Messenger RNA expression of Pabpnl1 and Mbd3l2 genes in oocytes and cleavage embryos

Fernando Henrique Biase, Ph.D.,[a,b] *Lúcia Martelli, M.D., Ph.D.,*[b] *Renato Puga, M.Sc.,*[b] *Silvana Giuliatti, Ph.D.,*[b] *Weruska Karyna Freitas Santos-Biase, M.Sc.,*[a] *Giovana Krempel Fonseca Merighe, Ph.D.,*[a] *and Flávio Vieira Meirelles, Ph.D.*[a]

[a]Departamento de Ciências Básicas, Faculdade de Zootecnia e Engenharia de Alimentos; and [b]Departamento de Genética, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brazil

Objective: To identify genes specifically expressed in mammalian oocytes using an in silico subtraction, and to characterize the mRNA patterns of selected genes in oocytes, embryos, and adult tissues.
Design: Comparison between oocyte groups and between early embryo stages.
Setting: Laboratories of embryo manipulation and molecular biology from Departamento de Genética (FMRP) and Departamento de Ciências Básicas (FZEA) - University of São Paulo.
Sample(s): Oocytes were collected from slaughtered cows for measurements, in vitro fertilization, and in vitro embryo culture. Somatic tissue, excluding gonad and uterus tissue, was collected from male and female cattle.
Main Outcome Measure(s): Messenger RNA levels of poly(A)-binding protein nuclear-like 1 (Pabpnl1) and methyl-CpG–binding domain protein 3–like 2 (Mbd3l2).
Result(s): Pabpnl1 mRNA was found to be expressed in oocytes, and Mbd3l2 transcripts were present in embryos. Quantification of Pabpnl1 transcripts showed no difference in levels between good- and bad-quality oocytes before in vitro maturation (IVM) or between good-quality oocytes before and after IVM. However, Pabpnl1 transcripts were not detected in bad-quality oocytes after IVM. Transcripts of the Mbd3l2 gene were found in 4-cell, 8-cell, and morula-stage embryos, with the highest level observed in 8-cell embryos.
Conclusion(s): Pabpnl1 gene expression is restricted to oocytes and Mbd3l2 to embryos. Different Pabpnl1 mRNA levels in oocytes of varying viability suggest an important role in fertility involving the oocyte potential for embryo development. (Fertil Steril® 2010;93:2507–12. ©2010 by American Society for Reproductive Medicine.)

Key Words: Bovine, embryo, gene expression

Messenger RNA synthesis and storage in mammalian oocytes after the resumption of meiosis is closely related to the ability of the oocyte to sustain proper early embryo development, both in vivo and in vitro. The correct equilibrium of mRNA synthesis and decay from diverse functional and structural genes is essential for the proper activation of the embryonic genome (1) and the further development of a healthy animal. Using the bovine model, some genes have been reported to have variations in their mRNA abundance owing to different parameters previously recognized as oocyte quality predictors (reviewed by Wrenzycki et al. [2]).

Genes preferentially expressed in oocytes (such as Mater, Zp1, Zp2, Zp3, Figα, and Gdf9) are expected to regulate most of the pathways required for oocyte growth, follicle formation and embryo cleavage (3). However, these oocyte-exclusive genes and their functions have not yet been extensively characterized. To this end, the mining of public sequence and library databases has been of great value for discovering gene expression patterns (4, 5), as well as new genes or gene variants (6, 7). The genes Oogenesin-2 and Oogenesin-3 were previously characterized as being specifically expressed in mouse oocytes (8). Further analyses were conducted on the same dataset and identified other genes (SpeerA, Fbxo12B, Tcl1, Fbxo12D) that were proposed to be specific expressed in mouse oocytes (9). However those studies could represent an underestimation of genes with this expression pattern, because a noncustom tool was used. The identification and comprehension of genes specifically expressed in oocytes may provide a better understanding of how the cytoplasm of the oocyte is able to reprogram the nucleus of a differentiated cell toward that of a totipotent cell.

We tested the hypothesis that the abundance of gene transcripts expressed specifically in oocytes varies according to the potential of the oocyte for sustaining early embryonic development. To address this hypothesis, the present study aimed to identify genes specifically expressed in oocytes applying an in silico approach using UniGene as a source database. After identification of candidate gene clusters, we aimed to characterize the mRNA expression patterns of poly(A)-binding protein nuclear-like 1 (Pabpnl1) and methyl-CpG–binding domain protein 3–like 2 (Mbd3l2) genes in bovine oocytes and early developing embryos and tissues.

MATERIALS AND METHODS

In Silico Search for Oocyte-Specific Transcripts

The files containing the bovine (*Bos taurus*, build #93) and mouse (*Mus musculus*, build #177) UniGene cluster data (ftp://ftp.ncbi.nih.gov/repository/UniGene/ [10]) were used as the information source for in silico gene identification. The search for putative oocyte-specific transcripts was performed in each UniGene entry based on information contained in the

Received January 28, 2009; revised August 18, 2009; accepted August 19, 2009; published online October 12, 2009.
F.H.B. has nothing to disclose. L.M. has nothing to disclose. R.P. has nothing to disclose. S.G. has nothing to disclose. W.K.F.S.B. has nothing to disclose. G.K.F.M. has nothing to disclose. F.V.M. has nothing to disclose.
Supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Brazil.
Reprint requests: Flávio Vieira Meirelles, Departamento de Ciências Básicas, USP-FZEA, Rua Duque de Caxias Norte, 225, Pirassununga–SP, 13635-900, Brazil (FAX: 55 19 3565 4117; E-mail: meirellf@usp.br or fernandobiase@hotmail.com).

RESTRICTED EXPRESSION and SOURCE lines. A Perl program was developed to identify clusters with "egg" as a term in the RESTRICTED EXPRESSION information and no tissues or cell lines listed in the SOURCE line other than eggs or oocytes. This program is available from the author upon request.

Oocyte Collection and in Vitro Maturation

The chemicals used for medium supplementation (for in vitro maturation [IVM], fertilization, and embryo cultures) were obtained from Sigma (St. Louis, MO) unless otherwise specified.

Oocytes were obtained from bovine (*Bos taurus*) ovaries collected at a slaughterhouse. Cumulus oocyte complexes (COCs) were manually aspirated from follicles with a 3–5 mm diameter using an 18-gauge needle. Under a stereomicroscope, COCs were morphologically classified and separated into two groups according to previous descriptions (11): COC-A) homogeneous ooplasm surrounded by $\geq$5 layers of compact cumulus cells; and COC-B) ooplasm that was nonhomogeneous and surrounded by <5 layers of noncompact cumulus cells. The morphology described above reflects a distinction in the competence of an oocyte to develop early embryos in an in vitro culture, where COC-A is representative of oocytes with higher competence than oocytes from COC-B [blastocyst development rate for grade A oocytes = 20.6%; for grade B oocytes = 12.9% (F. V. Meirelles, unpublished observations)]. Immediately after aspiration, the COCs of different morphologies were matured separately in vitro in TCM199–sodium bicarbonate medium (Gibco-BRL, Grand Island, NY) supplemented with 10% fetal bovine serum 50 μL of gentamycin, 50 μg/mL LH, 22 μg/mL pyruvate; 0.5 μg/mL follicle stimulant hormone; and 1 μg/mL E_2 in air atmosphere with 5% CO_2, 100% humidity, and temperature of 38°C.

The COCs were sampled at the time of recovery from ovaries [oocytes-GV (germinal vesicle)] and after 18 hours of IVM [oocytes-MII (metaphase II)]. Cumulus cells were removed from oocytes by vortexing in sterile phosphate-buffered saline solution. Groups of 20 immature and 20 mature oocytes of each COC morphology group (A or B) were collected in three different experiments, snap frozen in liquid nitrogen, and stored at −80°C.

In Vitro Fertilization and Bovine Embryo Culture

Groups consisting of 30 high-quality matured COCs (morphologically classified as grade A) were fertilized in vitro in 100 μL IVF medium [TALP, supplemented with heparin (10 μg/mL), pyruvate (22 μg/mL), gentamicin (50 μg/mL), bovine serum albumin (BSA) fraction V (6 mg/mL), penicilin (2 μmol/L), hypotaurin (1 μmol/L), and epinephrine (0.25 μmol/L)] (12) containing 2×10^6 spermatozoa/mL. After 18 hours, the presumptive zygotes had the cumulus cells removed by manual pipetting and were then cocultured with a cumulus cell layer in a 100-μL microdrop of IVF medium under mineral oil. In vitro fertilization and embryo cultures were incubated at 38.0°C in water-saturated atmosphere containing 5% CO_2. Embryos were collected at 27 (2 cells), 42 (4 cells), 48 (8 cells), 120 (morula), 160 (blastocyst), and 210 (hatched blastocyst) hours after insemination (hpi), snap frozen in liquid nitrogen, and stored at −80°C. Twenty embryos of each stage were collected in three independent IVF proceedings.

Oocyte and Embryo mRNA Extraction and Reverse Transcription Reactions

Messenger RNA from pools of 20 oocytes or embryos was extracted using the QuickPrep Micro mRNA Purification kit (Amersham Biosciences, Buckinghamshire, U.K.) according to the manufacturer's instructions. Briefly, extraction buffer was added to the tube containing the oocytes or embryos and, after mixing, an elution buffer was added. The mixture was centrifuged in a column containing oligo(dT) cellulose. After successive washings with salt buffers, the mRNA was recovered by centrifugation with elution buffer. The mRNA was concentrated by ethanol precipitation (using glycogen, sodium acetate, and an ethanol solution), and the pellet was resuspended in 10 μL of RNase-free water.

Immediately thereafter, 1 μL RNase-free water containing oligo($dTTP_{(12–18)}$) (1 μg) was added to each mRNA-containing tube. The tubes were heated to 70°C for 5 minutes and then cooled to 4°C for the addition of a mix of reagents (supplied buffer, 250 mmol/L; Tris, 375 mmol/L; KCl, 50 mmol/L; DTT, 50 mmol/L; $MgCl_2$, 3 mmol/L; dNTP, 10 mmol/L each; RNase inhibitor, 1 U/μL) and enzyme (ImProm-II Reverse Trascriptase; Promega, Madison, WI) to a final volume of 20 μL. The cDNA synthesis was performed at 50°C for 1 hour, followed by enzyme heat-inactivation at 70°C and storage at −20°C.

RNA Extraction From Different Tissues and Reverse Transcription Reactions

Total RNA was extracted from the following somatic and gonad tissues or cells: brain, corpus luteum, cumulus cells, fibroblast, heart, kidney, leukocyte, liver, lung, muscle, ovary stroma, spleen, stomach, testis, and uterus. For the evaluation of transcript expression in the ovary, oocytes were dissociated as much as possible through manual separation under a microscope. Therefore, it was considered that the tissue evaluated was composed mostly of cells from the stroma. RNA extraction was performed by homogenizing 100 mg of each tissue or cells with Trizol reagent (Invitrogen, Carlsbad, CA). After the addition of chloroform (0.2 mL), the tubes were centrifuged. The aqueous phase was transfered to a new tube, and RNA was precipitated with isopropyl alcohol (0.5 mL) and centrifugation. The RNA pellet was washed with 75% ethanol (1 mL) and then air dried, resuspended in RNase-free water, and stored at −85°C until further use. Reverse transcription from these tissues was performed using 1 μg of RNA as the template using the same procedures described above for oocyte and embryo mRNA reverse transcription.

Real-Time Polymerase Chain Reactions and Analysis

The genes chosen to validate the in silico subtraction results were Pabpnl1 and Mbd3l2, and glyceraldeyde 3-phosphate dehydrogenase (Gapdh) was used as a reference gene for polymerase chain reaction (PCR) data normalization.

The reactions were performed in a real-time PCR thermocycler (7500 Real Time PCR System; Applied Biosystems, Foster City, CA) using Sybr Green I as the double-strain DNA dye. Reactions were carried out using the LightCycler FastStart DNA Master Sybr Green I reaction mix (1×) (Roche Diagnostics Corporation, Indianapolis, IN), supplemented with $MgCl_2$ (5 mmol/L), and a specific pair of primers (Table 1) in a final volume of 20 μL. The cycle protocol initiated with denaturation at 95°C for 10 minutes, followed by 45 cycles at 95°C for 10 seconds, at a primer specific annealing temperature for 30 seconds and at 72°C for 45 seconds, and then finished with a standard dissociation curve. The cDNA equivalent of one oocyte or embryo was used as a template for triplicate reactions for each gene. The reactions for target and normalization genes were performed separately. For three different extracted pools of mRNA, a mix sufficient for nine reactions was set up with the produced cDNA. Each reaction mix was separated into three sets for the addition of gene specific primers with a subsequent separation for technical triplicates. Amplicons for both target genes were sequenced to assure the specificity of the primers.

The PCR efficiency was estimated for each sample reaction and each gene using the window-of-linearity method (13). For each pair of primers, the fluorescence threshold line was fixed at the average of the lower and higher fluorescence values used by the software to estimate the PCR efficiency.

Quantification of relative Pabpnl1 and Mbd3l2 levels between samples was calculated using the method described for different primer efficiencies (14), and the significance of the differences was estimated using a pair-wise fixed reallocation randomization test in the Relative Expression Software Tool (15). The hypothesis of "no difference between groups" was considered to be null (H_0), and the differences were regarded to be statistically significant when the probability of the alternative hypothesis ($P_{(H1)}$) was $<.05$. Results are shown as the average rate of expression and standard error of the three replicates.

RESULTS

The Perl routine developed for this study produced consistent results after sequential analysis, making it possible to identify UniGene IDs containing the term "egg" in the first information field (RESTRICTED EXPRESSION) and no other types of specific tissues in the second (SOURCE). The program retrieved 19 mouse UniGene

TABLE 1

Oligonucleotides and annealing temperatures used in polymerase chain reactions (PCRs), amplicon lengths, and melting temperatures.

Gene symbol	Primers 5′ = > 3′	GenBank gi	Annealing temperature	Fragment length	T_m [a]
Pabpnl1	AGCCGAGAAGACCCATCTGG CCTTCATCCAGGTTCTGCACC	76648613	62°C	231	89°C
Mbd3l2	TGGATTCAAGGACACCAGCATGTG TCTGTCACGACGCCTCTGCTTG	119894802	66°C	175	84°C
Gapdh	GGCGTGAACCACGAGAAGTATAA CCCTCCACGATGCCAAAGT	28189708	62°C	118	87°C

Note: Gapdh = glyceraldeyde 3-phosphate dehydrogenase; Mbd3l2 = methyl-CpG–binding domain protein 3–like 2; Pabpnl1 = poly(A)-binding protein nuclear-like 1.

[a] Melting temperature of PCR products.

Biase. Pabpnl1 and Mbd3l2 mRNA levels in cattle. Fertil Steril 2010.

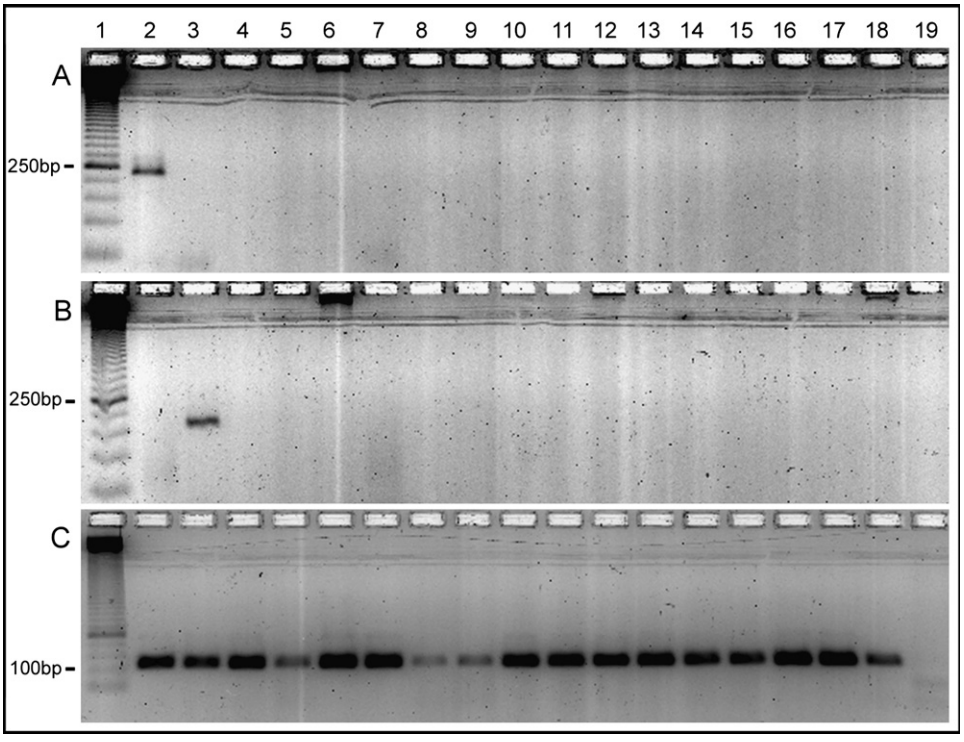
IDs, of which eight were annotated in GenBank (Astl, Mbd3l2, Obox1, Omt2b, Oog2, Pabpnl1, Tcl1b1, Tcl1b2). No cluster was found in the bovine database.

Sequences of the eight annotated UniGene IDs for mouse were compared against the bovine RefSeq and Genome sequences using a web-based BLAST tool (16). Functional prediction analysis using the protein Motif database (17) and a review of the literature were performed after identification of the corresponding bovine genes and sequences. We characterized mRNA expression of two genes in bovine oocytes, embryos, and other tissues.

Transcription profiles of the genes Pabpnl1 and Mbd3l2 were characterized in bovine tissues, oocytes, and embryos by real-time PCR. No mRNA expression of either gene was detected in somatic and gonad tissues. Transcripts of Pabpnl1 were observed in oocytes both before and after IVM, whereas Mbd3l2 transcripts were detected only in early embryos (Fig. 1).

FIGURE 1

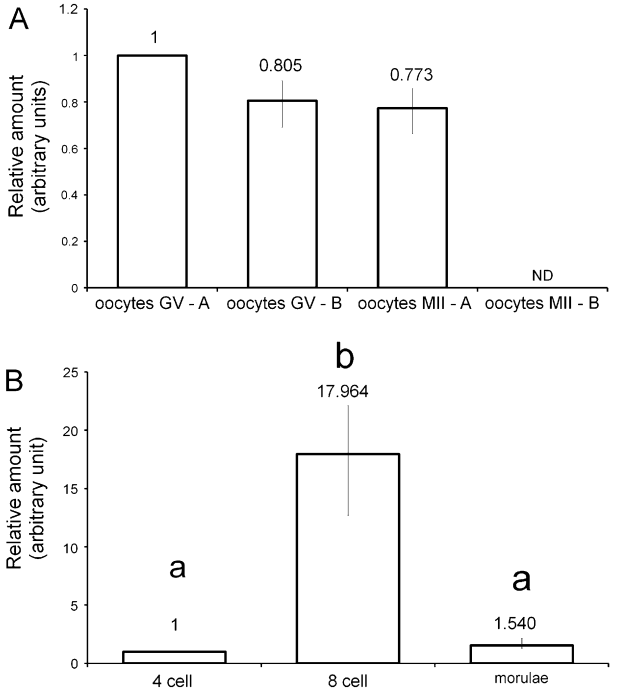
Representative reverse-transcription polymerase chain reaction detection of poly(A)-binding protein nuclear-like 1 (Pabpnl1) and methyl-CpG–binding domain protein 3–like 2 (Mbd3l2) transcripts in bovine tissues and cells. Agarose gel containing ethidium bromide showing the amplified fragments for the (**A**) Pabpnl1, (**B**) Mbd3l2, and (**C**) glyceraldeyde 3-phosphate dehydrogenase genes. Lanes: 1) 50-bp DNA marker; 2) immature and in vitro–matured oocytes; 3) preimplantation embryos; 4) brain; 5) corpus luteum; 6) cumulus cells; 7) fibroblast; 8) heart; 9) kidney; 10) leukocyte; 11) liver; 12) lung; 13) muscle; 14) ovary; 15) spleen; 16) stomach; 17) testis; 18) uterus, 19) control reaction without cDNA template.



Biase. Pabpnl1 and Mbd3l2 mRNA levels in cattle. Fertil Steril 2010.

FIGURE 2

Relative quantification of poly(A)-binding protein nuclear-like 1 (Pabpnl1) and methyl-CpG–binding domain protein 3–like 2 (Mbd3l2) transcripts. (**A**) Pabpnl1 expression in oocytes before maturation with germinal vesicle (GV), after in vitro maturation at metaphase II (MII) stage of high-quality (A) and low-quality (B) oocytes; ND=transcripts not detected. (**B**) Mbd3l2 expression in early bovine embryos. The vertical lines are the standard error; different letters above the numbers indicate statistical significance ($P<.05$).



Biase. Pabpnl1 and Mbd3l2 mRNA levels in cattle. Fertil Steril 2010.

A detailed analysis of expression of the Pabpnl1 gene was performed in oocytes with different developmental potential, before and after IVM (Fig. 2A). The transcript of this gene was detected in oocytes from grade A and B COCs before IVM; however, after IVM, it was detected only in oocytes from grade A COCs, and it was not found in poor-quality oocytes. Relative Pabpnl1 expression was not significantly different between oocytes recovered from grade A and B COCs before IVM, and the same was observed for oocytes recovered from grade A COCs before and after IVM.

The analysis of Mbd3l2 gene expression in embryos fertilized in vitro was performed at different hours after insemination (Fig. 2B). Transcripts for this gene were first detected in 4-cell embryos (42 hpi) and were up-regulated in 8-cell embryos (48 hpi, 17.9-fold; $P<.01$), and they then decreased in embryos at the morula stage (120 hpi, 0.08-fold; $P<.01$). No PCR amplification was detected in 2-cell embryos (27 hpi), blastocysts (160 hpi), or hatched blastocysts (210 hpi).

DISCUSSION

A computational approach was developed using a Perl routine to extract information about clusters of sequences expressed in different tissues from an organism of interest. This program was written to read and extract information from UniGene files (for example: Mm.seq.all.gz), independent of the species of interest. The results obtained from this Perl routine contained three UniGene identifications previously reported to be specifically expressed in mammal oocytes: Tcl1b1 (18, 19) and Oog2 and Tcl1b2 (18). However, our search did not find other genes previously described as specifically expressed in oocytes: Mater (20), Oosp1 (21), and Zar1 (22). This divergence between the description of genes in the literature and the UniGene databank may be caused by the high sensitivity of cDNA library construction and increases in sequence deposition to the databank by different laboratories, which leads to a better qualitative representation of a gene in a tissue. Although the script was written to investigate oocytes in this study, it is possible to retrieve information regarding other tissues with simple modifications. The lack of information in the bovine database compared with the mouse counterpart might be related to the extensive research that has been done with the murine model since the sequencing and analysis of the mouse genome to understand mammalian reproduction (23). However, the sequencing and analysis of the bovine genome revealed that the chromosomal arrangement is more similar between cattle and humans than between humans and rodents (24).

After a search regarding function in a protein motif database and a review of the literature, two genes putatively expressed only in oocytes were experimentally tested to address our hypothesis regarding developmental competence; however, we characterized mRNA expression of its orthologues using the bovine model. Transcripts of Pabpnl1 were detected in oocytes, but not in 16 tested bovine somatic tissues, testis, or early developing embryos. The expression in bovine oocytes confirms the previous findings for *Xenopus* and mouse oocytes (25, 26).

Expression of Pabpnl1 mRNA was also observed in mouse 2-cell embryos (27) and in *Xenopus* embryos and testis cells (25); however, no transcripts were detected in early developing bovine embryos or testis cells. This might be caused by an evolutionary discrepancy in expression patterns between amphibians and amniotes and between rodents and artiodactyla.

Transcripts of the Pabpnl1 gene were not detected in low-quality oocytes after IVM. In previous reports, we showed that low-competence oocytes had ~79% less global mRNA content compared with high-competence oocytes, and this global variation also reflected the difference in Gapdh mRNA levels (~84%, [28]). The absence of Pabpnl1 mRNA detected in low-quality oocytes is in accordance with the small amount of mRNA in these oocytes.

The degradation or translational repression of mRNA is highly associated with the adenylation status of the transcript, and polyA-binding proteins (PABP) have important functions in this process because they are protective against RNA deadenylation (29, 30). The Pabpnl1 protein was observed in mouse oocytes (25), where it has a function in mRNA protection (31) and may stimulate translation, as ePAB does (32). In bovine oocytes, the pattern of adenylation varies among genes; in some cases, however, mRNAs are highly adenylated in oocytes with high developmental competency compared with those that have low developmental competency (33). The down-regulation of the Pabpnl1 gene in low-competence oocytes reinforces the importance of the PABP family in mammalian oocytes and shows that the Pabpnl1 gene may be regulated in response to the developmental potential of the oocyte in bovines.

Transcripts of Mbd3l2 were not observed in bovine somatic tissues and oocytes, but they were detected in embryos at the 4-cell, 8-cell, and morula stages. The expression of Mbd3l2 in bovines, however, is not in accordance with the observed expression in human testis, prostate, thymus, and spleen tissues (4), the human ovarian teratocarcinoma cell line PA1, and the mouse embryonic

carcinoma cell line P19 (34). Microarray studies have detected its expression in mouse oocytes (26, 35), but this expression was restricted to the small and large antral follicle stages and was not found in the primary follicle stage (36). In the present experiment, the presence of Mbd3l2 transcripts was not detected in three replicates of 20 oocytes each, either because it is present at low frequency or because it was not present. The expression in embryos was considered to be a positive control reaction.

The presence of Mbd3l2 mRNA in bovine embryos was detected during the transition between maternal to embryonic control of development (embryonic genome activation [EGA] [37]). The mRNA expression of Mbd3l2 does not follow the major pattern of mRNA accumulation and degradation observed previously for in vitro–produced bovine embryos (28); however, it has been demonstrated that gene expression during cleavage is cell-stage dependent (38), and this gene showed a marked transient upregulation in transcription between the 4-cell stage and morula stage.

In mouse, EGA is programmed during the transition from 1- to 2-cell embryos (39), when Mbd3l2 is up-regulated (26). It was reported that in the CpG-methylated DNA binding complex (MeCP1), the protein Mbd3l2 interacts with the nucleosome remodeling and histone deacetylase complex (Mi2-NuRD) via substitution of methyl-binding domain 3 (MBD3) protein and release of the MBD2 of the CpG-methylated promoter. This finding led to the understanding that Mbd3l2 has a key function in reactivation of gene transcription (40). The temporally limited expression of Mbd3l2 transcripts is coincident with EGA in bovine and mouse (37), and it could indicate that this protein also promotes MeCP1 release from repressed genes during maternal to embryonic transcript transition.

In summary, a Perl program was written to analyze public information in the UniGene database and identify putative genes specifically expressed in oocytes compared with every other tissue represented in the database. One bovine oocyte-specific gene was identified (Pabpnl1) that showed differential expression in oocytes matured in vitro that had different competence for embryonic development. Because the Pabpnl1 mRNA was not detected in oocytes at the meiosis II stage, it is likely that Pabpnl1 is one of the important factors regulating gene expression at the translational level in oocytes. These results also suggest that measuring gene expression as temporally close as possible to the moment of fertilization may be more informative in elucidating the maternal cytoplasmic inheritance factors that drive the proper early development of the embryo. The gene Mbd3l2 showed an embryonically restricted transcription in bovines, as well as a temporally regulated expression during the early cleavages. Both genes, Pabpnl1 and Mbd3l2, should be considered for further functional studies to identify RNA and proteins that affect fertility through oocyte quality and the promotion of embryonic development.

Acknowledgments: The authors are grateful for valuable suggestions from the anonymous referees of this journal.

REFERENCES

1. Meirelles FV, Caetano AR, Watanabe YF, Ripamonte P, Carambula SF, Merighe GK, et al. Genome activation and developmental block in bovine embryos. Anim Reprod Sci 2004;82–83:13–20.
2. Wrenzycki C, Herrmann D, Niemann H. Messenger RNA in oocytes and embryos in relation to embryo viability. Theriogenology 2007;68(Suppl. 1):S77–83.
3. Dean J. Oocyte-specific genes regulate follicle formation, fertility and early mouse development. J Reprod Immunol 2002;53:171–80.
4. Jiang CL, Jin SG, Pfeifer GP. MBD3L1 is a transcriptional repressor that interacts with methyl-CpG-binding protein 2 (MBD2) and components of the NuRD complex. J Biol Chem 2004;279:52456–64.
5. Ferguson DA, Chiang JT, Richardson JA, Graff J. eXPRESSION: an in silico tool to predict patterns of gene expression. Gene Expr Patterns 2005;5: 619–28.
6. Kumar CG, Larson JH, Band MR, Lewin HA. Discovery and characterization of 91 novel transcripts expressed in cattle placenta. BMC Genomics 2007;8:113.
7. Choo KB, Chen HH, Cheng WT, Chang HS, Wang M. In silico mining of EST databases for novel pre-implantation embryo-specific zinc finger protein genes. Mol Reprod Dev 2001;59:249–55.
8. Dade S, Callebaut I, Mermillod P, Monget P. Identification of a new expanding family of genes characterized by atypical LRR domains. Localization of a cluster preferentially expressed in oocyte. FEBS Lett 2003;555:533–8.
9. Paillisson A, Dade S, Callebaut I, Bontoux M, Dalbies-Tran R, Vaiman D, et al. Identification, characterization and metagenome analysis of oocyte-specific genes organized in clusters in the mouse genome. BMC Genomics 2005;6:76.
10. Wheeler DL, Church DM, Lash AE, Leipe DD, Madden TL, Pontius JU, et al. Database resources of the National Center for Biotechnology Information: 2002 update. Nucleic Acids Res 2002;30:13–6.
11. Bilodeau-Goeseels S, Panich P. Effects of oocyte quality on development and transcriptional activity in early bovine embryos. Anim Reprod Sci 2002;71: 143–55.
12. Parrish JJ, Susko-Parrish J, Winer MA, First NL. Capacitation of bovine sperm by heparin. Biol Reprod 1988;38:1171–80.
13. Ramakers C, Ruijter JM, Deprez RH, Moorman AF. Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. Neurosci Lett 2003;339:62–6.
14. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 2001;29. e45.
15. Pfaffl MW, Horgan GW, Dempfle 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.
16. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol 1990;215:403–10.
17. Gaulton A, Attwood TK. Motif3D: Relating protein sequence motifs to 3D structure. Nucleic Acids Res 2003;31:3333–6.
18. Paillisson A, Dade S, Callebaut I, Bontoux M, Dalbies-Tran R, Vaiman D, et al. Identification, characterization and metagenome analysis of oocyte-specific genes organized in clusters in the mouse genome. BMC Genomics 2005;6:76.
19. Rajkovic A, Yan MSC, Klysik M, Matzuk M. Discovery of germ cell-specific transcripts by expressed sequence tag database analysis. Fertil Steril 2001;76:550–4.
20. Dalbies-Tran R, Papillier P, Pennetier S, Uzbekova S, Monget P. Bovine mater-like NALP9 is an oocyte marker gene. Mol Reprod Dev 2005;71:414–21.
21. Tremblay K, Vigneault C, McGraw S, Morin G, Sirard MA. Identification and characterization of a novel bovine oocyte-specific secreted protein gene. Gene 2006;375:44–53.
22. Pennetier S, Uzbekova S, Perreau C, Papillier P, Mermillod P, Dalbies-Tran R. Spatio-temporal expression of the germ cell marker genes MATER, ZAR1, GDF9, BMP15, and VASA in adult bovine tissues, oocytes, and preimplantation embryos. Biol Reprod 2004;71:1359–66.
23. Mouse Genome Sequencing Consortium. Initial sequencing and comparative analysis of the mouse genome. Nature 2002;420:520–62.
24. Bovine Genome Sequencing and Analysis Consortium. The genome sequence of taurine cattle: a window to ruminant biology and evolution. Science 2009;324:522–8.
25. Good PJ, Abler L, Herring D, Sheets MD. *Xenopus* embryonic poly(A) binding protein 2 (ePABP2) defines a new family of cytoplasmic poly(A) binding proteins expressed during the early stages of vertebrate development. Genesis 2004;38:166–75.
26. Zeng F, Baldwin DA, Schultz RM. Transcript profiling during preimplantation mouse development. Dev Biol 2004;272:483–96.
27. Seli E, Lalioti MD, Flaherty SM, Sakkas D, Terzi N, Steitz JA. An embryonic poly(A)-binding protein (ePAB) is expressed in mouse oocytes and early preimplantation embryos. Proc Natl Acad Sci U S A 2005;102:367–72.
28. Biase FH, Fonseca Merighe GK, Santos Biase WK, Martelli L, Meirelles FV. Global poly(A) mRNA expression profile measured in individual bovine oocytes and cleavage embryos. Zygote 2008;16: 29–38.
29. Meyer S, Temme C, Wahle E. Messenger RNA turnover in eukaryotes: pathways and enzymes. Crit Rev Biochem Mol Biol 2004;39:197–216.
30. Newbury SF, Muhlemann O, Stoecklin G. Turnover in the Alps: an mRNA perspective. Workshops on mechanisms and regulation of mRNA turnover. EMBO Rep 2006;7:143–8.
31. Voeltz GK, Ongkasuwan J, Standart N, Steitz JA. A novel embryonic poly(A) binding protein, ePAB, reg-

ulates mRNA deadenylation in Xenopus egg extracts. Genes Dev 2001;15:774–88.
32. Wilkie GS, Gautier P, Lawson D, Gray NK. Embryonic poly(A)-binding protein stimulates translation in germ cells. Mol Cell Biol 2005;25:2060–71.
33. 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.
34. Jiang CL, Jin SG, Lee DH, Lan ZJ, Xu X, O'Connor TR, et al. MBD3L1 and MBD3L2, two new proteins homologous to the methyl-CpG-binding proteins MBD2 and MBD3: characterization of MBD3L1 as a testis-specific transcriptional repressor. Genomics 2002;80:621–9.
35. Kocabas AM, Crosby J, Ross PJ, Otu HH, Beyhan Z, Can H, et al. The transcriptome of human oocytes. Proc Natl Acad Sci U S A 2006;103: 14027–32.
36. Pan H, O'Brien MJ, Wigglesworth K, Eppig JJ, Schultz RM. Transcript profiling during mouse oocyte development and the effect of gonadotropin priming and development in vitro. Dev Biol 2005;286:493–506.
37. Telford NA, Watson AJ, Schultz GA. Transition from maternal to embryonic control in early mammalian development: a comparison of several species. Mol Reprod Dev 1990;26:90–100.
38. Hamatani T, Carter MG, Sharov AA, Ko MS. Dynamics of global gene expression changes during mouse preimplantation development. Dev Cell 2004;6: 117–31.
39. Schultz RM. The molecular foundations of the maternal to zygotic transition in the preimplantation embryo. Hum Reprod Update 2002;8:323–31.
40. Jin SG, Jiang CL, Rauch T, Li H, Pfeifer GP. MBD3L2 interacts with MBD3 and components of the NuRD complex and can oppose MBD2-MeCP1-mediated methylation silencing. J Biol Chem 2005;280:12700–9.