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The human cumulus-oocyte complex gene expression profile

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Running Title: Gene expression profiling of oocytes

Key words: oocytes, germinal cells, microarray, cumulus

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BACKGROUND: The understanding of the mechanisms regulating human oocyte maturation is still rudimentary. We have identified transcripts differentially expressed between immature and mature oocytes, and cumulus cells.

METHODS: Using oligonucleotides microarrays, genome wide gene expression was studied in pooled immature and mature oocytes or cumulus cells from patients who underwent IVF.

RESULTS: In addition to known genes such as *DAZL*, *BMP15* or *GDF9*, oocytes upregulated 1514 genes. We show that PTTG3 and AURKC are respectively the securin and the Aurora kinase preferentially expressed during oocyte meiosis. Strikingly, oocytes overexpressed previously unreported growth factors such as TNFSF13/APRIL, FGF9, FGF14, and IL4, and transcription factors including OTX2, SOX15 and SOX30. Conversely, cumulus cells, in addition to known genes such as LHCGR or BMPR2, overexpressed cell-to-cell signaling genes including TNFSF11/RANKL, numerous complement components, semaphorins (SEMA3A, SEMA6A, SEMA6D) and CD genes such as CD200. We also identified 52 genes progressively increasing during oocyte maturation, comprising CDC25A and SOCS7.

CONCLUSION: The identification of genes up and down regulated during oocyte maturation greatly improves our understanding of oocyte biology and will provide new markers that signal viable and competent oocytes. Furthermore, genes found expressed in cumulus cells are potential markers of granulosa cell tumors.

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Introduction

The quality of oocytes obtained under controlled ovarian stimulation (COS) for assisted reproductive technology (ART) varies considerably. While most oocytes are amenable to fertilization, only half of those fertilized complete preimplantation development and even fewer implant. During follicle growth, the oocyte obtains the complement of cytoplasmic organelles and accumulates mRNAs and proteins that will enable it to be fertilized and to progress through the first cleavage divisions until embryonic genes start to be expressed. Transcriptional activity decreases as the oocyte reaches maximal size (Fair *et al.*, 1995) and later on the oocyte depends on stored RNAs for normal function during maturation, fertilization and early embryonic development (Moor *et al.*, 1998). After oocyte retrieval, the mature oocyte (MII) and some still immature oocytes (GV and MI) are surrounded by the cumulus oophorus. Several layers of cumulus cells surround the oocyte in antral follicle and play an important support and regulation role in oocyte maturation (Dekel and Beers, 1980; Larsen *et al.*, 1986).

Analysis of the oocyte maturation using microarray analysis techniques could detail the genes involved in this process and the specific checkpoints regulating acquisition of full competence for ovulation and fertilization. The understanding of the molecular processes involved in the development of a competent oocyte under COS conditions could guide the choice of ovarian hyperstimulation protocols and lead to improvements in oocyte quality, oocyte culture and manipulation. Some studies demonstrate that changes in gene expression during COS, such as *GDF9* or *Bone Morphogenic Protein-15* (*BMP15*) in oocyte, or *Pentraxin 3* (*PTX3*) in cumulus cell, can be monitored for selecting oocytes for fertilization and embryos for replacement (Elvin *et al.*, 1999; Yan *et al.*, 2001; Zhang *et al.*, 2005). Therefore, transcriptome studies in human oocytes and cumulus cells could contribute not only to elucidate the mechanisms of oocyte maturation, but could also provide valuables molecular

markers of abnormal gene expression in oocytes with reduced competence. The aims of the present study were to establish: (1) whole genome transcriptome of human immature and matures oocytes and cumulus cells, (2) specific gene expression signatures of immature and mature oocytes and cumulus cells and (3) genes whose expression progressively increase during oocyte maturation.

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Materials and Methods

Oocytes and cumulus cells

Oocytes and cumulus cells were collected from patients consulting in our center for conventional in vitro fertilization (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; Ipsen, Paris, France) and recombinant FSH (Puregon and Gonal F; Organon and Serono respectively) or Menopur (Ferring). 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 a mature oocyte (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) and 16 MII oocytes (6 patients, age 34 years ±4.5) were analyzed by DNA microarrays. All these oocytes were from couples referred to our center 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 RNA integrity was assessed by using an Agilent 2100 Bioanalyzer (Agilent, Palo Alto, CA). RNA quantity was also assessed for some samples using the Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies Inc., DE, USA). cRNA was prepared according to the manufacturer's protocol "small sample protocol II" starting from total RNA (ranging from ~4 ng pooled oocytes to 100 ng cumulus cells), and hybridized to HG-U133 plus 2.0 GeneChip pangenomic

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oligonucleotide arrays (Affymetrix, Santa Clara, CA, USA). HG-U133 plus 2.0 arrays contain 54 675 sets of oligonucleotide probes ("probeset") which correspond to ≈ 39 000 unique human genes or predicted genes. The GeneChip system is a robust microarray system with more than 3000 publications using this technology (http://www.affymetrix.com/community/publications/index.affx), little lab-to-lab variability and a good accuracy and precision (Irizarry *et al.*, 2005). Primary image analysis of the arrays was performed by using 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. The dataset was floored to 2, i.e. each signal value under 2 was given the value 2.

Statistical analysis

Samples were analyzed using a pair wise comparison using the GCOS 1.2 software (Affymetrix). Of interest, this algorithm provides the information of 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, "absent" when both perfect match and mismatch probes display a similar fluorescent signal, or "marginal" when the probeset does neither comply to the "present" nor to the "absent" call criteria. A gene was denoted as exclusively expressed in one category when this gene displayed a detection call "Present" in this given category and "Absent" or "Marginal" in all other three categories. A gene was considered as over- or underexpressed in a category when all three possible pair wise comparisons showed a significant change p-value ($P \leq 0.01$) according to the GCOS 1.2 software and a ratio ≥ 3 or ≤ 0.333 for the genes increased and decreased respectively. We also determined a list of genes whose expression progressively increased during oocyte maturation by selecting the probesets with a significant increase

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according to the GCOS 1.2 algorithm and matching the following ratio constraints: cumulus < GV (with GV/Cumulus ≥3), GV < MI (MI/GV ≥ 1.73) and MI < MII (MII/MI ≥ 1.73), where cumulus, GV, MI and MII stands for signals values in these samples. Note that 1.73 x 1.73 = 3. Gene annotation was based on Unigene Build 176.

For hierarchical clustering, data were filtered (15 000 genes with a significant expression ("present" detection call) in at least one sample and with the highest variation coefficient), log transformed, median centered, and processed with the CLUSTER and TREEVIEW software packages with the average linkage method and an uncentered correlation (Eisen *et al.*, 1998). Gene Ontology (GO) annotations (http://www.geneontology.org/) were obtained and analyzed via the FATIGO web site tool (http://www.fatigo.org/) using level three annotations. In some cases we used the GO annotations downloaded from the Affymetrix NetAffx database. Genes with a role in cell-to-cell communication function were obtained by filtering the genes on the following criteria: cellular component comprising the terms "membrane" or "extracellular". Bibliographical search was carried out in Pubmed using Boolean logic. For each gene ***G*** present in table 2 and 3, using its Hugo approved abbreviation or any of its aliases, we looked for publication matching the query « gene ***G*** AND (gamete or "germ cell" or "germ cells" or egg or eggs or oocytes or oocyte or meiosis) » for genes found preferentially expressed in oocytes, and the query « gene ***G*** AND (gamete or "germ cell" or "germ cells" or egg or eggs or oocytes or oocyte or cumulus or granulosa) » for genes overexpressed in cumulus cells.

The expression, including signal values, of all genes cited in Tables 2 and 3 can be examined on our web site as online supplemental data : http://amazonia.montp.inserm.fr/the_human_oocyte_transcriptome.html. Expression of these genes in various normal tissues transcriptome datasets, including ovarian and testis samples, is provided through the Amazonia! database web page (Manuscript in preparation).

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Results

Identification of genes expressed in human oocytes and cumulus cells

Total cRNA was synthetized from pools of GV, MI or MII stage oocytes, or cumulus cells, then labeled and hybridized to pan-genomic oligonucleotide microarrays. We analyzed the detection call (GCOS 1.2 software) of all 54 675 probes in oocytes and cumulus samples. Oocytes express in average 8728 genes. The lowest number of genes expressed was found in MII oocytes (n = 5 633) and highest in GV oocytes (n = 10 892) (Table 1). We found that expression variations between MI, MII or GV samples was low as illustrated by tight scatter plots and high correlation coefficients (0.63 – 0.92), as opposed to a marked difference of expression between the cumulus sample and the oocytes samples as illustrated by dispersed scatter plots and low correlation coefficients (0.39 – 0.50) (Figure 1A).

We visualized the respective gene expression across all samples using hierarchical clustering. Average linkage hierarchical clustering on 15 000 genes showed that oocytes cluster together, demonstrating a common gene expression, but are only distantly related to cumulus cells (Figure 1B). These results highlight that feminine germ cells and their nourishing neighbor cumulus cells display very different expression profile, in agreement with a very different but complementary biological function and with cell lineage disparity.

Specific transcription program in each sample type

We next examined which genes were specific to each sample category, using two different approaches. First we determined the genes that were only detected in one sample and not in the three other samples. These genes were called “exclusively expressed” (Table 1). As expected, cumulus cells have the largest number of exclusively expressed genes (n = 1829), likely because they display a very different transcriptome as compared to oocytes (n = 234 - 739). Second, we considered the probes that were overexpressed or underexpressed in one sample compared to all three other samples, with a fold ratio of at least three. Again, cumulus

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cells show the largest lists of genes, overexpressing 2600 and underexpressing 1514 genes as compared to oocytes. Using this rather stringent criteria (fold change of at least 3 between a given sample and the three other samples), we found very few genes over or under expressed in GV and MI oocytes. This shows that very few genes modify their expression between GV and MI oocytes, as opposed to MII oocytes that overexpress more than 400 genes and underexpress more than 800.

We compared functional Gene Ontology annotations of overexpressed genes versus under expressed genes in oocytes and cumulus cells. We observed that certain functional annotations were more represented in either oocytes or cumulus cells (Figure 2). There were significantly more genes involved in “Response to stimulus”, “Secretion”, “Extracellular matrix” in cumulus cells, suggesting that cumulus cells are more active in cell-to-cell communication processes. Conversely, genes annotated “Reproduction”, “Ubiquitin ligase complex”, “Microtubule associated complex”, “Microtubule motor activity”, “Nucleic acid binding”, “Ligase activity” were significantly more frequently associated with genes overexpressed in oocytes, in agreement with the major processes involved in meiosis and implying microtubules attachement to chromosomes and the ubiquitin ligase complex APC/C regulation.

Whole genome transcriptome of oocytes

We observed that 1514 genes were expressed with at least a 3-fold increase in oocytes, i.e. underexpressed in cumulus cells when compared to oocytes. Selected genes are highlighted in Table 2, which is also available as web supplemental data including the expression histogram for each gene (http://amazonia.montp.inserm.fr/the_human_oocyte_transcriptome.html). This list includes genes already recognized as specifically expressed in male and female germinal cells in mammals such as *DAZL*, the *RNA helicase DDX4/VASA* or *DPPA3/STELLA* (full

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names are listed in Table 2). Numerous well recognized actors of meiosis were highly expressed in oocytes: the components of the maturation-promoting factor (MPF) (*CDC2/CDK1, CCNB1, CCNB2)*, CDC25 phosphatases (*CDC25A*, *CDC25B* and *CDC25C)*, components of the spindle checkpoint (*BUB1*, *BUBR1*, *MAD2L1*/*MAD2*, *CENP-A, CENP-E*), *CDC20* which is a components of the anaphase promoting complex (APC/C), and a downstream target, the meiosis specific sister chromatid arm cohesin *STAG3* (Figure 3A). As expected, we observed the overexpression of genes known to be specific of oocytes such as the *Zona Pellucida* genes (*ZP 1*, *2, 3* and *4*), members of the transforming growth factor-beta superfamily such as *Growth differentiation factor 9* (*GDF9*), *Bone morphogenetic protein 6 and 15* (*BMP6 and BMP15*), *FGFR2,* the chromatin remodeling molecules histone deacetylase *HDAC9* and the oocyte-specific H1 histone *H1FOO* (Figure 3B). Thus, the data are in complete agreement with published studies. Interestingly, we show here that many genes, previously found expressed in oocytes in various animal models, are indeed highly expressed in human oocytes. Hence, our microarray data are of sufficient scope and accuracy to pave the way to a systematic gene expression exploration of oocyte and cumulus transcriptome.

We observed that several genes previously reported to be expressed in male germ cells are also highly expressed in human oocytes, in all maturation stages, such as *Aurora Kinase C* (*AURKC*), *SOX30,* or *Sperm Associated Antigen 16* (*SPAG16/PF20*). Still, the majority of the genes we found overexpressed in oocytes were not yet reported to be associated with gamete biology. Some of these previously unrecognized "oocytes genes" are listed in Table 2 and comprise several functional categories. After fertilization, the spindle checkpoint inhibition is released and the APC/C complex degrades the securins, resulting in entry into anaphase. We found that genes of the centromere protein *CENPH* that interacts with the spindle checkpoint, the anaphase promoting complex subunits *ANAPC1* and *ANPC10* are highly expressed in

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oocytes. Moreover, the securing genes *PTTG1* and *3* are 58 and 50 times more expressed in oocytes than in cumulus cells, respectively. We found several growth factors and growth factor receptors significantly overexpressed in oocytes (*IL-4, FGF9, FGF14, TNFSF13/APRIL*), transcription factors (*SOX15*, *OTX2*, *FOXR1*), three anti-apoptosis molecules (*BCL2L10*, *BNIP1*, *BIRC5/Survivin*), and the glucose transporter *SLC5A11*.

Whole genome transcriptome of cumulus cells

Conversely, we observed that 2600 genes are overexpressed in cumulus cells compared to all three oocytes samples. The cumulus sample we studied was obtained from a MII oocyte during ovulation. First, we observed a marked expression of the LH receptor *LHCGR* in cumulus cells, which primes these cells to respond to the LH surge. Second, we observed that genes overexpressed in MII cumulus cells comprise the main genes that are induced by the LH surge during ovulation (Table 3). We observed a very high expression of the progesterone receptors *PGRMC1* and *2*, and the steroidogenic acute regulatory (*STAR*) that are induced by LH. Similarly we found that eicosanoids biosynthesis enzymes such as the two P*rostaglandin Endoperoxyde Synthetase PTGS1* and *PTGS2/COX2* and the *Prostaglandin I2* (*Prostacyclin*) S*ynthase PTGIS*, the P*rostaglandin Receptor PTGER2*, and two downstream effector of this signalling pathway, I*nterleukin IL1beta* and *Pentaxin-Related 3 (PTX3)*, are also overexpressed in cumulus cells. These genes were mostly described in animal models, and we show here for the first time that the RNA expression of these genes is also highly induced in human cumulus cells obtained after ovulation. Two chemokines are highly produced by cumulus cells, *CXCL1/GRO-alpha* and *IL8*, in agreement with the invasion of the granulosa by leucocytes during ovulation. Interestingly, the metalloprotease *ADAMTS1*, as well as its target *Versican* whose cleavage has been shown to contribute to the proteolytic disintegration of the cumulus matrix, were also highly induced. The transcription factor *CEBPB*, induced after the gonadotrophin surge and mediating the upregulation of *inhibin alpha* (*INHA*), is

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found overexpressed in our post stimulation cumulus cells. Accordingly, *INHA*, as well as *INHBA/Activin A*, are 5 and 34 times more expressed in cumulus cells than in oocytes respectively. Another transcription factor characteristic of granulosa cells, *GATA6*, is also highly overexpressed in comparison to oocytes. We observed the upregulation of peroxiredoxins (*PRDX2, 4, 5 and 6*) that are part of a family of peroxidases involved in antioxidant protection and cell signaling and recently reported in bovine ovaries (Leyens *et al.*, 2004), as well as a lysosomal cysteine proteinase, *cathepsin K* (*CTSK*). Genes coding for protein found in follicular fluid such as *PAPPA* are also found overexpressed in cumulus cells. Thus, genes found overexpressed in cumulus cells by our whole genome transcriptome analysis recapitulates previous expression studies on post-LH surge granulosa cells carried out in various species.

Considering that cell-to-cell communication genes are a functional category that plays an essential role in the maturation of the cumulus-oocyte complex, we focused on genes filtered on the Gene Ontology cellular localization annotations “membrane” or “extracellular” (see material and method): 615 genes passed this filter. The most noticeable genes from this list were ligands (*BMP1*, *BMP8B*) or receptors (*BAMBI*, *BMPR2*) from the TGF superfamily, ligands (*TNFSF11/OPGL/RANKL*) or receptors from the TNFR superfamily (*TNFRSF1A*/*TNF-R*, *TNFRSF10B*/*DR5*, *TNFRSF12A*), components of the complement (*CFHL1*, *C7*, *IF*, *CFH*, *C1S*, *C1R*) and one inhibitor of the complement system (*CLU*), semaphorins (*SEMA3A*, *SEMA6A*, *SEMA6D)*, tetraspanins (*TM4SF1*, *TM4SF6*, *TM4SF8*, *TM4SF10*) and various CD members (*CD24, CD44, CD47, CD58, CD59, CD63, CD74, CD81, CDW92, CD99, CD151, CD200*). Table 3 lists these genes, references key publications relevant for feminine reproduction biology. Furthermore, components of the cumulus-oocyte complex signalling pathways were retrieved such as *connexin 43*. We found that this connexin was expressed at a high signal in both cumulus cells and in all oocytes categories, in line with

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its extracellular domains that provide strong and specific homophilic adhesion properties. Most interestingly, many of these genes were never before highlighted as expressed in granulosa cells.

Differences in genes expression variation during oocyte maturation

An important feature of our work is that we established a transcriptome for each of the three stages of oocyte maturation: GV, MI and MII. We were thus able to identify genes whose expression gradually increased during oocyte maturation (see material and method). Fifty two probesets were retrieved, including the phosphatase *CDC25A, PCNA* and *SOCS7*. However, most of the resulting genes are poorly characterized or only predicted coding sequences. All these genes are candidate marker for oocyte cytoplasmic and/or nuclear maturation.

Discussion

We undertook to establish the molecular transcriptome phenotype of the human oocyte and its surrounding cumulus cells by using oligonucleotide microarrays covering most of the genes identified in human. Relying on a recently developed technique of double in vitro transcription, that amplifies more than 100 000 times the initial RNA input, we were able to establish the expression profile of pooled oocytes from distinct maturation stages, and from cumulus cells of MII oocytes. Thus, for the first time, we report in human samples, the variation of gene expression during oocyte nuclear maturation, and that of their neighboring cumulus cells, at whole genome scale. A global analysis of the number of genes detected in each sample category showed a progressive decrease of the number of genes expressed during oocyte nuclear maturation, with the lowest number of genes expressed found in MII oocytes compared with GV or MI oocytes. This is in agreement with the significant decrease, both in quantity and in diversity, of maternal RNAs observed in mouse oocytes (Bachvarova *et al.*,

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1982)(Wang *et al.*, 2004). Indeed, GV and MI oocytes over or under expressed few genes compared with the other samples (Table 1), reflecting a very similar expression profile. By contrast, MII oocytes differed markedly, underexpressing specifically many genes (n = 803), which may be explained by the RNA content decrease. In addition, MII oocytes overexpress 444 genes, which may be due to a specific expression pattern related to the near completion of meiosis, or to the longer in vitro incubation time secondary to the IVF procedure (21 or 44 hours post-insemination).

Hierarchical clustering demonstrated that oocytes expression profiles where markedly different from cumulus cells (Figure 1). We compared the oocytes samples to the cumulus cells and we found that 1514 genes were upregulated in oocytes whereas 2600 genes were upregulated in cumulus cells. Analyzing these lists of genes, we observed that oocyte markedly overexpressed genes involved in meiosis process such as MPF, APC/C and spindle checkpoint complexes. Full completion of meiosis is only accomplished after fecundation because metaphase exit is prevented by the activity of cytostatic factor (CSF) that will only be relieved by gamete fusion. As expected, *EMI1*, which was recently found to be part of CSF, is highly expressed in all oocytes samples, as well as *MOS*. We also found that the two major cyclin-dependent kinase inhibitors *CDKN1A/p21* and *CDKN1B/p27,* acting at the G1-S transition, were found markedly downregulated in oocytes as compared to cumulus cells (see Table 3). The separation of sister chromatids at the metaphase-to-anaphase transition is activated by proteases called separases that are activated by the destruction of the inhibitory chaperone securins. Interestingly, we found two securins highly expressed in all oocytes pools: *PTTG1* and 3. These securins are expressed at least 15 times more in oocytes than in cumulus cells, CD34+ sorted bone marrow cells, B lymphocytes or mesenchymal stem cells (data not shown). *PTTG1* expression was reported in mice oocytes, but not human oocytes, whereas PTTG3 marked expression in oocytes was not previously noted. Considering that

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post-ovulation oocytes are germinal cells that have just escaped the very long meiosis I arrest and are due to the second meiosis arrest, securins, that are crucial to these processes, must be expressed at a high level. We propose that *PTTG1* and 3 play this role in oocytes (Figure 3). The metaphase-to-anaphase transition is associated with a rapid drop of securin protein level mediated by the proteases of the separase family. Degradation of securins leads to the destruction of cohesins, a ring structure formed by a multisubunit complex that holds sister chromatids together. We confirm the specific upregulation of the meiosis-specific cohesin subunit *STAG3* in human oocytes, whereas the mitotic cohesin *STAG2* is markedly downregulated in oocytes compared to cumulus cells or other somatic cells (data not shown). Thus, as for the securins, two homologs of an essential component of the cell division machinery are differentially expressed between human oocytes and somatic cells, implying that one homolog (the cohesin *STAG2*) is operating during mitosis, whereas the other homolog (the cohesin *STAG3*) is replacing the first one during the very specialized cell division process of meiosis.

The high conservation of many of the molecular determinants of gametogenesis in the animal kingdom, sometimes from yeast to mammals, suggests that genes found in mammals oocytes should be expressed in human oocytes. We provide here the unambiguous demonstration for many genes that they are indeed strongly overexpressed in the three pools of oocytes (Table 2). These genes include *CENPA*, *CENPE*, *PTTG1, FBXO5/EMI1* or *BMP6*. These results underscore the consistency of our approach. Furthermore, the inventory of human genes essential for nuclear and cytoplasmic oocyte maturation is an important step toward the comprehensive understanding of oocyte biology.

Although female and male gametes differ in many aspects, they share a common meiosis machinery. Indeed, we see here that genes reported to be expressed specifically in spermatozoa are also highly overexpressed in oocytes in comparison with somatic cumulus

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cells. This is the case for *Aurora Kinase C* (*AURKC*), *Sperm Associated Antigen 16* (*SPAG16/PF20*) and *SOX30* (Osaki *et al.*, 1999; Horowitz *et al.*, 2005; Yan *et al.*, 2005). Three aurora kinases have been identified (*AURA/STK6*, *AURKB* and *AURKC*) that share a conserved catalytic domain and play a role in centrosome separation and maturation, spindle assembly and segregation, and cytokinesis (Giet *et al.*, 2005). Whereas *AURA* and *AURKB* are involved in mitosis in somatic cells, *AURKC* was only found highly expressed in testis, suggesting a tissue specific role in meiosis. It is therefore of special interest to observe that *AURKC* is also 49 times more expressed in pure oocytes samples than in somatic cells. Since *AURKB* and *AURKC* have a similar cellular localization and a similar biological activity such as *SURVIVIN/BIRC5* binding, we propose that *AURKC* is replacing *AURKB* during meiosis in both male and female gametes. In line with this proposition, our data shows that in oocytes samples, *AURKB* expression is close to background whereas survivin/BIRC5, a known partner of the *AURKC* complex (Yan *et al.*, 2005) is also strongly overexpressed.

We found the specific upregulation in oocytes of two methyltransferase enzymes (*DNMT1* and *3B*), one histone deacetylase (*HDAC9*) and an oocyte specific histone (*H1FOO*). Interestingly, the C*hromosome Condensation Protein G* (*HCAP-G*) which is a components of the condensin complex that mediates genome-wide chromosome condensation at the onset of mitosis and directly interacts with *DNMT3B* (Geiman *et al.*, 2004) is also found preferentially expressed in oocytes, suggesting that this condensin is essential to the nuclear maturation of oocytes. Keeping in line with epigenetic modifications of the genome, we screened our list of oocytes genes for imprinted genes. Of note, one paternally imprinted gene, *MEST*, was highly overexpressed in all three oocytes samples as compared to cumulus cells, while other paternally imprinted gene such as *IGF2* or *NNAT* were not.

We noted the overexpression of two pro-apopototic genes in oocytes (*BNIP1* and *BCL2L10*). These findings strongly argue in favor of a model where the survival of oocytes is mediated

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by external signals provided by surrounding cumulus cells rather than by intrinsic cues such as overexpression of anti-apoptotic factors. Accordingly, we found many receptors for growth factors overexpressed on oocytes, including a BMP receptor (*BMPR2*), the receptor for the stem cell factor (*KIT*), a member of the EGF receptor familiy (*ERBB4*), and a frizzled receptors (*FZD3*) member of the WNT pathway. In addition we observed 6 poorly characterized G protein-coupled receptors in oocytes (*GPR37*, *GPR39*, *GPR51*, *GPR126*, *GPR143*, *GPR160*). The fact that oocytes overexpress these growth factors receptors strongly suggests that the ligands of these receptors are involved in conveying surviving and maturation cues from the oophorus cumulus to the oocytes. Conversely, oocytes express many growth factors. Among the genes, we noted the remarkable overexpression of a ligand from the TNF superfamily, *TNFSF13/APRIL* that we found 131 times more expressed in oocytes than in cumulus cells. We did not see a significant expression of the two TNF receptors for *APRIL*, *TNFRSF13B/TACI* and *TNFRSF17/BCMA* (data not shown). But it was recently described that APRIL's binding to proteoglycan was necessary for the survival signal conveyed by this cytokine to targets cells (Ingold *et al.*, 2005). Since cumulus cells overexpress several proteoglycan such as *CSPG2/VERSICAN* (Table 3) and *SYNDECAN4* (data not shown), *APRIL* could mediate a comparable trophic signal from the oocyte to the surrounding cumulus cells.

We also focused our analysis on genes which expression increased progressively during oocyte meiosis. We postulate that they could be interesting candidate genes for oocyte maturation. Indeed, if these genes fail to be upregulated in MII-stage oocytes, it is likely that the maturation process was defective. Genes increasing progressively during oocyte maturation comprise *SOCS7*. This gene is part of a family of proteins negatively regulating intracellular signal transduction cascades (Krebs and Hilton, 2000). Its overexpression in MII-stage oocytes may indicate the shutting down of specific cytokine signalling. For this

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category, it must also be noted that many genes are still not characterized and remain without any hint about their function (20 out of 48 genes, = 42%). It is not a surprise if so many genes from this list have escaped bioinformatics or biological functional investigations to date, because (i) MII-stage oocytes are a very rare cell type, (ii) it is a very specialized cell type expressing numerous genes that may not be found in any other tissue type, including genes devoided of any molecular motif found in other tissues, and (iii) we used here pangenomic microarray to study here for the first time gene expression of this cell type without any selection bias. It will be essential to describe in detail the function of these genes to obtain further insights in oocyte biology.

In order to decipher the tight relationship weaved between the oocyte and its surrounding follicle cells, we also analyzed the transcriptome profile of cumulus cells. Indeed, 24% of the 2600 genes overexpressed in cumulus cells are annotated either “membrane” or “extracellular”, demonstrating a strong bias towards genes involved in cell-to-cell communication processes. The signalling pathways involved comprise the progesterone and its receptors, eicosanoids and several enzymes involved in their biosynthesis and chemokines. We showed in this study that cumulus cells up regulated hormonal receptors and hormones such as *LHCGR*, *Inhibin alpha*, *Inhibin beta A*, *GNRH1* and *progesterone receptor membrane component1 and 2*.

Interestingly, cumulus cells overexpress *BMPR2* which is the receptor for *GDF9* which is overexpressed by oocytes, demonstrating a typical intercellular communication process. In addition to the inhibins *INHA* and *INHBA*, we also observed the overexpression of *BMP1* and *BMP8B*, as well as the pseudoreceptor *BAMBI*, lacking an intracellular serine/threonine kinase domain and thus negatively regulating TGF-beta signalling. Another important growth factor superfamily found to be overexpressed in cumulus is the TNF superfamily. The marked

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overexpression of *TNFSF11/OPGL/RANKL* (80 times more expressed in cumulus cells than in oocytes) is intriguing and awaits further investigations. Magier et al. suggested a positive effect of cumulus cells on fertilization, a protective effect and a possible beneficial effect on further embryo development (Magier *et al.*, 1990). In addition, Platteau et al. (Platteau *et al.*, 2004) suggested that the exogenous luteinizing hormone activity may influence treatment outcome in IVF but not in ICSI. We provide here molecular evidence for cumulus cells expression by of hormones and growth factors that could mediate these functions.

Another puzzling observation is the increased expression of seven complement factors or closely related genes. Whether this overexpression is involved in the cellular destruction process taking place in the antrum during ovulation needs to be considered. Finally, cumulus cells express several other cell surface gene families such as semaphorins, first identified for their role in neuron guidance, tetraspanins, with one member, *CD9*, directly involved in fertilization (Le Naour *et al.*, 2000), and many other CD molecules with various function (Table 3). Very interestingly, some genes overexpressed in granulosa cells are also found expressed in ovarian tumors. We found for example a high expression in cumulus cells of *CD24* and *CD99* which are expressed in ovarian tumors and have been proposed as either diagnostic tools (Choi *et al.*, 2000) or as prognostic tools (Kristiansen *et al.*, 2002). These findings suggest that many of the genes overexpressed in cumulus samples, including the cell surface markers of cumulus cells listed in Table 3, could provide ovarian cancer markers.

We pooled oocytes according to their maturation stage for this first, exploratory, whole genome transcriptome analysis. This strategy leveled down differences that would be associated with different IVF settings such as maternal age, sperm exposure or in vitro incubation time length. In order to describe the expression modifications that may be related to specific conditions, we are currently analyzing the transcriptome of oocytes pooled according to the hormonal profile at day 3, maternal age or ovarian hyperstimulation protocol.

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Nevertheless, to appreciate variations in gene expression according to each patients idiosyncrasy, we will need to achieve reliable transcriptome analysis from single oocytes.

In conclusion, DNA microarray provided us with the opportunity to analyze human oocytes and cumulus cells expression profiles on a genome scale and permitted a significant progress to understand the molecular events involved in the process governing oocyte maturation. Many of the genes described here may well provide markers to monitor health, viability and competence of oocytes. In addition, underpinning oocyte growth factors receptors should help to design optimal in vitro culture conditions for oocyte and early embryo development.

Acknowledgments

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Tables

Table 1 : genes expressed in oocytes and cumulus cells

	GV	MI	MII	Cumulus
Expressed genes (1)	10 869	9 682	5 633	10 610
Exclusive genes (2)	739	326	234	1 829
Genes overexpressed (3)	104	4	444	2600
Genes underexpressed (4)	6	5	803	1514

1: genes (based on Unigene Build 176) that had at least one probe with a detection call "Present".

2: genes (based on Unigene Build 176) that had a detection call "Present" only in one sample category.

3: genes significantly overexpressed in one sample compared to all other samples, with a fold ratio of at least three.

4: genes significantly underexpressed in one sample compared to all other samples, with a fold ratio of at least 0.333.

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Table 2 : genes significantly overexpressed in oocytes

Gene Symbol	Gene Title	Fold ratio	Probeset	Chromosomal Location	Species (1)	References
Gamete markers						
DAZL	deleted in azoospermia-like	976.3	206588_at	chr3p24.3	h	(Nishi *et al.*, 1999; Cauffman *et al.*, 2005)
DDX4/VASA	DEAD (Asp-Glu-Ala-Asp) box polypeptide 4	1181.5	221630_s_at	chr5p15.2-p13.1	h	(Castrillon *et al.*, 2000)
DPPA3/STELLA	developmental pluripotency associated 3	10389.1	231385_at	chr12p13.31	h	(Saitou *et al.*, 2002)
Maturation promoting factor and related factors						
CCNB1	cyclin B1	157.0	228729_at	chr5q12	h	(Heikinheimo *et al.*, 1995)
CCNB2	cyclin B2	308.8	202705_at	chr15q22.2	b	(Wu *et al.*, 1997)
CDC2	cell division cycle 2, G1 to S and G2 to M	18.4	210559_s_at	chr10q21.1	m	(Kalous *et al.*, 2005)
CDC25A	cell division cycle 25A	90.8	1555772_a_at	chr3p21	m	(Wickramasinghe *et al.*, 1995)
CDC25B	cell division cycle 25B	9.7	201853_s_at	chr20p13	m	(Lincoln et al., 2002)
CDC25C	cell division cycle 25C	75.2	205167_s_at	chr5q31	c	(Gall *et al.*, 2002)
Spindle checkpoint						
BUB1	BUB1 budding uninhibited by benzimidazoles 1 homolog	20.6	209642_at	chr2q14	h	(Steuerwald *et al.*, 2001)
BUB1B / BUBR1	BUB1 budding uninhibited by benzimidazoles 1 homolog beta	117.8	203755_at	chr15q15	x	(Abrieu *et al.*, 2000)
CENPA	centromere protein A	88.1	204962_s_at	chr2p24-p21	m	(Schatten *et al.*, 1988)
CENPE	centromere protein E	113.9	205046_at	chr4q24-q25	m	(Duesbery *et al.*, 1997)
CENPH	centromere protein H	14.3	231772_x_at	chr5p15.2	NR	
MAD2L1/MAD2	MAD2 mitotic arrest deficient-like 1	53.5	203362_s_at	chr4q27	m	(Wassmann *et al.*, 2003)
APC/C complex, securins, cohesins						
ANAPC1/APC1	anaphase promoting complex subunit 1	6.8	218575_at	chr2q12.1	NR	
ANAPC10/APC10	anaphase promoting complex subunit 10	7.0	207845_s_at	chr4q31	NR	
CDC20	CDC20 cell division cycle 20	273.9	202870_s_at	chr1p34.1	m	(Chang *et al.*, 2004)
PTTG1	pituitary tumor-transforming 1	58.2	203554_x_at	chr5q35.1	m	(Yao et al., 2003)
PTTG3	pituitary tumor-transforming 3	50.4	208511_at	chr8q13.1	NR	
STAG3	stromal antigen 3	76.9	219753_at	Hs.323634	h	(Prieto *et al.*, 2004)

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Epigenetic remodeling						
DNMT1	DNA (cytosine-5-)-methyltransferase 1	49.8	201697_s_at	chr19p13.2	h	(Huntriss *et al.*, 2004)
DNMT3B	DNA (cytosine-5-)-methyltransferase 3 beta	24.2	220668_s_at	chr20q11.2	h	(Huntriss *et al.*, 2004)
HDAC9	histone deacetylase 9	342.6	1552760_at	chr7p21.1	m	(De La Fuente *et al.*, 2004)
H1FOO	H1 histone family, member O, oocyte-specific	414.5	1553064_at	chr3q21.3	h	(Tanaka et al., 2003)
HCAP-G	chromosome condensation protein G	260.1	218663_at	chr4p15.33	NR	
Meiosis, miscellaneous						
AKAP1	A kinase (PRKA) anchor protein 1	72.1	210625_s_at	Hs.78921	r	(Carr *et al.*, 1999)
MCM3	MCM3 minichromosome maintenance deficient 3	21.2	201555_at	chr6p12	x	(Kubota *et al.*, 1995)
MOS	v-mos Moloney murine sarcoma viral oncogene homolog	72.5	221367_at	chr8q11	h	(Pal *et al.*, 1994)
SPAG16	sperm associated antigen 16	393.4	240898_at	chr2q34	NR	
TUBB4Q	tubulin, beta polypeptide 4, member Q	866.5	211915_s_at	chr4q35	NR	
FBXO5/EMI1	F-box protein 5	414.2	234863_x_at	chr6q25-q26	m	(Paronetto *et al.*, 2004)
AURKC	aurora kinase C	49.1	211107_s_at	chr19q13.43	NR	
Extracellular matrix, growth factors, cell surface, signalling						
BMP15	bone morphogenetic protein 15	31.0	221332_at	chrxp11.2	h	(Aaltonen *et al.*, 1999)
BMP6	bone morphogenetic protein 6	38.3	206176_at	chr6p24-p23	m	(Lyons *et al.*, 1989)
GDF9	growth differentiation factor 9	83.0	221314_at	chr5q31.1	h	(Aaltonen *et al.*, 1999)
FGFR2	fibroblast growth factor receptor 2	8.4	208228_s_at	chr10q26	m	(Haffner-Krausz *et al.*, 1999)
FGF9	fibroblast growth factor 9 (glia-activating factor)	43.8	206404_at	chr13q11-q12	NR	
FGF14	fibroblast growth factor 14	28.1	221310_at	chr13q34	NR	
KIT	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog		205051_s_at	chr4q11-q12	h	(Liu, 2006)
IL4	interleukin 4	52.5	207538_at	chr5q31.1	NR	
TNFSF13 / APRIL	tumor necrosis factor superfamily, member 13	131.6	210314_x_at	chr17p13.1	NR	
ERBB4	v-erb-a erythroblastic leukemia viral oncogene homolog 4	32.3	206794_at	chr2q33.3-q34	NR	
FZD3	frizzled homolog 3	17.9	219683_at	chr8p21	NR	
GPR37	G protein-coupled receptor 37 (endothelin receptor type B-like)	64.4	209631_s_at	chr7q31	NR	
GPR39	G protein-coupled receptor 39	1234.0	229105_at	chr2q21-q22	NR	
GPR51	G protein-coupled receptor 51	5.7	209990_s_at	chr9q22.1	NR	
GPR126	G protein-coupled receptor 126	11.7	213094_at	chr6q24.1	NR	
GPR143	G protein-coupled receptor 143	94.1	206696_at	chrxp22.3	NR	

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GPR160	G protein-coupled receptor 160	11.3	223423_at	chr3q26.2	NR	
ZP1	zona pellucida glycoprotein 1 (sperm receptor)	86.6	237335_at	11q12.2	h	(Lefievre *et al.*, 2004)
ZP2	zona pellucida glycoprotein 2 (sperm receptor)	1558.8	207933_at	chr16p12	h	(Hinsch *et al.*, 1998)
ZP3	zona pellucida glycoprotein 3 (sperm receptor)	87.6	204148_s_at	chr7q11.23	h	(Grootenhuis *et al.*, 1996)
ZP4	zona pellucida glycoprotein 4	52.2	231756_at	chr1q43	h	(Eberspaecher *et al.*, 2001)
SLC5A11	solute carrier family 5 (sodium/glucose cotransporter), member 11	144.7	237254_at	chr16p-p11	NR	
SOCS7	suppressor of cytokine signaling 7	26.3	2265772_at	chr17q12	NR	
Transcription factors						
SOX15	SRY (sex determining region Y)-box 15	127.2	206122_at	chr17p13	NR	
SOX30	SRY (sex determining region Y)-box 30	618.5	207678_s_at	chr5q33	NR	
OTX2	orthodenticle homolog 2 (Drosophila)	5022.8	242128_at	chr14q21-q22	NR	
FOXR1	forkhead box R1	344.1	237613_at	chr11q23.3	NR	
Imprinted gene						
MEST	mesoderm specific transcript homolog	39.2	202016_at	chr7q32	h	(Salpekar *et al.*, 2001)
Apoptosis						
BNIP1	BCL2/adenovirus E1B 19kDa interacting protein 1	20.8	207829_s_at	chr5q33-q34	NR	
BIRC5	baculoviral IAP repeat-containing 5 (survivin)	23.2	202094_at	chr17q25	NR	
BCL2L10	BCL2-like 10 (apoptosis facilitator)	623.3	236491_at	chr15q21	m	(Burns *et al.*, 2003)

1: h = Homo sapiens; m = Mus musculus (mouse); r = Rattus norvegicus (rat); b = Bos Taurus (cow); c = Capra hircus (goat); p = Sus scrofa (pig); x = Xenopus laevis (frog). NR: not reported: a research in Pubmed with each synonym for this gene (as listed by LocusLink) and one of the following keywords did not retrieve any significant result: oocyte, germ cell, gamete, egg, meiosis. Expression values of all the genes described in this Table can be accessed on our web site (see material and method) or can be downloaded as supplemental data.

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Table 3 : genes significantly overexpressed in cumulus oophorus cells

Gene Symbol	Gene Title	Fold ratio	Probeset	Chromosomal Location	Species (1)	References (2)
Hormone and hormone receptors						
LHCGR	luteinizing hormone/choriogonadotropin receptor	47.4	207240_s_at	chr2p21	NR	
PGRMC1	progesterone receptor membrane component 1	13.4	201121_s_at	chrxq22-q24	r	(Park and Mayo, 1991)
PGRMC2	progesterone receptor membrane component 2	7.4	213227_at	chr4q26	h	(Tokuyama *et al.*, 2001)
STAR	steroidogenic acute regulator	33.0	204548_at	chr8p11.2	h	(Devoto *et al.*, 2001)
GNRH1	Gonadotropin-releasing hormone 1 (luteinizing-releasing hormone)	26.7	207987_s_at	8p21-p11.2	h	(Leung *et al.*, 2003)
Prostaglandins biosynthesis						
PTGS1	prostaglandin-endoperoxide synthase 1	5.0	215813_s_at	chr9q32-q33.3	NR	
PTGS2/COX-2	prostaglandin-endoperoxide synthase 2	18.7	204748_at	chr1q25.2-q25.3	r	(Sirois *et al.*, 1993; Davis *et al.*, 1999)
PTGIS	prostaglandin I2 (prostacyclin) synthase	10.8	208131_s_at	chr20q13.11-13	NR	
PTGER2	prostaglandin E receptor 2	5.4	206631_at	chr14q22	h	(Narko *et al.*, 2001)
BMP and BMPR superfamily						
BAMBI	BMP and activin membrane-bound inhibitor homolog	9.3	203304_at	chr10p12.3-11.2	NR	
BMP1	bone morphogenetic protein 1	10.6	202701_at	chr8p21	NR	
BMP8B	bone morphogenetic protein 8b	19.4	235275_at	chr1p35-p32	NR	
BMPR2	bone morphogenetic protein receptor, type II	7.2	225144_at	chr2q33-q34	r	(Vitt *et al.*, 2002)
INHA	inhibin, alpha	5.2	210141_s_at	chr2q33-q36	h	(Jaatinen *et al.*, 1994)
INHBA	inhibin, beta A (activin A, activin AB alpha polypeptide)	34.8	210511_s_at	chr7p15-p13	h	(Rabinovici *et al.*, 1992)
TNF and TNFR superfamily						
TNFSF11/OPGL/RANKL	tumor necrosis factor (ligand) superfamily, member 11	79.9	210643_at	chr13q14	NR	
TNFRSF1A/ TNF-R	tumor necrosis factor receptor superfamily, member 1A	14.9	207643_s_at	chr12p13.2	NR	
TNFRSF10B/ DR5	tumor necrosis factor receptor superfamily, member 10b	5.9	209295_at	chr8p22-p21	NR	
TNFRSF12A	tumor necrosis factor receptor superfamily, member 12A	4.6	218368_s_at	chr16p13.3	NR	
Complement						
CFHL1	complement factor H-related 1	105.8	215388_s_at	chr1q32	NR	
C7	complement component 7	135.8	202992_at	chr5p13	NR	

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IF	I factor (complement)	34.7	203854_at	chr4q25	NR	
CFH	complement factor H	40.0	213800_at	chr1q32	NR	
C1S	complement component 1, s subcomponent	28.0	208747_s_at	chr12p13	NR	
C1R	complement component 1, r subcomponent	9.1	212067_s_at	chr12p13	NR	
CLU	clusterin (complement lysis inhibitor)	140.9	208791_at	chr8p21-p12	r	(Hurwitz *et al.*, 1996)
Secreted (other)						
CXCL1	chemokine (C-X-C motif) ligand 1	8.0	204470_at	chr4q21	h	(Karstrom-Encrantz *et al.*, 1998)
IL1B	interleukin 1, beta	11.7	39402_at	chr2q14	h	(de los Santos *et al.*, 1998)
IL8	interleukin 8	12.9	202859_x_at	chr4q13-q21	h	(Runesson *et al.*, 2000)
TIMP1	tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor)	25.3	201666_at	chrxp11.3-23	h	(O'Sullivan *et al.*, 1997)
TIMP3	tissue inhibitor of metalloproteinase 3 (Sorsby fundus dystrophy, pseudoinflammatory)	19.9	201150_s_at	chr22q12.3	NR	
PAPPA	pregnancy-associated plasma protein A, pappalysin 1	54.3	224942_at	chr9q33.2	h	(Stanger *et al.*, 1985)
PTX3	pentaxin-related gene, rapidly induced by IL-1 beta	25.4	206157_at	chr3q25	h	(Zhang *et al.*, 2005)
CD molecules						
CD24	CD24 antigen	126.9	208650_s_at	chr6q21	h	(Hourvitz *et al.*, 2000)
CD44	CD44 antigen	17.0	212063_at	chr11p13	NR	
CD47	CD47 antigen	4.6	213857_s_at	chr3q13.1	NR	
CD58	CD58 antigen / LFA3	18.6	216942_s_at	chr1p13	h	(Hattori *et al.*, 1998)
CD59	CD59 antigen p18-20	16.4	228748_at	chr11p13	NR	
CD63	CD63 antigen	7.7	200663_at	chr12q12-q13	r	(Espey and Richards, 2002)
CD74	CD74 antigen	5.9	209619_at	chr5q32	NR	
CD81	CD81 antigen	6.1	200675_at	chr11p15.5	NR	
CDW92	CDW92 antigen	4.7	224596_at	chr9q31.2	NR	
CD99	CD99 antigen	4.1	201029_s_at	chrxp22.32	h	(Gordon *et al.*, 1998)
CD151	CD151 antigen	8.4	204306_s_at	chr11p15.5	NR	
CD200	CD200 antigen	22.9	209583_s_at	chr3q12-q13	NR	
Membrane bound (other)						
ADAMTS1	a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 1	58.2	222162_s_at	chr21q21.2	m	(Russell *et al.*, 2003)
CSPG2	chondroitin sulfate proteoglycan 2 (versican)	5.5	211571_s_at	chr5q14.3	m	(Russell *et al.*, 2003)
SEMA3A	semaphorin 3A	43.2	206805_at	chr7p12.1	NR	
SEMA6A	semaphorin 6A	9.8	225660_at	chr5q23.1	NR	

HAL author manuscript inserm-00130809, version 1

SEMA6D	semaphorin 6D	38.5	226492_at	chr15q21.1	NR	
TM4SF1	transmembrane 4 superfamily member 1	83.8	215034_s_at	chr3q21-q25	NR	
TM4SF6	transmembrane 4 superfamily member 6	13.2	209108_at	chrxq22	NR	
TM4SF8	transmembrane 4 superfamily member 8	5.4	200973_s_at	chr15q24.3	NR	
TM4SF10	transmembrane 4 superfamily member 10	7.5	209656_s_at	chrxp11.4	NR	
Transcription factors						
CEBPB	CCAAT/enhancer binding protein (C/EBP), beta	7.4	212501_at	chr20q13.1	p	(Gillio-Meina *et al.*, 2005)
GATA6	GATA binding protein 6	56.8	210002_at	chr18q11.1-11.2	h	(Suzuki *et al.*, 1996)
Other						
PRDX2	peroxiredoxin 2	6.2	39729_at	chr19p13.2	b	(Leyens *et al.*, 2004)
PRDX4	peroxiredoxin 4	31.2	201923_at	chrxp22.11	b	(Leyens *et al.*, 2004)
PRDX5	peroxiredoxin 5	5.3	1560587_s_at	chr11q13	b	(Leyens *et al.*, 2004)
PRDX6	peroxiredoxin 6	23.2	200844_s_at	chr1q25.1	b	(Leyens *et al.*, 2004)
CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	17.0	202284_s_at	chr6p21.2	m	(Jirawatnotai *et al.*, 2003)
CDKN1B	cyclin-dependent kinase inhibitor 1B (p27, Kip1)	17.0	209112_at	chr12p13.1-p12	m	(Robker and Richards, 1998)
CTSK	cathepsin K (pycnodysostosis)	11.8	202450_s_at	chr1q21	r	(Oksjoki *et al.*, 2001)

1: h = Homo sapiens; m = Mus musculus (mouse); r = Rattus norvegicus (rat); b = Bos Taurus (cow); c = Capra hircus (goat); p = Sus scrofa (pig); x = Xenopus laevis (frog). NR: not reported: a research in Pubmed with each synonym for this gene (as listed by LocusLink) and one of the following keywords did not retrieve any significant result: oocyte, germ cell, gamete, egg, cumulus, granulosa.

Expression values of all the genes described in this Table can be accessed on our web site (see material and method) or can be downloaded as supplemental data.

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Legends to Figures

Figure 1 : Global gene expression variation

(A) Scatter plots. Each sample was plotted against all other samples to visualize the expression variation. Only the 26 662 probes with at least one sample with a "Present" detection call were included. All signal values were floored to 2. Red circles : probes overexpressed in the sample specified on the left side; green circles : probes overexpressed in the sample specified at the bottom of each plot; grey circles : probes whose expression do not vary significantly between the two samples. For each couple of sample, the Pearson correlation coefficient was computed ("r"), based on the signal of probes with at least one sample with a "Present" detection call. GV (germinal vesicle), MI (metaphase I); MII (metaphase II).

(B) Hierarchical clustering. The expression signature of oocyte and cumulus cells were visualized by hierarchical clustering on the 15 000 probesets with the highest variation coefficient. The colors indicate the relative expression levels of each gene, with red indicating an expression above median, green indicating expression under median and black representing median expression. Cluster (a) was a group of genes overexpressed in oocyte (GV-MI-MII), including genes such as DAZL, GDF9, BMP15, ZP1,2,3,4. Cluster (b) was group of genes overexpressed in cumulus cells, including genes such as CD24, Activin A, PAPPA, TNTSF11, LHCGR and INHA.

Figure 2 : Differential Gene Ontology annotations between oocytes and cumulus cells

We compared the frequency of level 3 Gene Ontology (GO) annotations of genes overexpressed in oocytes to those of genes overexpressed in cumulus cells. The statistical analysis was made using the Fatigo web site (http://www.fatigo.org/) using Unigene cluster

HAL author manuscript inserm-00130809, version 1

ID. Histograms show the percentage of genes with the specified GO annotation in the group of genes overexpressed in oocytes (purple) or in cumulus (green). *P* : *P*-value.

Figure 3 : Schematic representation of selected genes involved in meiosis and cumulus-oocyte-complex

(**A**) **Meiosis**. Actors of meiosis in oocytes: components of the Maturation-Promoting Factor (MPF), components of the spindle checkpoint, components of the Anaphase Promoting Complex (APC/C), the downstream targets such as the securin *PTTG3,* and regulators. Genes in pink are upregulated in oocytes. Genes that are specific to meiosis are highlighted by an orange hexagon. Genes in white did not display a significant modification in gene expression between oocytes and somatic cells (cumulus cells). See table 2 for full name and references.

(**B**) **Cumulus-oocyte-complex**. Genes overexpressed in oocytes (pink) or overexpressed in cumulus (green) that are involved in the cumulus-oocyte-complex interactions. Oocytes genes included members of the transforming growth factor-beta superfamily such as *Growth Differentiation Factor 9* (*GDF9*), *Fibroblast Growth Factor 9* and *15* (*FGF9*, *15*), *Bone Morphogenetic Protein 6* and *15* (*BMP6*, *15*). Conversely, in cumulus cells, the genes overexpressed included hormonal receptors such as *Luteinizing Hormone/ Choriogonadotropin Receptor (LHCGR)*, *Progesterone Receptor Membrane Component 1* and *2* (*PGRMC1, 2*), *Interleukin IL1beta*, *chemokines* (*IL8*) and *CD24* antigen, *Inhibin Alpha* (*INHA*), *Activin A* (*INHBA*). Genes in red are upregulated in the oocytes compared to cumulus cells. Genes in green are upregulated in the cumulus cells compared to oocytes. Genes shown in blue are expressed in oocytes and cumulus cells such as *Gap Junction protein Alpha* (*GJA1*). See table 3 for complete list of full names and references.

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Figure 4 : Expression histograms of selected genes in oocytes and cumulus cells

Histograms show signal values of 20 genes which are differentially expressed between oocyte (purple) and cumulus cells (green). Gene expression is measured by pan genomic HG-U133 Plus 2.0 Affymetrix oligonucleotides microarrays and the signal intensity for each gene is shown on the Y axis as arbitrary units determined by the GCOS 1.2 software (Affymetrix). GV : germinal vesicle; MI : metaphase I; MII : metaphase II; C : cumulus.

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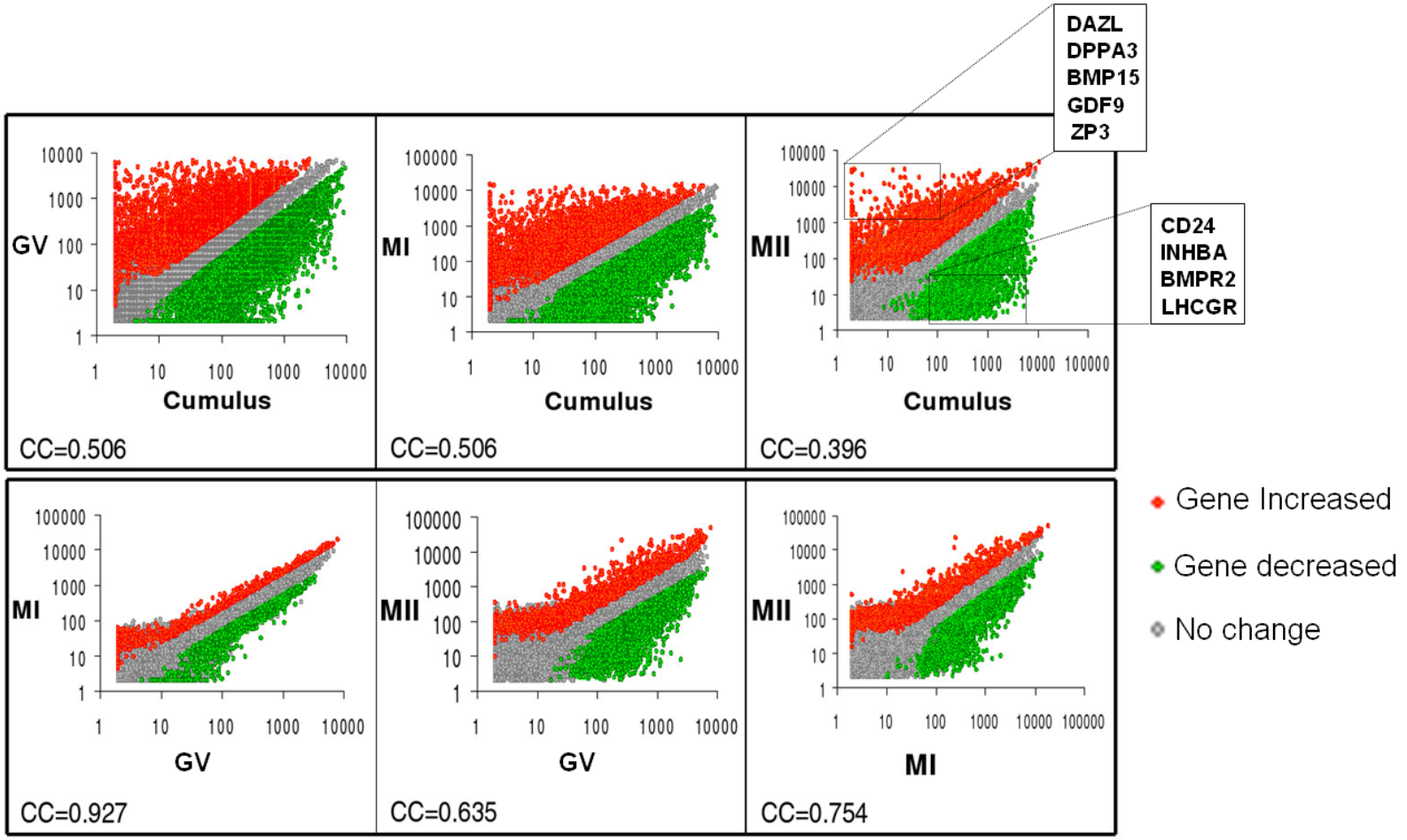


Figure 1A
Assou et al.

Figure 1 : Global gene expression variation
(A) Scatter plots. Each sample was plotted against all other samples to visualize the expression variation. Only the 26 662 probes with at least one sample with a “Present” detection call were included. All signal values were floored to 2. Red circles : probes overexpressed in the sample specified on the left side; green circles : probes overexpressed in the sample specified at the bottom of each plot; grey circles : probes whose expression do not vary significantly between the two samples. For each couple of sample, the Pearson correlation coefficient was computed (“r”), based on the signal of probes with at least one sample with a “Present” detection call. GV (germinal vesicle), MI (metaphase I); MII (metaphase II).

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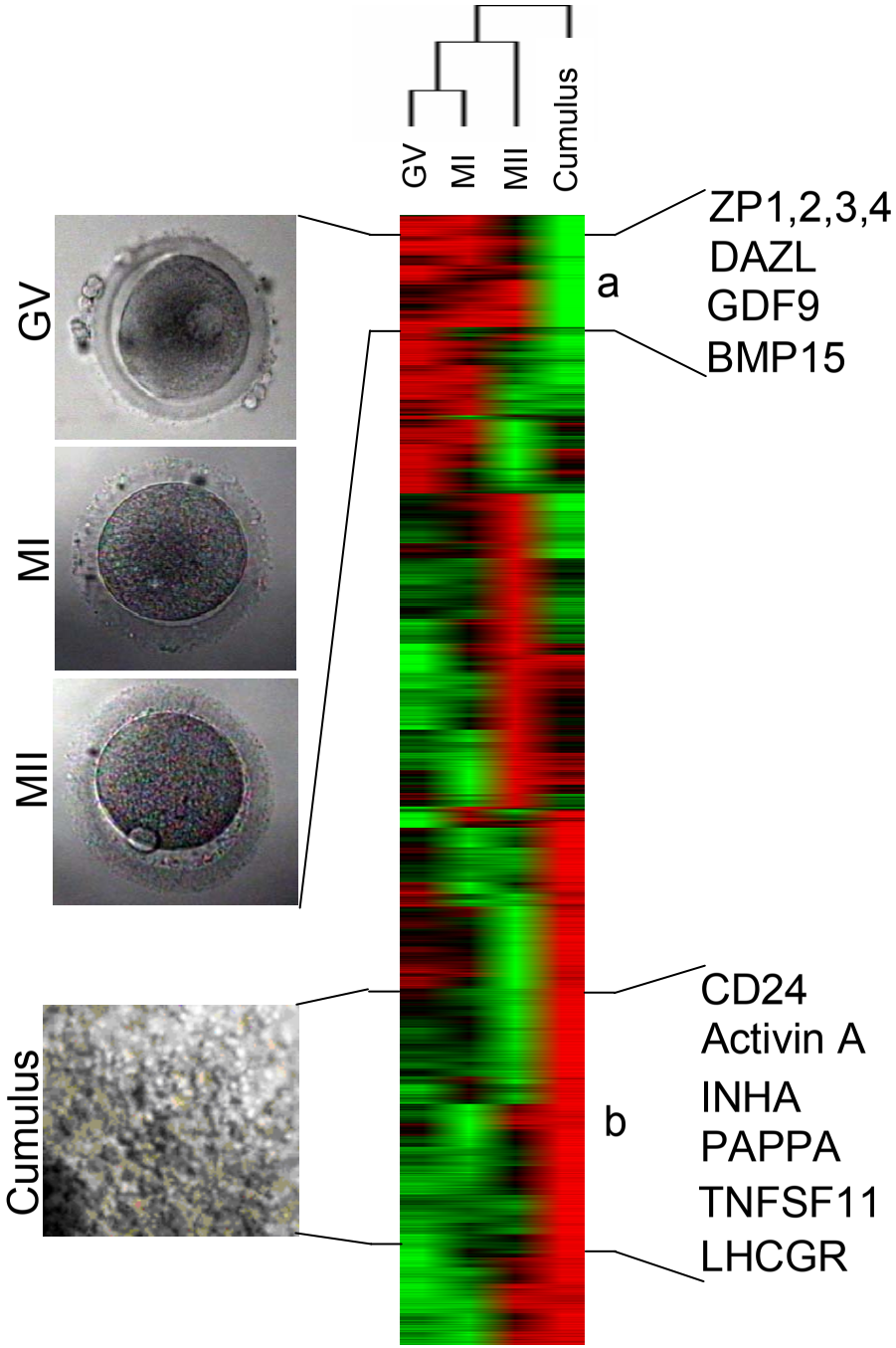


Figure 1B
Assou et al.

Figure 1 B: Global gene expression variationAssou et al.
(B) Hierarchical clustering. The expression signature of oocyte and cumulus cells were visualized by hierarchical clustering on the 15 000 probesets with the highest variation coefficient. The colors indicate the relative expression levels of each gene, with red indicating an expression above median, green indicating expression under median and black representing median expression. Cluster (a) was a group of genes overexpressed in oocyte (GV-MI-MII), including genes such as DAZL, GDF9, BMP15, ZP1,2,3,4. Cluster (b) was group of genes overexpressed in cumulus cells, including genes such as CD24, Activin A, PAPPA, TNTSF11, LHCGR and INHA.

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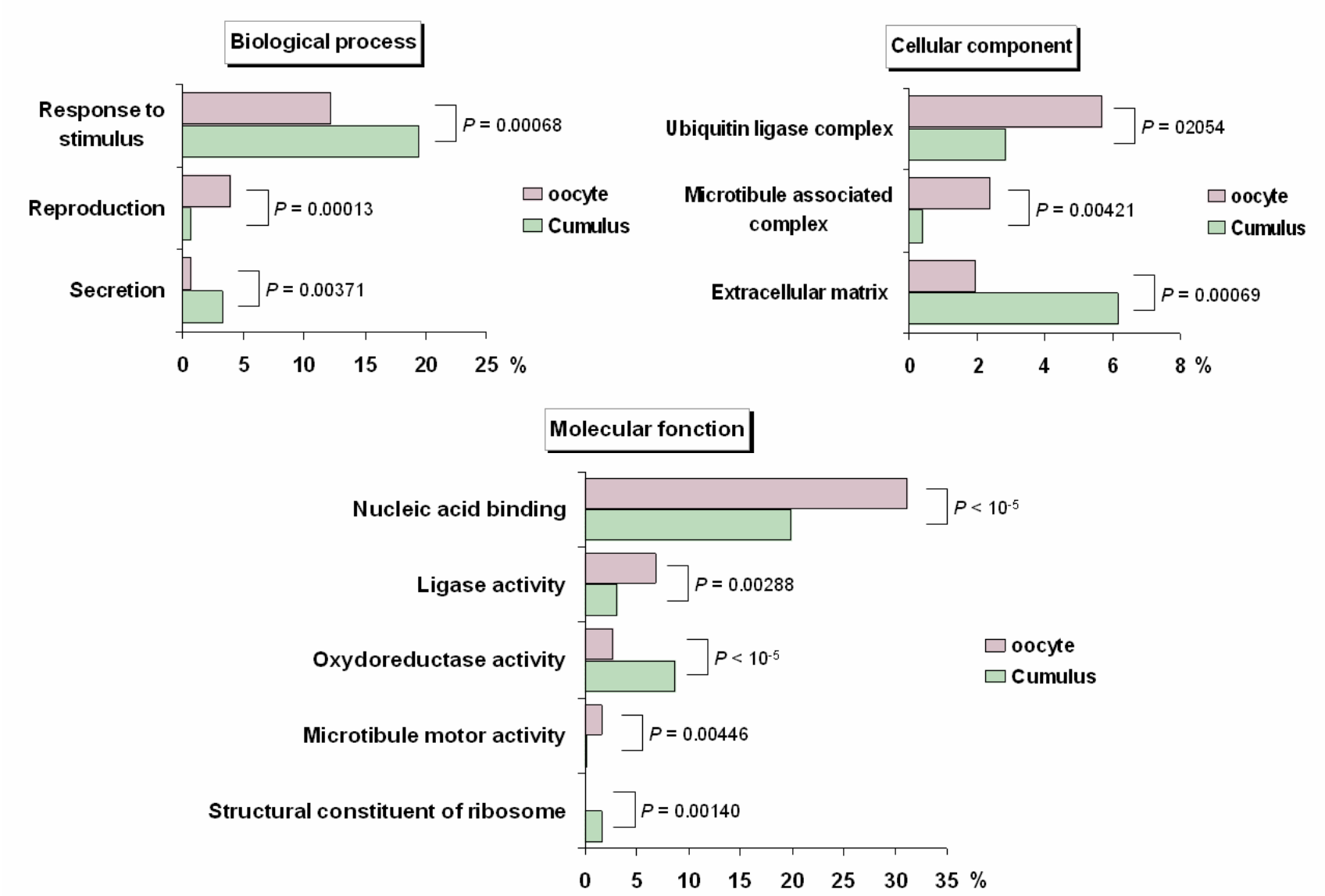


Figure 2

Assou et al.

Figure 2 : Differential Gene Ontology annotations between oocytes and cumulus cells
We compared the frequency of level 3 Gene Ontology (GO) annotations of genes overexpressed in oocytes to those of genes overexpressed in cumulus cells. The statistical analysis was made using the Fatigo web site (http://www.fatigo.org/) using Unigene cluster ID. Histograms show the percentage of genes with the specified GO annotation in the group of genes overexpressed in oocytes (purple) or in cumulus (green). *P* : *P*-value.

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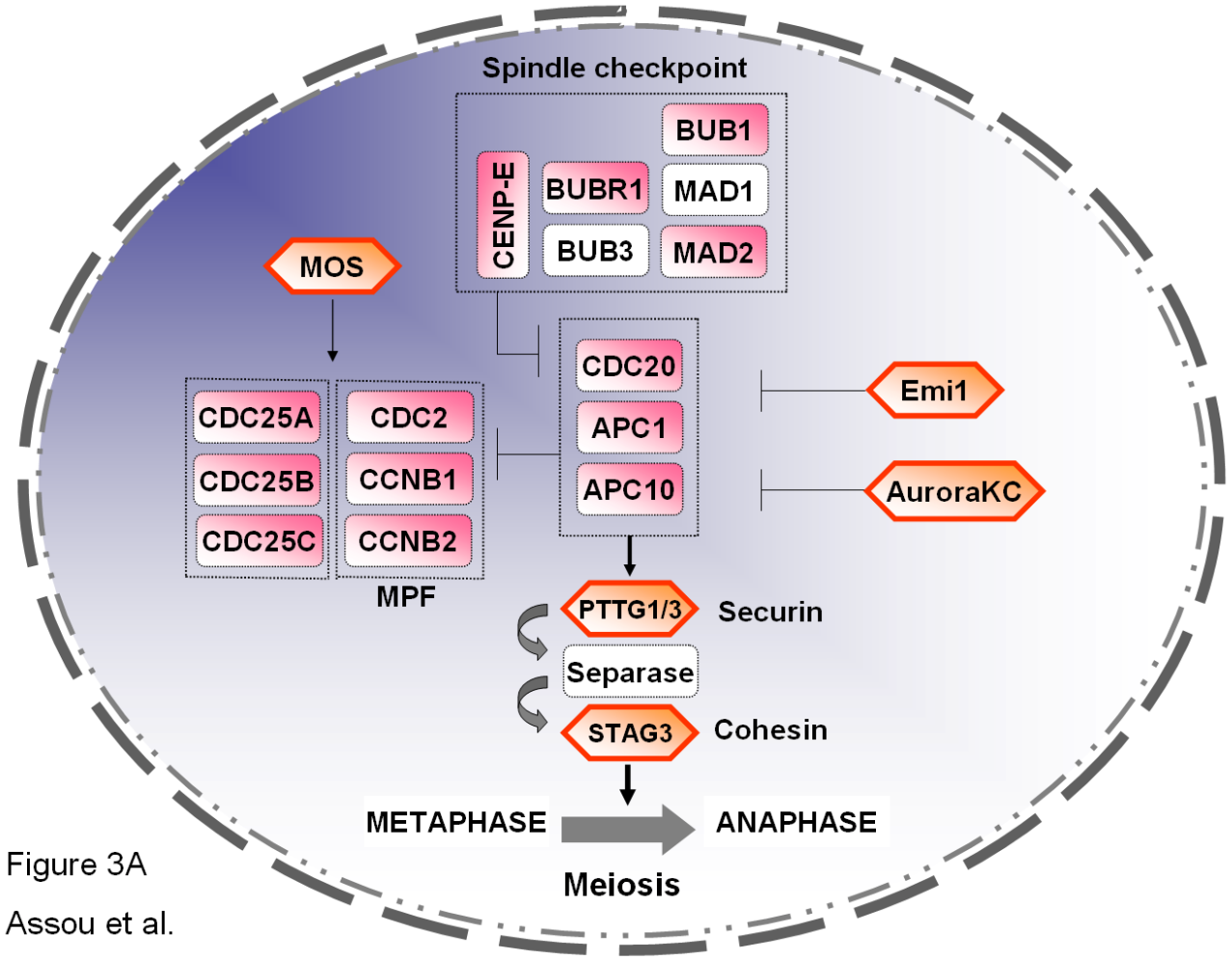


Figure 3 : Schematic representation of selected genes involved in meiosis and cumulus-oocyte-complex

(**A**) **Meiosis**. Actors of meiosis in oocytes: components of the Maturation-Promoting Factor (MPF), components of the spindle checkpoint, components of the Anaphase Promoting Complex (APC/C), the downstream targets such as the securin *PTTG3,* and regulators. Genes in pink are upregulated in oocytes. Genes that are specific to meiosis are highlighted by an orange hexagon. Genes in white did not display a significant modification in gene expression between oocytes and somatic cells (cumulus cells). See table 2 for full name and references.

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Figure 3B

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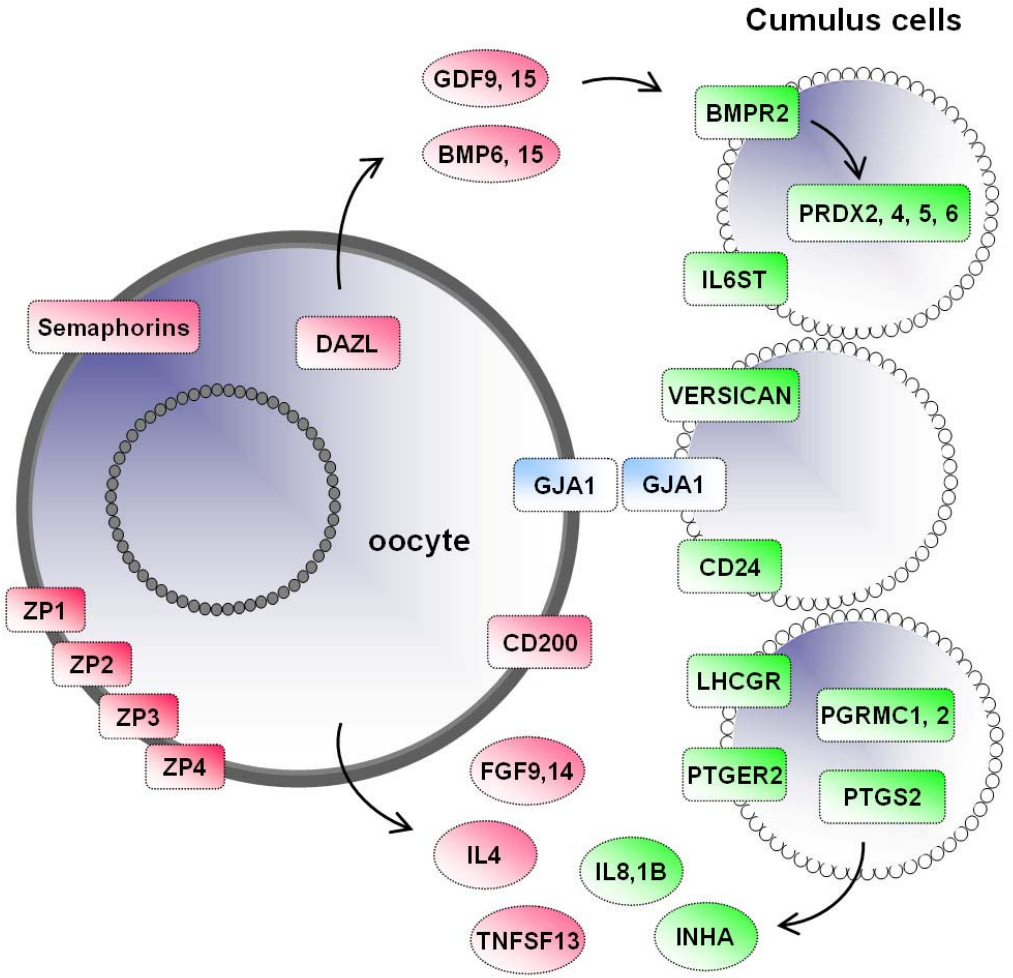


Figure 3 (**B**) **Cumulus-oocyte-complex**. Genes overexpressed in oocytes (pink) or overexpressed in cumulus (green) that are involved in the cumulus-oocyte-complex interactions. Oocytes genes included members of the transforming growth factor-beta superfamily such as *Growth Differentiation Factor 9* (*GDF9*), *Fibroblast Growth Factor 9* and *15* (*FGF9*, *15*), *Bone Morphogenetic Protein 6* and *15* (*BMP6*, *15*). Conversely, in cumulus cells, the genes overexpressed included hormonal receptors such as *Luteinizing Hormone/ Choriogonadotropin Receptor (LHCGR)*, *Progesterone Receptor Membrane Component 1* and *2* (*PGRMC1, 2*), *Interleukin IL1beta*, *chemokines* (*IL8*) and *CD24* antigen, *Inhibin Alpha* (*INHA*), *Activin A* (*INHBA*). Genes in red are upregulated in the oocytes compared to cumulus cells. Genes in green are upregulated in the cumulus cells compared to oocytes. Genes shown in blue are expressed in oocytes and cumulus cells such as *Gap Junction protein Alpha* (*GJA1*). See table 3 for complete list of full names and references.

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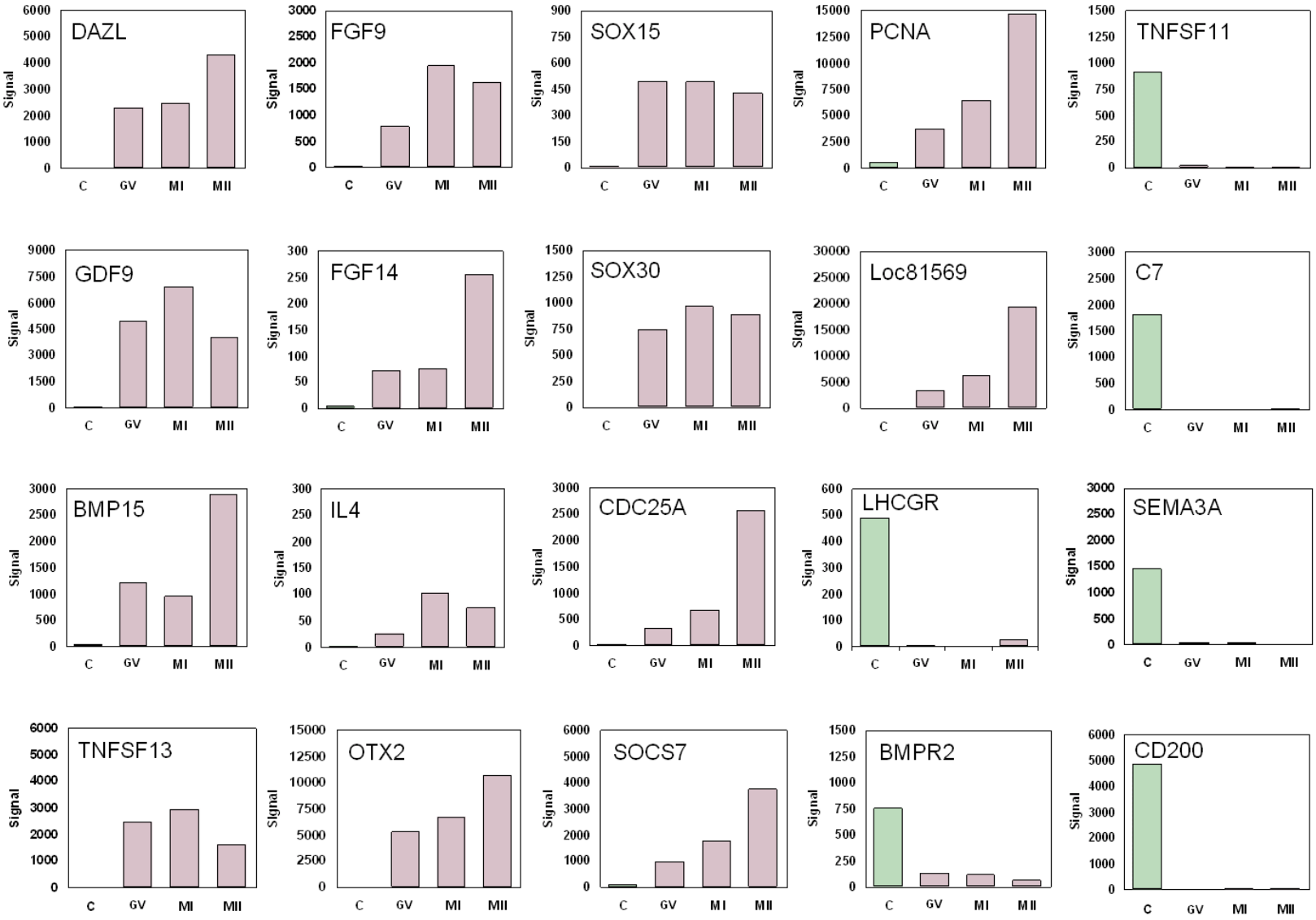


Figure 4

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Figure 4 : Expression histograms of selected genes in oocytes and cumulus cells
Histograms show signal values of 20 genes which are differentially expressed between oocyte (purple) and cumulus cells (green). Gene expression is measured by pan genomic HG-U133 Plus 2.0 Affymetrix oligonucleotides microarrays and the signal intensity for each gene is shown on the Y axis as arbitrary units determined by the GCOS 1.2 software (Affymetrix). GV : germinal vesicle; MI : metaphase I; MII : metaphase II; C : cumulus.