Journal of Reproduction and Development, Vol. 55, No. 6, 2009

—Full Paper—

Gene Expression in Early Expanded Parthenogenetic and *In Vitro* Fertilized Bovine Blastocysts

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Abstract. Mammalian oocytes can undergo artificial parthenogenesis *in vitro* and develop to the blastocyst stage. In this study, using real-time PCR, we analyzed the expression of genes representative of essential events in development. *In vitro* matured oocytes were either fertilized or activated with ionomycin + 6-DMAP and cultured in simple medium. The pluripotency-related gene *Oct3/4* was downregulated in parthenotes, while the *de novo* methylation *DNMT3A* gene was unchanged. Among the pregnancy recognition genes, *IFN-t* was upregulated, *PGRMC1* was downregulated and *PLAC8* was unchanged in parthenotes. Among the metabolism genes, *SLC2A1* was downregulated, while *AKR1B1*, *COX2*, *H6PD* and *TXN* were upregulated in parthenotes; there was no difference in *SLC2A5*. Among the genes involved in compaction/blastulation, *GJA1* expression increased in parthenotes, but no differences were detected within *ATP1A1* and *CDH1*. Expression of $p66^{shc}$ and the *Bax/Bcl2* ratio were higher in parthenotes, and there was no difference in *p53*. Parthenotes and embryos may differ in the way they stimulate apoptosis, with a preponderant role for $p66^{shc}$ within parthenotes. Differentially affected functions may also include pluripotency, *de novo* methylation and early embryonic signalling.

Key words: Bovine, Embryo, Gene expression, Parthenote

(**J. Reprod. Dev. 55:** 607–614, 2009)

Parthenogenesis, a form of reproduction not spontaneous in mammals, is a process by which the oocyte develops without the male gamete. Mammalian parthenotes are unable to develop to term *in utero* (developing for a maximum of 48 days in the cow; [1]), and this is thought to be due to alterations in their genomic imprinting, a mechanism that provides complementary, but not equivalent, specific expression between maternal and paternal genomes [2]. During fertilization, the spermatozoon triggers multiple and rhythmic oscillations of intracellular free calcium that release the oocyte from this blocked stage [3]. These intracellular calcium patterns can be mimicked by a number of chemicals that activate the oocyte without sperm, yielding parthenogenetic embryos [4]. In cattle, production of reconstructed embryos by somatic cell nuclear transfer (SCNT) [5] and intracytoplasmic sperm injection (ICSI) [6] depends on successful oocyte activation. Therefore, procedures involving SCNT and ICSI could benefit from a better knowledge of mechanisms that differ between embryos and parthenotes.

Contrary to the research in mice and humans, most studies have failed to detect putatively imprinted genes in bovine early development [7, 8], suggesting that monoallelic expression may not develop until after Day 21 of development, as occurs in ovine species [9]. In fact, a recent study found imprinted expression of bovine *Mest-1* in Day-21 but not Day-14 embryos, while 7 other putatively imprinted genes were shown to be not imprinted at the two stages analyzed [10]. However, gene expression does differ between blastocysts from embryos and parthenotes in domestic species [11, 12], and we have hypothesized that the female genotype carried by parthenotes may account for some of these differences [11]. In a previous gene expression study, we used blastocysts, an endpoint normally used in parthenogenetic experiments *in vitro* [4], that were *in vitro* cultured up to the expanded stage for 8 days in a serum-free medium with BSA. Under such conditions, a cohort of blastocysts expand early on Day 7, and show improved survival after cryopreservation compared with their Day-8 counterparts [13–15]. Embryos that blastulate early show a higher probability of producing live births and therefore are considered to be more viable than embryos showing delayed blastulation [16, 17]. In this respect, Day-7 and Day-8 blastocysts differ in expression of developmentally important genes [18]. An expression analysis covering genes representative of essential events during development could help to understand the mechanisms underlying parthenogenesis. Therefore, in the present work, we analyzed the expression of genes involved in compaction / blastocyst formation, metabolism, pluripotency, de novo methylation, apoptosis and pregnancy recognition in parthenogenetic and IVF Day-7 bovine blastocysts.

Materials and Methods

All reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise indicated.

In vitro embryo production

Embryos and parthenotes were produced using cumulus-oocyte complexes (COCs) from slaughterhouse ovaries as previously

Accepted for publication: July 19, 2009
Published online in J-STAGE: August 24, 2009
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reported [11]. Briefly, oocytes were *in vitro* matured for 24 h and subjected to either *in vitro* fertilization (IVF) or parthenogenetic activation (PA). IVF was accomplished by co-incubating oocytes and swim-up separated, frozen/thawed sperm cells together for 18–20 h in fertilization medium (Fert-TALP) containing heparin. Cumulus cells were detached from fertilized oocytes after IVF using a vortex.

For PA, cumulus cells were removed by incubation in hyaluronidase and vortexing in M199-BSA. Matured oocytes were activated with 5 mM ionomycin for 4 min followed by 2 mM 6-DMAP for 4 h.

Both presumptive zygotes and parthenotes were washed 3 times in culture medium, consisting of synthetic oviduct fluid containing aminoacids, citrate and myo-inositol (mSOF), as described by Holm *et al*. [19], modified by addition of 6 g/l BSA and no serum. *In vitro* culture (IVC) was performed in 400 μl of medium layered under mineral oil at 38.7 C in 5% CO_2, 5% O_2 and humidified air. Blastocyst development was monitored on Day 7 (Day 0=onset of IVF or PA).

Gene expression

RNA extraction: For gene expression analysis, fully expanded blastocysts were picked up from the culture medium on Day 7. Pools of 15 embryos were used in order to mimize individual variations. Embryos were snap frozen in LN_2 and then stored at –80 C. Isolation of mRNA was performed using a Dynabeads mRNA Direct Kit (Dynal, Oslo, Norway), according to the manufacturer's instructions. Samples were lysed in 50 μl lysis/binding buffer (Dynal). Hybridization was performed with 10 μl Dynabeads oligo (dT)25 for 5 min. Poly A RNA-bead complexes were separated from the binding buffer and rinsed in buffer A and B (Dynal) using a magnetic separator. Poly A RNA was finally eluted in 30 μl of 10 mM Tris-HCL.

Reverse transcription: Reverse transcription was achieved using First Strand cDNA Synthesis Kit for RT-PCR (AMV, Roche, Barcelona, Spain) with oligo d(T)25, according to the manufacturer's instructions. The RNA purified from embryos was mixed with poly(T) primer and heated to 70 C for 5 min to denature the secondary RNA structure. Thereafter, the RT mix was completed with the addition of RT enzyme (5 units), RNase inhibitor (10 units) and 20 nMol of each dNTP in a total volume of 40 μl to prime the RT reaction and to produce cDNA. The tubes were then incubated at 25 C for 10 min to allow annealing. The RNA was subsequently reverse transcribed at 42 C for 60 min and the uncubated for 5 min at 95 C to denature the RT enzyme. The samples were then cooled at 4 C.

Real-time PCR

Quantification was performed using a real-time polymerase chain reaction (qRT-PCR) method. Briefly, a Rotor-Gene 2000 Real Time Cycler™ (Corbett Research, Sydney, Australia) and SYBR Green (Molecular Probes, Eugene, OR, USA), as a double-stranded DNA-specific fluorescent dye, were used to determine the mtDNA copy number. The PCR reaction mixture (25 ml) contained 1 × PCR buffer, 3 mM $MgCl_2$, 2 U Taq Express (MWGAG Biotech, Ebersberg, Germany), 100 mM of each dNTP, and 0.2 mM of each primer. In addition, the double-stranded DNA dye, SYBR Green I (1:3000 of the 10000× stock solution), was included in each reaction. The PCR protocol included an initial step of 94 C (2 min), followed by 40 cycles of 94 C (15 sec), 56–59 C (30 sec) and 72 C (10 sec). Fluorescent data were acquired at 83 C after the elongation step. The melting protocol consisted of a hold temperature of 40 C for 60 sec and then heating from 50 to 94 C, holding at each temperature for 5 sec while monitoring the fluorescence. Product identity was confirmed by ethidium bromide staining and 2% agarose gel electrophoresis. Experiments were conducted to contrast relative levels of each transcript and histone *H2Az (H2A)* in every sample. PCR was performed by adding a 2-μl aliquot of each sample to the PCR mix containing the specific primers to amplify each gene. Primer sequences, sizes of the amplified fragments of all transcripts and GenBank accession numbers are shown in Table 1. The comparative CT method was used to quantify expression levels [20]. Quantification was normalized to the endogenous control, *H2A*. Fluorescence was acquired in each cycle to determine the threshold cycle or the cycle during the log-linear phase of the reaction at which fluorescence increased above the background for each sample. Within this region of the amplification curve, a difference of one cycle is equivalent to doubling of the amplified PCR product. According to the comparative CT method, the ΔCT value was determined by subtracting the *H2A.z* CT value for each sample from the CT value of the sample for each gene. Calculation of ΔΔCT involved using the highest sample ΔCT value (i.e., the sample with the lowest target expression) as an arbitrary constant to subtract from all other ΔCT sample values. Fold changes in the relative gene expression of the target were determined using the formula 2-ΔΔCT.

Statistical analysis

Embryonic development was analyzed using the GLM procedures of the SAS software (1999; SAS Institute, Cary, NC, USA), which performs ANOVA for unbalanced data. Differences in mRNA expression assayed by quantitative RT-PCR were analyzed by one-way repeated-measures ANOVA (followed by multiple pair-wise comparisons using the Student-Newman-Kleus method). Gene expression was expressed as arbitrary units.

Results

The proportions of embryos developing to the blastocyt and expanded blastocyst stages are shown in Table 2; parthenotes developed at rates higher than IVF embryos during blastulation. Representative pictures of IVF embryos and parthenotes in culture at Day 7 are shown in Figs. 1 and 2, respectively.

Figure 3 shows the genes involved in metabolism and compaction and blastocyst formation. Among the metabolism related genes, the *H6PD, AKR1B1, COX-2* and *TXN* genes were upregulated in parthenotes, while *SLC2A1* was upregulated in IVF embryos; there was no difference in *SLC2A5*. Within the compaction and blastocyst formation genes, *GJA1* was upregulated in IVF embryos, while *CDH1* and *ATP1A1* did not differ between the IVF embryos and parthenotes.

Figure 4 shows the genes involved in pluripotency/*de novo* methylation, apoptosis and pregnancy recognition. The pluripo-

Table 1. Primer sequences, sizes of the amplified fragments of transcripts and GenBank accession numbers

Gen symbol	MGI official name	Primers sequence (5'–3')	Fragment size	GenBank accesion no.
H2az	Histone H2az	5'- AGGACGACTAGCCATGGACGTGTG 5'- CCACCACCAGCAATTGTAGCCTTG	212	NM_016750
POU5F1	POU class 5 homeobox 1 (Oct3/4)	5'-CGAGTATCGAGAACCGAGTG 5'-CAGGGTTCTCTCCCTAGCTC	440	NM_174580.1
DNMT3A	DNA cytosine-5-methyltransferase 3 alpha	5'-CTGGTGCTGAAGGACTTGGGC 5'-CAGAAGAAGGGGCGGTCATC	318	XM_001252215.1
SHC1 SHC ($p66^{shc}$)	SHC (Src homology 2 domain-containing) transforming protein 1	5'-GTGAGGTCTGGGGAGAAGC 5'-GGTTCGGACAAAGGATCACC	334	NM_001075305
Tp53	Tumor protein p53	CTCAGTCCTCTGCCATACTA GGATCCAGGATAAGGTGAGC	364	U74486
BAX	BCL2-associated X protein	5'- CTACTTTGCCAGCAAACTGG 5'- TCCCAAAGTAGGAGAGGA	158	NM_173894.1
BCL2L1	BCL2-like 1	5'- GGAGCTGGTGGTTGACTTTC 5'- CTAGGTGGTCATTCAGGTAAG	517	BC147863.1
PLAC8	Placenta-specific 8	5'-CGGTGTTCCAGAGGTTTTTCC 5'-AAGATGCCAGTCTGCCAGTCA	163	NM_016619
IFN-τ	Interferon-tau (IFN-τ)	5'- GCCCTGGTGCTGGTCAGCTA 5'- CATCTTAGTCAGCGAGAGTC	564	AF238612
PGRMC1	Progesterone receptor membrane component 1	5'-TGTGTGTCACAAATCCAGAAAG 5'-AATCATGCAGTTAGGTCAATCG	265	AF254804
SLC2A1	Solute carrier family 2 (facilitated glucose 1 transporter), member	5'-AGCGTCATCTTCATCCCAGC 5'-CCACAATGCTCAGGTAGGAC	540	M60448
H6PD	Glucose-6-phosphate dehydrogenase	5'-TTGCGGCCGCCGTCCTCTATGTG 5'-GCCMGGCTTCTTGGTCATCATC	220	XM_583628
SLC2A5	Solute carrier family 2 (facilitated glucose transporter), member 5	5'-AGTCATCTCCATCATCGTCCT 5'-GTACCCGCCACCATGTAGGCAG	531	AF308830
AKRIBI	Aldose reductase mRNA	5'-CGTGATCCCCAAGTCAGTGA 5'-AATCCCTGTGGGAGGCACA	152	M314631
COX-2	Prostaglandin G/H synthase-2 (PGHS-2)	5'-ATCTACCCGCCTCATGTTCCT 5'-GGATTAGCCTGCTTGTCTGGA	187	AF031698
TXN	Thioredoxin	5'-ATGGTGAAACAGATTGAGAGCA 5'-CGTTGGAATACTTTTCAGAGAGAGAA	154	AF104105
GJA1	Gap junction protein, alpha 1, 43 kDa	5'- TGCCTTTCGTTGTAACACTCA 5'- AGAACACATGAGCCAGGTACA	142	AY382593.1
CDH1	Cadherin 1, type 1, E-cadherin	5'- GACACTGGAGGTATCAGCGCAC 5'- TGATCTGGACCAGCGACTTAGG	193	AY508164.1
ATP1A1	ATPase, Na+/K+ transporting, alpha 1 polypeptide	5'- ACCTGCTGGGCATCCGAGTGAC 5'- GGGAAGGCACAGAACCACCAG	333	BC123864.1

Table 2. Day-7 blastocyst development of *in vitro* matured bovine oocytes fertilized or parthenogenetically activated with ionomycin + 6-DMAP

	N	Blastocysts	Expanded blastocysts
Parthenotes	389	34.4 ± 2.5^{x}	23.8 ± 2.1^{x}
IVF embryos	545	22.4 ± 2.4^{y}	13.3 ± 2.0^{y}

The data are presented as proportions (LSM ± SEM) of the cultured oocytes (N). $^{x, y}$ $P<0.02$.

tency-related gene *POU5F1 (Oct3/4)* was downregulated in parthenotes, while there was no difference in the *de novo* methylation gene, *DNMT3A*. Among the growth arrest and apoptotic genes, the stress sensor *SHC1SHC* (*$p66^{shc}$*) and pro-apoptotic *Bax* were upregulated in parthenotes, while *p53* and *Bcl2* remained unchanged. The resulting *Bax/Bcl2* ratio was higher in parthenotes, suggesting a pro-apoptotic status. Among the pregnancy recognition genes, *TP1 (IFN-τ)* doubled its expression level in parthenotes, while *PGRMC1* reached significantly increased levels within IVF embryos and there was no change in *PLAC8*.

Discussion

Comparison of the transcription profiles of Day-7 IVF embryos

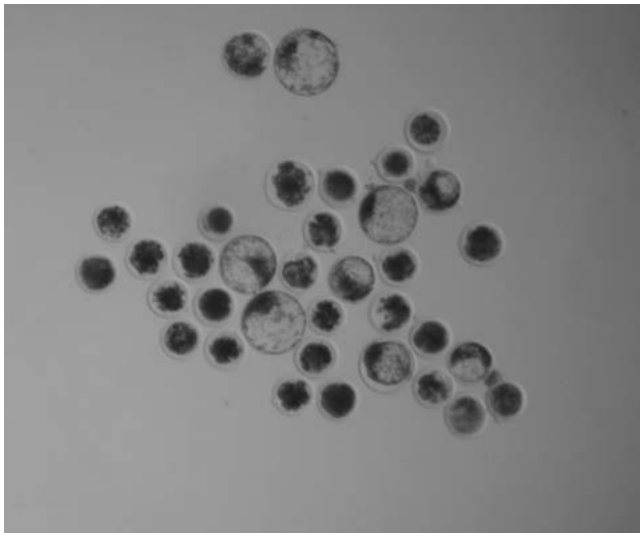

Fig. 1. Day-7 *in vitro* fertilized bovine embryos cultured in modified synthetic oviduct fluid (60×).

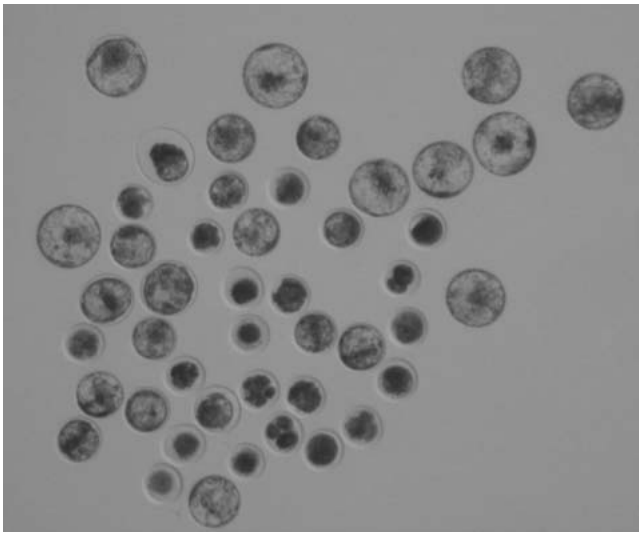

Fig. 2. Day-7 *in vitro* fertilized bovine parthenotes cultured in modified synthetic oviduct fluid (60×).

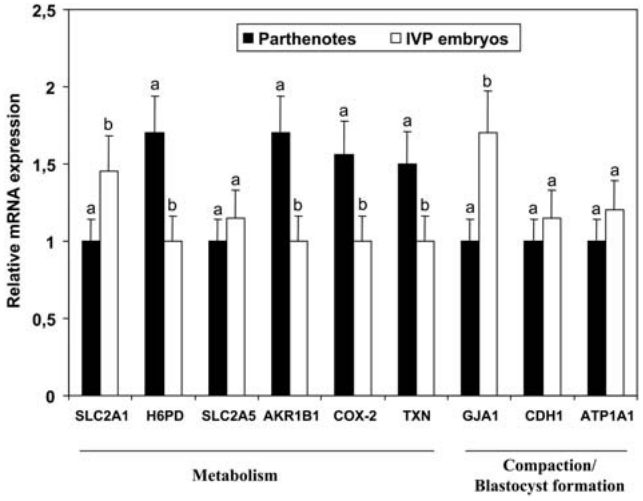

Fig. 3. Relative mRNA transcription of genes involved in metabolism and compaction/blastocyst formation in bovine *in vitro* fertilized and parthenogenetic blastocysts (superscripts indicate significant differences within a gene; $P<0.05$).

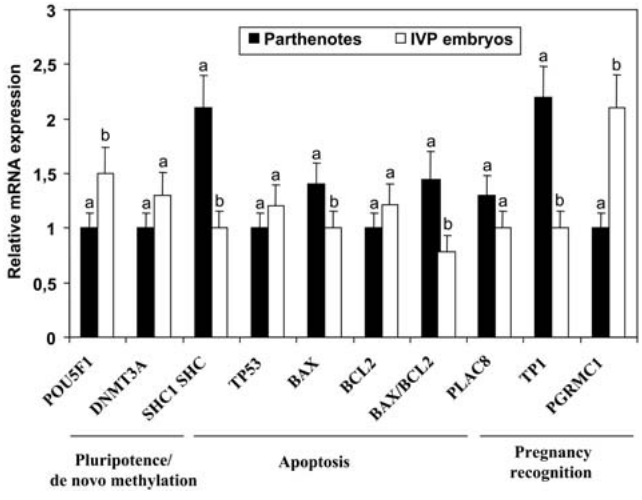

Fig. 4. Relative mRNA transcription of genes involved in pluripotency/ *de novo* methylation, apoptosis and pregnancy recognition in bovine *in vitro* fertilized and parthenogenetic blastocysts (superscripts indicate significant differences within a gene; $P<0.05$).

and parthenotes revealed great similarities to the differences described between their Day-8 counterparts [11]. All the genes analyzed for metabolism and compaction/blastulation in the Day-7 blastocysts matched exactly the expression patterns recorded for both types of Day-8 blastocysts. The enzyme of the aldose reductase gene (*AKR1B1*) exerts two activities, namely, metabolism of progesterone, which is important for implantation, and synthesis of PGF2α and, subsequently, termination of pregnancy. *AKR1B1* has been detected in IVF bovine embryos [21, 22], and its expression has been associated with embryos that fail to establish pregnancies or lead to resorption [22]. In somatic cells, *AKR1B1* is reported to be involved in hyperosmotic stress [23, 24]. It is possible that *AKR1B1* participates in apoptotic mechanisms in the early embryo. As the enzyme AKR1B1 converts glucose to sorbitol, certain glucose-linked signs of sexual dimorphism, such as altered developmental kinetics and sex-based stress resistance [25], could be altered. Therefore upregulation of AKR1B1 may swell the pro-apoptotic genotype of parthenotes, which has been found to not correspond to an increase in TUNEL-detected DNA damage in Day-8 blastocysts [11].

Similar to increased *AKR1B1*, we found enhancement of *H6PD*, which codes for the pentose phosphate pathway rate-limiting rate enzyme. Expression of *H6PD* is increased in female embryos [20, 25–27], and involved in metabolic differences between male and female embryos [27–29]. However, in contrast with our results, Day-7 parthenotes have been reported to not overexpress *H6PD* [26], although in the latter study, the exact blastocyst stage at which the embryos were analyzed was not described [26]. In somatic cells, overexpression of *H6PD* induces expression of pro-oxidative enzymes [30, 31], which is consistent with the overexpression of thioredoxin (*TXN*) in our parthenotes. The TXN protein plays a role in mouse implantation and responds to oxidative stress in mouse and bovine embryos [32, 33]. The *TXN* gene is downregulated in embryos that fail to result in pregnancies and those leading to resorption [22], while addition of the protein to pig embryo culture improves blastocyst formation, cell numbers and apoptosis [34]. The upregulation of *TXN* in parthenotes could be beneficial to the short-term survival of these blastocysts.

Prostaglandin G/H synthase-2 (*COX2*) is markedly inducible in specialized cell types [35]. Inhibition of COX in embryo recipients increases pregnancy rates after transfer and lowers PGF2α release [36]. Expression of *COX2* is more abundant in blastocysts resulting in calf delivery compared with those resulting in resorption [22]. Gene expression of *COX2* increases throughout the implantation window, which indicates an important role for the PGs released by the embryo in mediating interactions with the uterus [37, 38]. Our parthenogenetic blastocysts showed higher levels of *COX2*, an activity that should not limit the ability of such embryos to proceed during implantation.

Among the glucose transporters analyzed, expression of *SLC2A1* was reduced in parthenotes, while there was no change in *SLC2A5* (a fructose transporter). *In vivo* derived embryos show higher *SLC2A1* mRNA abundance than IVP embryos [39–41]. However, the expression of both *SLC2A1* and *SLC2A5* decreases in culture under low oxygen tension [39, 42], a condition that improves embryonic development. Embryonic respiration rates and expression of *SLC2A1* and *H6PD* mRNAs are correlated, and *SLC2A1* is downregulated in female blastocysts [27]. Therefore, downregulation of *SLC2A1* in parthenotes might reflect an absence of pro-oxidative conditions, such as those described to occur in female blastocysts.

Intercellular structures are altered in IVP bovine embryos [43], leading to less pronounced compaction than in *in vivo* derived embryos [44]. Expression of genes responsible for compaction and cell-to-cell adhesion, such as *GJA1 (CX43)* and *CDH1 (E-CADHERIN)*, is diminished [45–47]. Low expression of *CX43* in blastocysts has been associated with low quality and reduced survival after cryopreservation [47, 48]. *E-CADHERIN* intervenes in cell to cell adhesion as it is associated with compaction [49, 50] and blastocyst formation [51]. In our study, the levels of *E-CADHERIN* did not differ between the IVF blastocysts and parthenotes, suggesting that the *E-CADHERIN* contribution to the compaction process and blastocyst formation is not altered in parthenotes. However, the *CX43* levels are diminished in parthenotes; however, even if the level of *CX43* is altered, its function could be compensated by coexpressed connexins [52].

The composition and accumulation of fluid in the blastocoele is regulated by Na/K-ATPase. Embryonic Na/K-ATPase activity increases when a nascent blastocoele cavity forms and then decreases to the baseline level at full expansion [53]. Mouse embryos with targeted disruption in the Na/k-ATPase α1-subunit progress through blastulation, but they do not expand and eventually die [54]. We found lower *ATP1A1* expression in fully expanded blastocysts than in blastocysts that hatched [55]. According to their *ATP1A1* expression levels, our parthenotes did not seem to differ essentially from IVF embryos during blastulation.

The parthenotes in the present study exhibited a pro-apoptotic gene expression profile, as defined by their increased *BAX* expression and *BAX/BCL2* ratio. This condition is consistent with a higher apoptotic phenotype observed in Day-8 blastocysts produced under the same conditions and generally higher apoptotic indexes found in bovine parthenotes, compared with IVF embryos [56–58]. *BAX* and *BCL2* are pro- and anti-apoptotic genes, respectively, and their balance indicates the tendency of a cell to undergo apoptosis [59]. However, although their pro-apoptotic profile is equivalent (i.e., *BAX/BCL2* ratio), our Day-7 parthenotes showed significantly increased *BAX* and no increase in *BCL2* compared with the IVF embryos, which is contrary to the results of our previous study of Day-8 parthenotes [11]. Likewise, the mRNA for $p66^{shc}$ was more abundant in parthenotes, although there was no difference in *p53* between the Day-7 parthenotes and IVF embryos. Again, this is contrary to our previous results for Day-8 parthenogenetic blastocysts, which show downregulated *p53* expression [11]. Within IVF bovine embryos, the stress-related protein $p66^{shc}$ is upregulated in early arrest, while *p53* seems to not be involved in the same function [60–62]. In contrast, within embryos developed in the uterus, developmental arrest has been described as being both apoptosis-dependent [63] and apoptosis-independent [64]. It is conceivable that gene expression upstream *BAX* differs between parthenotes and embryos, particularly in the way they channel apoptotic stimuli through $p66^{shc}$ and *p53* [11, 65, 66].

The increase in the *BAX/BCL2* ratio in parthenotes compared with the IVF embryos is consistent with reduced expression of the pluripotency gene *OCT3/4*, such as that observed in Day-8 parthenotes [11]. In agreement with our data, pig parthenotes treated with melatonin showed decreased *BAX* expression, while also showing increased *BCL2L1* and *OCT3/4* expression [12]. Knockdown of *OCT3/4* mRNA causes a reduction in ICM cells in bovine IVF embryos [67]. However, a reduction in *OCT3/4* gene expression might not affect ICM viability, as embryos with different development potentials, such as those produced by nuclear transfer, *in vivo*-derived and IVF embryos do not differ in terms of *OCT3/4* expression [68]. *DNMT3A* is a *de novo* methyltransferase, that sets up the methyl-CG landscape of the genome early in development. The *DNMT3A* levels in the present study did not vary, although the Day-8 parthenotes in our previous study showed lower methylation activity than the IVF embryos [11], which is in agreement with the low expression of *DNMT3A* reported in female blastocysts and XX ES cell lines [69, 70]. Sagirkaya *et al*. [71] found that *DNMT3A* expression changes with culture conditions, and we suggest that changes in *DNMT3A* expression could also be related to develop-

ment timing. The distinct difference in gene expression level of *DNMT3A* between Day-7 and Day-8 parthenotes compared with IVF embryos is nevertheless intriguing, as no imprinted genes have been found until now during bovine early embryonic development [7, 8].

PLAC8, an invasion specific gene, is more abundant in blastocysts resulting in calf delivery compared with those resulting in resorption [22] and is more abundant in the endometria of pregnant cows compared with non-pregnant cows [72, 73]. This suggests a potential role in placental development and the feto-maternal interface. Our work suggests that, unlike in their Day-8 counterparts [11], expression of *PLAC8* is not defective in Day-7 parthenotes.

Interferon-tau (IFN-τ) is produced by the trophoblasts of ruminant species before placentation is initiated. The rapid upregulation of *IFN-τ* expression at a time when the corpus luteum is wavering on the point of regression is essential if pregnancy is to be maintained [74]. Differential blastocyst loss is attributed to differences in IFN-τ secretion. Thus, female bovine embryos [75–77] and parthenotes [75] express higher IFN-τ than males. The above findings are consistent with our increased *IFN-τ* mRNA abundance in Day-7 and Day-8 [11] parthenotes. *PGRMC1* is a membrane-bound progesterone receptor that mediates the anti-apoptotic effects of progesterone in granulosa cells [78–80]. The role of *PGRMC1* in development is unclear, but it seems to be related to pregnancy recognition, a function compatible with parthenogenetic development in the bovine. The reduced expression of *PGRMC1* in the Day-7 parthenotes compared with the IVF embryos suggests that this activity can limit the viability of parthenotes.

The results of this study show that 7 out of 18 genes analyzed (i.e., *SLC2A5*, *E-CADHERIN*, *ATP1A1*, *DNMT3A*, *TP53*, *BCL2* and *PLAC8*) were equally expressed in the IVF embryos and parthenotes. The expressions of three genes (i.e., *IFN-τ*, *H6PD* and *SLC2A1*) varied in the parthenotes and IVF embryos, as has been reported to occur in male and female embryos. In addition, possible sexually dimorphic expression of *AKR1B1* merits further research. There are outstanding differences in pregnancy recognition, as judged by the gene panel studied, between Day-7 IVF embryos and parthenotes, as it is intriguing that there is weaker signalling through *PGRMC1*, although an increase in *IFN-τ* is typical from the female genotype carried by parthenotes. The differences found between the parthenotes and IVF embryos seem to be associated with the genes involved in placenta formation more than ICM pluripotency, which would support parthenotes as a potential source of stem cells even though they are unable to develop to term.

The pro-apoptotic gene expression profile in parthenotes is more pronounced than within IVF embryos, although this is inconsistent with the similar incidence of DNA damage found in Day-8 IVF embryos and parthenotes [7]. In addition, parthenotes and embryos could differ in terms of the stress signalling that triggers growth arrest and eventually apoptosis, $p66^{shc}$ being preponderant in parthenotes over *p53*. The lower expression of *CX43* in parthenotes does not correspond to morphological or functional differences between both blastocyst types within Day-8 embryos [7]. Lower expression of *OCT3/4* in parthenotes may indicate low pluripotency in such blastocysts. However, *DNMT3A* did not increase in the Day-7 parthenotes, but has previously been shown to be decreased in Day-8 parthenotes [7] and Day-7 female embryos [68]. It is therefore unclear whether *DNMT3A* activity in parthenotes depends on the female genotype. The differences in gene expression may explain important traits of parthenotes, especially concerning cell death and possibly the female genotype. Genes that differ between parthenotes and IVF embryos are potential candidates to be imprinted.

Acknowledgements

We are grateful to J Rodriguez, for recovery of ovaries. The present study received grant support from the Spanish Ministry of Science and Innovation, projects AGL2008-01530 and AGL2006-04799. M Muñoz received a grant from the Programa Ramon y Cajal (RYC08-03454).

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