Research article

**Microarray-based analysis of fish egg quality after natural or controlled ovulation**

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**Abstract**

**Background:** The preservation of fish egg quality after ovulation-control protocols is a major issue for the development of specific biotechnological processes (e.g. nuclear transfer). Depending on the species, it is often necessary to control the timing of ovulation or induce the ovulatory process. The hormonal or photoperiodic control of ovulation can induce specific egg quality defects that have been thoroughly studied. In contrast, the impact on the egg transcriptome as a result of these manipulations has received far less attention. Furthermore, the relationship between the mRNA abundance of maternally-inherited mRNAs and the developmental potential of the egg has never benefited from genome-wide studies. Thus, the present study aimed at studying the rainbow trout (*Oncorhynchus mykiss*) egg transcriptome after natural or controlled ovulation using 9152-cDNA microarrays.

**Results:** The analysis of egg transcriptome after natural or controlled ovulation led to the identification of 26 genes. The expression patterns of 17 of those genes were monitored by real-time PCR. We observed that the control of ovulation by both hormonal induction and photoperiod manipulation induced significant changes in the egg mRNA abundance of specific genes. A dramatic increase of Apolipoprotein C1 (APOC1) and tyrosine protein kinase HCK was observed in the eggs when a hormonal induction of ovulation was performed. In addition, both microarray and real-time PCR analyses showed that prohibitin 2 (PHB2) egg mRNA abundance was negatively correlated with developmental success.

**Conclusion:** First, we showed, for the first time in fish, that the control of ovulation using either a hormonal induction or a manipulated photoperiod can induce differences in the egg mRNA abundance of specific genes. While the impact of these modifications on subsequent embryonic development is unknown, our observations clearly show that the egg transcriptome is affected by an artificial induction of ovulation.

Second, we showed that the egg mRNA abundance of prohibitin 2 was reflective of the developmental potential of the egg.

Finally, the identity and ontology of identified genes provided significant hints that could result in a better understanding of the mechanisms associated with each type of ovulation control (i.e. hormonal, photoperiodic), and in the identification of conserved mechanisms triggering the loss of egg developmental potential.
Background

Fish egg quality can be defined as the ability of the egg to be fertilized and subsequently develop into a normal embryo. The egg's potential to produce a viable and normal embryo can be affected by many environmental and biological factors acting at various steps of the oogenetic process (see [1,2] for review). The determinism of egg quality has also been shown to be under the influence of genetic factors [3-5]. While the effects of many experimental factors have been studied, the mechanisms by which they trigger egg quality losses are far less documented. Yolk composition as a result of a specific diet has been intensively studied in several fish species in relationship with egg developmental capacities [6-8]. Hormones of maternal origin supplied to the egg by the egg also have a significant effect on embryonic development as shown by several studies [9]. In contrast, the putative role of non-yolk cytoplasmic components accumulated during oogenesis, such as structural and regulatory proteins, cortical alveoli content and messenger RNAs (mRNAs), has received far less attention [1]. Nevertheless, maternal mRNAs that accumulate in the oocyte during oogenesis are essential for early embryonic development [10,11]. Like in other animals, some maternal mRNAs are involved in embryonic germ cells formation in fish [12], but other oocyte mRNAs, such as those involved in growth regulation, could be necessary to ensure a normal early development [13]. Thus, in bovine two-cell embryos, a relationship between embryonic developmental competence, assessed in terms of time of first cleavage, and the expression of IGF1 mRNA was reported [14]. In addition, other studies showed a relationship between variation of maternal RNA polyadenylation levels and developmental competence of mammalian oocytes, thus pointing out a relationship between maternal mRNA stability and embryonic developmental capacities [15]. In fish, the possibility that specific oocyte mRNAs might be affected when egg quality is experimentally decreased has been seriously suggested by a previous work dealing with the effect of egg post-ovulatory ageing on the mRNA levels of many genes (~40) in rainbow trout eggs [16].

In fish, it is often useful or necessary to control the timing of spawning or induce the ovulatory process. These techniques are used for biotechnical, experimental or economical reasons to obtain out of season egg production and/or synchronous egg production within a group of females or, for some species, to obtain eggs from captive fish. The effects of these manipulations on fish egg quality have been thoroughly studied [1,17]. However, the impact on egg transcriptome as a result of these manipulations has received far less attention despite recent efforts to study the ovarian or follicular transcriptome during oogenesis [18-20]. In the present study, we analyzed the transcriptome of unfertilized rainbow trout (Onchorhyncus mykiss) eggs after natural or controlled ovulation. Two different protocols of controlled ovulation that are widely used in laboratories and fish farms were carried out: (i) a hormonal induction of ovulation using intraperitoneal GnRH-analog injection, and (ii) a specific photoperiod regime designed to advance the spawning period. In addition, a third group was not subjected to any specific manipulation to allow egg collection after natural spontaneous ovulation. For each individual female, egg samples were collected and either subjected to a microarray analysis or transferred in an experimental hatchery after fertilization for monitoring developmental success (e.g. embryonic survival, malformations). Thus, the present study aimed at (i) analyzing the effect of ovulation control processes on egg transcriptome and (ii) analyzing possible links between egg transcriptome and egg developmental potential.

Results

Egg quality

Both hormonal induction and photoperiodic manipulation of ovulation had a negative impact on egg quality. The percentage of normal (i.e. without morphological abnormalities) alevins monitored at yolk-sac resorption (YSR) was used to characterize the egg quality of each individual female. The higher percentage of normal alevins at YSR, 84 ± 5%, was observed after natural (N) ovulation (Figure 1). In contrast, significantly lower percentages were observed after hormonal induction (HI) of ovulation (65 ± 9%) or photoperiodic manipulation (PM) of ovulation (37 ± 16%) (Figure 1).

Transcriptomic analysis

After signal processing, 8423 clones out of 9152 were kept for further analysis. SAM analysis was performed using the expression data of those 8423 clones. Twenty six genes exhibiting a differential mRNA abundance among at least 2 of the 3 experimental groups were identified (Table 1, Figure 2) with a false discovery rate (FDR) of 3.4%. The ontologies of those genes are presented in Table 2. Thirty one genes putatively linked to egg quality were identified (Table 3, Figure 3) with a FDR of 30%. The ontologies of those genes are presented in Table 4.

Real-time PCR analysis

From the 57 (26+31) genes identified in the transcriptomic analysis, 32 were ultimately kept for real-time PCR analysis (Table 5). Real-time PCR data corresponding to the remaining 25 was not used in the analysis because of methodological reasons (e.g. low expression, poor PCR efficiency, double amplification).
Genes exhibiting a differential egg mRNA abundance among experimental groups

Among the 26 genes exhibiting a differential mRNA abundance between experimental groups, 17 were studied by real-time PCR. Among those 17 genes, 7 were found to be differentially expressed in the real-time PCR study (Figure 4). The identity of those 7 genes is presented below. Only the informative alignments obtained using the full rainbow trout coding sequence (CDS) or a substantial part of the CDS are presented (Figures 3, 4). For clarity reasons, the official human protein symbol was used in the text.

Clone # 1RT65F10_D_C05 exhibited significant sequence similarity with mouse Apolipoprotein C-I precursor (APOC1, Table 1) and was significantly more abundant in eggs of the HI group than in eggs of the N group while intermediate levels were observed in eggs of the PM group. The mRNA abundance in the HI group was 13 times higher than in the N group while it was 2 times higher than in the PM group (Figure 4). After performing a Blast search in the GenBank database, the complete rainbow trout amino acid sequence deduced from the EST sequence exhibited 54% sequence identity at the amino acid level with the zebrafish (Danio rerio) cognate protein (Figure 5A). A sequence identity of 33 and 26% was observed with mouse and human proteins respectively (Figure 5A). The number of amino acids deduced from the trout EST is consistent with the number of amino acids present in mammalian and zebrafish sequences.

Clone 1RT68D18_D_B09 that exhibited sequence similarity with mouse Hemopoietic cell kinase (HCK, Table 1). The deduced partial amino acid sequence generated from the corresponding UniGene cluster exhibited 40% and 38% identity with mouse and human HCK proteins respectively.

Clone tcay0027.b.13 exhibited sequence similarity with human pyruvate carboxylase (PYC, Table 1) and was more abundant in eggs of the PM group than in eggs of the N group, while intermediate levels were observed in eggs of the HI group (Figure 4). This sequence did not include a full CDS. After performing a Blast search using this partial coding sequence, the amino acid sequence identity with cognate vertebrate forms was above 80%.

Clone 1RT139F11_B_C06 exhibited sequence similarity with ribosomal protein RPL24 and was more abundant in...
Table 1: Genes exhibiting differential egg mRNA abundance among experimental groups identified from the microarray analysis.

| Clones          | GenBank    | Sigenae contig       | Symbol                      | swissprot_hit_description                                                                 | Score | UniGene      |
|-----------------|------------|----------------------|-----------------------------|--------------------------------------------------------------------------------------------|-------|--------------|
| tcac0001.c.18   | BX082249   | tcac0001c.c.18.5.1.s.om.8 | YEAST (P53230) Hypothetical 44.2 kDa protein in RME1-TFC4 intergenic region | 283                                           |       |              |
| IRT159P21_B_H11 | CA388269   | CA388269.1.s.om.8     |                             |                                               |       |              |
| tcbk0051.c.02   | BX878405   | tcay0028b.c.19.3.1.s.om.8 |                             |                                               |       |              |
| IRT65F10_D_C05  | CA353171   | tcb0001c.m.15.5.1.s.om.8 | APOC1                       | MOUSE (P34928) Apolipoprotein C-I precursor (Apo-C1) (Apo-C1) | 123   | Omy.10219    |
| IRT121B08_D_A04 | CA359367   | CA359367.1.s.om.8     | CTNNBL1                     | HUMAN (Q8WY74) Beta-catenin-like protein 1 (Nuclear-associated protein) | 950   | Omy.23137    |
| IRT56C04_H_C02  | CA351228   | CA351228.1.s.om.8     | DAB2                        | MOUSE (P90878) Disabled homolog 2 (DOK-2) | 215   |              |
| IRT64F24_D_C12  | CA358202   | CA358202.1.s.om.8     | DBNL                        | MOUSE (Q62418) Dreblike protein (SH3 domain-containing protein) | 720   |              |
| IRT8E10_C_C05   | CA345343   | tcaov0028b.g.03.3.1.s.om.8 | DDAH2                       | MOUSE (Q99LD8) NG, NG-dimethylarginine dimethylaminohydrolase 2 | 629   | Omy.23405    |
| IRT68D18_B_D09  | CA34327    | tcb0017c.p.21.5.1.s.om.8 | HCK                         | MOUSE (P08103) Tyrosine-protein kinase HCK (EC 2.7.1.112) | 324   | Omy.9448     |
| tcaov0037.m.11  | BX319623   | tcaov0032l.02.3.1.s.om.8 | HNRPK                       | HUMAN (Q8NCC3) 1-O-acylceramide synthase precursor (EC 2.3.1.-) | 816   | Omy.9525     |
| tcaov0030.n.02  | BX316222   | tcaov005c.v.03.3.1.s.om.8 | HSPA9B                      | HUMAN (P38646) Stress-70 protein, mitochondrial precursor | 901   | Omy.26983    |
| IRT62C23_A_B12  | CA387140   | tcb0011c.o.01.5.1.s.om.8 | ING1                        | HUMAN (Q9UK53) Inhibitor of growth protein 1 | 435   | Omy.24666    |
| IRT121G15_A_D08 | CA362639   | tcaov007b.n.06.3.1.s.om.8 | LYPA3                       | HUMAN (Q8NCC3) 1-O-acylceramide synthase precursor (EC 2.3.1.-) | 466   | Omy.19659    |
| tcbk0023.o.24   | BX875550   | tcb0005c.o.10.5.1.s.om.8 | MR-1                        | ARATH (O24496) Hydroxacyclgluthione hydrolase cytoplasmic (EC 3.1.2.6) | 1171  | Omy.26818    |
| IRT131K20_C_F10 | CA383630   | CA383630.1.s.om.8     | MYO1B                       | MOUSE (P46735) Myosin Ib (Myosin 1 alpha) (MMI-alpha) (MMIA) | 985   |              |
| tcbao0025.m.15  | BX863839   | tcb0008b.e.10.3.1.s.om.8 | NTAN1                       | HUMAN (Q96AB6) Protein N-terminal asparagine amidohydrolase (EC 3.5.1) | 510   |              |
| tcbk0049.m.03   | BX884905   | tcb0002c.v.19.5.1.s.om.8 | OSBP5                       | MOUSE (Q9ER64) Oxyester binding protein-related protein 5 | 344   | Omy.14649    |
| tcbk0050.a.20   | BX886190   | tcb0005c.a.20.5.1.s.om.8 | PCH2                        | CHICK (P27607) Prostaglandin G/H synthase 2 precursor | 1636  | Omy.20943    |
| tcbk0055.m.20   | BX880138   | tcb0005c.m.20.5.1.s.om.8 | PKPI                        | HUMAN Plakophilin 1 | 337   |              |
| tcaov0027.b.13  | BX313624   | tcaov0027b.b.13.5.1.s.om.8 | PYC                         | HUMAN (P11498) Pyruvate carboxylase, mitochondrial precursor (EC 6.4.1.1) | 1021  |              |
| IRT6N13_B_G07   | CA360456   | CA351353.1.s.om.8     | RBM5                        | HUMAN (P52756) RNA-binding protein 5 (Putative tumor suppressor LUCA15) | 296   |              |
| tcbk0027.b.05   | BX887647   | tcb00025c.k.02.5.1.s.om.8 | RL10                        | HUMAN (P27635) 60S ribosomal protein L10 | 1039  | Omy.4144     |
| IRT139F11_B_C06 | CA384643   | tcaov0034b.h.11.3.1.s.om.8 | RPL24                       | GILM1 (Q9DFQ7) 60S ribosomal protein L24 | 529   | Omy.9444     |
| IRT6M02_C_G01   | CA343072   | tcb0004c.h.08.5.1.s.om.8 | RPN2                        | HUMAN (P04844) Dolichyl-diphosphooligosaccharide-protein glycosyltransferase 63 kDa subunit precursor (Ribophorin II) | 643   | Omy.24414    |
| tcad0007.p.12   | BX078856   | tcaov005c.e.12.3.1.s.om.8 | SEC22                       | YARLI (Q6C880) Protein transport protein SEC22 | 237   | Omy.913      |
| IRT56L15_B_F08  | CA351681   | CA351681.1.s.om.8     | TPH                         | XENLA (Q92142) Tryptophan 5-hydroxylase (EC 1.14.16.4) | 725   |              |

Genes subsequently studied by real time PCR are bolded. For each gene, clone name, GenBank accession number, official human symbol and corresponding UniGene cluster are indicated. The Sigenae contig name [60] used for Blast comparison against the Swiss-Prot database is shown. Resulting best hit and corresponding score are indicated.
Table 2: Ontologies of the genes exhibiting differential egg mRNA abundance among experimental groups identified from the microarray analysis.

| Symbol  | Biological Process (P)                                                                 | Cellular component (C)                        | Molecular Function (F)                                                                 |
|---------|--------------------------------------------------------------------------------------|-----------------------------------------------|--------------------------------------------------------------------------------------|
| APOC1   | negative regulation of lipoprotein lipase activity                                  | chylomicron                                  | enzyme activator activity                                                           |
|         | negative regulation of binding                                                      |                                               | lipid binding                                                                        |
| CTNNBL1 | induction of apoptosis                                                               | nucleus                                      | protein binding                                                                      |
|         | cell proliferation                                                                   |                                               | actin binding                                                                        |
| DAB2    | Rac protein signal transduction                                                      | Lamellipodium                                | enzyme activator activity                                                           |
|         | activation of JNK activity                                                          | Cytoplasm                                    | protein binding                                                                      |
|         |                                                                                     | cell cortex                                  | hydrolase activity                                                                   |
| DBNL    | anti-apoptosis                                                                       |                                               | protein-tyrosine kinase activity                                                    |
|         | arginine catabolism                                                                 |                                              | protein binding                                                                      |
|         | nitric oxide mediated signal transduction                                           |                                              | single-stranded DNA binding                                                         |
|         | protein amino acid phosphorylation                                                  |                                              | ATPase activity                                                                      |
|         | mesoderm development                                                                |                                              | ATP binding                                                                          |
|         |                                                                                     |                                              | DNA binding                                                                         |
| DDAH2   |                                                                                     |                                              | phospholipid binding                                                                |
|         |                                                                                     |                                              | lysophospholipase activity                                                          |
| HCK     | anti-apoptosis                                                                       |                                               | motor activity                                                                       |
|         |                                                                                     |                                              | protein N-terminal asparagine amidohydrolase activity                                |
| HNRPK   |                                                                                     |                                              | protein binding                                                                      |
|         |                                                                                     |                                              | oxyysterol binding                                                                  |
| HSPA9B  | hemopoiesis                                                                          | mitochondrial matrix                         | Peroxidase activity                                                                 |
|         |                                                                                     |                                              | prostaglandin-endoperoxide synthase activity                                         |
| ING1    | negative regulation of cell proliferation                                          | nucleus                                      | structural constituent of epidermis                                                 |
|         | negative regulation of cell growth                                                 |                                              | signal transducer activity                                                          |
|         |                                                                                     |                                              | intermediate filament binding                                                       |
|         |                                                                                     |                                              | ATP binding                                                                          |
|         |                                                                                     |                                              | biotin binding                                                                       |
| LYPA3   | fatty acid catabolism                                                                | lysosome                                     | pyruvate carboxylase activity                                                       |
|         |                                                                                     |                                              | RNA processing                                                                      |
| MR-1    |                                                                                     |                                              | structural constituent of ribosome                                                  |
| MYO1B   | nervous system development                                                          | Cytoskeleton                                 | structural constituent of ribosome                                                  |
|         |                                                                                     | brush border                                 | structural constituent of ribosome                                                  |
| NTAN1   | memory                                                                               | Nucleus                                      | structural constituent of ribosome                                                  |
|         | adult locomotory behavior                                                            |                                              | structural constituent of ribosome                                                  |
| OSBPL5  | cholesterol metabolism                                                               | cytoplasm                                    | structural constituent of ribosome                                                  |
|         | cholesterol transport                                                               | integral to membrane                         | structural constituent of ribosome                                                  |
| PGH2    | physiological process                                                                | Nucleus                                      | structural constituent of ribosome                                                  |
|         | keratinocyte differentiation                                                         |                                              | structural constituent of ribosome                                                  |
|         | cyclooxygenase pathway                                                               |                                              | structural constituent of ribosome                                                  |
| PKP1    | signal transduction                                                                 | desmosome                                    | structural constituent of ribosome                                                  |
|         | cell adhesion                                                                        | nucleus                                      | structural constituent of ribosome                                                  |
| PYC     |                                                                                     | intermediate filament                        | structural constituent of ribosome                                                  |
|         |                                                                                     |                                              | structural constituent of ribosome                                                  |
| RBMS5   | RNA binding                                                                          | nucleus                                      | structural constituent of ribosome                                                  |
| RPL10   | Spermatogenesis                                                                      | cytosolic large ribosomal subunit (sensu Eukaryota) | structural constituent of ribosome                                                  |
| RPL24   | protein biosynthesis                                                                | mitochondrial large ribosomal subunit        | structural constituent of ribosome                                                  |
| RPN2    | protein modification                                                                 | mitochondrial large ribosomal subunit        | structural constituent of ribosome                                                  |
| SEC22   | ER to Golgi vesicle-mediated transport                                               | oligosaccharyl transferase complex           | structural constituent of ribosome                                                  |
| TPH1    | serotonin biosynthesis from tryptophan                                               | endoplasmic reticulum membrane               | transporter activity                                                                 |
| YGIW    | protein import into mitochondrial matrix                                            | cytoplasm                                    | tryptophan 5-monoxygenase activity                                                   |
|         |                                                                                     | mitochondrion                                | Protein binding                                                                     |
Table 3: Genes exhibiting differential mRNA abundance in eggs of varying quality identified from the microarray analysis

| Symbol         | Genbank   | GenBank contig | Symbol     | Swissprot hit description               | Score  | UniGene |
|----------------|-----------|----------------|------------|------------------------------------------|--------|---------|
| tcb001.c.08    | CA378261  | NO CONTIG      | tcb010c.08 | HUMAN (Q95394) Phosphoacetylglucosamine mutase (EC 5.4.2.3) | 1065   | Omy.22147 |
| tcbr0001.b.08  | CA362248  | NO CONTIG      | tcbr0001.b.08 | HUMAN (Q9H553) Alpha-1,3-mannosyltransferase ALG2 (EC 2.4.1.-) | 662    |         |
| tcbr0001.c.08  | CA345100  | NO CONTIG      | tcbr0001.c.08 | HUMAN (P04114) Apolipoprotein B-100 precursor | 617    | Omy.8599 |
| tcay0008.m.21  | BX301016  | NO CONTIG      | tcay0008.m.21 | MOUSE (P23359) Bone morphogenetic protein 7 precursor (BMP-7) | 1154   | Omy.19556 |
| tcbk0001.p.13  | BX873334  | NO CONTIG      | tcbk0001.p.13 | HUMAN (Q8EVM) Probable transcription factor CST | 234    | Omy.20281 |
| tcay0007.20    | BX300279  | NO CONTIG      | tcay0007.20 | RAB1 (Q5FWS4) Protein C6orf188 homolog | 468    | Omy.26998 |
| tcb0030.i.17   | BX867113  | NO CONTIG      | tcb0030.i.17 | RAB7 (Q29425) Cullin-5 (CUL-5) | 1694   | Omy.21358 |
| tca0001.c.17   | BX861936  | NO CONTIG      | tca0001.c.17 | HUMAN (Q9Y6J8) Dual specificity protein phosphatase 24 | 688    |         |
| tca0001.c.10   | BX861936  | NO CONTIG      | tca0001.c.10 | MOUSE (Q8U20) FYVE, RhoGEF and PH domain containing protein 5 | 145    | Omy.10646 |
| tca0001.c.10   | BX861936  | NO CONTIG      | tca0001.c.10 | HUMAN (Q9Y6J8) Dual specificity protein phosphatase 24 | 688    |         |
| tca0001.c.10   | BX861936  | NO CONTIG      | tca0001.c.10 | MOUSE (Q8U20) FYVE, RhoGEF and PH domain containing protein 5 | 145    | Omy.10646 |

Genes subsequently studied by real time PCR are bolded. For each gene, clone name, GenBank accession number, official human symbol and corresponding UniGene cluster are indicated. The Sigenae contig name [60] used for Blast comparison against the Swiss-Prot database is shown. Resulting best hit and corresponding score are indicated.
Table 4: Ontologies of the genes exhibiting differential mRNA abundance in eggs of varying quality identified from the microarray analysis.

| Symbol | Biological Process (P) | Cellular component (C) | Molecular Function (F) |
|--------|------------------------|------------------------|------------------------|
| AGM1   | glucosamine metabolism | extracellular region   | phosphoacetylglucosamine mutase activity |
| APOB   | circulation, signal transduction | endoplasmic reticulum, microsome | lipid transport activity, receptor binding |
| BMP7   | BMP signalling pathway, organ morphogenesis, mesoderm formation | Extracellular space | cytokine activity, protein binding |
| CUL5   | cell cycle arrest, cell proliferation | cytoplasm, endoplasmic reticulum | calcium channel activity, protein binding, receptor activity |
| DCPS   | mRNA catabolism | cytoplasm, Golgi apparatus | pyrophosphatase activity, small GTPase binding, protein binding |
| FGD5   | cytoskeleton organization and biogenesis | cytoplasm, cytoplasm | protein binding |
| GMCL1  | nuclear membrane organization and biogenesis | nucleolus, nuclear lamina | protein binding |
| GTF2B  | mRNA transcription from RNA polymerase II promoter | transcription factor complex | general RNA polymerase II transcription factor activity, protein binding |
| HCFC1  | positive regulation of progression through cell cycle | cytoplasm, nucleus | identical protein binding, transcription coactivator activity, transcription factor activity |
| KHK    | carbohydrate catabolism | cytoplasm | ketohexokinase activity, microtubule motor activity |
| KIF4A  | organelle organization and biogenesis | cytoplasm, spindle microtubules | calcium ion binding, oxidoreductase activity, phospholipase A2 activity, structural molecule activity, phosphatidylinositol binding, Rho guanyl-nucleotide exchange factor activity |
| LAMB2  | synaptic transmission, electron transport | basal lamina, membrane | protein binding |
| MCF2L  | Rho protein signal transduction | membrane, lamellipodium | protein binding, protein kinase activity, specific transcriptional repressor activity |
| NEK1   | response to DNA damage stimulus, response to ionizing radiation | cytoplasm, nucleus | protein kinase activity, protein binding |
| PDCL3  | phototransduction | cytoplasm | protein binding |
| PDGFRA | cell proliferation, extracellular matrix organization and biogenesis, male genitalia development, organ morphogenesis | integral to plasma membrane | platelet-derived growth factor binding, protein dimerization activity, protein serine/threonine kinase activity |
| PHB2   | signal transduction, negative regulation of transcription | mitochondrial inner membrane, nucleus | estrogen receptor binding, protein binding |
| RAB3IP | | cytoplasm, nucleus | protein Binding, GTPase binding, ATP binding, protein binding |
| TGFB2R | regulation of cell proliferation, Cell fate commitment, protein amino acid phosphorylation, protein amino acid dephosphorylation | integral to membrane | protein tyrosine kinase activity |
| TLE1   | signal transduction, regulation of transcription, DNA-dependent organ morphogenesis | nucleus | protease binding, protein binding |
| VWF    | cell adhesion, response to wounding | extracellular space | protein binding |
eggs of the HI group than in eggs of the PM group (Figure 4). This clone included a full CDS and the deduced amino acid sequence exhibited very strong (above 95%) sequence identity with cognate fish proteins (Figure 5C).

For 5 genes (HRNPK, RBM5, DAB2, PGH2 and SEC22, Table 1) similar expression profiles were observed in real-time PCR and microarray analyses. However, no statistical differences between groups were observed in the real-time PCR experiment (Figure 4).

For 3 genes (PKP1, DBNL and LYPA3, Table 1) the consistency between real-time PCR and microarray data was limited to 2 of the 3 experimental groups. In addition, no statistical differences between groups were observed in the real-time PCR analysis (Figure 4).

For the 2 remaining clones (BX082249 and CA388269, Table 1), no correlation was observed between real-time PCR and microarray data (data not shown).

Genes exhibiting a quality-dependent mRNA abundance in the eggs
Among the 31 genes identified as linked to egg quality, 15 were analyzed by real-time PCR. Among those 15 genes, the mRNA abundance of 1 gene was found to be significantly correlated with egg quality. This clone (PHB2) exhibited significant sequence similarity with rat prohibitin 2 (Table 3). Its mRNA abundance in the eggs was negatively correlated ($R = -0.47, p < 0.05$) with the percentage normal alevins at yolk-sac resorption. In addition the mRNA abundance of this gene was significantly higher in eggs exhibiting the lowest developmental potential (Figure 6). An amino acid sequence was generated from nucleotide sequences of Omy.9050 UniGene cluster. This deduced amino acid sequence exhibited 83% identity with zebrafish sequence and 76% identity with human and rat sequences (Figure 6).
Discussion

Microarray analysis efficiency and reliability

The hybridization of radiolabeled cDNAs with cDNAs deposited onto nylon membranes has been used for several decades. However, the use of nylon cDNA microarrays is not very common in comparison to glass slide microarrays. Nevertheless, this technology has successfully been used for several years [21]. In our laboratory, we have successfully used this technology to identify differentially expressed genes during oocyte maturation and ovulation [18]. In the present study, we have used the same methodology and have identified a group of 26 genes exhibiting differential egg mRNA abundance after natural controlled ovulation with a false discovery rate of 3.4%. Using real-time PCR, the egg mRNA abundance of 17 genes was analyzed. Among those 17 genes, only 2 exhibited expression patterns totally inconsistent with microarray data. In contrast, the expression patterns of the other genes were very similar to microarray data, even though observed differences were not always significant.

It is noteworthy that the 2 genes exhibiting inconsistent expression patterns between PCR and microarray experiments correspond to uncharacterized proteins. Indeed, one of the genes (CA388269) had no significant hit in the Swiss-Prot database while the other one (BX082249) had a significant hit with a hypothetical yeast protein (Table 1). To conclude, the overall consistency of PCR and microarray data suggests that the microarray analysis performed in the present study is robust and reliable.

Genes exhibiting a differential mRNA abundance after natural or controlled ovulation

Hormonal induction of ovulation

Among identified genes, APOC1 and HCK were the most affected by a hormonal induction of ovulation. Thus, the egg mRNA abundance of those 2 genes was dramatically increased after hormonal induction of ovulation in comparison to natural ovulation (Figure 4). Human APOCs are protein constituents of chylomicrons, very low density lipoproteins, and high-density lipoproteins [22]. The
Real-time PCR analysis of gene mRNA abundance (mean ± SEM) in unfertilized eggs collected after natural ovulation (n = 4), hormonally-induced ovulation (n = 11) and photoperiod-manipulated ovulation (n = 14).

**Figure 4**
Real-time PCR analysis of gene mRNA abundance (mean ± SEM) in unfertilized eggs collected after natural ovulation (n = 4), hormonally-induced ovulation (n = 11) and photoperiod-manipulated ovulation (n = 14). Different letters indicate significant differences between groups at p < 0.05. The official human symbol is indicated for all studied genes.
human APOC1 protein is predominantly expressed in liver and adipose tissue [23]. APOC1 may modulate the activity of plasma enzymes involved in lipid metabolism. Besides, APOC1 has also been reported to interfere with the APOE-dependent hepatic uptake of lipoprotein remnants by the low density lipoprotein receptor (LDLr) and LDLr-related protein [24]. Interestingly, it was previously shown in rainbow trout that the same clone of the APOC1 gene was significantly up-regulated in the ovary at the time of oocyte maturation [18]. This could be related to the arrest of lipoproteins uptake by the oocyte at the end of vitellogenesis concomitantly with a decrease of the expression of vitellogenin receptor [25]. It is therefore possible that the hormonal induction of ovulation induces an artificial over abundance of some hormonally-dependent genes, such as APOC1, in the eggs. However, the possible consequences of such an over abundance on lipid metabolism of the embryo is so far unknown.

Similarly to APOC1, the egg mRNA abundance of HCK gene was also dramatically increased after hormonal induction of ovulation. HCK, hemopoietic cell kinase, belongs to Src-family tyrosine kinases and is expressed in cells of myelomonocytic lineage, B lymphocytes, and embryonic stem cells. It was previously shown that the conventional progesterone receptor could interact, in a progestin-dependent manner, with various signaling molecules, including Src tyrosine kinases [26]. Indeed, these authors used downregulated HCK as a general model of the c-Src family tyrosine kinases to investigate the mechanism of activation by conventional progesterone receptor. In addition, the participation of the conventional progesterone receptor in African clawed frog (Xenopus laevis) oocyte maturation process was seriously suggested by two independent studies [27,28]. Besides, Src tyrosine kinase activation has been shown to be one of the earliest transcription-independent responses of Xenopus oocytes to
Figure 6

(A) Amino acid sequence alignment of deduced rainbow trout prohibitin 2 (PHB2) with human, rat and zebrafish forms. For each target species, the GenBank accession number of the protein is indicated. (B) Real-time PCR analysis of PHB2 mRNA abundance (mean ± SEM) in eggs of low (n = 10), intermediate (n = 9) and high (n = 10) developmental potential estimated by the percentage normal alevins at yolk-sac resorption. Different letters indicate significant differences between groups at p < 0.05.
progesterone during in vitro induced maturation; a period when oocyte mRNA content remains stable [29]. Interestingly, we observed a dramatic over abundance of HCK mRNA in the eggs after hormonal induction of ovulation. To date, the significance of this over abundance as a result of hormonally-induced ovulation is unknown. However, it further demonstrates that the egg mRNA abundance of specific genes can be dramatically affected by a hormonal induction of ovulation.

In addition to APOC1 and HCK, eggs obtained after hormonal induction of ovulation were also characterized by higher NTAN1 and lower MR-1 mRNA abundance. However, the fold difference observed for those 2 genes was less important. In mice it has been shown that NTAN1 encodes an N-terminal amidohydrolase specific for N-terminal asparagines, which is involved in ubiquitin-proteasome proteolysis termed as the N-end rule pathway [30]. N-end rule pathway determines metabolic instability of different proteins that contain a destabilizing N-terminal residue [31]. More specifically, a recent study suggested that an over expression of NTAN1 using recombinant NTAN1 adenovirus vector resulted in a marked decrease in the microtubule-associated protein 2 (MAP2) expression in hippocampal neurons in rat [32]. Regardless of the specific target of NTAN1 in the oocyte, an increased expression of this enzyme should participate in protein turnover, and its regulation might be important for the normal development of the oocyte. The second gene, MR-1, is a newly identified protein that interacts with contractile proteins and exists in human myocardial myofibrils [33].

Finally, the egg mRNA abundance of RPL24 was higher after hormonal induction of ovulation. However, this difference was only significant in comparison with the PM group. The 60S ribosomal protein L24 (RPL24) is one of the forty seven 60S ribosomal proteins present in eukaryotic organisms and often used as markers for phylogenetic studies and comparative genomics. Those ribosomal proteins have been sequenced recently in catfish (Ictalurus punctatus) and high similarities with mammalian ribosomal protein were found [34]. 60S ribosomal subunit proteins in translational initiation in combination with 40S ribosomal subunit [35]. An insertional mutagenesis study carried out in zebrafish (Danio rerio) reported this gene to be essential for early embryonic development. Mutation of this gene resulted in small head/eyes mutants [36]. Interestingly, when monitoring embryonic development in the present study, we noticed that many embryos originating from eggs of hormonally-induced females exhibited small eyes at eyeing stage. Precise quantification of this phenomenon would be necessary to stress its relationship with RPL24 over abundance in the eggs.

Photoperiodic control of ovulation
Four genes exhibited differential egg mRNA abundance after photoperiod treatment in comparison to natural ovulation. Similarly to eggs obtained after hormonal induction of ovulation, eggs of the PM group also exhibited increased levels of APOC1 and HCK. The differential abundance of both genes was high but less pronounced than after hormonally-induced ovulation. In addition eggs obtained after photoperiod manipulation of ovulation were also characterized by higher MYO1B and PYC mRNA abundance. According to the gene ontology analysis, MYO1B is a cytoskeleton protein involved in nervous system development (Table 2). It is also expressed in a wide variety of tissues including rat neonatal tissues [37,38]. The class I myosin, MYO1B, is a calmodulin- and actin-associated molecular motor widely expressed in mammalian tissues [39]. MYO1B can interact on the dynamic actin filament populations and might play a role in intracellular membrane trafficking [40]. Myosin light chain has been recently suggested to participate in anchoring the 26S proteasome, a 26S multiprotein complex that catalyses the breakdown of polyubiquitinated proteins, to the actin cytoskeleton of goldfish oocyte [41]. Degradation of proteins mediated by ubiquitin-proteasome pathway plays important roles in the regulation of eukaryotic cell cycle [42] and can be involved in oocyte maturation and further embryonic cell cleavages.

Pyruvate carboxylase (PYC) is a mitochondrial biotin-dependent carboxylase. In the adipose tissue and liver PYC participates in the citrate shuttle by which NADPH equivalents are transported out of mitochondria to the cytosol for lipogenesis [43]. Five alternative forms of rat pyruvate carboxylase cDNAs have been identified in liver, kidney, brain, and adipose tissue and these are expressed in a tissue-specific manner [44-46]. In red Seabream (Pagrus major), PYC mRNA was detected by Northen blot analysis in heart, liver, muscle and ovary [47]. Interestingly, it was previously shown that a photoperiod manipulation of spawning date was associated with a significantly higher occurrence of yolk-sac resorption defects [48]. Together, these observations suggest a putative link between an abnormal stockpiling of PYC mRNA in the egg and problems in the processing and/or use of yolk-sac lipidic stores. Indeed, it was previously reported that non viable gilthead sea bream eggs have lower pyruvate carboxylase activity than viable eggs [49].

Genes exhibited an egg mRNA abundance correlated with egg’s developmental potential
From microarray data, 30 genes were identified as exhibiting an egg mRNA abundance correlated with egg’s developmental potential. However, the false discovery rate was elevated and those genes were considered as candidate genes requiring PCR validation. Nevertheless, it is note-
worthy that the ontological analysis of this group showed that 5 genes are involved in the regulation of transcription and others in cell proliferation/development and cytoskeleton organization and biogenesis. In addition, the correlation was confirmed for 1 of the 15 genes analyzed by real-time PCR: prohibitin 2 (PHB2). In animals and yeast, prohibitins have been shown to play important roles in cell cycling and senescence. One of prohibitin 2 major role is to be a chaperone-like regulator of the AAA protease in the mitochondrial matrix that assists in the assembly of inner membrane complex [50]. In Caenorhabditis elegans, PHB proteins were showed to be essential during embryonic development and are required for somatic and germ line differentiation in the larval gonad [51]. Moreover, deletions of the Saccharomyces cerevisiae homologues, PHB1 and PHB2, result in a decreased replicative lifespan, and a defect in mitochondrial membrane potential. The prohibitin protein has been immunolocalized in mammalian oocytes and embryos and suggested to have an antiproliferative activity [52]. Besides, a higher immunoreactivity level was found in the nucleus of embryo that failed to develop normally in comparison to morphologically normal ones. In the present study, we observed a higher prohibitin 2 mRNA abundance in eggs exhibiting the lowest developmental potential. This differential abundance in eggs of varying quality suggests that prohibitin 2 plays a role in the developmental potential of the embryo. Further studies are needed to unravel the link between an overabundance of prohibitin 2 mRNA in the eggs and a reduced egg developmental potential. Thus, this overabundance could be the result of a reduced prohibitin 2 synthesis during oogenesis.

Conclusion
In the present study we successfully used rainbow trout cDNA microarrays to analyze egg transcriptome after natural and controlled ovulation and in relationship with the developmental potential of the eggs. We showed that the control of ovulation using either a hormonal induction or a manipulated photoperiod could induce differences in the egg mRNA abundance of specific genes.

In addition, we showed that the egg mRNA abundance of prohibitin 2 (PHB2) was negatively correlated with the developmental potential of the egg.

Furthermore, the identity and ontology of identified genes provided significant hints that could result in a better understanding of the mechanisms associated with each type of ovulation control (e.g. hormonal, photoperiodic) or conserved mechanisms triggering a loss of egg developmental potential.

Methods

Animals
Investigations were conducted according to the guiding principles for the use and care of laboratory animals and in compliance with French and European regulations on animal welfare. Three groups of male and female rainbow trout (Oncorhynchus mykiss) were obtained from our experimental fish farm (Sizun, France) and maintained until reproductive season under natural photoperiod and water temperature conditions. A first set of egg samples was collected from females undergoing natural (N) ovulation. Four weeks before expected ovulation fish (25 females) were transferred in a controlled recirculated water system (12°C) under natural photoperiod and water temperature conditions. A second set of egg samples was collected from females subjected to a hormonal induction (HI) of ovulation. Four weeks before expected ovulation fish were transferred in a controlled recirculated water system (12°C) under natural photoperiod in INRA experimental facilities (Rennes, France). A third set of egg samples was collected from females subjected to a photoperiod manipulation (PM) of ovulation. After a first reproduction, fish (17 females) were isolated in light-proofed tanks and exposed to an artificial photoperiod. Beginning on January 15th, all fish were held under constant light (24L:0D) for 490°C.day. Then, beginning on March 27th, they were held under short photoperiod (8L:16D) until ovulation (1230°C.day). Light was supplied by 4 neon tubes (58 Watts).

Gamete collection
In order to avoid excessive post-ovulatory ageing, unfertilized eggs were collected by manual stripping 5 days after detected ovulation. Two batches of 5 mL of eggs (approximately 100 to 200 eggs per batch) were used for fertilization. At each egg collection day, fresh sperm samples were collected from 10 mature males originating from the same group in order to fertilize eggs with a pool of sperms. Sperm samples were obtained by manual pressure on the abdomen and kept at 4°C for a short time before use.

Fertilization and early development
Fertilization was performed under previously described standardized conditions [16]. The two batches of 5 mL of eggs were fertilized with 5 µl of pooled semen. Fertilized eggs were transferred into compartmentalized incubation trays supplied by recirculated water (10°C). Water temperature and chemistry were routinely monitored and maintained constant over the entire incubation period. Dead eggs and embryos were periodically removed and survival rates were estimated as percentages of the initial number of eggs used for fertilization. Survival at the com-
pletion of yolk sac resorption (YSR, 550°C.day) was monitored. The occurrence of noticeable morphologicalmalformations at YSR was also monitored. Survival andmalformation data were used to calculate the proportion ofnormal alevins at YSR expressed as a percentage of theinitial number of eggs.

**RNA extraction**
Extractions were performed as previously described [53]with minor modifications. Total RNA was extracted from20 unfertilized eggs using 9 mL of TRIzol (Invitrogen) in13 mL sterile polypropylene tubes. Because of high eggvitellogenic content, each RNA was subsequently repurifiedusing a Nucleospin RNA 2 kit (Macherey Nagel) inorder to obtain genomic-grade RNA quality. For each eggsample, three RNA extracts were obtained, pooled andprecipitated with sodium acetate (3 M, pH5.2, Prolabo) toincrease RNA concentration. Thus, any RNA sample usedfor transcriptomic analysis originated from 60 unferti-

**cDNA microarrays**
Nylon micro-arrays (7.6 x 2.6 cm) were obtained fromINRA-GADIE (Jouy-en-Josas, France) resource center [54].A set of 9152 distinct rainbow trout cDNA clones originatingfrom 2 pooled-tissues library [55,56] were spotted in
duplicates after PCR amplification. PCR products were
spotted onto Hybond N+ membranes as previously described [57]. This rainbow trout generic array was deposited in Gene Expression Omnibus (GEO) database (Platform# GPL3650) [58].

**Microarray hybridization**
Four RNA samples originating from naturally ovulating
females, 11 RNA samples originating from hormonally-
induced females and 14 RNA samples originating from
photoperiod-manipulated females were used for microarrayhybridization according to the following procedure.

Hybridizations were carried out as previously described[21], with minor modifications. At INRA genomic facility(Rennes). A first hybridization was performed using a33P-labelled oligonucleotide (TAATACGACTCACTATAGGG which is present at the extremity of each PCR product) to monitor the amount of cDNA in each spot. After stripping (3 hours 68°C, 0.1× SSC, 0.2% SDS), arrays were prehybridized for 1 h at 65°C in hybridization solution (5× Denhardt's, 5× SSC, 0.5% SDS). Complex probes were prepared from 3 µg of RNA by simultaneous reverse transcription and labelling for 1 hour at 42°C in the presence of 50 µCi [alpha-33P] dCTP, 5 µM dCTP, 0.8 mM each dATP, dTTP, dGTP and 200 units M-MLV SuperScript RNase H-reverse transcriptase (GIBCO BRL) in 30 µL finalvolumes. RNA was degraded by treatment at 68°C for 30 min. Neutralization step was done by adding 10 µl 1 M Tris-HCl plus 3 µl 2 N HCl. Arrays were incubated with thecorresponding denatured labeled cDNAs for 18 h at 65°C inhybridization solution. After 3 washes (1 hours 68°C,0.1× SSC 0.2% SDS), arrays were exposed 65 hours tophosphor-imaging plates before scanning using a FUJIBAS 5000. Signal intensities were quantified usingArrayGauge software (Fujifilm Medical Systems, Stanford,CT) and deposited in GEO database (Series# GSE5928)[58].

**Microarray signal processing**
Spots with low oligonucleotide signal (lower than three
times the background level) were excluded from the analy-

sis. After this filtering step, signal processing was per-
formed using the vector oligonucleotide data to correct
each spot signal by the actual amount of DNA present in
each spot. After correction, signal was normalized by
dividing each gene expression value by the median value ofthe array.

**Microarray data analysis**
Statistical analysis was performed using Significance Analy-

sis of Microarray (SAM) software [59]. For each compa-
rison, the lowest false discovery rate (FDR) was used toidentify differentially abundant genes. A first analysis was
performed in order to identify differentially abundant tran-
scripts between N group and the two other experi-
mental groups (HI and PM). A second analysis was per-
formed in order to identify differentially abundant tran-
scripts in relation with egg quality, estimated by per-
centage of normal alevins at YSR within the complete data set or inside each experimental group (HI and PM).

**Identity of microarray cDNA clones**
Rainbow trout sequences originating from INRA AGENAE[55] and USDA [56] EST sequencing programs were used togenerate publicly available contigs [60]. The 8th ver-
sion (Om.8, released January 2006) was used for BlastX[61] comparison against the Swiss-Prot database (January2006) [62]. The score of each alignment was retrievedafter performing BlastX comparison. This was performedautomatically for each EST spotted onto the membrane andused to annotate the 9152 clones of the microarray.

**Data mining**
For all the clones identified as differentially abundantafter a SAM analysis (Table 1, 3) the official human gene
symbol was retrieved [63] and used in the text, figures and tables for clarity reasons. In addition, the accessionnumber of the corresponding rainbow trout cluster (Uni-
Gene Trout, January 2006), if any, was retrieved from theUniGene database [64]. For all genes identified as differ-
entially abundant in the transcriptomic analysis, ontolo-
gies were obtained using the AmiGO tool [65]. Finally, for
the differentially abundant genes identified in the real-time PCR analysis, a BlastX search was performed against the GenBank NR database. When possible, this was done using the contig sequence generated from all the ESTs present in the corresponding UniGene cluster. Subsequently, the amino acid sequence deduced from the trout contig sequence was aligned with cognate vertebrate forms.

Real-time PCR analysis
Real-time PCR was performed using all RNA samples used for microarray analysis (N = 29). Reverse transcription and real time PCR were performed as previously described [66]. Briefly, 2 μg of total RNA were reverse transcribed using 200 units of Moloney murine Leukemia virus (MMLV) reverse transcriptase (Promega, Madison, WI) and 0.5 μg dT15 Oligonucleotide (Promega) per μg of total RNA according to manufacturer’s instruction. RNA and dNTPs were denatured for 6 min at 70°C then chilled on ice for 5 min before the reverse transcription master mix was added. Reverse transcription was performed at 37°C for 1 hour and 15 min followed by a 15 min incubation step at 70°C. Control reactions were run without MMLV reverse transcriptase and used as negative controls in the real-time PCR study. Real-time PCR experiments were conducted using an I-Cycler IQ (Biorad, Hercules, CA). Reverse transcription products were diluted to 1/25, and 5 μl were used for each real-time PCR reaction. Triplicates were run for each RT product. Real-time PCR was performed using a real-time PCR kit provided with a SYBR Green fluorophore (Eurogentec, Belgium) according to the manufacturer’s instructions and using 600 nM of each primer. After a 2 min incubation step at 50°C and a 10 min incubation step at 95°C, the amplification was performed using the following cycle: 95°C, 20 sec; 60°C, 1 min, 40 times. The relative abundance of target cDNA within sample set was calculated from a serially diluted oocyte cDNA pool using the I-Cycler IQ software. After amplification, a fusion curve was obtained using the following protocol: 10 sec holding followed by a 0.5°C increase, repeated 80 times and starting at 55°C. The level of CyclinA2 RNAs was monitored using the same sample set to allow normalization. Cyclin A2 was used for normalization because its mRNA abundance was shown to be elevated and highly stable in rainbow trout eggs collected 5 days after ovulation [16]. Statistical analyses were performed using Statistica 7.0 software (Statsoft, Tulsa, OK). Differences between groups were analyzed using non-parametric U tests.

Authors’ contributions
EB performed egg quality monitoring, real-time PCR study, microarray analysis and drafted the manuscript. AF and JB coordinated the study and participated in real-time PCR and microarray data analysis, and in manuscript writing. All authors read and approved the final manuscript.

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References
1. Brooks S, Tyler CR, Sumpter JP: Egg quality in fish: what makes a good egg? In Reviews in Fish Biology and Fisheries Volume 7. Chapman & Hall, 1997:387-416.
2. Kjorsvik E, Mangor-Jensen A, Homeford I: Egg quality in fishes. Advances in Marine Biology 1990, 26:71-113.
3. Gall GAE, Gross SJ: A genetics analysis of the performance of three rainbow trout broodstocks. Aquaculture 1978, 15:113-127.
4. Gall GAE, Huang N: Heritability and selection schemes for rainbow trout: Female reproductive performance. Aquaculture 1988, 73:57-66.
5. Su GS, LiJedahl L, Gall GAE: Genetic and environmental variation of female reproductive traits in rainbow trout (Oncorhynhus mykiss). Aquaculture 1997, 154.
6. Izquierdo MS, Fernandez-Palacios H, Tacon AGJ: Effect of broodstock nutrition on reproductive performance of fish. Aquaculture 2001, 197:23-42.
7. Craik J, Harvey S: Egg quality in rainbow trout: the relation between egg viability, selected aspects of egg composition, and time of stripping. Aquaculture 1984, 40:115-134.
8. Craik JCA: Egg quality and egg pigment content in salmonid fishes. Aquaculture 1985, 47:61-88.
9. Mac Cormick MI: Experimental test of the effect of maternal hormones on larval quality of a coral reef fish. Oecologia 1999, 118:412-422.
10. Dworkin MB, Dworkin-Rastl E: Functions of maternal mRNA in early development. Mol Reprod Dev 1990, 26:261-297.
11. Nagler J: In vitro treatment with cycloheximide or actinomycin D inhibits early embryonic development in rainbow trout (Oncorhynhus mykiss). Fish Physiology & Biochemistry 2000, 22:61-66.
12. Hashimoto Y, Maegawa S, Nagai T, Yamaoka T, Yamaha E, Suzuka Y, Inoue K: Localized maternal factors are required for zebrafish germ cell formation. Dev Biol 2004, 268:152-161.
13. Yang BY, Green M, Chen TT: Early embryonic expression of the growth hormone family protein genes in the developing rainbow trout, Oncorhynhus mykiss. Mol Reprod Dev 1999, 53:127-134.
14. Lonergan P, Gutierrez-Adan A, Pintado B, Fair T, Ward F, Fuente JD, Boland M: Relationship between time of first cleavage and the expression of IGF-I growth factor, its receptor, and two housekeeping genes in bovine two-cell embryos and blastocysts produced in vitro. Mol Reprod Dev 2000, 57:146-152.
15. Brevini TA, Lonergan P, Cillo F, Francisci C, Favetta LA, Fair T, Gandolfi F: Evolution of mRNA polyadenylation between oocyte maturation and first embryonic cleavage in cattle and its relation with developmental competence. Mol Reprod Dev 2002, 63:510-517.
16. Aegerter S, Jalabert B, Bobe J: Large scale real-time PCR analysis of mRNA abundance in rainbow trout eggs in relationship with egg quality and post-ovulatory ageing. Mol Reprod Dev 2005, 72:337-385.
17. Arabaci M, Diller I, Sari M: Induction and synchronisation of ovulation in rainbow trout, Oncorhynhus mykiss, by administration of emulsified buserelin (GnRHa) and its effects on egg quality. Aquaculture 2004, 237:475-484.
18. Bobe J, Monfort J, Nguyen T, Fosster A: Identification of new participants in the rainbow trout (Oncorhynhus mykiss) oocyte maturation and ovulation processes using cDNA microarrays. Reprod Biol Endocrinol 2006, 4:39.
19. Knoll-Gellida A, Andre M, Gattegno T, Forgue J, Adamon A, Babin P: Molecular phenoyx-peptide and proteomic profiling, and comparison with the transcriptomes of other animals. BMC Genomics 2006, 7:46.
20. von Schalburg KR, Rise ML, Brown GD, Davidson WS, Koop BF: A comprehensive survey of the genes involved in maturation and development of the rainbow trout ovary. *Biol Reprod* 2005, 72:687-699.

21. Bertucci F, Bernard K, Loriod B, Chang YC, Granjeaud S, Birnbaum D, Nguyen C, Peck K, Jordan BR: Sensitivity issues in DNA array-based expression measurements and performance of yeast microarrays for small samples. *Hum Mol Genet* 1999, 8:1715-1722.

22. Shulman RS, Herbert PN, Wehrly KF, Friedricksson DS: The complete amino acid sequence of C-1 (apoLp-Ser), an apolipoprotein from human very low density lipoproteins. *J Biol Chem* 1975, 250:

23. Jong MC, Hofer MH, Havekes LM: Role of ApoCos in lipoprotein metabolism: functional differences between ApoC1, ApoC2, and ApoC3. *Arterioscler Thromb Vasc Biol* 1999, 19:

24. Berbee JF, van der Hoogt CC, Sundaramaran D, Havekes LM, Rensen PC: Severe hypertriglyceridaemia in human APOC1 transgenic mice is caused by apoC1-induced inhibition of LPL. *J Lipid Res* 2005, 46:297-306.

25. Hirama N, Chapman RW, Lindzey JK, Haynes MR, Sullivan CV: The mitochondrial prohibitin complex is essential for early vertebrate development. *BMC Genomics* 2007, 8:55 http://www.biomedcentral.com/1471-2164/8/55

26. Boonyaratanakornkit V, Scott MP, Ribon V, Sherman L, Anderson SM, Shulman RS, Herbert PN, Wehrly K, Fredrickson DS: Expression and characterization of vitamin D receptor from perch white perch (Morone americana). *Biol Reprod* 2004, 70:1720-1730.

27. Boonyaratanakornkit V, Scott MP, Riban V, Sherman L, Anderson SM, Hiramatsu N, Chapman RW, Lindzey JK, Haynes MR, Sullivan CV: The mitochondrial prohibitin complex is essential for early vertebrate development. *BMC Genomics* 2007, 8:55 http://www.biomedcentral.com/1471-2164/8/55

28. Tian J, Kim S, Heilig E, Ruderman JV: Identification of XPR-1, a pro-esterase receptor required for Xenopus oocyte activation. *Proc Natl Acad Sci U S A* 2000, 97:12607-12612.

29. Yama K, Booth RA, Sheng Y, Liu XJ: The classical progestosterone receptor mediates Xenopus oocyte maturation through a nongenomic mechanism. *Proc Natl Acad Sci U S A* 2000, 97:12607-12612.

30. Tian J, Kim S, Heilig E, Ruderman JV: Identification of XPR-1, a pro-esterase receptor required for Xenopus oocyte activation. *Proc Natl Acad Sci U S A* 2000, 97:14338-14343.

31. Li TB, Liu XH, Feng S, Hu Y, Yang WX, Han Y, Wang YG, Gong LM: Characterization of MR-1, a novel myofibrillarogenesis regulator in the complete set of 47 60S ribosomal proteins. *Gene* 2003, 305:151-160.

32. Yi S, Sitaramaiah V, Kominami E, Okochi T, Sugiyama T, Introna L: Comparative proteome analysis of changes in the 26S proteasome during oocyte maturation in goldfish. *Proteomics* 2006, 6:4195-4202.

33. Abe S, Charmann C, Miyamoto K, Minamino Y, Nouda M: Isolation and identification of 3-methylcrotonyl coenzyme A carboxylase cDNAs and pyruvate carboxylase, and their expression in red seabream (Pagrus major) organs. *Mar Biotechnol (NY)* 2004, 6:272-280.

34. Bonnet E, Fostier A, J. B Characterization of rainbow trout egg quality: a case study using four different breeding protocols, with emphasis on the incidence of embryonic malformations. *Biol Reprod* 2004, 71:282-290.

35. Wilkie GS, Dickson KS, Gray NK: Regulation of mRNA translation by 5' and 3' UTR-binding factors. *Trends Biochem Sci* 2003, 28:182-188.

36. Golling G, Amsterdam A, Sun Z, Antonelli M, Maldonado E, Chen W, Burgess S, Halil M, Arzt K, Farrington S, Lin SY, Nissen RM, Hopkins M: Identification and characterization of novel murine T cell-expressed genes essential for early vertebrate development. *Nat Genet* 2002, 31:135-140.

37. Ruppert C, Krosczewski R, Bahler M: Identification, characterization and cloning of myl1, a mammalian myosin-I. *J Cell Biol* 1993, 120:1393-1403.

38. Sherr EH, Joyce MP, Greene LA: Mammalian myosin I alpha, beta, and gamma: widely expressed genes of the myosin I family. *J Cell Biol* 1993, 120:1403-1416.

39. Stafford WF, Walker ML, Trinick JA, Collucio LM: Mammalian class I myosin, Myosin I, is monomeric and cross-links actin filaments as determined by hydrodynamics and electron microscopy. *Biophys J* 2005, 88:384-391.

40. Tang N, Ostap EM: Motor domain-dependent localization of myosin-1 (myl1). *Curr Biol* 2001, 11:1311-1315.

41. Hasegawa R, Dohra H, Tokunaga T: Comparative proteome analysis of changes in the 26S proteasome during oocyte maturation in goldfish. *Proteomics* 2006, 6:4195-4202.

42. Huo LJ, Fan HY, Zhong ZS, Chen DY, Schatten H, Sun QY: Ubiquitin-proteasome pathway modulates mouse oocyte meiotic maturation and fertilization via regulation of MAPK cascade and cyclin B1 degradation. *Mech Dev* 2004, 121:1275-1287.

43. Leh D, Moran SM, MacDonald MJ: The sequence of the rat pyruvate carboxylase-encoding cDNA. *Gene* 1995, 165:331-332.

44. Jirapakdee S, Booker GW, Cassidy AI, Wallace JC: The rat pyruvate carboxylase gene structure. Alternate promoters generate multiple transcripts with the S-end heterogeneity. *J Biol Chem* 1997, 272:20522-20530.

45. Jirapakdee S, Walker ME, Wallace JC: Identification of novel alternatively spliced pyruvate carboxylase mRNAs with divergent S'-translated regions which are expressed in a tissue-specific manner. *Biochem Biophys Res Commun* 1996, 233:695-700.

46. Jitrapakdee S, Booker GW, Cassidy AI, Wallace JC: Cloning, sequencing and expression of rat liver pyruvate carboxylase. *Biochem J* 1996, 316 (Pt 2):631-637.