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Identifying new human oocyte marker genes: a microarray approach

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Running Title: Profiling COC's markers

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Abstract

Efficiency in classical IVF (cIVF) techniques is still impaired by poor implantation and pregnancy rates after embryo transfer. This is mostly due to a lack of reliable criteria for the selection of embryos with sufficient development potential. Several studies have provided evidence that some genes' expression levels could be used as objective markers of oocytes and embryos competence and of their capacity to sustain a successful pregnancy. These analyses usually used reverse transcription-polymerase chain reaction to look at small sets of pre-selected genes. However, microarray approaches permit to identify a wider range of cellular marker genes. Thus they allow the identification of additional and perhaps more suited genes that could serve as embryo selection markers. Microarray screenings of circa 30 000 genes on U133P Affymetrix™ gene chips made it possible to establish the expression profile of these genes as well as other related genes in human oocytes and cumulus cells. In this study, we identified new potential regulators and marker genes such as *BARD1*, *RBL2*, *RBBP7*, *BUB3* or *BUB1B*, which are involved in oocyte maturation.

Introduction

The quality of oocytes obtained under ovarian stimulation for classical IVF (cIVF) varies considerably. Whilst most oocytes are capable of being fertilized, only half of those fertilized complete preimplantation development and fewer still implant.

After oocyte retrieval several layers of cumulus oophorus cells still surround mature oocytes (metaphase II, MII) and immature oocytes (germinal vesicle (GV) and MI). Granulosa cell derived cumulus cells surround the oocyte in the antral follicle and play an important role in regulating oocyte maturation (Dekel *et al.*, 1980; Larsen *et al.*, 1986). Ebner et al. (Ebner *et al.*, 2006) demonstrated that, in vitro, the culture of human oocytes with attached cumulus cells may improve preimplantation embryo development.

Gene expression alterations in oocytes and their supporting cells can be correlated with defects or variations in the ovulation or maturation processes. Gene expression in granulosa cells is altered in patients with empty follicle syndrome (Inan *et al.*, 2006). A number of studies suggest that changes in gene expression, such as *GDF9* or *Bone Morphogenic Protein-15* (*BMP15*) in oocytes, or *Pentraxin 3* (*PTX3*) in cumulus cells, can be monitored for selecting oocytes for fertilization and embryos for implantation (Elvin *et al.*, 1999; Yan *et al.*, 2001; Zhang *et al.*, 2005).

Therefore, gene expression studies in human oocyte and cumulus cells could contribute not only to identify factors involved in the oocyte maturation pathway, but could also provide valuable molecular markers of abnormal gene expression in oocytes with reduced competence.

Specific gene expression screenings for caspase and cell death proteins (Spanos *et al.*, 2002), FSH receptor and LH receptor (Patsoula *et al.*, 2003) or cell adhesion molecules (Bloor *et al.*, 2002) have also been attempted to determine the status of embryos. More recently, Wells et al (2005a, 2005b) analyzed by quantitative polymerase chain reaction (Q-PCR) a panel of cell division and DNA damage marker genes (*BRCA1* & *2*, *ATM*, *TP53*, *RB1*, *BUB1*, *MAD2* and *APC*.) to establish a correlation between their expression levels and the quality grade of preimplantation embryos (Wells *et al.*, 2005b). The aim of the present study, based in parts on data obtained by Assou et al. (2006), was to apply a microarray approach to identify new potential regulators and marker genes which are involved in human oocyte maturation as well as in cumulus cell function.

Materials and Methods

Oocytes and cumulus cells

Oocytes and cumulus cells were collected from patients consulting in our centre for cIVF or for intracytoplasmic sperm injection (ICSI). This study has received institutional review board approval. Patients were stimulated with a combination of gonadotropin-releasing hormone agonist (GnRH-a) (Decapeptyl PL 3) and recombinant FSH (Puregon or Gonal F) or hMG (Menopur). Ovarian response was evaluated by serum estradiol level and daily ultrasound examination to observe follicle development. Retrieval of oocytes occurred 36 hours after hCG administration and was performed under ultrasound guidance. Cumulus cells were removed from one or two mature oocytes (MII) 21 hours post insemination. Immature oocytes (GV and MI) and unfertilized MII oocytes were collected 21 hours or 44 hours post insemination or post microinjection by ICSI. Cumulus cells and oocytes were frozen at -80°C in RLT buffer (RNeasy kit, Qiagen, Valencia, CA, USA) before RNA extraction. Pools of 20 GV (7 patients, age 30 years ±4.6), 20 MI (6 patients, age 30.1 years ±6.7), 2 pools of 16 (6 patients, age 34 years ±4.5) and 21 MII oocytes (8 patients, age 33.2 years± 6.4) and 2 pools of cumulus cells (2 patients, age 31 and 37) were separately analyzed on 6 Affymetrix™ DNA microarrays. All these oocytes or cumulus cells were from couples referred to our centre for cIVF (tubal infertility) or for ICSI (male infertility).

Complementary RNA (cRNA) preparation and microarray hybridization

RNA was extracted using the micro RNeasy Kit (Qiagen) and the total RNA quantity was measured with a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies Inc., DE, USA) and RNA integrity was assessed with an Agilent 2100 Bioanalyzer (Agilent, Palo Alto, CA, USA). cRNA was prepared with two rounds of amplification according to the manufacturer's protocol "small sample protocol II" starting from total RNA (ranging from ~4 ng for pooled oocytes to 100 ng for cumulus cells), and hybridized to HG-U133 plus 2.0

GeneChip pangenomic oligonucleotide arrays (Affymetrix™, Santa Clara, CA, USA). HG-U133 plus 2.0 arrays contain 54 675 sets of oligonucleotide probes (“probeset”) which correspond to ≈ 30 000 unique human genes or predicted genes. Primary image analysis of the arrays was performed with the GeneChip Operating Software 1.2 (GCOS) (Affymetrix™), resulting in a single value for each probe set (“signal”). Data from each different array experiment were scaled to a target value of 100 by GCOS using the “global scaling” method. This algorithm determines whether a gene is expressed with a defined confidence level or not (“detection call”). This “call” can either be “present” (when the perfect match probes are significantly more hybridized than the mismatch probes, p-value < 0.04), “marginal” (for p-values > 0.04 and <0.06) or “absent” (p-value > 0.06). A gene was denoted as “absent” in a sample when all its probeset displayed an “absent” or “marginal” detection call for this sample. The dataset was floored to 2, i.e. each signal value under 2 was given the value 2.

Statistical analysis

Samples were analyzed by pair wise comparison using the GCOS 1.2 software (Affymetrix™).

For hierarchical clustering, we used the probesets included in table 1 (for a gene, the probeset with the highest signal in one of the samples). Signal values lower than 2 were arbitrarily floored to the value of 2. Data were log transformed, mean centred, and processed with the CLUSTER and TREEVIEW software packages with the average linkage method and un-centered correlation (Eisen *et al.*, 1998).

Gene search

We search through the gene annotation lists (Unigene Build 190) to identify related genes based on their description. The gene annotation lists included the following terms: gene symbol; gene name; the Gene Ontology “biological process”, “Cellular component” and “Molecular function”; genetic pathway. We filtered the genes with the following criteria: lists comprising the terms “retinoblastoma” (for *RB1*), “bub” for *BUB1*, “atm” OR “atr” for *ATM*, “tp53” for *TP53*, “brca” for *BRCA1 & 2*, “mad” for *MAD2L1*, and “adenomatosis” for *APC* identified the genes presented in this study.

Database

The expression, including signal values, of all genes cited in Table 1 can be examined on our web site as online supplemental data: Expression of these genes in various normal tissues transcriptome datasets, including ovarian and testis samples, is provided through the “Amazonia!” database web page: http://amazonia.montp.inserm.fr/

Results

Analysis of "marker genes" expression in human oocytes and cumulus cells

We evaluated the gene expression level of *BRCA1* & 2, *ATM*, *TP53*, *RB1*, *BUB1*, *MAD2*, *APC* and *ACTB* in cumulus cells, in unfertilized MII oocytes and in immature oocytes GV and MI stages. These genes are presented in bold type at the top of each section in Table 1. For cumulus cells and MII oocytes, the presented average signal values were calculated from two independent sample chip hybridization experiments for each. All genes were detected in cumulus cell and oocyte samples with the following exceptions: *BRCA1* was absent in cumulus cells, *TP53* was absent in MI oocytes, and *RB1* was absent in MII oocytes. In addition, *ACTB* and MAD2L1 were present in all samples and presented the highest signal levels (circa 20 fold higher on average). The signal fold increase between cumulus cell average signal and all oocyte average signals (Figure 1) indicates that *RB1* is down-regulated in oocytes. On the other hand, *BUB1*, *BRCA1* & 2 and MAD2L1 are down-regulated in cumulus cells whereas *ATM and APC* are slightly up regulated in oocytes. Although weaker in GV and MI oocytes, *ACTB* expression varied little between samples.

Expression profile of new marker genes

We used the "marker genes" names or symbol names as a keyword list to search the GeneNote annotations associated with each probeset present on the chip in order to identify eight groups of genes related to the marker genes cited above (*β-Actin* was not included in the search). Thus, 149 probesets were retrieved, of which one for each of the 40 corresponding genes is listed in Table 1. The signal values of the probesets for each sample are indicated (highest sample signal in bold type, value in grey when absent). The highest fold change between sample-pairs is also indicated (Table 1). Three of these genes (*TP53I-11*, *TP53I-13* and *APCDD1* – full names can be found in Table 1) were never detected in our samples and are listed at the bottom of the table. In general retrieved genes corresponded to proteins belonging to the regulatory pathway, to interacting partners or to paralogous proteins of the “marker genes”.

The hierarchical clustering analysis of the probesets signal values from Table 1 across all samples showed that oocytes cluster together and suggested that some gene expression levels could be specific to the degree of oocyte nuclear maturation. As expected, the cumulus cell lineage is the most distant from oocytes. The main expression groups are for genes over-expressed in cumulus cells or in oocytes. For the latter, sub-groups of genes specific to MII or to GV and MI oocytes are also apparent (Figure 2). The lesser distinction was observed between GV and MI oocytes.

RB1 group

The *RB1* profile was also found for *RBL1*, *RBBP6* and *RBBP9*, which were also absent in MII oocytes. However, the *RB1* pathway was not completely down-regulated in oocytes. On the contrary, the highest expression levels were detected with *RBBP7*, *RBBP4*, *RAP140*, and *RBBP8* in oocyte samples. *RBBP8* gene was highest in GV oocytes, *RBBP4* in MI oocytes and *RBBP7* and *RBL2* in MII oocytes.

BUB1 group

TBC1D1, BUB1B and *BUB3* displayed patterns similar to that of *BUB1*. *BUB1B* and *BUB3* mimicked *BUB1* expression but at much higher levels (6 and 3 fold respectively), although *BUB3* was high in cumulus cells as well.

ATM group

ATR and the *ATM/ATR* substrate *ASCIZ* differed from *ATM* in that their expression was high in cumulus and strongest in immature oocytes, particularly in GV oocytes.

TP53 group

Unlike *TP53* for which the highest expression levels were found in MII oocytes, many targets or *TP53* partners were not expressed in MII oocytes: Two targets of *TP53* (*TP53TG3*, *TP53INP1*) were found at higher levels in oocytes whereas four partners (*TP53RK*, *PERP*, *TP53BP1* & *2*) and one target (*TP53I3*) were specific or over expressed in cumulus cells. Among the genes expressed in oocytes, *RPRM*, *RRM2B* and *TP53INP2* were evenly expressed across the four samples (no significant change). *TP53TG3* and *TP53INP1* were up-regulated in GV and MII oocytes respectively.

BRCA1 and BRCA2 group

BRCA1 and *BRCA2* partners remained mostly confined to oocytes with the strongest expression generally found in immature oocytes or cumulus cells. *BRCA1* was stronger in GV oocytes and *BRCA2* in MII oocytes. The cofactor *COBRA1* was highest in cumulus cells but was actually not significantly different between all samples, whereas *BRCC3* and BAP1 low expression was turned down in oocytes compared to cumulus cells. *BRIP1* was only found in GV and MI oocytes. Expression of *BARD1* represented the strongest signals, increasing from GV to metaphase MI and MII oocytes. *BRAP* and BCCIP were similar but with reduced expression in MII oocytes.

MAD2 group

Genes related to *MAD2L1* did not match its high expression levels. *MAD2L2* is similar but found at much lower levels. *MAD2L1BP* and *MAD1L1* appeared GV specific and were not found or low in MII.

APC group

Following our search criteria, only one gene related to *APC, APC2,* was expressed in our sample although at low levels and only in cumulus cells: We found no expression for the *APCDD1* gene.

Discussion

Marker gene expression in cumulus cells and immature oocytes

Recently, we reported the expression of circa 30 000 human genes in our cumulus-oocyte complex gene expression profiling studies (Assou *et al.*, 2006). Some of them were previously described as potential markers for the evaluation of human oocyte or embryo quality, based on their expression pattern in preimplantation embryos (Wells *et al.*, 2005a, b). These genes were *BRCA1* & 2, *ATM*, *TP53*, *RB1*, *BUB1*, *MAD2*, *APC* and *ACTB*, which are involved in cell cycle checkpoint and DNA repair control.

The analysis of cumulus cell expression provided additional information on the gene expression profile of oocyte supporting cells. Overall, we observed similar expression patterns in all oocyte stages for these genes, but differences were nevertheless observed. Wells et al. (2005a, b) reported the strongest signal for *APC* in a “typical” oocyte and used this value as a 100% scale reference for all the genes tested. In our study, *ACTB* and *MAD2L1* were present in all samples and presented the highest signal levels (circa 20 fold higher on average). Apart from these two genes, the strongest signals came from *RB1* in cumulus cells (signal value = 920), *BRCA1* in GV oocytes (988), *BUB1* in MI oocytes (888) and *BRCA2* in MII oocytes (575). The variations in levels of expression (e.g. for the *MAD2L1* and *APC* genes) could be due to the detection methods (microarray vs. quantitative RT-PCR). The use of specific marker genes to normalize expression data should help in the comparison of expression values measured in different laboratories. The genes for glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) or beta-2-microglobuline (*B2M*) are commonly used as ubiquitously expressed reference markers. The IkappaB kinase alpha gene (*CHUK*) was also recently proposed as a better internal standard for oocytes and pre-embryo cells (Falco *et al.*, 2006). *MAD2L1* was already observed at very high levels in single oocyte microarray analyses (Bermudez *et al.*, 2004). High *MAD2L1* expression in our MII oocytes could also reflect that these unfertilized oocytes are blocked in pro-metaphase II (Wassmann *et al.*, 2003). However, these variations do not interfere with the quality of these genes as oocyte and embryo fitness markers. Thus, for this set of genes expression was similar to that of previous reports with the additional detection of *RB1* in cumulus cells.

The analysis of expression in immature oocytes (GV and MI) and in unfertilized MII oocytes provided supplemental insights into the *BRCA1* and *BRCA2* expression profiles: the two genes are co-expressed but expression of the former is down-regulated whereas the latter increased slightly during oocyte maturation.

Identification of new marker genes.

Transcriptional control

The restriction of *RB1* expression to cumulus cells was intriguing for a gene usually found in most tissues. Expression of other factors interacting with RB1, in particular *RBL1*, which is strictly restricted to cumulus cells, further suggest that regulation by RB1 is involved in these cells. Although it is absent from oocytes and preimplantation embryos (Wells *et al.*, 2005a), its expression was detected in hatching blastocysts (Wells *et al.*, 2005b). Finding high *RBBP8* expression in GV oocytes is consistent with its binding to and modulation of *BRCA1* expression (Yu *et al.*, 2000; Yu *et al.*, 2004). The high expression levels of *RBBP7*, *RBBP4* and *RBL2* in MI and MII oocytes suggested that this regulation pathway could be active during oocyte maturation. For *TP53* genes, the strongest signals were observed in MII oocytes for *RRM2B* and *TP53INP1*, which are both induced by TP53, with TP53INP1 having the same positive action as *TP53* on catalases and proteasome endopeptidases (Tomasini *et al.*, 2005). TP73, a potential TP53INP1 activator and TP53 homolog was not expressed in our microarrays.

DNA repair markers

ATM and ATR both phosphorylate BRCA1 (Gatei *et al.*, 2001) and were differentially expressed during maturation with *ATR* appearing mostly in immature oocyte.

Expression of *BARD1* is interesting because it displayed the strongest signals in its group and was co-expressed in oocytes with both *BRCA1* and *BRCA2*. BARD1 is an important regulator of BRCA1 activity: binding of BARD1 with BRCA1 maintains both proteins in the nucleus thus preventing apoptosis (Fabbro *et al.*, 2004b). *BARD1* is very similar to *BRCA1* and both proteins induce apoptosis when they are confined to the cytoplasm (Schuchner *et al.*, 2005). BARD1 is also a key factor in DNA repair (reviewed by Henderson, 2005). The BRCA1-BARD1 complex is also required for ATM/ATR (*ataxia-telangiectasia-mutated/Rad3-related*)-mediated phosphorylation of P53 (Ser-15) (Fabbro *et al.*, 2004a).

Cell cycle checkpoint markers

The expression profile and the interaction networks of BUB and MAD2 genes also suggest that they could also provide new marker genes. The proteins BUB3 and BUB1B interact with CDC20 at checkpoint activation (Tang *et al.*, 2001). MAD2L2 negatively regulates the

CDC20/Anaphase promoting complex APC (Chen *et al.*, 2001). MAD2L1 together with BUB1B inhibits CDC20/APC to prevent premature separation of sister chromatids (Fang, 2002). MAD2L1BP and MAD1L1 bind MAD2L1 and are crucial for localization of MAD2L1 to kinetochores where it binds to CDC20 (Sironi *et al.*, 2001).

Identifying new oocyte or embryo marker genes

Gene expression is a first step in the identification of potential marker genes and it has been undertaken by different research groups using microarray approaches (Bermudez *et al.*, 2004; Richards *et al.*, 2005; Assou *et al.*, 2006). The Affymetrix™ GeneChip is a reliable microarray system (http://www.Affymetrix™.com/community/publications/index.affx), presenting little inter-laboratory variability (Irizarry *et al.*, 2005). Different criteria can be used to pre-select candidate marker genes. Stronger expression may be easier to detect but variation in expression could be less visible or less relevant. Genes that are more specific reflect tighter regulation and could provide better reporter genes because variations may be more readily detectable. However, after their identification on the basis of gene expression profiles and the verification of their patterns by Q-PCR, understanding the function of factors in regulating pathways will be the next validation step to select marker genes. In the end, experimental data linking their expression levels with oocyte or embryo quality status will determine their practical value. The list of genes presented here was filtered on the basis of keywords and not pathway oriented. In the case of *APC* related genes, the search criteria were clearly not appropriate. Analyzing other genes like *CTNNB1* (highly expressed in oocytes in Bermudez *et al.*, 2004), *AXIN2*, *WNT1* or *WNT8A*, which are partners of *APC* in the WNT signalling pathway could be more relevant. Likely, other *ATM/ATR* targets, not initially reported with our search criteria, may represent alternate markers to *ATM*: *H2AFX*'s and *CHEK1*'s profiles are similar to that of *ATM* but with stronger signals, and *CHEK2* was only detected in GV and MI oocytes (data not shown). The fact that some genes analyzed in this study were not detected at all or only in some samples raises different interpretations. They may be truly absent, their expression levels could be below the detection threshold of the microarray approach or the lack of detection could reflect sample or experimental discrepancies. We favour the two former possibilities because only a couple of signal values are sporadic and could result from the latter explanation. Indeed, most absent signals were either duplicated in separate experiments (Cum and MII samples) or were concomitantly observed within groups of related samples such as immature oocytes or all oocytes. Therefore, absent signals reported here should be viewed as very low or absent transcripts.

With the present study, some factors are clearly put in perspective as potential markers of oocyte competence (Figure 3). Their expression profiles suggest different roles played in supporting cumulus cells or in oocytes during successive maturation stages. *RBL1* appears as a very specific marker in cumulus cells. However, most of the genes presented here are preferentially expressed in oocytes and different criteria should be used to identify cumulus cell markers that could reflect oocyte quality. A number of oocyte factors interact within the *BRCA1* regulation pathway and are co-expressed in MII oocytes. RBBP8, BRAP and ATR bind and modulate BRCA1 activity (Li *et al.*, 1999) and are co-expressed with BRCA1. RBBP8 also binds RB1 and BARD1 (Yu *et al.*, 2000). RBBP4 and RBBP7 bind BRCA1 like RB1 (Yarden *et al.*, 1999). The interaction of BUB1B and BUB3 with RBL2 (Cam *et al.*, 2004) also link them to the RB1 and BRCA1 regulation pathways. *BUB1B* or *RBBP7* were already observed as highly expressed genes in previous studies (Bermudez *et al.*, 2004; Assou *et al.*, 2006). Thus we identified genes with relevant expression patterns to serve **as a resource for potential** new oocyte markers. **Interestingly, the factors encoded by these genes** intersect in **common** regulatory pathways..

Our aim was to show the relevance of the microarray approach to identify and bring forward new potential regulators and marker genes. Such study is qualitative and partly quantitative within the limits of the microarray approach. The adjunction of additional independent series could strengthen these results further. However, once a discrete number of genes as been selected, the validation of differential expression by Q-PCR is more reliable, faster and more cost-effective. Thus, Q-PCR analyses on specific genes presented herein will be the focus of future studies. Finally, the account of oocyte and cumulus cells gene expression profiles should be strengthened by functional analyses since protein activity often depends on post-translational modification and interactions with other partners. Proteomics approaches and interactome analyses may have the last word to determine the real activity of genes and proteins.

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Figures Legends

Figure 1. Marker genes up or down-regulated in cumulus cells and oocytes.

Histogram representation of the fold increase in the signal between cumulus cell and oocyte average signals for nine genes (cumulus versus oocyte in grey bars and oocyte versus cumulus in white bars). Probe set values are from table 1.

Figure 2. Hierarchical clustering of 46 genes signal values across cumulus cell and oocyte samples.

Signal values were floored (minimal signal value = 2), log transformed and mean centered. Average linkage with un-centered correlation was evaluated using the Cluster software (Eisen et al. 1998). On the right side, genes are clustered by their preferential expression in the four samples. Red and green mark over- and under-expression, respectively and black colour represents mean values. GV = germinal vesicle; M = metaphase.

Figure 3. New marker genes involved in oocyte maturation.

Factors identified in this study with restricted expression in immature germinal vesicle (GV) and metaphase I (MI) oocytes, in unfertilized metaphase II (MII) oocytes and in cumulus cells. Factors in darker shade are shared by MI and MII oocytes.

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Table 1. Affymetrix™ GeneChip signal values of 46 genes expressed in oocytes and cumulus cells.

Reference[a]	Symbol[b]	Gene name[b]	Cum[c,d]	GV[c]	MI[c]	MII[c,d]	Highest fold increase in[e]	
203132_at	**RB1**	**retinoblastoma 1 (including osteosarcoma)**	**920.2**	48.7	39.2	*9.0*	x 23.5	in Cum / MI
212781_at	RBBP6	retinoblastoma binding protein 6	**327.7**	39.1	13.1	55.9	x 25.0	in Cum / MII
205296_at	RBL1	retinoblastoma-like 1 (p107)	**30.9**	*9*	*2*	*9.7*	nd	Cum only
226696_at	RBBP9	retinoblastoma binding protein 9	**129.4**	27.3	34.9	*49.0*	x 4.7	in Cum / GV
205169_at	RBBP5	retinoblastoma binding protein 5	40.9	94.1	165.5	**616.9**	x 15.1	in MII / Cum
210371_s_at	RBBP4	retinoblastoma binding protein 4	315.9	1131.8	**2914.8**	1679.2	x 9.2	in MI / Cum
201092_at	RBBP7	retinoblastoma binding protein 7	1765.1	3678.4	6372.3	**9743.3**	x 5.5	in MII / Cum
209284_s_at	RAP140	retinoblastoma-associated protein 140	549.0	2344.6	**2874.2**	2054.3	x 5.2	in MI / Cum
203344_s_at	RBBP8	retinoblastoma binding protein 8	356.4	**1904.8**	873.3	152.5	x 12.5	in GV / MII
211950_at	RBAF600	retinoblastoma-associated factor 600	**529.1**	494.9	122.7	*246.8*	x 4.3	in Cum / MI
212331_at	RBL2	retinoblastoma-like 2 (p130)	529.6	279.5	238.1	**657.7**	—	—
209642_at	**BUB1**	**BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)**	42.5	821	**887.9**	251.4	x 20.9	in MI / Cum
203755_at	BUB1B	BUB1 budding uninhibited by benzimidazoles 1 homolog beta (yeast)	33.0	3802	**6299.9**	1033.1	x 190.9	in MI / Cum
212350_at	TBC1D1	TBC1 (tre-2/USP6, BUB2, cdc16) domain family, member 1	117.5	527.3	**649.8**	621.4	x 5.5	in MI / Cum
209974_s_at	BUB3	BUB3 budding uninhibited by benzimidazoles 3 homolog (yeast)	1220.8	2362	**2624.7**	629.2	x 4.2	in MI / MII
200801_x_at	**ACTB**	**actin, beta**	**5347.3**	705.8	1809.2	4294.6	x 7.6	in Cum / GV
210858_x_at	**ATM**	**ataxia telangiectasia mutated (comp. groups A, C and D)**	101.6	98.6	165	**562.0**	x 5.7	in MII / GV
209903_s_at	ATR	ataxia telangiectasia and Rad3 related	449.4	**781.7**	511.6	205.4	x 3.8	in GV / MII
201855_s_at	ASCIZ	ATM/ATR-Substrate Chk2-Interacting Zn2+-finger protein	392.6	**1369.3**	712.4	*117.4*	x 3.5	in GV / Cum
201746_at	**TP53**	**tumour protein p53 (Li-Fraumeni syndrome)**	63.2	27.5	*29.7*	**163.8**	x 6.0	in MII / GV
210609_s_at	TP53I3	tumour protein p53 inducible protein 3	**363.6**	*51.6*	*6.2*	*33.9*	nd	Cum only
222392_x_at	PERP	PERP, TP53 apoptosis effector	**131.3**	*12.1*	*2.5*	*42.4*	nd	Cum only
203050_at	TP53BP1	tumour protein p53 binding protein, 1	**445.4**	132.1	208.8	*8.0*	x 3.4	in Cum / GV
225402_at	TP53RK	TP53 regulating kinase	**112.5**	*7.2*	*2.4*	*22.5*	nd	Cum only
203120_at	TP53BP2	tumour protein p53 binding protein, 2	**611.4**	158.8	200.6	*101.7*	x 3.8	in Cum / GV
220167_s_at	TP53TG3	TP53TG3 protein	118.0	**571.3**	457.7	247.4	x 4.8	in GV / Cum
225912_at	TP53INP1	tumour protein p53 inducible nuclear protein 1	707.4	438	553.6	**1770.8**	x 4.0	in MII / GV
219370_at	RPRM	reprimo, TP53 dependant G2 arrest mediator candidate	*49.1*	*34.5*	**73.3**	*200.6*	nd	MI only
210886_x_at	TP53AP1	TP53 activated protein 1	**69.7**	33.9	44.8	*65.5*	x 2.1	in Cum / GV
223342_at	RRM2B	ribonucleotide reductase M2 B (TP53 inducible)	789.8	848.4	1429.7	**2028.9**	—	—

224836_at	TP53INP2	tumour protein p53 inducible nuclear protein 2	**430.6**	362.8	394.4	183.2	—	—
204531_s_at	**BRCA1**	**breast cancer 1, early onset**	*11.4*	**988.2**	226.9	106.0	x 9.3	in GV / MII
205345_at	BARD1	BRCA1 associated RING domain 1	61.0	1214.7	2897.5	**4351.0**	x 71.3	in MII / Cum
235609_at	BRIP1	BRCA1 interacting protein C-terminal helicase 1	*10.6*	80.6	**127.3**	*75.0*	—	—
213473_at	BRAP	BRCA1 associated protein	125.7	521.9	**687.2**	151.2	x 5.5	in MI / Cum
230922_x_at	BRCC3	BRCA1/BRCA2-containing complex, subunit 3	**115.9**	27.5	4.4	*32.7*	x 26.3	in Cum / MI
201419_at	BAP1	BRCA1 associated protein-1 (ubiquitin carboxy-terminal hydrolase)	**131.8**	*95.6*	*30.3*	*30.6*	nd	Cum only
202757_at	COBRA1	cofactor of BRCA1	**210.8**	199	175.5	177.4	—	—
208368_s_at	**BRCA2**	**breast cancer 2, early onset**	5.9	270.9	246.3	**575.2**	x 97.5	in MII / Cum
227322_s_at	BCCIP	BRCA2 and CDKN1A interacting protein	541.6	1239.8	**1594.8**	343.1	x 4.6	in MI / MII
203362_s_at	**MAD2L1**	**MAD2 mitotic arrest deficient-like 1 (yeast)**	81.7	3217	4035.1	**9559.6**	x 117.1	in MII / Cum
203094_at	MAD2L1BP	MAD2L1 binding protein	140.7	**405.1**	310.7	44.8	x 9.0	in GV / MII
204857_at	MAD1L1	MAD1 mitotic arrest deficient-like 1 (yeast)	*11.1*	**78.4**	32.1	*8.2*	x 2.4	in GV / MI
223234_at	MAD2L2	MAD2 mitotic arrest deficient-like 2 (yeast)	130.7	100.4	110.7	**294.6**	—	—
203525_s_at	**APC**	**adenomatosis polyposis coli**	210.8	**841.5**	590.3	131.3	x 6.4	in GV / MII
227965_at	**APC2**	**adenomatosis polyposis coli 2**	**18.6**	*8.1*	*2*	*30.5*	nd	Cum only

Genes undetected in cumulus cells and oocytes: **TP53I11**, tumour protein p53 inducible protein 11; **TP53I13**, Tumour protein p53 inducible protein 13; **APCDD1**, adenomatosis polyposis coli down-regulated 1.

a) Affymetrix probeset reference; **b)** symbol name and gene name (Unigene build 190). **c)** for each probeset line, sample with highest signal in bold type or italicized and greyed when "Absent". **d)** for cumulus cells and MII oocytes samples, the values represent the average value derived from independent samples hybridized to two DNA chip arrays. **e)** best fold increase (>2 and p<0.001) found for the pair-wise comparison indicated in the last column and not determined (nd) between "Absent" genes or when not significant (—). Abbreviations: Cum = cumulus cells, GV = germinal vesicle, MI & MII = metaphase I & II oocytes respectively