplasm (n=37) were selected for the experiment. The selected oocytes were denuded with 0.1% hyaluronidase, pooled in groups of ten, and rapidly frozen in liquid nitrogen for further RNA extraction.

In vitro maturation

In the IN VITRO-OPU (n=40) and IN VITRO-Abattoir groups (n=40), only immature cumulus-oocyte complexes (COCs) with more than three compact layers of cumulus cells and oocytes with homogeneous cytoplasm were selected and matured in vitro. IVM was performed in tissue culture medium (medium 199; Gibco Life Technologies, Grand Island, NY, USA) supplemented with 20 µg/ml1 FSH (Pluset, Serono, Italy), 0.36 mM sodium pyruvate, 10 mM sodium bicarbonate, and 50 mg/ml1 streptomycin-penicillin in a humidified atmosphere with 5% CO2, in
air at 38.5°C for 24 h. Only oocytes with expanded cumulus cell were denuded, pooled, and stored in the same way of the oocytes matured in vivo.

**Total RNA extraction and reverse transcription (RT)**

Total RNA was extracted from three pools of 10 oocytes per group with the RNeasy Micro Kit (Qiagen, Hilden, Germany) according to manufacturer instructions and treated with DNase to prevent DNA contamination. Elution was performed with 12 µL of RNase-free water. In order to isolate poly(A)+ RNA, the samples (8 µL, equivalent to 6.7 oocytes) were submitted to reverse transcription with the SuperScript III First-Strand Synthesis Supermix (Invitrogen, Carlsbad, CA, USA) kit according to manufacturer instructions using oligo(dT)20 primers, dNTP mix, Superscript III RT, RNaseOUT, MgCl2, and RT buffer in a final volume of 20 µL (equivalent to 0.33 oocyte/µL). The samples were first incubated at 65°C for 5 min and then at 50°C for 50 min. The reaction was terminated at 85°C for 5 min and the samples were then chilled in ice. After that, RNase H was added to the samples and incubated at 37°C for 20 min.

RNA and cDNA from each pool and group were quantified on a spectrophotometer (Nanodrop 2000, Wilmington, DE, USA) using 1 µL of sample. Samples presenting 260/280 ratios between 1.7 and 2.0 were considered appropriate for expression analysis.

**Relative quantification by real-time polymerase chain reaction (PCR)**

Relative quantification was performed in triplicate using real-time polymerase chain reaction (ABI Prism 7300 Sequence Detection Systems, Foster City, CA, USA). The reactions were prepared using a mixture of SYBR Green PCR Master Mix (Applied Biosystems), 0.1µM primers, nucleic acid-free water, and cDNA. The volume of RT (with cDNA) in the PCR reactions was calculated based on oocyte-equivalents. Due to the restriction of RNA amount in the oocytes, only one housekeeping gene was used as an endogenous reference for relative quantification. The beta-ACTIN (Actb) gene was chosen as the endogenous reference since it displayed a low coefficient of variation (2.2%) among the samples in the present experiment. Polymerase chain reaction for the Bax, Bcl-2, Prdx1 and Hsp70.1 genes was performed with 1 µL of RT reaction (equivalent to 0.33 oocyte/PCR reaction) whereas for the Gdf9, Mater, Zar1, Hmgn1 and Actb genes, PCR was performed with 0.33 µL of RT reaction (equivalent to 0.1 oocyte/PCR reaction). The cDNA template was denatured at 95°C for 10 min, followed by 45 cycles at 95°C for 15s, the gene-specific primer annealing temperature for 30s (Table 1) and elongation at 60°C for 30s. After each PCR run, melting curve analysis was performed to confirm that a single specific product was generated. Negative controls, comprising the PCR reaction mixture without nucleic acids, were also run with each group of samples. Primer efficiency was calculated using the LinRegPCR software (Ramakers et al., 2003) for each reaction. The mean primer efficiency was 1.84, 1.63, 1.80, 1.84, 1.81, 1.87, 1.88, 1.85, and 1.84 for Actb, Bax, Bcl-2, Prdx1, Hsp70.1, Gdf9, Mater, Zar1, and Hmgn1, respectively.

**Statistical analysis**

The Comparative Ct quantification method on the REST software package (Pfaffl et al., 2002) was used to perform relative quantification analysis based on primer efficiency. Data from the IN VIVO-OPU group were used as calibrator and set to one. Analysis was performed by a pair-wise fixed reallocation randomization test. p<0.05 was considered significant and the relative expression values were presented as mean values ± SEM.

**RESULTS**

Full cumulus cell expansion was observed at the end of in vitro and in vivo maturation, but oocytes matured in vivo had a more gelatinous matrix around the oocyte. After denudation, only oocytes with a homogenous cytoplasm...
were used for relative quantification of Bax, Bcl-2, Prdx1, Hsp70.1, Gdf9, Mater, Zar1, and Hmgn1 transcripts. The oocytes collected from the same donors included in the IN VITRO-OPU group had decreased (p<0.05) relative abundance of Prdx1, Hsp70.1, Gdf9, and Mater transcripts when compared to the oocytes in the IN VIVO-OPU group (Figure 1). A similar result was observed when the comparison was performed between oocytes from different cows matured in vivo or in vitro. The oocytes in the IN VITRO-Abattoir group had decreased abundance of Prdx1, Hsp70.1, Gdf9, and Mater transcripts than the oocytes in the IN VIVO-OPU group (Figure 2). The abundance of Zar1 transcripts was also lower in the oocytes in the IN VITRO-Abattoir group (Figure 2). When both in vitro maturation groups (IN VITRO-OPU vs. IN VITRO-Abattoir) were compared, lower (p<0.05) amounts of Prdx1, Hsp70.1, Gdf9, and Zar1 transcripts were found in the oocytes in the IN VITRO-Abattoir group (Figure 3).

**DISCUSSION**

In vitro maturation is a critical step to produce developmentally competent oocytes for the in vitro production of embryos of domestic species, and may become an important tool in human assisted reproductive technology procedures. However, in vitro maturation of mammalian oocytes has been associated with decreased developmental ability, possibly due to cellular and molecular disturbances caused by the in vitro environment (Gilchrist & Thompson, 2007; Krisher, 2013). On the other hand, in vivo maturation improved the quality and developmental competence of bovine oocytes (Blondin et al., 2002; Dieleman et al., 2002). However, the effect of IVM on mRNA abundance is controversial (Zheng et al., 2005; Jones et al., 2008; Lee et al., 2008; Wells & Patrizio, 2008), with discrepancies arising from individual oocyte donor-related effects. Differently from other studies, we compared the abundance of polyadenylated transcripts between in vivo and in vitro matured bovine oocytes obtained from the same donors, and found that IVM affected the relative abundance of specific transcripts even in same-donor oocytes, reinforcing the idea that the IVM environment may affect the amount of mRNA stored in the ooplasm. However, the oocytes matured in vivo included in our study were not obtained in natural conditions, since follicle growth and LH surge were stimulated with exogenous hormones. Thus, the difference observed in the present study between oocytes matured in vivo and in vitro might not fully represent all possible differences between oocytes derived from a single ovulation of a natural estrus cycle and oocytes submitted to in vitro maturation. We also found that the relative abundance of some transcripts may also differ when in vitro maturation is performed with oocytes obtained from pools with different donors.

In vitro maturation decreased the amount of polyadenylated transcripts of genes associated to maternal effects and to stress in both IVM groups (IN VITRO-OPU and IN VITRO-Abattoir), when compared to in vivo maturation (IN VIVO-OPU). Maternal-effect genes Mater and Zar1 play an important role in the development of mouse oocytes after fertilization (Tong et al., 2000; Wu et al., 2003), while Gdf9 is an oocyte-secreted factor present in oocyte-somatic cell interactions involved in oocyte developmental competence (Gilchrist et al., 2008). The lower abundance of transcripts encoding these genes seen in oocytes matured in vitro may be associated with decreased oocyte competence after IVM, when compared to oocytes submitted to in vivo maturation (van de Leemput et al., 1999; Humblot et al., 2005). Peroxiredoxins and HSPs are proteins involved in cell defense against oxidative stress (Martindale & Holbrook, 2002; Immenschuh & Baumgart-Vogt, 2005). In vitro culture conditions are known to increase the production of reactive oxygen species and thus cause oxidative damage to oocytes (Combelles et al., 2009; Morado et al., 2009). The lower abundance of Prdx1 and Hsp70.1 transcripts found after in vitro maturation can implicate in oocytes more sensitive to stressful conditions imposed by in vitro environment, which may contribute to the low developmental competence after fertilization.

The reasons behind the effects of in vitro maturation on the abundance of transcripts in the ooplasm have not been entirely elucidated, and may involve a combination of factors including a more stressful environment (Morado et al., 2009) requiring mRNAs for the synthesis of specific proteins, decreased ability of the oocyte transcription machinery (Bettgowda & Smith, 2007) to synthesese new mRNAs, and degradation or deadenylation of transcripts during in vitro maturation (Thelie et al., 2009). A RNA-seq study showed that some polyadenylated transcripts decreased in abundance during oocyte maturation, while others associated to cell-cycle progression, cytoskeletal organization, and macromolecule metabolism increased (Reyes et al., 2015). Despite these variations, polyadenylated mRNAs are relevant in meiotic resumption and further early embryo development (Piccioni et al., 2005; Brevini et al., 2007; Evsikov & Marín de Evsikova, 2009). Changes to polyadenylated transcripts may interfere with oocyte competence (Gandolfi & Gandolfi, 2001). However, the effects of in vitro maturation found in the present study may be specific for genes encoding high-demand proteins such as maternal-effect and antioxidant proteins, since we were unable to find differences in the abundance of transcripts encoded by genes related to apoptosis (Bax and Bcl-2) and chromatin unfolding (Hmgn1).

Interestingly, differences on relative abundance were found between the IN VITRO-OPU and IN VITRO-Abattoir groups, with lower amounts of Prdx1, Hsp70.1, Gdf9, and Zar1 transcripts in the oocytes collected post-mortem at the abattoir. These oocytes also had lower amounts of the same transcripts when compared to the oocytes obtained from in vivo maturation followed by OPU (IN VIVO-OPU). Oocytes from the same donors were included in the IN VIVO-OPU and IN VITRO-OPU groups, while the IN VITRO-Abattoir group featured oocytes harvested from different cows. A possible reason for the low content of some specific transcripts in the oocytes collected post-mortem at the abattoir is the fact that these oocytes were harvested from donors with a different genetic background than the subjects included in the IN VIVO-OPU and IN VITRO-OPU groups. A recent study showed that cattle breed might affect oocyte mRNA abundance (Ticianelli et al., 2017). These findings highlight the need to compare maturation systems for efficiency using oocytes from the same donors in an attempt to avoid the misinterpretation of findings.

In general terms, this study showed that in vitro maturation might alter the abundance of key transcripts stored in the oocyte cytoplasm when compared to in vivo maturation induced by exogenous hormones, even in oocytes from the same donors. Therefore, IVM optimization is still required to improve molecular maturation regardless of oocyte origin.

**CONFLICT OF INTERESTS**

The authors have no conflict of interest to declare.
Figure 1. Relative abundance of transcripts from different genes of in vivo-matured (IN VIVO-OPU) and in vitro-matured (IN VITRO-OPU) bovine oocytes derived from the same donors. Transcript level of in vivo-matured oocytes was used as calibrator (relative abundance = 1.00). Data show as mean ± SEM. (*) Asterisk indicates difference between IN VIVO-OPU and IN VITRO-OPU groups ($p<0.05$)

Figure 2. Relative abundance of transcripts from different genes of in vivo-matured (IN VIVO-OPU) and in vitro-matured (IN VITRO-Abattoir) bovine oocytes derived from different donors. Transcript level of in vivo-matured oocytes was used as calibrator (relative abundance = 1.00). Data show as mean ± SEM. (*) Asterisk indicates difference between IN VIVO-OPU and IN VITRO-Abattoir groups ($p<0.05$)

ACKNOWLEDGEMENTS

This study received support from the National Council for Scientific and Technological Development (CNPq) and the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

Corresponding author:
Luiz Sergio Almeida Camargo
Embrapa Gado de Leite
Juiz de Fora, MG, Brazil
E-mail: luiz.camargo@embrapa.br
Figure 3. Relative abundance of transcripts from different genes of in vitro-matured bovine oocytes derived from different donors, collected by means of OPU (IN VITRO-OPU) or by post-mortem follicular aspiration (IN VITRO-Abattoir). Transcript level of IN VITRO-OPU oocytes was used as calibrator (relative abundance = 1.00). Data are show as mean ± SEM. (*) Asterisk indicates difference between IN VITRO-OPU and IN VITRO-Abattoir groups ($p<0.05$)

REFERENCES

Ata B, Shalom-Paz E, Chian RC, Tan SL. In vitro maturation of oocytes as a strategy for fertility preservation. Clin Obstet Gynecol. 2010;53:775-86. PMID: 21048444 DOI: 10.1097/GRF.0b013e3181f9718f

Banwell KM, Thompson JG. In vitro maturation of Mammalian oocytes: outcomes and consequences. Semin Reprod Med. 2008;26:162-74. PMID: 18302108 DOI: 10.1055/s-2008-1042955

Bettgebowda A, Smith GW. Mechanisms of maternal mRNA regulation: implications for mammalian early embryonic development. Front Biosci. 2007;12:3713-26. PMID: 17485333 DOI: 10.2741/2346

Blondin P, Bousquet D, Twagiramungu H, Barnes F, Sirard MA. Manipulation of follicular development to produce developmentally competent bovine oocytes. Biol Reprod. 2002;66:38-43. PMID: 11751261 DOI: 10.1095/biolreprod.66.1.38

Brevini-Gandolfi TA, Favetta LA, Mauri L, Luciano AM, Cillo F, Gandolfi F. Changes in poly(A) tail length of maternal transcripts during in vitro maturation of bovine oocytes and their relation with developmental competence. Mol Reprod Dev. 1999;52:427-33. PMID: 10092123 DOI: 10.1002/(SICI)1098-2795(199904)52:4<427::AID-MRD12>3.0.CO;2-G

Brevini TA, Cillo F, Antonini S, Tosetti V, Gandolfi F. Temporal and spatial control of gene expression in early embryos of farm animals. Reprod Fertil Dev. 2007;19:35-42. PMID: 17389133 DOI: 10.1071/RD06119

Combelles CM, Gupta S, Agarwal A. Could oxidative stress influence the in-vitro maturation of oocytes? Reprod Biomed Online. 2009;18:864-80. PMID: 19490793 DOI: 10.1016/S1472-6483(10)60038-7

Coticchio G, Dal Canto M, Mignini Renzini M, Guglielmo MC, Brambillasca F, Turchi D, Novara PV, Fadini R. Oocyte maturation: gamete-somatic cells interactions, meiotic resumption, cytoskeletal dynamics and cytoplasmic reorganization. Hum Reprod Update. 2015;21:427-54. PMID: 25744083 DOI: 10.1093/humupd/dmv011

Dieleman SJ, Hendriksen PJ, Viuff D, Thomsen PD, Hyttel P, Knijn HM, Wrenzycki C, Kruij T, Niemann H, Gadella BM, Bevers MM, Vos PL. Effects of in vivo prematuration and in vivo final maturation on developmental capacity and quality of pre-implantation embryos. Theriogenology. 2002;57:5-20. PMID: 11775980 DOI: 10.1016/S0093-691X(01)00655-0

Evsikov AV, Marin de Eviskova C. Gene expression during the oocyte-to-embryo transition in mammals. Mol Reprod Dev. 2009;76:805-18. PMID: 19363788 DOI: 10.1002/mrd.21038

Ferreira EM, Vireque AA, Adona PR, Meirelles FV, Ferriani RA, Navarro PA. Cytoplasmic maturation of bovine oocytes: structural and biochemical modifications and acquisition of developmental competence. Theriogenology. 2009;71:836-48. PMID: 19121865 DOI: 10.1016/j.theriogenology.2008.10.023

Gandolfi TA, Gandolfi F. The maternal legacy to the embryo: cytoplasmic components and their effects on early development. Theriogenology. 2001;55:1255-76. PMID: 11327683 DOI: 10.1016/S0093-691X(01)00481-2
Gilchrist RB, Thompson JG. Oocyte maturation: emerging concepts and technologies to improve developmental potential in vitro. Theriogenology. 2007;67:6-15. PMID: 17092551 DOI: 10.1016/j.theriogenology.2006.09.027

Gilchrist RB, Lane M, Thompson JG. Oocyte-secreted factors: regulators of cumulus cell function and oocyte quality. Hum Reprod Update. 2008;14:159-77. PMID: 18175787 DOI: 10.1093/humupd/dmn040

Humbert P, Holm P, Lonergan P, Wrenzycki C, Lequarré AS, Joly CG, Herrmann D, Lopes A, Rizos D, Niemann H, Callesen H. Effect of stage of follicular growth during super-ovulation on developmental competence of bovine oocytes. Theriogenology. 2005;63:1149-66. PMID: 15710200 DOI: 10.1016/j.theriogenology.2004.06.002

Immenschuh S, Baumgart-Vogt E. Peroxiredoxins, oxidative stress, and cell proliferation. Antioxid Redox Signal. 2005;7:768-77. PMID: 15890023 DOI: 10.1016/j.ars.2005.7.768

Jones GM, Cram DS, Song B, Magli MC, Gianaroli L, Lacham-Kaplan O, Findlay JK, Jenkin G, Trounson AO. Gene expression profiling of human oocytes following in vivo or in vitro maturation. Hum Reprod. 2008;23:1138-44. PMID: 18346995 DOI: 10.1093/humrep/den085

Katz-Jaffe MG, McCallie BR, Preis KA, Filipovits J, Gardiner DK. Transcriptome analysis of in vivo and in vitro matured bovine MI oocytes. Theriogenology. 2009;71:939-46. PMID: 19150733 DOI: 10.1016/j.theriogenology.2008.10.024

Kasum M, von Wolff M, Franulić D, Čehić E, Klepac-Pulanić T, Orešković S, Joly CG, Herrmann D, Lopes A, Rizos D, Niemann H, Callesen H, Lequarré AS, Joly CG, Herrmann D, Lopes A, Rizos D, Niemann H, Callesen H. Effect of stage of follicular growth during super-ovulation on developmental competence of bovine oocytes. Theriogenology. 2005;63:1149-66. PMID: 15710200 DOI: 10.1016/j.theriogenology.2004.06.002

Krisher RL. In vivo and in vitro environmental effects on mammalian oocyte quality. Annu Rev Anim Biosci. 2013;1:393-417. PMID: 25387025 DOI: 10.1146/annurev-animal-031412-103647

Lambertini M, Ginsburg ES, Partridge AH. Update on fertility preservation in young women undergoing breast cancer therapy. Curr Opin Obstet Gynecol. 2004;16:191-200. PMID: 15180694 DOI: 10.1097/00001703-200206000-00009

Lonergan P, Gutiérrez-Adán A, Rizos D, Pintado B, de la Fuente J, Boland MP. Relative messenger RNA abundance in bovine oocytes collected in vitro or in vivo before and 20 hr after the preovulatory luteinizing hormone surge. Mol Reprod Dev. 2003;66:297-305. PMID: 14502609 DOI: 10.1002/mrd.10357

Martindale JL, Holbrook NJ. Cellular response to oxidative stress: signaling for suicide and survival. J Cell Physiol. 2002;192:1-15. PMID: 12115731 DOI: 10.1002/jcp.10119

Meirelles FV, Caetano AR, Watanabe YF, Ripamonti P, Car- ambula SF, Merighe GK, Garcia SM. Genome activation and developmental block in bovine embryos. Anim Reprod Sci. 2004;82-83:13-20. PMID: 15271440 DOI: 10.1016/j.anireprosci.2004.05.012

Ménézé YJ, Hérubel F. Mouse and bovine models for human IVF. Reprod Biomed Online 2002;4:170-5. PMID: 12470581 DOI: 10.1016/S1472-6483(10)61936-0

Morado SA, Cetica PD, Beconi MT, Dalvit GC. Reactive oxygen species in bovine oocyte maturation in vitro. Reprod Fertil Dev. 2009;21:608-14. PMID: 19383267 DOI: 10.1071/FR08198

Piccioni F, Zappavigna V, Verrotti AC. Translational regulation during oogenesis and early development: the cap-poly(A) tail relationship. C R Biol. 2005;328:863-81. PMID: 16286077 DOI: 10.1016/j.crvi.2005.05.006

Pitcon HM. Oocyte maturation in vitro. Curr Opin Obstet Gynecol. 2002;14:295-302. PMID: 12032386 DOI: 10.1097/00001703-200206000-00009

Pfaffl MW, Horgan GW, Dempflle L. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res. 2002;30:e36. PMID: 11972351 DOI: 10.1093/nar/30.9.e36

Ramakers C, Ruijter JM, Depraet RH, Moorman AF. Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. Neurosci Lett. 2003;328:69-72. PMID: 12082367 DOI: 10.1016/j.neulet.2003.09.011

Reinblatt SL, Buckett W. In vitro maturation for patients with polycystic ovary syndrome. Semin Reprod Med. 2004;22:121-6. PMID: 15181090 DOI: 10.1002/mrd.10243-4

Reyes JM, Chitwood JL, Ross PJ. RNA-Seq profiling of single bovine oocyte transcript abundance and its modulation by cytoplasmic polyadenylation. Mol Reprod Dev. 2015;82:103-14. PMID: 25560149 DOI: 10.1002/mrd.22445

Sauerbrunn-Cutler MT, Veja M, Keltz M, McGovern PG. In vitro maturation and its role in clinical assisted reproductive technology. Obstet Gynecol Surv. 2015;70:45-57. PMID: 25616347 DOI: 10.1097/OGX.000000000000150
Schultz RM. The molecular foundations of the maternal to zygotic transition in the preimplantation embryo. Hum Reprod Update. 2002;8:323-31. PMID: 12206467 DOI: 10.1093/humupd/8.4.323

Shalom-Paz E, Almog B, Shehata F, Huang J, Holzer H, Chian RC, Son WY, Tan SL. Fertility preservation for breast-cancer patients using IVM followed by oocyte or embryo vitrification. Reprod Biomed Online. 2010;21:566-71. PMID: 20822957 DOI: 10.1016/j.rbmo.2010.05.003

Shirasawa H, Terada Y. In vitro maturation of human immature oocytes for fertility preservation and research material. Reprod Med Biol. 2017;16:258-67. PMID: 29259476 DOI: 10.1002/rmb2.12042

Smitz JE, Thompson JG, Gilchrist RB. The promise of in vitro maturation in assisted reproduction and fertility preservation. Semin Reprod Med. 2011;29:24-37. PMID: 21207332 DOI: 10.1055/s-0030-1268701

Suikkari AM. In-vitro maturation: its role in fertility treatment. Curr Opin Obstet Gynecol. 2008;20:242-8. PMID: 18460938 DOI: 10.1097/GCO.0b013e3282f88e33

Suikkari AM, Söderström-Anttila V. In-vitro maturation of eggs: is it really useful? Best Pract Res Clin Obstet Gynaecol. 2007;21:145-55. PMID: 17291833 DOI: 10.1016/j.bpobgyn.2006.09.003

Thelie A, Papillier P, Perreau C, Uzbekova S, Hennenquet-Antier C, Dalbies-Tran R. Regulation of bovine oocyte-specific transcripts during in vitro oocyte maturation and after maternal-embryonic transition analyzed using a transcriptomic approach. Mol Reprod Dev. 2009;76:773-82. PMID: 19343788 DOI: 10.1002/mrd.21031

Ticianelli JS, Emanuelli IP, Satrapa RA, Castilho ACS, Loureiro B, Sudano MJ, Fontes PK, Pinto RFP, Razza EM, Surjus RS, Sartori R, Assumpção MEQA, Visintin JA, Barros CM, Paula-Lopes FF. Gene expression profile in heat-shocked Holstein and Nelore oocytes and cumulus cells. Reprod Fertil Dev. 2017;29:1787-802. PMID: 27802411 DOI: 10.1071/RF16154

Tomek W, Torner H, Kanitz W. Comparative analysis of protein synthesis, transcription and cytoplasmic polyadenylation of mRNA during maturation of bovine oocytes in vitro. Reprod Domest Anim. 2002;37:86-91. PMID: 11975745 DOI: 10.1046/j.1439-0531.2002.00336.x

Tong ZB, Gold L, Pfeifer KE, Dorward H, Lee E, Bondy CA, Dean J, Nelson LM. Mater, a maternal effect gene required for early embryonic development in mice. Nat Genet. 2000;26:267-8. PMID: 11062459 DOI: 10.1038/81547

van de Leemput EE, Vos PL, Zeinstrra EC, Bevers MM, van der Weijden GC, Dieleman SJ. Improved in vitro embryo development using in vivo matured oocytes from heifers superovulated with a controlled preovulatory LH surge. Theriogenology. 1999;52:335-49. PMID: 10734399 DOI: 10.1016/S0093-691X(99)00133-8

Wells D, Patrizio P. Gene expression profiling of human oocytes at different maturational stages and after in vitro maturation. Am J Obstet Gynecol. 2008;198:455.e1-9. PMID: 18395038 DOI: 10.1016/j.ajog.2007.12.030

Wu X, Viveiros MM, Eppig JJ, Bai Y, Fitzpatrick SL, Matzuk MM. Zygote arrest 1 (Zar1) is a novel maternal-effect gene critical for the oocyte-to-embryo transition. Nat Genet. 2003;33:187-91. PMID: 12539046 DOI: 10.1038/ng1079

Zheng P, Patel B, McMenamin M, Moran E, Paprocki AM, Kihara M, Schramm RD, Latham KE. Effects of follicle size and oocyte maturation conditions on maternal messenger RNA regulation and gene expression in rhesus monkey oocytes and embryos. Biol Reprod. 2005;72:890-7. PMID: 15590902 DOI: 10.1095/biolreprod.104.035881