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Growth Hormone and Gene Expression of In Vitro-Matured Rhesus Macaque Oocytes

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Abstract

Growth hormone (GH) in rhesus macaque in vitro oocyte maturation (IVM) has been shown to increase cumulus expansion and development of embryos to the 9–16 cell stage in response to 100 ng/ml recombinant human GH (r-hGH) supplementation during IVM. Although developmental endpoints for metaphase II (MII) oocytes and embryos are limited in the macaque, gene expression analysis can provide a mechanism to explore GH action on IVM. In addition, gene expression analysis may allow molecular events associated with improved cytoplasmic maturation to be detected. In this study, gene expression of specific mRNAs in MII oocytes and cumulus cells that have or have not been exposed to r-hGH during IVM was compared. In addition, mRNA expression was compared between in vitro and in vivo-matured MII oocytes and germinal vesicle (GV)- stage oocytes. Only two of 17 genes, insulin-like growth factor 2 (*IGF2*) and steroidogenic acute regulator (*STAR*), showed increased mRNA expression in MII oocytes from the 100 ng/ml r-hGH treatment group compared with other IVM treatment groups, implicating insulin-like growth factor (IGF) and steroidogenesis pathways in the oocyte response to GH. The importance of *IGF2* is notable, as expression of *IGF1* was not detected in macaque GV-stage or MII oocytes or cumulus cells.

Keywords

non-human primate; growth hormone; oocyte; gene expression

INTRODUCTION

In order for an oocyte to be developmentally competent it must complete nuclear and cytoplasmic maturation. This includes possessing the molecular components needed to complete meiosis upon fertilization, enter the mitotic cycle and initiate transcription of the embryonic genome during development in addition to supporting homeostatic and metabolic processes (Zheng et al. 2005a). High levels of mRNA, proteins, substrates and nutrients are

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accumulated in the oocyte during maturation and are associated with oocyte developmental competence (Song and Wessel 2005; Watson 2007). In vitro maturation can alter the abundance of certain MII oocyte mRNAs compared with MII oocytes matured in vivo (Lee et al. 2008; Zheng et al. 2005b). While in vitro-matured MII oocytes are capable of successful fertilization and oocyte activation, their capacity to support early development is decreased, an effect usually attributed to insufficient cytoplasmic maturation (Krisher 2004; Sirard et al. 2006; Watson 2007). The molecular events associated with successful cytoplasmic maturation and high developmental competence are largely unknown and warrant investigation.

Current culture conditions for non-human primate IVM are inadequate, and further research is needed in order to understand the environment surrounding the oocyte and to reproduce and optimize those conditions in culture, as an aid to improving human IVM. There is evidence for a role for GH in oocyte maturation in a variety of mammalian species. Growth hormone has stimulatory effects on oocyte maturation in cattle, rats, horses, pigs and rabbits (Apa et al. 1994; Izadyar et al. 1997; Marchal et al. 2003; Yoshimura et al. 1993). The GH receptor (GHR) is present in rhesus macaque GV-stage and MII oocytes and on the surface of cumulus cells from GV-stage cumulus oocyte complexes (COCs) (Nyholt de Prada and VandeVoort, 2008). In addition, GH increases cumulus expansion during macaque IVM, as well as the percentage of macaque embryos developing to the 9–16 cell stage (Nyholt de Prada and VandeVoort, 2008). The presence of GHR and increased cumulus expansion in rhesus macaque COCs support the hypothesis that GH is involved in oocyte maturation. These studies relied on the developmental capability of embryos after in vitro fertilization (IVF) to assess oocyte competence, though this endpoint may not be sensitive enough to detect subtle changes or improvements that occur in relation to cytoplasmic maturation. Examining gene expression patterns provides a more complete evaluation of the effects of altered IVM conditions, such as incorporation of GH.

The expression of specific mRNAs in MII oocytes and cumulus cells that have or have not been exposed to r-hGH during IVM was analyzed in this study. Immature, FSH-primed COCs cultured with 0, 10 or 100 ng/ml r-hGH were analyzed after in vitro maturation (IVM). In addition, mRNA expression profiles were compared with MII oocytes from FSH- and human chorionic gonadotropin- (hCG) primed females (in vivo matured) and GV-stage oocytes from FSH-primed females (FSH-primed). We report here effects of GH during IVM on the expression of *IGF2*, *STAR*, and other genes related to cell survival and transcriptional regulation.

RESULTS

We hypothesized that r-hGH supplementation during IVM would improve cytoplasmic maturation by altering gene expression in selected genes. The genes selected for this study have key roles in cell cycle progression, DNA repair, cell survival, the IGF pathway, transcriptional regulation, steroidogenesis, intercellular communication, metabolic pathways or are up-regulated following GH exposure in other cell types (Table 1).

Insulin-like growth factor pathway

Insulin-like growth factor 2 (*IGF2*) mRNA levels were increased up to 3-fold in MII oocytes from the 100 ng/ml r-hGH IVM treatment group compared with the control and 10 ng/ml r-hGH IVM treatment groups (Figure 1). Expression of insulin-like growth factor 1 (*IGF1*) was not detected in any of the oocyte or cumulus cell samples. The insulin-like growth factor 1 receptor (*IGF1R*) mRNA and insulin-like growth factor 2 receptor (*IGF2R*) mRNA expression increased by up to 5-fold in all IVM MII oocyte groups compared with GV-stage oocytes (Figure 1).

Steroidogenesis and intercellular communication

Gap junction protein, alpha 1 (*GJA1*) mRNA expression appeared to increase with maturation, but was only significantly higher in IVM MII oocytes from the 100 ng/ml r-hGH treatment group compared with GV-stage oocytes (Figure 2). Steroidogenic acute regulator (*STAR*) mRNA expression was greater than 4-fold elevated in IVM MII oocytes from the 100 ng/ml r-hGH treatment group compared with all other MII oocyte groups (Figure 2). The *GJA1* and *STAR* mRNAs were variably elevated in cumulus cells with 10 ng/ml r-hGH treatment.

Cell survival, Cell cycle, DNA repair, post- transcriptional regulation and the cytoskeleton

Expression of stem-loop binding protein (*SLBP*) mRNA was not statistically different between any oocyte or cumulus cell groups (Figure 3). Endonuclease G (*ENDOG*), heat shock 27kDa protein 1 (*HSPB1*) and *YY1* mRNA expression in IVM MII oocytes was not significantly different amongst the r-hGH treatment groups and the control group (Figure 3). *ENDOG* and *YY1* mRNA was variably elevated in cumulus cells with 10 ng/ml r-hGH treatment, though this did not reach statistical significance. Heat shock 70kDa protein 4 (*HSPA4*) mRNA expression was > 1.5-fold higher in MII oocytes from in vivo-matured compared with IVM MII oocytes in the control and 10 ng/ml r-hGH treatment group (Figure 3). The expression level of *HSPA4* mRNA in the 100 ng/ml r-hGH treatment group was between the IVM control and in vivo-matured MII oocytes levels and was not statistically significant from either. The GV-stage oocytes had *HSPA4* expression levels of >12-fold higher than all MII oocytes, regardless of treatment group.

Metaphase II oocytes in the 100 ng/ml r-hGH IVM treatment group had more than 1.5-fold lower t-complex 1 (*TCPI*) mRNA expression compared with the 10 ng/ml r-hGH group (Figure 4). Isocitrate dehydrogenase 1 (*IDH1*) (Figure 4), ataxia telangiectasia mutated (*ATM*), breast cancer 1 (*BRCA1*) and ubiquitin B (*UBB*) mRNAs were not significantly different amongst MII oocytes or cumulus cells from different r-hGH treatment groups (Figure 4). In general, the pattern of mRNA expression levels of MII oocytes was not paralleled by cumulus cell gene expression.

DISCUSSION

This study is the first to investigate differences in gene expression between macaque oocytes and cumulus cells following IVM with or without varying levels of GH supplementation. We reported that 100 ng/ml r-hGH added to IVM medium did not affect nuclear maturation, however r-hGH did improve subsequent embryo development in the macaque (Nyholt de Prada and VandeVoort, 2008). Here we show changes in gene expression correlating with this effect and therefore potentially indicating improved cytoplasmic maturation (*IGF2*, *STAR*, *GJA1*, *HSPA4*, *TCPI*). Notably, *IGF2* expression is implicated in mediating the GH response in macaque IVM. Although correlations have been shown between GH supplementation during IVM and IGF gene expression in other species, this is the first report of this association in the macaque (Apa et al. 1994, Barreca 1993).

Out of 17 genes assayed in this study, only two genes, *IGF2* and *STAR*, showed increased expression in MII oocytes from the 100 ng/ml r-hGH treatment group compared with the other IVM treatment groups. Since *IGF2* and *STAR* mRNA levels were only increased after the 100 ng/ml r-hGH IVM treatment, we concluded the lower dose was insufficient to stimulate changes in MII oocyte gene expression. In addition the increased expression of these genes may be associated with improved developmental competence (Nyholt de Prada and VandeVoort, 2008).

According to the somatomedin hypothesis, the effects of GH are mediated by IGF (Le Roith et al. 2001). The expression of *IGF1R* and *IGF2R* mRNA is increased in mature compared with immature rhesus macaque oocytes providing support for the hypothesis that IGF1 and/or IGF2 play a role in macaque IVM. The presence of insulin-like growth factor 1 receptor mRNA in human oocytes and granulosa cells is similar to the macaque as demonstrated here (Lighten et al. 1997; Wang et al. 2006; Zhou and Bondy 1993). Further support for the somatomedin hypothesis is increased expression of *IGF2* in the high GH treatment group, suggesting this dosage is effective in activating the IGF signaling pathway that may be responsible for mediating the effects of GH on oocyte maturation and developmental competence. The lack of *IGF1* expression suggests that the any involvement of the IGF pathway on macaque oocyte maturation after GH stimulation is dependent on *IGF2* mediation. Similarly, the importance of *IGF2* mRNA in humans has been demonstrated. In the human, *IGF2* mRNA has been reported as more abundant than *IGF1* and potentially more important in follicular development (el-Roeiy et al. 1993; Zhou and Bondy 1993). Higher levels of *IGF2* mRNA in human follicular fluid were correlated with increased follicular diameter and improved embryo development (Thierry van Dessel et al. 1996; Wang et al. 2006) and the effect of GH on human granulosa cell culture has been reported to be mediated in part by IGF2 (Barreca et al. 1993). In an earlier study, macaque *IGF1* mRNA was not detected in GV-stage or MII oocytes (Zheng et al. 2007) and the absence of *IGF1* in rhesus cumulus cells and GV-stage or MII oocytes in the present study supports this finding and strengthens the role of *IGF2* in macaque oocyte maturation. While mediation of GH by IGF2 is suggested in the macaque and human, IGF1 mediates the GH response during IVM in the rat and GH acts via a direct mechanism in the bovine (Apa et al. 1994; Izadyar et al. 1997). The expression of *IGF2* mRNA provides a possible avenue for IGF mediated GH effects. Improvement in embryo development in the 100 ng/ml r-hGH treatment group may thus be mediated in part by the increased *IGF2* expression observed in the current study however the mechanism of GH action on oocyte maturation warrants further investigation.

In addition to increasing *IGF2* levels, the addition of r-hGH to IVM culture increased *STAR* expression in the 100 ng/ml r-hGH treatment group. Increased *STAR* expression indicates GH supplementation may aid in steroidogenesis as well. Steroidogenic acute regulator facilitates transport of cholesterol to the inner mitochondrial membrane and is the rate-limiting step in steroidogenesis (Christenson and Strauss 2000). Elevated expression of *STAR* occurs after the LH surge in human granulosa cells, and higher levels of expression are associated with nuclear status in human cumulus cells (Feuerstein et al. 2007; Rimón et al. 2004; Sasson et al. 2004). Incomplete follicular maturation is observed in *STAR*-deficient mice (Hasegawa et al. 2000). Growth hormone has been associated with increased *STAR* mRNA levels in sheep luteal tissues and rat leydig cells indicating the importance of GH in regulating steroidogenesis in the reproductive tissues of both sexes (Juengel et al. 1995; Kanzaki and Morris 1999). Steroidogenic acute regulator mRNA expression was not detected in macaque GV-stage oocytes, suggesting *STAR* expression is elevated during oocyte maturation in the rhesus macaque to quantifiable levels. Expression of *STAR* mRNA was higher in MII oocytes in the 100 ng/ml r-hGH treatment group compared with all other groups of MII oocytes. The higher expression of *STAR* mRNA in the 100 ng/ml r-hGH treatment group indicates that GH may influence oocyte maturation and developmental competence by aiding steroidogenesis. The expression of *STAR* MII oocytes matured in vivo did not match the 100 ng/ml r-hGH treatment group, indicating the higher levels of *STAR* mRNA induced by GH does not parallel in vivo conditions. These results are supported by a separate study where *STAR* mRNA was expressed at higher levels in IVM versus in vivo-matured macaque MII oocytes (Lee et al. 2008). Higher levels of *STAR* in IVM versus in vivo-matured oocytes may reflect poor oocyte quality but may also represent the need of IVM oocytes to utilize steroidogenesis to compensate for sub-optimal culture conditions. The ability of GH to increase *STAR* expression may aid in optimizing steroidogenesis in

vitro which may be unnecessary or redundant in vivo. The high expression of *STAR* in MII oocytes can be interpreted differently and further research is necessary to elucidate the downstream pathway and overall effect on MII oocyte quality.

Together the increased expression of *IGF2* and *STAR* mRNA in the 100 ng/ml r-hGH IVM group provide some insight into the role of GH on oocyte maturation and development. While increased expression of *IGF2* and *STAR* in the macaque oocyte was apparent in the presence of GH, other genes exhibited differences between oocyte groups that are not easily interpreted. Expression levels of *GJA1*, *HSPA4* and *TCPI* showed at least one difference between a GH treatment group and another oocyte group. While some of these differences may be attributed to the presence of GH during IVM, those distinctions are not adequately supported with the current data.

Germinal vesicle-stage macaque oocytes expressed lower levels of *GJA1* mRNA compared with IVM MII oocytes from the 100 ng/ml r-hGH treatment group. It seems likely that the increased expression in the 100 ng/ml r-hGH treatment group was a result of both oocyte maturation and GH supplementation as the other IVM treatment groups were not statistically different. Gap junction protein, alpha 1 provides routes for the diffusion of molecules and functions in intercellular communication (Grazul-Bilska et al. 1997). The resumption of meiosis is generally associated with a reduction in *GJA1* protein as seen in porcine cumulus cells, bovine COCs, equine COCs and mouse ovarian follicles (Marchal et al. 2003; Norris et al. 2008; Shimada et al. 2001; Sutovsky et al. 1993). The reduction in gap junctions between somatic cells is sufficient to initiate resumption of meiosis although another meiosis-stimulatory mechanism may act in parallel (Norris et al. 2008). While expression of *GJA1* mRNA and protein have been studied in cumulus cells during oocyte maturation in a variety of species we found no quantitative difference between *GJA1* mRNA in macaque cumulus cells in the various IVM treatment groups. The expression pattern of *GJA1* in oocytes before and after maturation is lacking making comparisons with our data difficult. The addition of 100 ng/ml r-hGH to IVM culture increased *GJA1* mRNA expression in macaque MII oocytes compared with GV-stage oocytes potentially indicating the presence of more gap junctions and altered intercellular communication within the COC. The presence of more gap junctions and altered intercellular communication may be a contributing factor to improved cytoplasmic maturation and developmental competence but is speculative at this stage.

Expression of *HSPA4* mRNA was higher in macaque in vivo-matured MII oocytes compared with IVM (control and 10 ng/ml r-hGH) MII oocytes, however, it should be noted that all MII oocytes had low levels of *HSPA4* mRNA. Heat shock proteins function in regulating protein translocation and folding and protecting the cell from stress-induced damage (Beere and Green 2001; Jurisicova and Acton 2004). Synthesis of *HSPA4* during oocyte maturation is important as mature porcine oocytes were unable to induce *HSPA4* synthesis (Lanska et al. 2006). As IVM MII oocytes are generally of lower quality than in vivo-matured MII oocytes, the lower *HSPA4* expression in IVM MII oocytes suggests an inadequate response to stress or alternatively inadequate storage of *HSPA4* mRNA for early embryogenesis and development (Krisher 2004; Rizos et al. 2002; Sirard et al. 2006; van de Leemput et al. 1999). The expression of *HSPA4* in the 100 ng/ml r-hGH treatment group was somewhere between the IVM control and in vivo-matured MII oocyte groups, demonstrating GH might be regulating *HSPA4* expression to the levels observed in an in vivo-matured MII oocyte.

Expression of *TCPI* was higher in the 100 ng/ml r-hGH treatment group compared with the 10 ng/ml treatment group. T-complex 1 is involved in actin and tubulin folding, implicating a cytoskeletal function (Sternlicht et al. 1993; Valpuesta et al. 2002; Yaffe et al. 1992;

Yokota et al. 2000). The importance of *TCP1* in mitotic spindle assembly has also been noted in yeast, demonstrating the importance of this gene in microtubule-mediated processes (Ursic and Culbertson 1991). Higher expression of *TCP1* from the 100 ng/ml r-hGH treatment group suggests that differences in GH dose may result in differences at the cytoskeletal level and ultimately the ability of the maturing oocyte to undergo microtubule mediated processes such as meiosis.

In general, the pattern of mRNA expression levels of MII oocytes was not paralleled by cumulus cell gene expression. Previous results indicated that the addition of 10 ng/ml r-hGH to IVM medium increased cumulus expansion in the macaque COC while 100 ng/ml r-hGH did not (Nyholt de Prada and VandeVoort, 2008). Cumulus expansion cannot be correlated with gene expression in the current study and additional genes should be investigated to elucidate the pathway involved in cumulus expansion.

The mRNA expression analysis presented here explored the potential benefits of supplementing IVM media with r-hGH at the molecular level. The addition of 100 ng/ml r-hGH to IVM medium increased mRNA expression levels of *IGF2* and *STAR* in macaque MII oocytes, supporting the notion that GH mediation by the IGF pathway and involvement of steroidogenesis improves oocyte developmental competence. Furthermore, the importance of *IGF2* is implicated, as expression of *IGF1* was not detected in macaque oocytes or cumulus cells. Expression levels of *GJA1*, *HSPA4* and *TCP1* showed at least one difference between a GH treatment group and another oocyte group. While some of these differences may be attributed to the presence of GH during IVM, additional research is needed to support the role of GH in altering gene expression of *GJA1*, *HSPA4* and *TCP1*.

MATERIALS AND METHODS

Hormone injections and immature oocyte collection

Adult female rhesus macaques (*Macaca mulatta*) were housed and cared for at the California National Primate Research Center as described previously (Nyholt de Prada and VandeVoort, 2008). Females were administered FSH for the collection of immature oocytes. The gonadotropin releasing hormone (GnRH) antagonist, Antide (Ares-Serono, Randolph, MA), was administered subcutaneously (0.5 mg/kg body wt.) once daily in the morning on days when recombinant macaque FSH (r-mFSH) was given to prevent endogenous gonadotropin secretion. Details regarding hormonal stimulation, oocyte aspiration and oocyte collection have been described (Nyholt de Prada and VandeVoort, 2008).

In vitro oocyte maturation with various r-hGH concentrations

Retrieved immature COCs from six females (105 COCs total) were put randomly into 70 μ l drops of M1A medium (Boatman 1987; Nyholt de Prada et al. 2008) with either 0, 10 or 100 ng/ml of r-hGH (R and D Systems, Minneapolis, MN) and incubated in a humidified atmosphere of 5% CO₂ in air for 28–30 hours at 37°C (2–14 COCs per group per female). After 28–30 hours of incubation in IVM medium, COCs were placed in 10 mg/ml hyaluronidase (MP Biomedicals, Solon, OH) in Tyrodes lactate (TL)-HEPES medium containing 0.1 mg/ml polyvinyl alcohol (PVA) that was pre-equilibrated to 37°C. Cumulus oocyte complexes were stripped of cumulus cells using a Stripper® (MidAtlantic Diagnostics Inc., Mount Laurel, NJ). Denuded oocytes were visually assessed, and only oocytes with polar bodies were used for this study. Cumulus cells separated from COCs in each treatment were also used for this study (50–100 cells).

Hormone injections and mature oocyte collection

For collection of mature COCs, females were given recombinant hCG in addition to the hormonal treatment outlined for immature COC collection. Hormonal stimulation for the collection of mature oocytes along with oocyte aspiration and oocyte collection has been described (Nyholt de Prada and VandeVoort, 2008; VandeVoort and Tarantal 2001). The COCs were put in pre-equilibrated 70 μ l drops of chemically-defined, protein-free hamster embryo culture medium 9 (HECM-9) under oil and incubated at 37°C in a humidified atmosphere of 5% CO₂ in air (McKiernan and Bavister 2000). After 6–8 hours of incubation, COCs were placed in TL-PVA pre-equilibrated to 37°C, and a Stripper® was used to ensure all cumulus cells were removed. Denuded oocytes were examined visually, and only oocytes with polar bodies were used.

Quantitative reverse transcription – polymerase chain reaction

Quantitative gene expression studies of small numbers of oocytes and embryos have been made possible with a novel reverse transcription-polymerase chain reaction (RT-PCR) approach (Rambhatla et al. 1995). This approach enables comparison of gene expression patterns and relative copy number of many different mRNAs from a common set of samples, which is crucial for non-human primate studies where resources are limited. This methodology allows for quantitative comparison of gene expression in MII oocytes that have been matured in vitro versus in vivo, providing insight into molecular differences among oocyte quality. In addition, gene expression analysis of MII oocytes matured in the presence of specific growth factors allows us to investigate at the molecular level the potential benefits of supplementing IVM media. The Primate Embryo Gene Expression Resource (PREGER; <http://www.preger.org>) has developed a novel RT-PCR approach to support gene expression analysis of non-human primate oocytes (Zheng et al. 2004a; Zheng et al. 2004b; Zheng et al. 2005b).

Denuded MII oocytes were briefly put in an acid Tyrodes solution (TL-PVA, pH 2.0) to remove the zona pellucida and then rinsed in TL-PVA. Zona-free oocytes were added in a minimal volume to the RT buffer (Rambhatla et al. 1995). Between 50 and 100 cumulus cells were put into a 1.5 ml tube and centrifuged at $6,000 \times g$ for 5 minutes. Supernatant was discarded, and pelleted cells were washed with 100 μ l of Dulbecco's phosphate buffered saline (DPBS) and centrifuged again for 5 minutes. Supernatant was discarded, and the RT buffer was added directly to pelleted cells. A quantitative RT-PCR was performed as described (Rambhatla et al. 1995; Zheng et al. 2004b). This method (Brady et al. 1990; Brady and Iscove 1993) uniformly amplifies the entire mRNA population, preserving relative abundances of cDNA sequences. Quantitative amplification and dot blotting (QADB) was used as described (Rambhatla et al. 1995; Zheng et al. 2004b). The sensitivity and reliability of the QADB method have been discussed extensively elsewhere (Latham 2006; Rambhatla et al. 1995; Zheng et al. 2004b).

The samples used in these experiments were added to the PREGER resource. The COCs were collected from six FSH-primed females and five FSH- and hCG-primed females. The COCs from FSH-primed females were used for IVM. One to two oocytes were collected per sample and one to three samples of oocytes were collected for each treatment per female (0 ng/ml r-hGH, 14 samples; 10 ng/ml r-hGH, 12 samples; 100 ng/ml r-hGH, eight samples). A cumulus cell sample was collected for each treatment group per female. Cumulus oocyte complexes from FSH- and hCG-primed females were collected (10 samples). Germinal vesicle- stage oocytes from two FSH-stimulated females were added to the sample set from the PREGER resource (six samples, one oocyte each).

Complementary DNA probes and hybridization

Plasmids containing cDNA inserts were obtained from Open Biosystems (Integrated Sciences, Australia). Primers and the amplified cDNA regions are listed in Table 2. Probe preparation was performed as described for the majority of genes (Latham 2006; Rambhatla et al. 1995; Zheng et al. 2004b). Complementary DNA probes for IGF1 and IGF2R were obtained by RT-PCR of RNA from rhesus macaque liver and the identities of the amplified cDNAs were confirmed by sequencing. Blot preparation, hybridization and quantitative analyses were performed as described (Latham 2006; Rambhatla et al. 1995; Zheng et al. 2004b). Data are expressed as the mean $\pm$ SEM cpm bound value for each treatment group of oocytes or cumulus cells.

Statistical analysis

Differences in gene expression between treatment groups and cell types were evaluated with two-sample t-tests assuming equal variances. The analysis of oocyte mRNA expression encompassed five different oocyte groups. In the cumulus cell analysis three IVM treatment groups were compared. Assuming the different groups were independent, the expression levels of each gene were tested in each of the four possible comparisons for oocytes or the two possible comparisons for cumulus cells. Non-human primate oocytes are both expensive and difficult to obtain and the limited sample size in this study may limit the differences observed, therefore, all statistical differences that pass the uncorrected marginal significance value of $P < 0.05$ are reported. In addition, comparisons that achieve statistical significance after applying the Bonferroni correction for the performance of four or two tests are reported; $P < 0.0125$ or $P < 0.025$ respectively (Armitage and Berry 1994).

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REFERENCES

- Apa R, Lanzone A, Miceli F, Mastrandrea M, Caruso A, Mancuso S, Canipari R. Growth hormone induces in vitro maturation of follicle- and cumulus-enclosed rat oocytes. *Mol Cell Endocrinol.* 1994; 106(1–2):207–212. [PubMed: 7895909]
- Armitage, P.; Berry, G. *Statistical Methods in Medical Research.* Oxford: Blackwell Sciences; 1994.
- Barreca A, Artini PG, Del Monte P, Ponzani P, Pasquini P, Cariola G, Volpe A, Genazzani AR, Giordano G, Minuto F. In vivo and in vitro effect of growth hormone on estradiol secretion by human granulosa cells. *J Clin Endocrinol Metab.* 1993; 77(1):61–67. [PubMed: 8325961]
- Beere HM, Green DR. Stress management - heat shock protein-70 and the regulation of apoptosis. *Trends Cell Biol.* 2001; 11(1):6–10. [PubMed: 11146277]
- Boatman, DE. In vitro growth of non-human primate pre- and peri- implantation embryos. In: Bavister, BD., editor. *The Mammalian Preimplantation Embryo: regulation of growth and differentiation in vitro.* New York: Plenum Press; 1987. p. 273-308.
- Brady G, Barbara M, Iscove NN. Representative in vitro cDNA amplification from individual hemopoietic cells and colonies. *Meth Mol Cell Biol.* 1990; 2:17–25.
- Brady G, Iscove NN. Construction of cDNA libraries from single cells. *Methods Enzymol.* 1993; 225:611–623. [PubMed: 8231874]
- Christenson LK, Strauss JF 3rd. Steroidogenic acute regulatory protein (StAR) and the intramitochondrial translocation of cholesterol. *Biochim Biophys Acta.* 2000; 1529(1–3):175–187. [PubMed: 11111087]

- el-Roeiy A, Chen X, Roberts VJ, LeRoith D, Roberts CT Jr, Yen SS. Expression of insulin-like growth factor-I (IGF-I) and IGF-II and the IGF-I, IGF-II, and insulin receptor genes and localization of the gene products in the human ovary. *J Clin Endocrinol Metab.* 1993; 77(5):1411–1418. [PubMed: 8077342]
- Feuerstein P, Cadoret V, Dalbies-Tran R, Guerif F, Bidault R, Royere D. Gene expression in human cumulus cells: one approach to oocyte competence. *Hum Reprod.* 2007; 22(12):3069–3077. [PubMed: 17951581]
- Grazul-Bilska AT, Reynolds LP, Redmer DA. Gap junctions in the ovaries. *Biol Reprod.* 1997; 57(5): 947–957. [PubMed: 9369157]
- Hasegawa T, Zhao L, Caron KM, Majdic G, Suzuki T, Shizawa S, Sasano H, Parker KL. Developmental roles of the steroidogenic acute regulatory protein (StAR) as revealed by StAR knockout mice. *Mol Endocrinol.* 2000; 14(9):1462–1471. [PubMed: 10976923]
- Izadyar F, Van Tol HT, Colenbrander B, Bevers MM. Stimulatory effect of growth hormone on in vitro maturation of bovine oocytes is exerted through cumulus cells and not mediated by IGF-I. *Mol Reprod Dev.* 1997; 47(2):175–180. [PubMed: 9136119]
- Juengel JL, Meberg BM, Turzillo AM, Nett TM, Niswender GD. Hormonal regulation of messenger ribonucleic acid encoding steroidogenic acute regulatory protein in ovine corpora lutea. *Endocrinology.* 1995; 136(12):5423–5429. [PubMed: 7588291]
- Juriscicova A, Acton BM. Deadly decisions: the role of genes regulating programmed cell death in human preimplantation embryo development. *Reproduction.* 2004; 128(3):281–291. [PubMed: 15333779]
- Kanzaki M, Morris PL. Growth hormone regulates steroidogenic acute regulatory protein expression and steroidogenesis in Leydig cell progenitors. *Endocrinology.* 1999; 140(4):1681–1686. [PubMed: 10098503]
- Krisher RL. The effect of oocyte quality on development. *J Anim Sci.* 2004; 82 E-Suppl:E14–E23. [PubMed: 15471793]
- Lanska V, Chmelikova E, Sedmikova M, Petr J, Rajmon R, Jeseta M, Rozinek J. Expression of heat shock protein70 in pig oocytes: heat shock response during oocyte growth. *Anim Reprod Sci.* 2006; 96(1–2):154–164. [PubMed: 16414213]
- Latham KE. The Primate Embryo Gene Expression Resource in embryology and stem cell biology. *Reprod Fertil Dev.* 2006; 18(8):807–810. [PubMed: 17147927]
- Le Roith D, Bondy C, Yakar S, Liu JL, Butler A. The somatomedin hypothesis: 2001. *Endocr Rev.* 2001; 22(1):53–74. [PubMed: 11159816]
- Lee SM, Koh HJ, Park DC, Song BJ, Huh TL, Park JW. Cytosolic NADP(+)-dependent isocitrate dehydrogenase status modulates oxidative damage to cells. *Free Radic Biol Med.* 2002; 32(11): 1185–1196. [PubMed: 12031902]
- Lee YS, Latham KE, Vandevoort CA. Effects of in vitro maturation on gene expression in rhesus monkey oocytes. *Physiol Genomics.* 2008; 35(2):145–158. [PubMed: 18697858]
- Lighten AD, Hardy K, Winston RM, Moore GE. Expression of mRNA for the insulin-like growth factors and their receptors in human preimplantation embryos. *Mol Reprod Dev.* 1997; 47(2):134–139. [PubMed: 9136113]
- Marchal R, Caillaud M, Martoriati A, Gerard N, Mermillod P, Goudet G. Effect of growth hormone (GH) on in vitro nuclear and cytoplasmic oocyte maturation, cumulus expansion, hyaluronan synthases, and connexins 32 and 43 expression, and GH receptor messenger RNA expression in equine and porcine species. *Biol Reprod.* 2003; 69(3):1013–1022. [PubMed: 12773434]
- McKiernan SH, Bavister BD. Culture of one-cell hamster embryos with water soluble vitamins: pantothenate stimulates blastocyst production. *Hum Reprod.* 2000; 15(1):157–164. [PubMed: 10611206]
- Mtango NR, Latham KE. Ubiquitin proteasome pathway gene expression varies in rhesus monkey oocytes and embryos of different developmental potential. *Physiol Genomics.* 2007; 31(1):1–14. [PubMed: 17550997]
- Norris RP, Freudzon M, Mehlmann LM, Cowan AE, Simon AM, Paul DL, Lampe PD, Jaffe LA. Luteinizing hormone causes MAP kinase-dependent phosphorylation and closure of connexin 43

- gap junctions in mouse ovarian follicles: one of two paths to meiotic resumption. *Dev.* 2008; 135(19):3229–3238.
- Nyholt de Prada JK, VandeVoort CA. Growth hormone and in vitro maturation of rhesus macaque oocytes and subsequent embryo development. *Journal of assisted reproduction and genetics.* 2008; 25:145–158. (Citation listed in PubMed: de Prada JK and VandeVoort CA). [PubMed: 18278582]
- Rambhatla L, Patel B, Dhanasekaran N, Latham KE. Analysis of G protein alpha subunit mRNA abundance in preimplantation mouse embryos using a rapid, quantitative RT-PCR approach. *Mol Reprod Dev.* 1995; 41(3):314–324. [PubMed: 8588930]
- Rimon E, Sasson R, Dantes A, Land-Bracha A, Amsterdam A. Gonadotropin-induced gene regulation in human granulosa cells obtained from IVF patients: modulation of genes coding for growth factors and their receptors and genes involved in cancer and other diseases. *Int J Oncol.* 2004; 24(5):1325–1338. [PubMed: 15067357]
- Rizos D, Fair T, Papadopoulos S, Boland MP, Lonergan P. Developmental, qualitative, and ultrastructural differences between ovine and bovine embryos produced in vivo or in vitro. *Mol Reprod Dev.* 2002; 62(3):320–327. [PubMed: 12112595]
- Sasson R, Rimon E, Dantes A, Cohen T, Shinder V, Land-Bracha A, Amsterdam A. Gonadotrophin-induced gene regulation in human granulosa cells obtained from IVF patients. Modulation of steroidogenic genes, cytoskeletal genes and genes coding for apoptotic signalling and protein kinases. *Mol Hum Reprod.* 2004; 10(5):299–311. [PubMed: 15026540]
- Sawa C, Yoshikawa T, Matsuda-Suzuki F, Delehouzee S, Goto M, Watanabe H, Sawada J, Kataoka K, Handa H. YEAF1/RYPB and YAF-2 are functionally distinct members of a cofactor family for the YY1 and E4TF1/hGABP transcription factors. *J Biol Chem.* 2002; 277(25):22484–22490. [PubMed: 11953439]
- Shimada M, Maeda T, Terada T. Dynamic changes of connexin-43, gap junctional protein, in outer layers of cumulus cells are regulated by PKC and PI 3-kinase during meiotic resumption in porcine oocytes. *Biol Reprod.* 2001; 64(4):1255–1263. [PubMed: 11259274]
- Sirard MA, Richard F, Blondin P, Robert C. Contribution of the oocyte to embryo quality. *Theriogenology.* 2006; 65(1):126–136. [PubMed: 16256189]
- Song JL, Wessel GM. How to make an egg: transcriptional regulation in oocytes. *Differentiation.* 2005; 73(1):1–17. [PubMed: 15733063]
- Sternlicht H, Farr GW, Sternlicht ML, Driscoll JK, Willison K, Yaffe MB. The t-complex polypeptide 1 complex is a chaperonin for tubulin and actin in vivo. *Proc Natl Acad Sci U S A.* 1993; 90(20):9422–9426. [PubMed: 8105476]
- Sutovsky P, Flechon JE, Flechon B, Motlik J, Peynot N, Chesne P, Heyman Y. Dynamic changes of gap junctions and cytoskeleton during in vitro culture of cattle oocyte cumulus complexes. *Biol Reprod.* 1993; 49(6):1277–1287. [PubMed: 8286609]
- Thierry van Dessel HJ, Chandrasekhar Y, Yap OW, Lee PD, Hintz RL, Faessen GH, Braat DD, Fauser BC, Giudice LC. Serum and follicular fluid levels of insulin-like growth factor I (IGF-I), IGF-II, and IGF-binding protein-1 and -3 during the normal menstrual cycle. *J Clin Endocrinol Metab.* 1996; 81(3):1224–1231. [PubMed: 8772603]
- Thomas MJ, Seto E. Unlocking the mechanisms of transcription factor YY1: are chromatin modifying enzymes the key? *Gene.* 1999; 236(2):197–208. [PubMed: 10452940]
- Ursic D, Culbertson MR. The yeast homolog to mouse Tcp-1 affects microtubule-mediated processes. *Mol Cell Biol.* 1991; 11(5):2629–2640. [PubMed: 1901944]
- Valpuestra JM, Martin-Benito J, Gomez-Puertas P, Carrascosa JL, Willison KR. Structure and function of a protein folding machine: the eukaryotic cytosolic chaperonin CCT. *FEBS Lett.* 2002; 529(1):11–16. [PubMed: 12354605]
- van de Leemput EE, Vos PL, Zeinstra EC, Bevers MM, van der Weijden GC, Dieleman SJ. Improved in vitro embryo development using in vivo matured oocytes from heifers superovulated with a controlled preovulatory LH surge. *Theriogenology.* 1999; 52(2):335–349. [PubMed: 10734399]
- VandeVoort CA, Tarantal AF. Recombinant human gonadotropins for macaque superovulation: Repeated stimulations and post-treatment pregnancies. *J Med Primatol.* 2001; 30:304–307. [PubMed: 11990529]

- Wang TH, Chang CL, Wu HM, Chiu YM, Chen CK, Wang HS. Insulin-like growth factor-II (IGF-II), IGF-binding protein-3 (IGFBP-3), and IGFBP-4 in follicular fluid are associated with oocyte maturation and embryo development. *Fertil Steril*. 2006; 86(5):1392–1401. [PubMed: 17070193]
- Watson AJ. Oocyte cytoplasmic maturation: a key mediator of oocyte and embryo developmental competence. *J Anim Sci*. 2007; 85(13 Suppl):E1–E3. [PubMed: 17322120]
- Yaffe MB, Farr GW, Miklos D, Horwich AL, Sternlicht ML, Sternlicht H. TCP1 complex is a molecular chaperone in tubulin biogenesis. *Nature*. 1992; 358(6383):245–248. [PubMed: 1630491]
- Yokota SI, Yanagi H, Yura T, Kubota H. Upregulation of cytosolic chaperonin CCT subunits during recovery from chemical stress that causes accumulation of unfolded proteins. *Eur J Biochem*. 2000; 267(6):1658–1664. [PubMed: 10712596]
- Yoshimura Y, Nakamura Y, Koyama N, Iwashita M, Adachi T, Takeda Y. Effects of growth hormone on follicle growth, oocyte maturation, and ovarian steroidogenesis. *Fertil Steril*. 1993; 59(4):917–923. [PubMed: 8458516]
- Zheng P, Patel B, McMenamin M, Moran E, Paprocki AM, Kihara M, Schramm RD, Latham KE. Effects of follicle size and oocyte maturation conditions on maternal messenger RNA regulation and gene expression in rhesus monkey oocytes and embryos. *Biol Reprod*. 2005a; 72(4):890–897. [PubMed: 15590902]
- Zheng P, Patel B, McMenamin M, Paprocki AM, Schramm RD, Nagl NG Jr, Wilsker D, Wang X, Moran E, Latham KE. Expression of genes encoding chromatin regulatory factors in developing rhesus monkey oocytes and preimplantation stage embryos: possible roles in genome activation. *Biol Reprod*. 2004a; 70(5):1419–1427. [PubMed: 14724134]
- Zheng P, Patel B, McMenamin M, Reddy SE, Paprocki AM, Schramm RD, Latham KE. The primate embryo gene expression resource: a novel resource to facilitate rapid analysis of gene expression patterns in non-human primate oocytes and preimplantation stage embryos. *Biol Reprod*. 2004b; 70(5):1411–1418. [PubMed: 14724133]
- Zheng P, Schramm RD, Latham KE. Developmental regulation and in vitro culture effects on expression of DNA repair and cell cycle checkpoint control genes in rhesus monkey oocytes and embryos. *Biol Reprod*. 2005b; 72(6):1359–1369. [PubMed: 15703371]
- Zheng P, Vassena R, Latham KE. Effects of in vitro oocyte maturation and embryo culture on the expression of glucose transporters, glucose metabolism and insulin signaling genes in rhesus monkey oocytes and preimplantation embryos. *Mol Hum Reprod*. 2007; 13(6):361–371. [PubMed: 17416905]
- Zhou J, Bondy C. Anatomy of the human ovarian insulin-like growth factor system. *Biol Reprod*. 1993; 48(3):467–482. [PubMed: 7680905]

Figure 1. Expression of insulin-like growth factor pathway mRNAs after in vitro maturation with growth hormone

Values are mean $\pm$ SEM. Differences between groups are indicated with an asterisk $P < 0.05$, or two asterisks $P < 0.0125$. Treatment groups are as follows: GV-stage oocytes from FSH-primed females (GV, FSH); Control oocytes matured in control IVM medium that reached the MII stage (MII, IVM, Control); oocytes matured in IVM medium + 10 or 100 ng/ml r-hGH that reached the MII stage (MII, IVM, GH-10 or GH-100, respectively); MII oocytes from FSH- and hCG-primed females (MII, hCG); cumulus cells matured in control IVM medium (CC, IVM, Control); cumulus cells matured in IVM medium supplemented with 10 or 100 ng/ml r-hGH (CC, IVM, GH-10 or GH-100, respectively).

Values are mean $\pm$ SEM. Differences between groups are indicated with an asterisk $P < 0.05$, or two asterisks $P < 0.0125$. Treatment groups are as follows: GV-stage oocytes from FSH-primed females (GV, FSH); Control oocytes matured in control IVM medium that reached the MII stage (MII, IVM, Control); oocytes matured in IVM medium + 10 or 100 ng/ml r-hGH that reached the MII stage (MII, IVM, GH-10 or GH-100, respectively); MII oocytes from FSH- and hCG-primed females (MII, hCG); cumulus cells matured in control IVM medium (CC, IVM, Control); cumulus cells matured in IVM medium supplemented with 10 or 100 ng/ml r-hGH (CC, IVM, GH-10 or GH-100, respectively).

Figure 3. Expression of cell survival mRNAs and mRNAs involved in transcriptional and translational regulation after in vitro maturation with growth hormone

Values are mean $\pm$ SEM. Differences between groups are indicated with an asterisk $P < 0.05$, or two asterisks $P < 0.0125$. Treatment groups are as follows: GV-stage oocytes from FSH-primed females (GV, FSH); Control oocytes matured in control IVM medium that reached the MII stage (MII, IVM, Control); oocytes matured in IVM medium + 10 or 100 ng/ml r-hGH that reached the MII stage (MII, IVM, GH-10 or GH-100, respectively); MII oocytes from FSH- and hCG-primed females (MII, hCG); cumulus cells matured in control IVM medium (CC, IVM, Control); cumulus cells matured in IVM medium supplemented with 10 or 100 ng/ml r-hGH (CC, IVM, GH-10 or GH-100, respectively).

Figure 4. Expression of mRNAs involved in cell cycle, DNA repair, metabolic pathways or maintaining cytoskeletal structure after in vitro maturation with growth hormone
 Values are mean $\pm$ SEM. Differences between groups are indicated with an asterisk $P < 0.05$, or two asterisks $P < 0.0125$. Treatment groups are as follows: GV-stage oocytes from FSH-primed females (GV, FSH); Control oocytes matured in control IVM medium that reached the MII stage (MI, IVM, Control); oocytes matured in IVM medium + 10 or 100 ng/ml r-hGH that reached the MII stage (MI, IVM, GH-10 or GH-100, respectively); MII oocytes from FSH- and hCG-primed females (MI, hCG); cumulus cells matured in control IVM medium (CC, IVM, Control); cumulus cells matured in IVM medium supplemented with 10 or 100 ng/ml r-hGH (CC, IVM, GH-10 or GH-100, respectively).

Table 1

Functional descriptions of genes studied.

Symbol	Name	Other Names	Function/Role
<i>ATM</i>	Ataxia telangiectasia mutated		Cell cycle and DNA repair
<i>BRCA1</i>	Breast cancer 1, early onset		Cell cycle and DNA repair
<i>ENDOG</i>	Endonuclease G		Cell survival
<i>GJA1</i>	Gap junction protein, alpha 1, 43 kDa	Connexin 43	Intercellular communication
<i>HSPA4</i>	Heat shock 70kDa protein 4	HSP70	Cell survival
<i>HSPB1</i>	Heat shock 27kDa protein 1	HSP27	Cell survival
<i>IDH1</i>	Isocitrate dehydrogenase 1 (NADP+) soluble	IDH, IDP	Metabolic pathway, TCA cycle
<i>IGF1</i>	Insulin-like growth factor 1		Insulin-like growth factor pathway, cell growth and development
<i>IGF1R</i>	Insulin-like growth factor 1 receptor		Insulin-like growth factor pathway, receptor for IGF1 and IGF2
<i>IGF2</i>	Insulin-like growth factor 2	Somatomedin A	Insulin-like growth factor pathway, cell growth and development
<i>IGF2R</i>	Insulin-like growth factor 2 receptor		Insulin-like growth factor pathway, receptor for IGF2
<i>SLBP</i>	Stem loop binding protein		Post-transcriptional regulation
<i>STAR</i>	Steroidogenic acute regulatory protein		Steroidogenesis
<i>TCP1</i>	T-complex 1		Cytoskeletal
<i>UBB</i>	Ubiquitin B		Cell cycle
<i>YY1</i>	YY1 transcription factor		Transcriptional regulation

Table 2

Information and sequences of specific primers used for PCR.

Gene	Oligos Sequences	GenBank accession number	Size of PCR product(bp)	Product bp range	Annealing temperature (°C)
<i>ATM</i>	Up5' - CCTGCCTTAGCCTCACAAAG-3' Low 5' - AATGTTTAAAGCATGGGCCAG-3'	BC007023	391	1100–1490	60.0
<i>BRCA1</i>	Up5' - CAAACATGCCACAGATCAAC-3' Low 5' - AAGCTCATCTTGGGGTCCCT-3'	BC115037	322	5519–5840	59.9
<i>ENDOG</i>	Up5' - AGAGGCTGATGGAAATCCT-3' Low5' - CACACCCACAGTCTCACTGG-3'	BC016351	309	830–1138	60.1
<i>GJA1</i>	Up5' - TCCTTTGGAGTGACCAGCAA-3' Low5' - TGCAATGAAGCTGAACATGA-3'	BC026329	301	2586–2886	59.2
<i>HSPA4</i>	Up5' - GATCATTTTGGATGCTGTA-3' Low5' - TCTTGTCTGAATCCGAAGGC-3'	BC110861	328	2311–2638	60.1
<i>HSPB1</i>	Up 5' – GTCCTTGGATGTCAACCACT-3' Low 5' - CTTTACTTGGCGCAGTCTC-3'	NM_001540	330	398–727	60.0
<i>IDH1</i>	Up5' - CCCGTTTGTGAGGTAGAGC-3' Low5' - AAAACAGGCCAGCAGAAAGTC-3'	BC012846	313	1928–2240	59.6
<i>IGF1</i>	Up5' - CCCCTGCTACTTTGAAACCA-3' Low5' - AGGAATGACACATTACACCAACA-3'	XM_001094016	330	7147–7476	59.0
<i>IGF1R</i>	Up 5' - GTTGAGGTGAGAGGTTTGCC-3' Low 5' - TTGCCTTAGCTTCAGCCATT-3'	BC010607	486	2790–3171	59.9
<i>IGF2</i>	Up5' - TTGCTCTACCCACCCAAAGAC-3' Low5' - TGTGGGGATAATTGGGGATA-3'	BC000531	353	1000–1352	60
<i>IGF2R</i>	Up5' - TTCAGGTTCTCATGATACCACC-3' Low5' - ATCAAAACAGCCAGCCAAAC-3'	XR_012149	377	8393–8769	59.0
<i>SLBP</i>	Up5' - TTGCTTGTCAGGTGGACTGT-3' Low5' - TAAATCGCTCCACTCTGTG-3'	BC014908	300	1265–1564	59.2
<i>STAR</i>	Up5' - AGGGATGAGGCTCTGGATT-3' Low5' - TATTCTGGAAGGCACCCAC-3'	BC010550	338	1105–1442	60
<i>TCP1</i>	Up5' - GTTAATGCTGCCCGGAGCTC-3'	BC000665	300	1485–1784	60.1

Gene	Oligos Sequences	GenBank accession number	Size of PCR product(bp)	Product bp range	Annealing temperature (°C)
YY1	Low5' -GGCTCCAGAGTGAAACAGCAT-3'				
	Up 5' - GGAGGTGATAAACTTTGCTTGC-3'	BC037308	392	1810-2201	60.0
	Low 5' - GCCCTTTCTGTGTACATGGGA-3'				

Primers for *UBB* were published previously (Mango and Latham 2007).