Reproduction in Domestic Animals

Reprod Dom Anim doi: 10.1111/j.1439-0531.2010.01617.x
ISSN 0936-6768

Expression of Maternal Transcripts During Bovine Oocyte *In Vitro* Maturation is Affected by Donor Age

R Romar[1], T De Santis[2], P Papillier[3], C Perreau[3], A Thélie[3], ME Dell'Aquila[2], P Mermillod[3] and R Dalbiès-Tran[3]

[1]*Faculty of Veterinary Science, Department of Physiology, University of Murcia, Murcia, Spain;* [2]*Department of Animal Production, University of Bari, Valenzano-Bari, Italy;* [3]*Physiologie de la Reproduction et des Comportements, UMR 85 Institut National de la Recherche Agronomique/UMR6175 Centre National de la Recherche Scientifique/Université François Rabelais de Tours/Haras Nationaux, Nouzilly, France*

Contents

The primary objective of this study was to compare expression of maternal transcripts in bovine oocyte populations with differential developmental competence: oocytes from prepubertal and pubertal animals; and oocytes from small (3–4 mm) and large (6–10 mm) follicles from pubertal animals. All transcripts were examined in oocytes prior to and after *in vitro* maturation (IVM). Genes were selected based on their known maternal effect in mouse (*ZAR1*, *STELLA*, *HSF1*, *MATER*/NLRP5 and its paralogue *NLRP9*), or their identification as markers of oocyte maturation, either involved in redox metabolism (*PRDX1*, *PRDX2*) or meiotic progression (*AURKA*). Total or polyadenylated forms of the transcripts were followed by reverse transcription coupled to real-time PCR. Six polyadenylated transcripts were found significantly reduced after maturation irrespective of donor age or follicle diameter ($p < 0.05$). Within these six polyadenylated transcripts, ZAR1, NLRP9, HSF1, PRDX1 and PRDX2 were significantly reduced in oocytes from prepubertal animals compared to adult animals ($p < 0.05$). A younger age was also associated with lower abundance (total form) of PRDX2/PRDX1 irrespective of maturation. Total HSF1, PRDX1 and polyadenylated NLRP9 showed a tendency (p values from 0.053 to 0.08) for a higher detection in oocytes from small follicles, thus encouraging further investigation of the follicle diameter model. However, at the present time, follicle size did not significantly affect expression of transcripts examined. In conclusion, this study demonstrates differences in the maternal store of RNA and its regulation during IVM which is dependent on donor age.

Introduction

Early embryonic failures are often related to poor oocyte quality because of the predominant role of maternal factors, at least until the embryo fully activates its own genome. *In vitro* experiments on the mammalian oocyte demonstrate the ability to accomplish nuclear competence and to sustain embryo development which is acquired successively during meiosis. Growth of the antral follicle and oocyte maturation are critical phases in generating a developmentally competent oocyte, thus the notion of cytoplasmic pre-maturation and maturation was developed; however, the underlying molecular events remain largely unknown (Bonnet et al. 2008; Ferreira et al. 2009). Transcription is very active in the growing oocyte, although the fully grown oocyte is essentially transcriptionally quiescent. Gene expression in early stage embryos relies mostly on post-transcriptional control of maternal transcripts accumulated during oocyte maturation. Therefore, suboptimal store of maternal RNA or inadequate regulation of their stability and polyadenylation are believed to be negatively impact subsequent embryo development.

Previous grouping of bovine oocyte populations based on their developmental competence (Blondin and Sirard 1995, Sun et al. 2008) makes this species a pertinent scientific model to address this hypothesis. Mature oocytes, collected by ovum pick-up shortly before ovulation, represent the optimal model of oocyte quality; up to 70% develop into blastocysts after *in vitro* fertilization (IVF), whereas only 30–40% of oocytes after *in vitro* maturation (IVM) (van de Leemput et al. 1999; Dieleman et al. 2002; Rizos et al. 2002; Humblot et al. 2005). Oocyte developmental competence appears to be acquired during antral follicular growth, as the proportion of competent oocytes increases with antral follicle diameter > 3 mm (Pavlok et al. 1992; Lonergan et al. 1994; Blondin and Sirard 1995; Hagemann et al. 1999; Machatkova et al. 2004; Kauffold et al. 2005; Lequarre et al. 2005; Beker-van Woudenberg et al. 2006). Oocyte quality is also influenced by donor age: oocytes from prepubertal animals display a suboptimal developmental competence after IVF, parthenogenesis and nuclear transfer compared to oocytes from adult animals (Levesque and Sirard 1994; Revel et al. 1995; Damiani et al. 1996; Gandolfi et al. 1998; Khatir et al. 1998; Majerus et al. 2000; de Paz et al. 2001; Salamone et al. 2001; Kauffold et al. 2005). Biochemical and molecular parameters of these differential populations of oocytes have been investigated.

A multi-parametric study on follicle diameter showed no significant differences at the oocyte level (Lequarre et al. 2005). In this study, oxygen, pyruvate uptake and lactate release were evaluated, and the corresponding follicle growth was not associated with distortion of neither the transcriptome nor the pattern of protein neosynthesis during IVM, although the transcriptome was analysed only in immature oocytes and onto a low density array of 1176 genes not including maternal effect genes. Thus, variation of a subset of developmentally important transcripts cannot be excluded. Prepubertal calf oocytes have been reported to differ from adult cow oocytes in several aspects: calf oocytes undergo nuclear maturation at a slower rate (Khatir et al. 1998), are smaller in diameter, metabolize glutamine and pyruvate at a lower rate, exhibit an earlier decline in protein synthesis (Gandolfi et al. 1998); and contain more microvilli on their cell surface, more endocytic vesicles and fewer mitochondria than cow oocytes (de Paz et al. 2001). Whether these features are related to developmental competence remains unknown.

The introduction of molecular biology has prompted the search for molecular markers of oocyte quality. Through the use of real-time PCR, transcript levels of selected target genes in oocytes were compared during *in vitro* vs *in vivo* maturation (Rizos et al. 2002; Jakobsen et al. 2006; Thelie et al. 2007), or in oocytes from growing follicles (Mourot et al. 2006; Racedo et al. 2008), or in oocytes selected by brilliant cresyl blue (Mota et al. 2009). Interestingly, two studies (Mourot et al. 2006; Thelie et al. 2007) reported a differential expression of the peroxiredoxin1 (PRDX1) transcript, whose protein product is involved in response to oxidative stress.

Maternal effect genes are obvious candidates as markers of developmental competence, as their aberrant expression would impair embryo development without abhorrently altering oogenesis or folliculogenesis. Such genes have been mostly characterized in mice by knock-out strategies. Oocytes from maternal effect gene-null mice are ovulated and fertilized, even though the resulting embryos did not develop beyond a few cell divisions (for reviews, see Zheng and Dean 2007, Sun et al. 2008). An example is heat-shock transcription factor 1 (*HSF1*) (Christians et al. 2000). Besides the bovine orthologues Zygote Arrest 1 (*ZAR1*) (Uzbekova et al. 2006), *STELLA* [also Developmental Pluripotency Associated 3 (Dppa3)] (Thelie et al. 2007), Maternal Antigen that Embryos Require [*MATER*; also *NACHT*, leucine-rich repeat and PYD containing 5 (*NALP5*)] (Pennetier et al. 2005, 2006) as well as its paralogue *NLRP9* (Dalbies-Tran et al. 2005) have been described as maternal effect genes.

Using real-time PCR, we have compared the expression of these developmentally important maternal effect genes in bovine oocyte populations with differential developmental competence: oocytes from 3 to 8 mm diameter follicles from prepubertal and pubertal animals; and oocytes from small (3–4 mm) and large (6–10 mm) follicles from pubertal animals. Oocytes in this study were selected from follicles of particular diameters because bovine oocytes must have reached a diameter of at least 110 μm to complete meiotic maturation which corresponds to a 2–3 mm diameter follicle (Fair et al. 1995). Immature oocytes, as well as in *in vitro*-matured oocytes, were evaluated to investigate inadequate RNA synthesis or storage, and the reduction in developmental competence associated with altered post-transcriptional regulation during maturation.

Materials and Methods

Unless otherwise indicated, all chemicals and reagents were purchased from Sigma (Sigma, Saint Quentin Fallavier, France).

Oocyte collection and IVM

Ovaries from 3 to 7 months old calves (displaying no sign of cyclic activity) and adult cows were obtained from a local slaughterhouse, and oocyte-cumulus complexes (OCC) were collected by aspiration from follicles approximately 3–8 mm in diameter. For follicle size study, follicles were dissected from cow ovaries, measured under the microscope using a graduated ocular, and separated into two groups based on their diameter, 3–4 mm (small) and 6–10 mm (large). The corresponding OCC were extracted, and those showing signs of atresia were discarded. Oocyte-cumulus complexes were washed several times in hepes-buffered TCM199. Ten OCC were submitted to mechanical treatment until separation of oocytes from surrounding somatic cells; absence of cumulus cells was checked visually under a microscope. Pools of 8–10 denuded immature oocytes were frozen in RNAlater solution (Ambion, Huntingdon, UK) at −80°C. The remaining OCC were allowed to undergo IVM for 22 h at 39°C in water-saturated air with 5% CO_2; maturation medium was TCM199 supplemented as previously described (Donnay et al. 2004). Groups of OCC (calf vs cow) were incubated in maturation medium drops under mineral oil in four-well plates (Nunc, Roskilde, Denmark) at a ratio of 1 OCC/10 μl medium. After IVM, oocytes with an expanded cumulus were denuded as described, washed, and pools of 8–10 oocytes showing the extrusion of first polar body were frozen in RNAlater. The whole process was replicated four times with independent batches of ovaries for each model.

Isolation of RNA, reverse-transcription and real-time PCR

For each experimental model (donor age, follicle size), RNA from four biological replicates for the four populations was isolated in parallel. One picogram of luciferase mRNA (Promega, Charbonnières-les-Bains, France) per oocyte was added as an exogenous standard. There is so far a lack of consensus on the choice of an internal standard in the specific model of oocyte maturation, as during the process many housekeeping transcripts undergo degradation and/or deadenylation. Therefore, normalization to an exogenous RNA has been widely applied in the field (Bettegowda et al. 2006; Pisani et al. 2008; Vigneault et al. 2009). Total DNAse-treated RNA was extracted using the PicoPure ARN Isolation Kit (Alphelys, Plaisir, France) following the manufacturer's instructions. It was divided into two equal parts, and reverse transcription was performed using either oligo$(dT)_{15}$ primers (Promega) or random hexamers (Promega) at 42°C for 50 min using Superscript-II reverse transcriptase (Invitrogen, Cergy-Pontoise, France) followed by 15 min inactivation at 70°C. Target cDNA were then quantified by real-time PCR using iQ SYBR green supermix (Bio-Rad, Marnes la Coquette, France) in a MyCycler system (Bio-Rad) using a cDNA amount equivalent to 0.2 oocyte and specific primers for each gene (Table 1) as previously published (Thelie et al. 2007). Forty PCR cycles (95°C for 30 s, 60°C for 30 s, 72°C for 20 s) were followed by acquisition of the melting curve. For each sample, the median value of PCR triplicates was considered and, the data (deduced from a standard curve, as was the PCR efficiency in the 85–105% range) was normalized to the median value for exogenous luciferase. Then, for each population the average of four biological replicates was calculated. Finally, for the 'animal age' and 'follicle diameter' differential models, expression was reported

Table 1. Sequences of the primers used for quantitative real-time PCR

Gene name or symbol (transcript variant)	GenBank accession no.	Primer orientation	Primer sequence (5′–3′)
LUCIFERASE		Forward	TCATTCTTCGCCAAAAGCACTCTG
		reverse	AGCCCATATCCTTGTCGTATCCC
MATER	NM_001007814	Forward	GCTGGAGGCGTGTGGACTG
		reverse	GGTCTGTAGATTAGAGGTGGGATGC
NLRP9	NM_001024664	Forward	GCGGCGGTGCTGTGTGAAG
		reverse	CTGCGTCTGCCCTCGTCATC
AURKA	DQ334808	Forward	TCGGGAGGACTTGGTTTCTT
		reverse	TGTGCTTGTGAAGGAACACG
STELLA (v1)	EF446904	Forward	TAGGACTACGCCCATTCACC
		reverse	TGCTGTAGGCTCAAACTGCTC
STELLA (v2)	EF446905	Forward	GCGGGGATGGCTACTCTTC
		reverse	TGCTGTAGGCTCAAACTGCTC
PRDX1	NM_174431	Forward	TCAAGCCTGATGTCCAGAAGAGC
		reverse	CCGTCCTGTCCCACACCAC
PRDX2	NM_174763	Forward	GATTATGGCGTGCTGAAGGAAGATG
		reverse	GAGCGTCCCACAGGCAAGTC
ZAR1	NM_001076203	Forward	TGCCGAACATGCCAGAAG
		reverse	TCACAGGATAGGCGTTTGC
HSF1	NM_001076809	Forward	AAGATTCGCCAGGACAGTGTTACC
		reverse	CGCCGTCGTTCAGCATCAGG

relative to immature adult oocytes and immature oocytes from large follicles, respectively. Indeed, as the two differential models compared in this article have been analysed in two sets of independent experiments, we cannot compare expression between the two models, only between the different populations within one differential model.

Statistical analysis

Data are presented as the mean ± SEM. Data were analyzed by one-way ANOVA with oocyte maturational stage, age of oocyte donor (calf and cow) and size of follicles (small and large) as fixed factors. When ANOVA revealed a significant effect, values were compared by the *post hoc* Tukey test. A p value <0.05 was taken to denote statistical significance.

Results

Using real-time PCR, we have analyzed expression of several target transcripts in oocytes collected from calves and cows, and from cow small (3–4 mm) and large (6–10 mm) follicles, as well as their regulation during IVM. We have used a double approach to analyze the transcripts abundance or total form (based on hexamer-primed RT) and their polyA-tail regulation [based on oligo(dT)-primed RT], as previously described (Thelie et al. 2007).

Effect of oocyte donor age

First, we analyzed the effect of donor age on transcript level before or after IVM (Fig. 1). We noticed a broader variability among the biological replicates of prepubertal immature oocytes. For all genes, similar profiles (stability or decrease) over maturation were observed oocytes originated both from prepubertal or adult animals. There was no significant change in abundance (total form) after IVM. However, after IVM the polyadenylated form of *MATER*, *ZAR1*, *NLRP9*, *HSF1*, *PRDX1* and *PRDX2* decreased in both age groups, while neither *AURKA* nor *STELLA* variants displayed significant change.

An effect of oocyte donor age was evidenced in several instances. Abundance of *PRDX* transcripts was higher in oocytes from adult animals than prepubertal animals, both before and after maturation for *PRDX2* whereas only in mature oocytes for *PRDX1*. Also polyadenylated transcripts for *ZAR1*, *NLRP9*, *HSF1*, *PRDX1* and *PRDX2* decreased to a lower level in calf oocytes when compared to cow, with the strongest difference for *PRDX2* (ratio of 50%).

Effect of follicle size

As observed previously, during maturation most polyadenylated forms (*MATER*, *ZAR1*, *NLRP9*, *HSF1*, *PRDX1*, *PRDX2*) fell close to or below half their initial level (Fig. 2), independently of follicle size; again no difference was observed for *AURKA* or *STELLA* transcripts. There was no significant effect of follicle diameter on any transcript, although total form of *PRDX1* in mature oocytes ($p = 0.08$), *HSF1* in immature oocytes ($p = 0.06$) and polyadenylated *NLRP9* transcript in immature oocytes ($p = 0.053$) tended to be higher in oocytes from small follicles.

Discussion

The different developmental ability between calf and cow oocytes (Levesque and Sirard 1994, Revel et al. 1995; Damiani et al. 1996; Gandolfi et al. 1998; Khatir et al. 1998; Majerus et al. 2000; de Paz et al. 2001; Salamone et al. 2001) as well as the effect of follicle size (Pavlok et al. 1992; Lonergan et al. 1994; Blondin and Sirard 1995; Revel et al. 1995; Damiani et al. 1996; Gandolfi et al. 1998; Hagemann et al. 1999; de Paz et al. 2001; Machatkova et al. 2004; Humblot et al. 2005; Lequarre et al. 2005; Racedo et al. 2008) on final embryo development ability is well established. Gene expression during maturation and early development

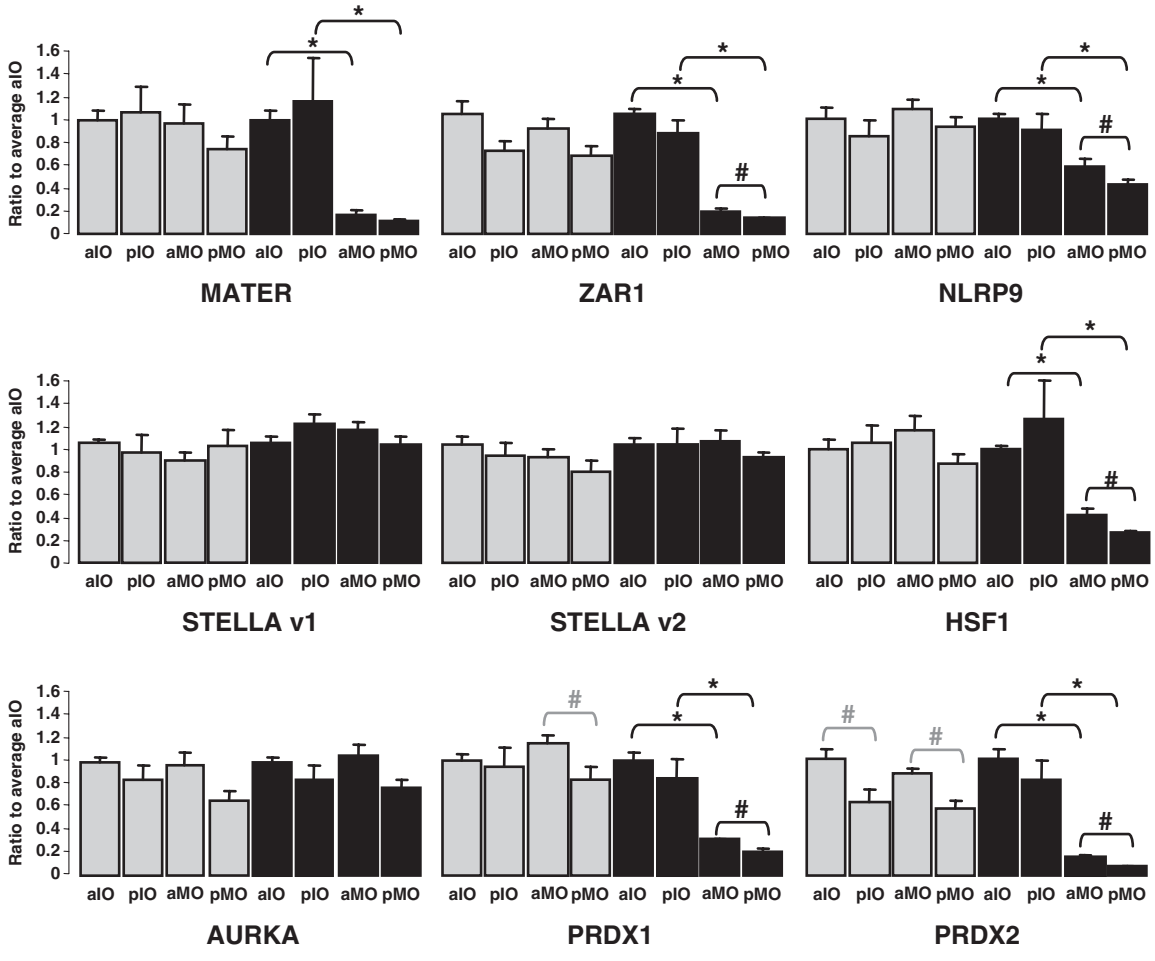

Fig. 1. Variation of transcripts during *in vitro* maturation of oocytes collected from adult (a) or prepubertal (p) animals. IO and MO refer to immature and *in vitro*-matured oocytes, respectively. Variation of total forms (grey bars) or polyadenylated forms (black bars). The ratio to the mean value in immature adult oocytes is represented (mean ± SEM). For each gene and form, *indicates a significant effect of maturation, and # a significant effect of animal age ($p < 0.05$)

relies mostly on RNA accumulated in the egg during oogenesis so it has been hypothesized that developmental competence may be mirrored by the oocyte transcriptome. Here, we investigate whether this difference was correlated to the expression and regulation of transcripts from known maternal effect genes and markers of oocyte maturation.

Regulation during IVM

We have described the distinct evolution of total and polyadenylated forms of several oocyte transcripts during IVM including *HSF1* which is studied in bovine for the first time. Maturation did not significantly affect the abundance of these nine maternal transcripts but the polyadenylated form of NLRP9 decreased by approximately 40% after IVM, PRDX1 by 70%, and MATER, ZAR1 and PRDX2 by 75–85%. The qualitative profiles (decrease or non-significant variation) we observed and the average ratios of polyadenylated transcripts in mature oocytes (relative to immature) are coincident to previous data with a different maturation medium (Thelie et al. 2007), suggesting that these transcripts are unaffected by the absence of serum. The present study confirms the profiles (decrease or non-significant variation) obtained for this set of genes. This consistency indicates the robustness of our approach, and has prompted us to apply the same methodology to compare quantitative data obtained from oocyte populations with differential developmental competence.

Effect of follicle diameter

We did not evidence a significant effect of follicle diameter on detection of this panel of transcripts in immature oocytes nor in their regulation during IVM. This was not unexpected, as transcription is already reduced during the corresponding phase, i.e. when the follicle grows from 3 mm to approximately 10 mm. In a medium-scale transcriptomic approach targeting approximately 1000 genes, oocytes from 3 to 4 mm follicles vs over 5 mm follicles displayed little difference (Lequarre et al. 2005). This study provides evidence that, in this respect, maternal-effect genes do not display a distinctive behaviour when compared to the majority of other genes. Heat-shock transcription factor 1 tended to be slightly more abundant in immature oocytes from smaller follicles, but in the absence of data in the

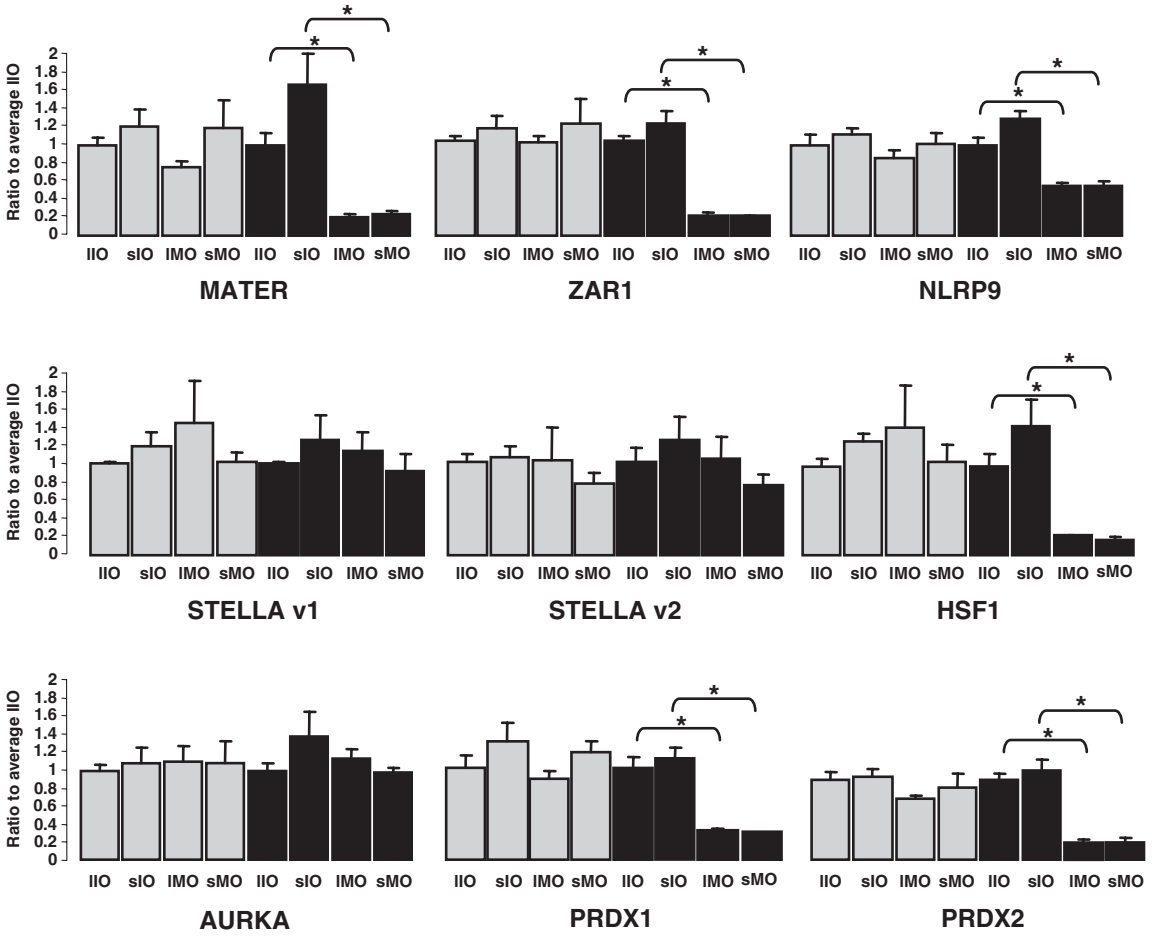

Fig. 2. Variation of maternal effect transcripts during *in vitro* maturation of cow oocytes collected from large (l) and small (s) follicles. IO and MO refer to immature and *in vitro*-matured oocytes, respectively. Variation of total forms (grey bars) or polyadenylated forms (black bars). The ratio to the mean value of immature oocytes from large follicle is represented (mean ± SEM). For each gene and form, *indicates a significant effect of maturation ($p < 0.05$). There was no significant effect of follicle diameter

literature, any interpretation would be very speculative. Among non-maternal-effect genes, our data for AURKA are in agreement with previous analysis revealing no variation throughout antral follicle growth from below 3 mm to over 8 mm (Mourot et al. 2006). Peroxiredoxin1, which tended to be more abundant in mature oocytes originating from smaller follicles, will be discussed in the next paragraph.

Effect of animal age

In this study, we noticed a marked variability among biological replicates of prepubertal immature oocytes that has also been reported when evaluating the content of polyadenylated transcripts in calf and cow oocytes (Lequarre et al. 2004). In our study, several polyadenylated transcripts displayed statistically significant differences. This is in agreement with a report of similar amounts of polyadenylated RNA in cow and calf immature oocytes (53 ± 4 vs 57 ± 8 pg, mean ± SEM), and a similar drop after IVM (to 25 ± 3 and 32 ± 7 pg, mean ± SEM) (Lequarre et al. 2004). Our data also corroborate available proteome analyses: protein patterns were mostly conserved in cow and calf oocytes, whether looking at constitutive or neo-synthesized proteins, but several proteins were detected preferentially or specifically in cow or calf oocytes (Gandolfi et al. 1998; Khatir et al. 1998).

Abundance (total RNA) was unaffected by animal age, except for the *PRDX* transcripts, which will be discussed later. When considering the polyadenylated forms, none of the selected transcripts displayed a significant difference between cow and calf immature oocytes. Indeed, the genes are not included in the partially available list of age-affected transcripts evidenced by a cDNA array approach (Patel et al. 2007). Lower detection (by semi-quantitative RT-PCR) of several transcripts in germinal vesicle stage oocytes from prepubertal vs adult ewes has also been reported (Leoni et al. 2007). On the other hand, among those polyadenylated transcripts undergoing a major drop during IVM, most decreased even further down in calf oocytes (*ZAR1*, *NLRP9*, *HSF1*, *PRDX1*, *PRDX2*). Although the following interpretation is a long shot, it could reveal a more active metabolism and more efficient deadenylation in calf oocytes. Thus, the control of maternal transcripts polyA tail would be deregulated in calf oocytes, affecting their developmental competence. Supporting this hypothesis, several transcripts from predicted developmentally incompetent oocytes

displayed shorter polyA tails when compared to competent oocytes, although this was observed not only in *in vitro*-matured oocytes but also in immature oocytes (Brevini-Gandolfi et al. 1999).

PRDX: markers of oocyte developmental competence?

The influence of oxidative stress onto oocyte maturation and embryonic development (Guerin et al. 2001; Ferreira et al. 2009) has led to investigate expression of the peroxiredoxin genes in gametes or embryos. In bovine, the transcripts are detected in oocytes (Dalbies-Tran and Mermillod 2003, Leyens et al. 2004a), and *PRDX1* and *PRDX2* transcripts were identified as markers of oocyte maturation, as their polyadenylated form exhibited a major decrease during the process (Dalbies-Tran and Mermillod 2003; Thelie et al. 2009). PRDX2 and PRDX6 proteins were reported to be degraded and synthesized, respectively, during IVM (Leyens et al. 2004b; Bhojwani et al. 2006). Peroxiredoxin1 abundance was differentially regulated during *in vitro* and *in vivo* maturation, degraded more severely in the latter case producing oocytes of optimal quality (Thelie et al. 2007). Here, there was an effect of animal age on the total and polyadenylated forms of PRDX1, with a lower detection in calf oocytes, i.e. less competent oocytes. In our second model, PRDX1 tended ($p = 0.08$) to be more abundant in mature oocytes originating from smaller follicles, that are less competent. However, Mourot et al. (2006) observed a higher level of PRDX1 in immature oocytes from follicles over 8 mm. Despite of distinct follicle classes being analysed in the two studies (3–4 mm and >6 mm in our case vs <3 mm, 3–5 mm, 5–8 mm and $>$ 8mm), it seems unlikely that this would result into observing opposite effects. The discrepancy might rather originate from targeting different variants or normalizing to an internal standard as normalizing implies that expression data are reported relative to an experimental group which is arbitrary decided by the own researcher. Overall, there does not appear to be a simple correlation between PRDX1 expression and developmental potential. Besides PRDX1, other PRDX transcripts were reported to vary between oocyte populations with uneven developmental potential. PRDX5 transcript was over-represented in immature calf oocytes (Patel et al. 2007), an opposite ratio than observed here for PRDX1 and PRDX2. Finally, PRDX6 transcript was decreased in cumulus-free matured oocytes, an alternative model of poor quality (Leyens et al. 2004b). Altogether, various *PRDX* transcripts appear to be affected in several models of developmental competence (*in vivo* maturation, animal age, follicle diameter, absence of cumulus cells). Their deregulation likely reflects/causes general alteration of the redox metabolism in oocytes of low developmental potential. However, their expression level is not directly correlated with oocyte quality and a precise range of suitable expression level needs to be defined before they can be used as predictive markers of embryo development.

In conclusion, this study reveals subtle variations in the maternal store of RNA and its regulation over IVM depending on donor age; this supports the hypothesis of a suboptimal molecular maturation of oocytes from prepubertal animals underlying poor developmental competence. It encourages further investigation of the follicle diameter model. Finally, it reinforces the peroxiredoxin genes as candidate markers of developmental potential.

Acknowledgements

Authors thank Gaël Rame for collecting ovaries, Dr Guylène Goudet, Cécile Douet, Sylvie Mugnier and Nadjem Lakhdari for their help in follicle dissection and Dr. Nana Satake for English review. This work was supported by grants from the Agence Nationale de la Recherche ANR-GANI-07-004-01, Apisgene, MEC and FEDER AGL2006-03495. RR's stay in Nouzilly was supported by Spanish Ministry of Education (Grant JC2007-0037).

Conflict of Interest

None of the authors have any conflict of interest to declare.

Authors contributions

RR, RDT and PM contributed to research design, acquisition and interpretation of data. TDS, PP, CP, AT, MEDA participated in the acquisition of data. RR and TDS analysed data. RR and RDT participated in the writing, drafting and critical reviewing of the manuscript.

References

Beker-van Woudenberg AR, Zeinstra EC, Roelen BA, Colenbrander B, Bevers MM, 2006: Developmental competence of bovine oocytes after specific inhibition of MPF kinase activity: effect of estradiol supplementation and follicle size. Anim Reprod Sci **92,** 231–240.

Bettegowda A, Patel OV, Ireland JJ, Smith GW, 2006: Quantitative analysis of messenger RNA abundance for ribosomal protein L-15, cyclophilin-A, phosphoglycerokinase, beta-glucuronidase, glyceraldehyde 3-phosphate dehydrogenase, beta-actin, and histone H2A during bovine oocyte maturation and early embryogenesis in vitro. Mol Reprod Dev **73,** 267–278.

Bhojwani M, Rudolph E, Kanitz W, Zuehlke H, Schneider F, Tomek W, 2006: Molecular analysis of maturation processes by protein and phosphoprotein profiling during in vitro maturation of bovine oocytes: a proteomic approach. Cloning Stem Cells **8,** 259–274.

Blondin P, Sirard MA, 1995: Oocyte and follicular morphology as determining characteristics for developmental competence in bovine oocytes. Mol Reprod Dev **41,** 54–62.

Bonnet A, Dalbiès-Tran R, Sirard MA, 2008: Opportunities and challenges in applying genomics to the study of oogenesis and folliculogenesis in farm animals. Reproduction **135,** 119–128.

Brevini-Gandolfi TA, Favetta LA, Mauri L, Luciano AM, Cillo F, Gandolfi F, 1999: 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 **52,** 427–433.

Christians E, Davis AA, Thomas SD, Benjamin IJ, 2000: Maternal effect of Hsf1 on reproductive success. Nature **407,** 693–694.

Dalbies-Tran R, Mermillod P, 2003: Use of heterologous complementary DNA array screening to analyze bovine oocyte transcriptome and its evolution during in vitro maturation. Biol Reprod **68,** 252–261.

Dalbies-Tran R, Papillier P, Pennetier S, Uzbekova S, Monget P, 2005: Bovine mater-like NALP9 is an oocyte marker gene. Mol Reprod Dev **71,** 414–421.

Damiani P, Fissore RA, Cibelli JB, Long CR, Balise JJ, Robl JM, Duby RT, 1996: Evaluation of developmental competence, nuclear and ooplasmic maturation of calf oocytes. Mol Reprod Dev **45,** 521–534.

Dieleman SJ, Hendriksen PJ, Viuff D, Thomsen PD, Hyttel P, Knijn HM, Wrenzycki C, Kruip TA, Niemann H, Gadella BM, Bevers MM, Vos PL, 2002: Effects of in vivo prematuration and in vivo final maturation on developmental capacity and quality of pre-implantation embryos. Theriogenology **57,** 5–20.

Donnay I, Verhaeghe B, Neirinckx G, 2004: Enriching a defined maturation medium improves subsequent embryonic development of bovine oocytes cultured in small and large groups. Reprod Fertil Dev **16,** 274.

Fair T, Hyttel P, Greve T, 1995: Bovine oocyte diameter in relation to maturational competence and transcriptional activity. Mol Reprod Dev **42,** 437–442.

Ferreira EM, Vireque AA, Adona PR, Meirelles FV, Ferriani RA, Navarro PAAS, 2009: Cytoplasmic maturation of bovine oocytes: structural and biochemical modifications and acquisition of developmental competence. Theriogenology **71,** 836–848.

Gandolfi F, Milanesi E, Pocar P, Luciano AM, Brevini TA, Acocella F, Lauria A, Armstrong DT, 1998: Comparative analysis of calf and cow oocytes during in vitro maturation. Mol Reprod Dev **49,** 168–175.

Guerin P, El Mouatassim S, Menezo Y, 2001: Oxidative stress and protection against reactive oxygen species in the preimplantation embryo and its surroundings. Hum Reprod Update **7,** 175–189.

Hagemann LJ, Beaumont SE, Berg M, Donnison MJ, Ledgard A, Peterson AJ, Schurmann A, Tervit HR, 1999: Development during single IVP of bovine oocytes from dissected follicles: interactive effects of estrous cycle stage, follicle size and atresia. Mol Reprod Dev **53,** 451–458.

Humblot P, Holm P, Lonergan P, Wrenzycki C, Lequarre AS, Joly CG, Herrmann D, Lopes A, Rizos D, Niemann H, Callesen H, 2005: Effect of stage of follicular growth during superovulation on developmental competence of bovine oocytes. Theriogenology **63,** 1149–1166.

Jakobsen AS, Avery B, Dieleman SJ, Knijn HM, Vos PL, Thomsen PD, 2006: Transcription of ribosomal RNA genes is initiated in the third cell cycle of bovine embryos. Mol Reprod Dev **73,** 196–205.

Kauffold J, Amer HA, Bergfeld U, Weber W, Sobiraj A, 2005: The in vitro developmental competence of oocytes from juvenile calves is related to follicular diameter. J Reprod Dev **51,** 325–332.

Khatir H, Lonergan P, Mermillod P, 1998: Kinetics of nuclear maturation and protein profiles of oocytes from prepubertal and adult cattle during in vitro maturation. Theriogenology **50,** 917–929.

van de Leemput EE, Vos PL, Zeinstra EC, Bevers MM, Van der Weijden GC, Dieleman SJ, 1999: Improved in vitro embryo development using in vivo matured oocytes from heifers superovulated with a controlled preovulatory LH surge. Theriogenology **52,** 335–349.

Leoni GG, Bebbere D, Succu S, Berlinguer F, Mossa F, Galioto M, Bogliolo L, Ledda S, Naitana S, 2007: Relations between relative mRNA abundance and developmental competence of ovine oocytes. Mol Reprod Dev **74,** 249–257.

Lequarre AS, Traverso JM, Marchandise J, Donnay I, 2004: Poly(A) RNA is reduced by half during bovine oocyte maturation but increases when meiotic arrest is maintained with CDK inhibitors. Biol Reprod **71,** 425–431.

Lequarre AS, Vigneron C, Ribaucour F, Holm P, Donnay I, Dalbies-Tran R, Callesen H, Mermillod P, 2005: Influence of antral follicle size on oocyte characteristics and embryo development in the bovine. Theriogenology **63,** 841–859.

Levesque JT, Sirard MA, 1994: Proteins in oocytes from calves and adult cows before maturation: relationship with their development capacity. Reprod Nutr Dev **34,** 133–139.

Leyens G, Knoops B, Donnay I, 2004a: Expression of peroxiredoxins in bovine oocytes and embryos produced in vitro. Mol Reprod Dev **69,** 243–251.

Leyens G, Verhaeghe B, Landtmeters M, Marchandise J, Knoops B, Donnay I, 2004b: Peroxiredoxin 6 is upregulated in bovine oocytes and cumulus cells during in vitro maturation: role of intercellular communication. Biol Reprod **71,** 1646–1651.

Lonergan P, Monaghan P, Rizos D, Boland MP, Gordon I, 1994: Effect of follicle size on bovine oocyte quality and developmental competence following maturation, fertilization, and culture in vitro. Mol Reprod Dev **37,** 48–53.

Machatkova M, Krausova K, Jokesova E, Tomanek M, 2004: Developmental competence of bovine oocytes: effects of follicle size and the phase of follicular wave on in vitro embryo production. Theriogenology **61,** 329–335.

Majerus V, Lequarre AS, Ferguson EM, Kaidi S, Massip A, Dessy F, Donnay I, 2000: Characterization of embryos derived from calf oocytes: kinetics of cleavage, cell allocation to inner cell mass, and trophectoderm and lipid metabolism. Mol Reprod Dev **57,** 346–352.

Mota GB, Batista RI, Serapião RV, Boité MC, Viana JH, Torres CA, De Almeida Camargo LS, 2009: Developmental competence and expression of the MATER and ZAR1 genes in immature bovine oocytes selected by brilliant cresyl blue. Zygote **26,** 1–8.

Mourot M, Dufort I, Gravel C, Algriany O, Dieleman S, Sirard MA, 2006: The influence of follicle size, FSH-enriched maturation medium, and early cleavage on bovine oocyte maternal mRNA levels. Mol Reprod Dev **73,** 1367–1379.

Patel OV, Bettegowda A, Ireland JJ, Coussens PM, Lonergan P, Smith GW, 2007: Functional genomics studies of oocyte competence: evidence that reduced transcript abundance for follistatin is associated with poor developmental competence of bovine oocytes. Reproduction **133,** 95–106.

Pavlok A, Lucas-Hahn A, Niemann H, 1992: Fertilization and developmental competence of bovine oocytes derived from different categories of antral follicles. Mol Reprod Dev **31,** 63–67.

de Paz P, Sanchez AJ, De la Fuente J, Chamorro CA, Alvarez M, Anel E, Anel L, 2001: Ultrastructural and cytochemical comparison between calf and cow oocytes. Theriogenology **55,** 1107–1116.

Pennetier S, Uzbekova S, Guyader-Joly C, Humblot P, Mermillod P, Dalbies-Tran R, 2005: Genes preferentially expressed in bovine oocytes revealed by subtractive and suppressive hybridization. Biol Reprod **73,** 713–720.

Pennetier S, Perreau C, Uzbekova S, Thelie A, Delaleu B, Mermillod P, Dalbies-Tran R, 2006: MATER protein expression and intracellular localization throughout folliculogenesis and preimplantation embryo development in the bovine. BMC Dev Biol **6,** 26.

Pisani LF, Antonini S, Pocar P, Ferrari S, Brevini TA, Rhind SM, Gandolfi F, 2008: Effects of pre-mating nutrition on mRNA levels of developmentally relevant genes in sheep oocytes and granulosa cells. Reproduction **136,** 303–312.

Racedo SE, Wrenzycki C, Herrmann D, Salamone D, Niemann H, 2008: Effects of follicle size and stages of maturation on mRNA expression in bovine in vitro matured oocytes. Mol Reprod Dev **75,** 17–25.

Revel F, Mermillod P, Peynot N, Renard JP, Heyman Y, 1995: Low developmental capacity of in vitro matured and fertilized oocytes from calves compared with that of cows. J Reprod Fertil **103,** 115–120.

Rizos D, Ward F, Duffy P, Boland MP, Lonergan P, 2002: Consequences of bovine oocyte maturation, fertilization or early embryo development in vitro versus in vivo: implications for blastocyst yield and blastocyst quality. Mol Reprod Dev **61,** 234–248.

Salamone DF, Damiani P, Fissore RA, Robl JM, Duby RT, 2001: Biochemical and developmental evidence that ooplasmic maturation of prepubertal bovine oocytes is compromised. Biol Reprod **64,** 1761–1768.

Sun QY, Liu K, Kikuchi K, 2008: Oocyte-specific knockout: a novel in vivo approach for studying gene functions during folliculogenesis, oocyte maturation, fertilization, and embryogenesis. Biol Reprod **79,** 1014–1020.

Thelie A, Papillier P, Pennetier S, Perreau C, Traverso JM, Uzbekova S, Mermillod P, Joly C, Humblot P, Dalbies-Tran R, 2007: Differential regulation of abundance and deadenylation of maternal transcripts during bovine oocyte maturation in vitro and in vivo. BMC Dev Biol **7,** 125.

Thelie A, Papillier P, Perreau C, Uzbekova S, Hennequet-Antier C, Dalbies-Tran R, 2009: Regulation of bovine oocyte-specific transcripts during in vitro oocyte maturation and after maternal-embryonic transition analyzed using a transcriptomic approach. Mol Reprod Dev **76,** 773–782.

Uzbekova S, Roy-Sabau M, Dalbies-Tran R, Perreau C, Papillier P, Mompart F, Thelie A, Pennetier S, Cognie J, Cadoret V, Royere D, Monget P, Mermillod P, 2006: Zygote arrest 1 gene in pig, cattle and human: evidence of different transcript variants in male and female germ cells. Reprod Biol Endocrinol **4,** 12.

Vigneault C, Gravel C, Vallee M, McGraw S, Sirard MA, 2009: Unveiling the bovine embryo transcriptome during the maternal-to-embryonic transition. Reproduction **137,** 245–257.

Zheng P, Dean J, 2007: Oocyte-specific genes affect folliculogenesis, fertilization, and early development. Semin Reprod Med **25,** 243–251.

Submitted: 22 Oct 2009; Accepted: 7 Mar 2010

Author's address (for correspondence): Dr R Romar, Faculty of Veterinary Science, Department of Physiology, University of Murcia, 30071 Murcia, Spain. E-mail: rromar@um.es