PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
MESTRADO EM CIÊNCIA ANIMAL

ADIÇÃO DE PROTEÍNA PLASMÁTICA *A* ASSOCIADA À PRENHEZ (PAPP-A) DURANTE A MATURAÇÃO *IN VITRO* AUMENTA O IGF-1 BIODISPONÍVEL E MODULA O PERFIL TRANSCRICIONAL DE COMPLEXOS CUMULUS-OÓCITOS E EMBRIÕES BOVINOS

ALAN BRUNHOLI GIROTO

Presidente Prudente – SP
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Dissertação apresentada a Pró-Reitoria de Pesquisa e Pós-Graduação, Universidade do Oeste Paulista, como parte dos requisitos para obtenção do título de Mestre em Ciência Animal - Área de Concentração: Fisiopatologia Animal.

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2018

636.208 982
G377a

Giroto, Alan Brunholi
Adição de proteína plasmática *a* associada à prenhez (PAPP-A) durante a maturação *in vitro* aumenta o IGF-1 biodisponível e modula o perfil transcricional de complexos cumulus-oócitos e embriões bovinos \ Alan Brunholi Giroto. -- Presidente Prudente, 2018.
62 f.: il.

Dissertação (Mestrado em Ciência Animal) -- Universidade do Oeste Paulista - Unoeste, Presidente Prudente, SP, 2018.
Bibliografia.
Orientador: Anthony César de Souza Castilho

1. Proteína de ligação do fator de crescimento semelhante à insulina. 2. Complexos cumulus-oócito. 3. Produção *in vitro* de embriões. 4. Criopreservação. 5. Bovinos. I. Título.

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Presidente Prudente, 25 de maio de 2018.

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DEDICATÓRIA

Primeiramente, dedico este trabalho à Deus, pois esta conquista foi apenas uma das vontades Dele.

Aos meus avôs Horácio Giroto e Antônio Brunholi.

Aos meus pais Terezinha e José, e ao meu irmão Alisson

Aos meus padrinhos Vera e Luis, exemplos de pessoas que sempre me apoiaram.

Dedico, também, a todos os meus amigos, aqueles que sempre estiveram ao meu lado.

AGRADECIMENTOS

Ao meu orientador, Prof. Dr. Anthony César de Souza Castilho, sem dúvidas um excelente mentor.

A todos os colegas de equipe: Patrícia Fontes, Priscila Helena, Fernanda Franchi, Marina Chaves e João Amoris. Pessoas maravilhosas que contribuíram de forma significativa.

Aos amigos Vitória Guilherme, Aline Sawa, Thaisy Dellaqua, Alexandre Mendes, Franciny Neves, Hugo de Rossi e Sara Nunes. Ajudaram ao máximo na realização deste trabalho.

Ao programa de Pós-Graduação em Ciência Animal e a Pró-Reitoria de Pesquisa e Pós Graduação da Universidade do Oeste Paulista - Unoeste.

Aos doutores Eduardo M. Razza, Caliê Castilho, Guilherme P. Nogueira, Marcos A. Maioli, Marcelo F. G. Nogueira, Gisele Z. Mingoti, Raquel Z. Puelker e Isabele P. Emanuelli.

À bolsa de estudo sob o processo nº 2016/22812-5, concedida pelo convênio Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) e Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

É muito melhor lançar-se em busca de conquistas grandiosas, mesmo expondo-se ao fracasso, do que alinhar-se com os pobres de espírito, que nem gozam muito nem sofrem muito, porque vivem numa penumbra cinzenta, onde não conhecem nem vitória, nem derrota." (Theodore Roosevelt)

RESUMO

Adição de proteína plasmática *A* associada à prenhez (PAPP-A) durante a maturação *in vitro* aumenta o IGF-1 biodisponível e modula o perfil transcricional de complexos cumulus-oócitos e embriões bovinos

A protéina sérica associada à prenhez (PAPP-A) é capaz de modular a biodisponibilidade do IGF-1 (fator de crescimento semelhante à insulina tipo 1) pela quebra das ligação com as proteínas de ligação do IGF (IGFBPs). O objetivo foi avaliar como a adição da PAPP-A durante a maturação *in vitro* (MIV) afetou a biodisponibilidade de IGF-1, a abundância de transcritos nos oócitos e células do cumulus e blastocistos, a produção embrionária e a sobrevivência pós aquecimento. Foi realizada a MIV de 24 h em complexos cumulus oócitos (CCOs – 20/grupo) oriundos de abatedouro, em meio TCM199 livre de soro, com adição de PAPP-A (100 ng/mL; grupo PAPP-A) ou sem (controle). O IGF-1 foi quantificado no meio de maturação e os CCOs maturados foram utilizados na fertilização e cultivo *in vitro*. Oócitos e células do cúmulus foram separados; e blastocistos foram congelados para a realização da expressão gênica. Taxa de clivagem e produção de blastocistos foram calculados como porcentagem e transformados para arco seno. Dados de expressão gênica foram normalizados com a média do grupo de controle. Os resultados foram analisados por test t, porém a criopreservação embrionária foi testada por Qui-quadrado (JMP software, SAS). Diferenças foram consideradas significativas quando $p \leq 0,05$. O grupo PAPP-A apresentou 27% mais concentração de IGF-1 biodisponível. Na produção *in vitro* de embriões, não encontrou-se diferença na taxa de clivagem, produção e sobrevivência embrionária. Apenas o gene *TXNRD1* mostrou maior expressão nos oócitos do grupo PAPP-A. No entanto, nas células do cumulus PAPP-A, o *VNN1* e *HDAC2* apresentaram maior expressão, enquanto os genes *AGPAT1*, *AGPAT9*, *FASN*, *CASP3*, *EGFR*, *HAS2*, *IMPDH1* e *MTIF3* apresentaram menor expressão. Nos blastocistos PAPP-A, os genes *CPT2*, *CASP9*, *DNMT3A*, *TFAM* e *KRT8* apresentaram maior expressão, enquanto os genes *ATF4*, *CASP3* e *IFITM3* apresentaram menor expressão. Em conclusão, a adição de PAPP-A durante a MIV aumentou o IGF-1 biodisponível, mas não influenciou a produção e a sobrevivência embrionária após desvitrificação. No entanto, o aumento do IGF-1 biodisponível pode melhorar a competência embrionária através da modulação da expressão gênica em oócitos, células do cúmulus e blastocistos.

Palavras-chave: proteína de ligação do fator de crescimento semelhante à insulina, complexos cumulus-oócito, produção *in vitro* de embriões, criopreservação, bovinos.

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SUMÁRIO

O presente manuscrito está redigido e submetido segundo as normas da revista *Molecular, Reproduction and Development*, exceto as imagens e tabelas dispostas ao longo do texto.

Addition of pregnancy-associated plasma protein-A (PAPP-A) during *in vitro* maturation increases bioavailable IGF-1 and modulates transcriptional profile of bovine cumulus-oocyte complexes and blastocysts

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Grants:

São Paulo Research Foundation (FAPESP; grant numbers: 2012/50533-2; 2013/11480-3; 2015/04505-5 and 2016/22812-5).

National Council for Scientific and Technological Development (CNPq; grant number: 403063/2016-7).

ABSTRACT

In this study we aimed to evaluate how PAPP-A addition during *in vitro* maturation affected the IGF-1 quantification, transcript abundance related to cumulus oocyte complexes (COCs) and blastocysts quality, embryonic yield, as well as post-warming survival. We matured COCs through a 24 h treatment of TCM199 serum-free medium, either with PAPP-A

supplementation (100 ng/mL; PAPP-A group) or without (control). Maturation medium was collected for IGF-1 quantification, and matured COCs were used for *in vitro* fertilization and culturing. The PAPP-A group exhibited 1.27 times higher IGF-1 concentrations than control. A comparison of *in vitro* embryo production across the groups found no difference in cleavage rate, embryonic yield, and survival, 3 and 24 h post-cryopreservation. In PAPP-A oocytes, only *TXNRD1* was up-regulated. However, in PAPP-A cumulus cells, *VNN1* and *HDAC2* were up-regulated, while *AGPAT1*, *AGPAT9*, *FASN*, *CASP3*, *EGFR*, *HAS2*, *IMPDH1*, and *MTIF3* were down-regulated. Finally, in PAPP-A blastocysts, *CPT2*, *CASP9*, *DNMT3A*, *TFAM*, and *KRT8* were up-regulated, while *ATF4*, *CASP3*, and *IFITM3* were down-regulated. We concluded that PAPP-A addition increased IGF-1 but did not influence embryonic yield and survival. Nevertheless, elevated IGF-1 could improve embryo competence through modulating expression of genes involved with lipid metabolism, oocyte competence and apoptosis in COCs and blastocysts.

Keywords: insulin-like growth factor-binding protein, cumulus-oocyte complexes, *in vitro* embryo production, cryopreservation, cattle.

1 INTRODUCTION

Insulin-like growth factor-1 (IGF-1) is one of several growth factors involved in oocyte maturation, specifically in influencing gonadotrophin action during folliculogenesis and meiosis progression (Mazerbourg & Monget, 2018; Sakaguchi et al., 2002). The IGF system is composed of IGF-1 and IGF-2, along with their receptors IGF-1R and IGF-2R (Kane, Morgan, & Coonan, 1997; Mazerbourg & Monget, 2018). In the bovine reproductive system, IGF-1 functions have been confirmed by IGF-1R presence in the oocyte (Armstrong et al., 2000; Satrapa, Castilho, et al., 2013; Satrapa, Razza, et al., 2013) and throughout stages of early embryonic development, including the zygote, 2–4 cell embryo, 8–16 cell embryo,

morula, and blastocyst (Yaseen, Wrenzycki, Herrmann, Carnwath, & Niemann, 2001). This system stimulates cell proliferation, differentiation, migration, survival, and metabolism (Duan et al., 2005). In the reproductive system, these processes are important to oocyte maturation (Sakaguchi et al., 2002; Sirotkin, Dukesová, Makarevich, Kubek, & Bulla, 2000; Xia, Tekpetey, & Armstrong, 1994) and embryonic development (Velazquez, Zaraza, Oropeza, Webb, & Niemann, 2009).

IGF binding proteins (IGFBPs) and pregnancy-associated plasma protein-A (PAPP-A) both modulate IGF action during embryonic development. The former group modulates the IGF action during cumulus-oocyte complexes (COCs) maturation and in subsequent growth stages (Harvey, Kind, & Thompson, 2007; Heyner, Shi, Garside, & Smith, 1993; Kaye & Harvey, 1995; Luciano, Modina, Gandolfi, Lauria, & Armstrong, 2000). Six IGFBP types (1–6) (Mazerbourg & Monget, 2018) have been reported in oocytes and bovine embryos (Lonergan, Rizos, Gutierrez-Adan, Fair, & Boland, 2003; Satrapa, Castilho, et al., 2013; Satrapa, Razza, et al., 2013; Sawai et al., 2005).

The PAPP-A is a protein produced by the placenta (Petry et al., 2017) and isolated in 1974 from normal human pregnancy serum (Lin, Galbert, Kiefer, Spellacy, & Gall, 1974; Petry et al., 2017). In women *PAPPA* knockout, incomplete fetal development and deformities were observed (Lawrence et al., 1999), and in rodents, the PAPPA knockout mouse subjected to ovarian stimulation ovulated fewer COCs (Nyegaard et al., 2010).

In cattle, PAPP-A has been found to increase IGF-1 hrough degrading IGFBPs, including IGFBP2, 4, and 5 in cattle (Mazerbourg, Bondy, Zhou, & Monget, 2003; Rivera & Fortune, 2003). A cross-breed comparison found higher *PAPPA* mRNA expression, but decreased *IGF1*, *IGF1R*, *IGFBP2*, and *IGFBP4* expression in Nelore cows than in Holstein cows (Satrapa, Castilho, et al., 2013). Corroborating with these previous findings, the

abundance of *PAPPA* mRNA was greater in oocytes and cumulus cells of Gir and Holstein × Gir hybrids than in Holstein cows (Lopes et al., 2017).

However, most studies only investigated the effects of direct exogenous IGF-1 addition during *in vitro* maturation (IVM) of bovine oocytes (Meiyu, Liu, & Roth, 2015; Sakaguchi et al., 2002; Zhandi, Towhidi, Nasr-Esfahani, Eftekhari-Yazdi, & Zare-Shahneh, 2009). We therefore know very little regarding how PAPP-A modulates IGF-1 during IVM and how this modulation affects *in vitro* embryo production in cattle and COCs or blastocyst quality.

Here, we hypothesized that exogenous PAPP-A addition would release IGF-1 from IGFBPs, thus increasing IGF-1 in the IVM medium. Therefore, our aim was to investigate PAPP-A action on IGF-1, along with any subsequent impact on embryo production and blastocyst viability post-cryopreservation. We were also interested in how PAPP-A influences bovine COC and blastocyst transcriptional profiles.

2 RESULTS

2.1 IGF-1 quantification in IVM medium and accompanying effects on *in vitro* production and survival post warming

After IVM, PAPP-A addition increased IGF-1 in IVM medium by 1.27-fold compared with control (p = 0.030; Table 1). However, cleavage and blastocyst rates did not differ between groups (p = 0.694 and p = 0.539, respectively; Table 1). After *in vitro* culture (IVC), expanded blastocysts were vitrified and then warmed. Re-expansion rate after 3 and 24 h did not differ between groups (p > 0.05; Table 1).

Table 1. IGF-1 quantification, blastocyst yield, and embryonic survival. In the IVM, IGF-1 data are represented as fold change. To IVP, cleavage and blastocyst rates are represented as mean (%) ± S.E.M and n = replicate; and blastocyst re-expansion is shown as percentages evaluated by chi-square test.

Groups	IVM	IVP		Blastocyst Re-expansion	
	IGF-1†	Cleavage	Blastocysts	3 hours	24 hours
Control (n = 360 COCs)	1 ± 0.0	83.7 ± 1.66	38 ± 1.79	56 (28/50)	54 (27/50)
PAPP-A (n = 360 COCs)	1.27 ± 0.09*	84.6 ± 1.61	38.2 ± 2.71	56 (37/66)	43.9 (29/66)

* $p \leq 0.05$ in the same column

† insulin-like growth factor 1

IVM, *in vitro* maturation; IVP, *in vitro*-embryo production; COCs, cumulus-oocyte complexes

2.2 Transcript profile in oocytes, cumulus cells, and blastocysts

We compared gene expression patterns between experimental and control groups using heat maps. In oocyte samples, only *TXNRD1* expression (based on mRNA abundance) differed significantly between treatments, being higher for PAPP-A-treated cells ($p \leq 0.05$; Figure 1).

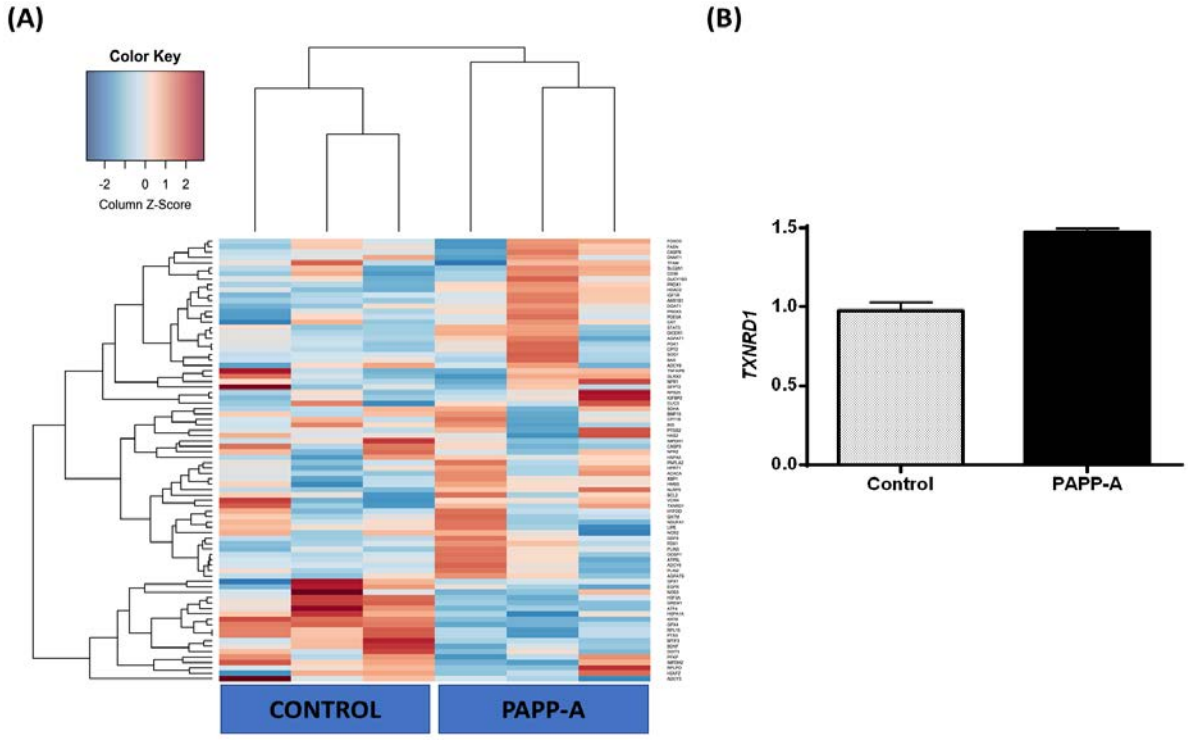

Figure 1. (A) Heat map showing gene expression differences between control and PAPP-A data in oocytes. Rows and columns are clustered by Euclidean distance and Ward-linkage hierarchical clustering. Color intensity represents the degree of between-group differences (based on Spearman's correlations). (B) Relative abundance of differentially expressed genes in oocytes ($p \leq 0.05$). Target genes were normalized with reference genes (*GAPDH*, *PPIA*, and *ACTB*), using the $2^{(-\Delta CT)}$ method. Data are means ± S.E.M. of three biological replicates tested by t test.

In cumulus cells, *VNN1* and *HDAC2* expression were higher in the PAPP-A group, whereas *AGPAT1*, *AGPAT9*, *EGFR*, *IMPDH1*, *FASN*, *HAS2*, *CASP3*, and *MTIF3* expression were lower ($p \leq 0.05$; Figure 2).

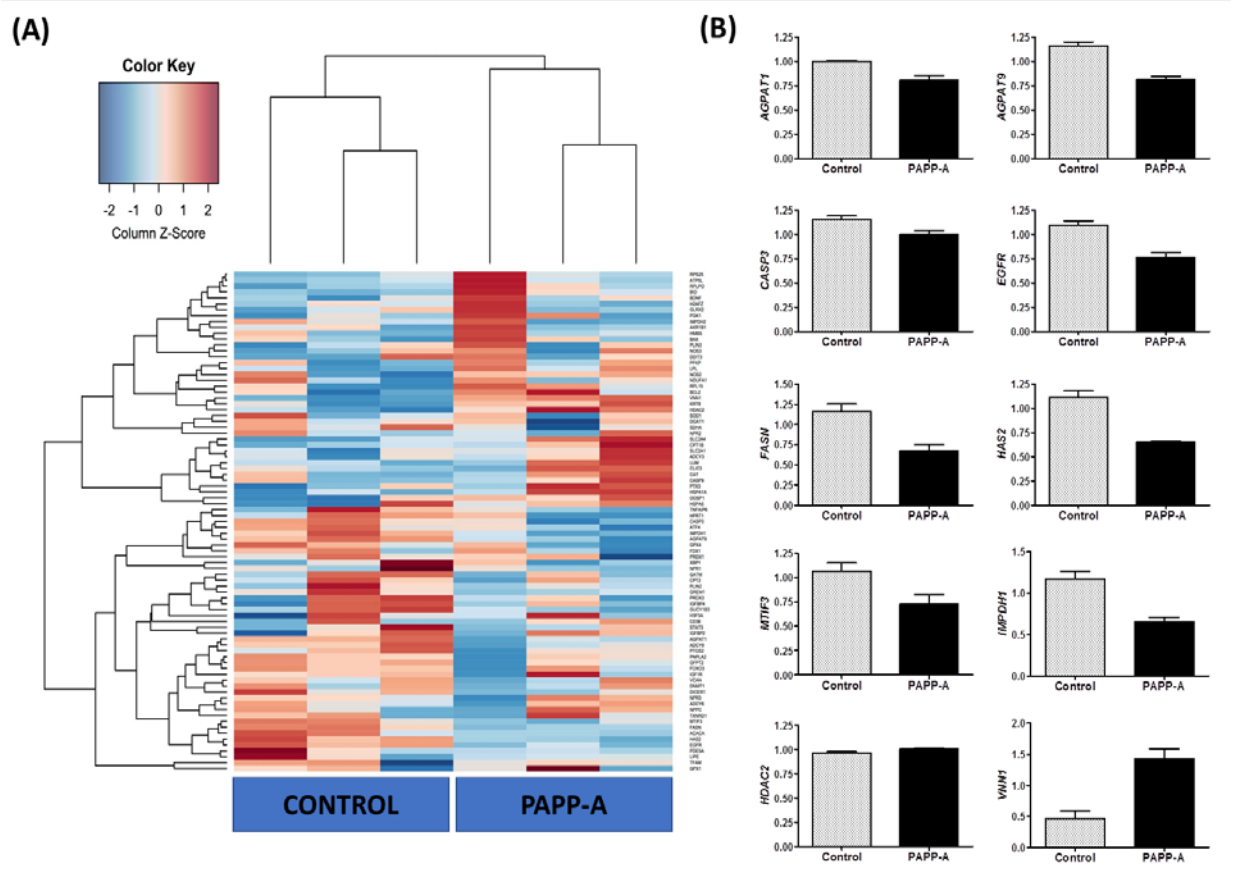

Figure 2. (A) Heat map showing gene expression differences between control and PAPP-A data in cumulus cells. Rows and columns are clustered by Euclidean distance and Ward-linkage hierarchical clustering. Color intensity represents the degree of between-group differences (based on Spearman's correlations). (B) Relative abundance of differentially expressed genes in cumulus cells ($p \leq 0.05$). Target genes were normalized with reference genes (*GAPDH*, *PPIA*, and *ACTB*), using the $2^{(-\Delta Ct)}$ method. Data are means ± S.E.M. of three biological replicates tested by t test.

In blastocysts, the PAPP-A group showed higher *DNMT3A*, *CASP9*, *CPT2*, *TFAM*, and *KRT8* expression, but lower *ATF4*, *IFITM3*, and *CASP3* expression ($p \leq 0.05$; Figure 3). To summary all data, a biological model was performed (Figure 4).

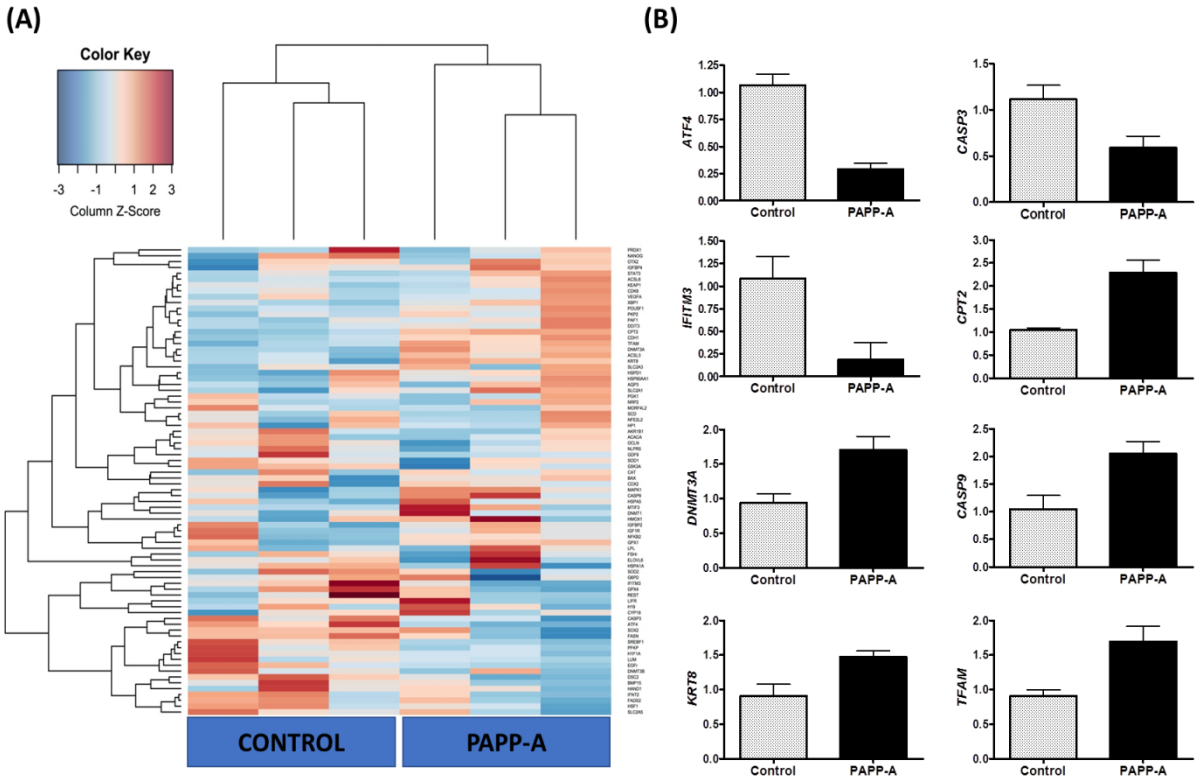

Figure 3. (A) Heat map showing gene expression differences between control and PAPP-A data in blastocysts. Rows and columns are clustered by Euclidean distance and Ward-linkage hierarchical clustering. Color intensity represents the degree of between-group differences (based on Spearman's correlations). (B) Relative abundance of differentially expressed genes in blastocysts ($p \leq 0.05$). Target genes were normalized with reference genes (*GAPDH*, *PPIA*, *ACTB* and *SDHA*), using the $2^{(-\Delta Ct)}$ method. Data are means ± S.E.M. of three biological replicates tested by t test.

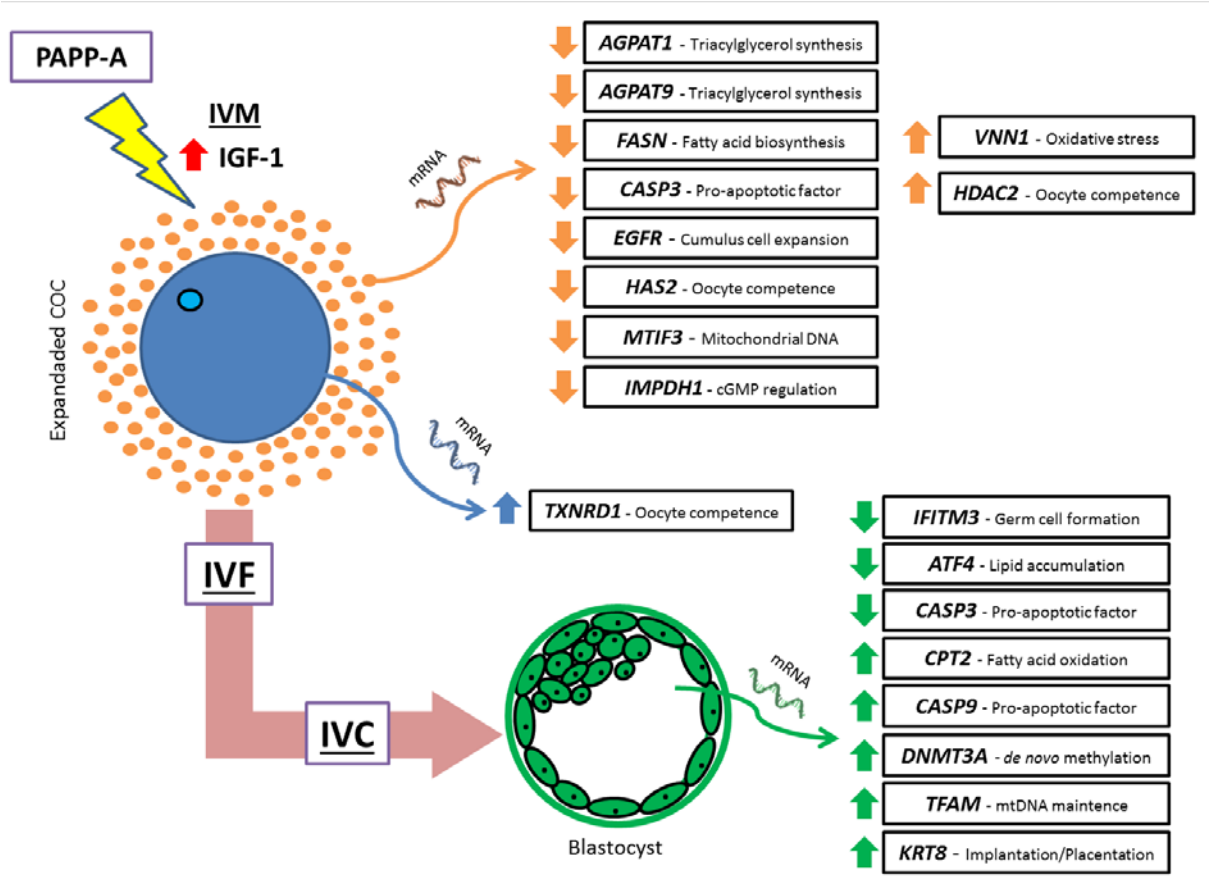

Figure 4. Effects of PAPP-A addition during *in vitro* bovine embryo production. In summary, we propose that the greater amount of IGF-1 into maturation medium by the PAPP-A action was able to promote a down-regulation in cumulus cells and an up-regulation in blastocysts of transcripts related to the quality of COCs and blastocysts, particularly, cellular pathways involved in lipid metabolism, apoptosis, and embryonic development. The yellow ray indicates the PAPP-A action on IVM of bovine COC. The orange, blue and green arrows indicates mRNA abundance levels in PAPP-A group (cumulus cells, oocytes and blastocysts; respectively). The red arrow indicates the IGF-1 into the IVM medium. PAPP-A: pregnancy-associated plasma protein-A, IGF-1: insulin-like growth factor 1, COC: cumulus-oocyte complex, IVM: *in vitro* maturation, IVF: *in vitro* fertilization and IVC: *in vitro* culture. The genes are represented in the bold boxes.

For a list of target genes that were not differentially expressed ($p > 0.05$) in tested cells, please see supplementary information (Table S1, S2, and S3).

3 DISCUSSION

To our knowledge, this study is the first to quantify IGF-1 after PAPP-A addition during IVM and examine the resultant effects on *in vitro* embryo production. Previous studies examining how exogenous IGF-1 addition influences cleavage rates and embryonic production have yielded inconsistent results. For instance, cleavage rates have been reported to increase (Palma, Müller, & Brem, 1997) or remain unaffected (Block, Wrenzycki, Niemann, Herrmann, & Hansen, 2008; Green & Day, 2013; Matsui, Takahashi, Hishinuma, & Kanagawa, 1995). Similarly, IGF-1 addition has both increased blastocyst rate (Green & Day, 2013; Matsui et al., 1995; Palma et al., 1997) and exerted no effect (Block et al., 2008). However, although we found that PAPP-A addition did no improve blastocyst production and cleavage rate, this result does not imply that embryonic quality would remain unaffected (Block et al., 2008).

In vitro embryos are more sensitive to cryopreservation because of lipid accumulation in their structure (Accorsi, Leão, Rocha-Frigoni, Perri, & Mingoti, 2016; Block et al., 2003; Meneghel et al., 2017) but the mechanism of this accumulation is unclear (Leão et al., 2015). Here, we found that PAPP-A treatment did not alter re-expansion rates of warmed blastocysts after 3 and 24 h. Although embryonic survival rate did not improve after warming, PAPP-A treatment increases IGF-1 (27% over) and modulates the expression of genes related to apoptotic effects, oocyte competence, and lipid metabolism in oocytes, cumulus cells and blastocysts.

The most interesting results were related to the regulation of genes associated with COC and blastocyst quality. Among oocytes, only one gene experienced altered expression (elevated transcript abundance) under PAPP-A treatment: *TXNRD1*, associated with oocyte competence and highly expressed in cells with strong proliferative activity (Jakupoglu et al., 2005). *TXNRD1* knockout mice showed embryonic lethality (Bondareva et al., 2007). On the

other hand, differences of gene expression were more apparent in cumulus cells, especially in genes related to lipid metabolism, apoptosis, oocyte competence, oxidative stress, meiosis resumption, and mitochondrial DNA transcription. In the PAPP-A group, *AGPAT1*, *AGPAT9*, *FASN*, *CASP3*, *EGFR*, *HAS2*, *MTIF3*, and *IMPDH1* were down-regulated, while *HDAC2* and *VNN1* were up-regulated. Additionally, lipid metabolism genes *AGPAT1*, *AGPAT9*, and *FASN* differed in transcript profiles under PAPP-A treatment. The first two genes participate in triacylglycerol synthesis and membrane-lipid biosynthesis (Körbes, Kulcheski, Margis, Margis-Pinheiro, & Turchetto-Zolet, 2016; Zeng et al., 2017). The third gene acts on long fatty acid biosynthesis (Ji et al., 2014; Z. Yang, Cappello, & Wang, 2015).

We also observed the modulation of three genes related to competence in cumulus cells, specifically the down-regulation of *EGFR* and *HAS2*, as well as the up-regulation of *HDAC2*. The first gene is involved in oocyte maturation and stimulates cumulus expansion through regulating hyaluronic acid synthesis (Bhardwaj, Ansari, Parmar, Chandra, & Sharma, 2016; Nagyova, 2012). Next, *HAS2* expression is correlated with oocyte developmental competence (Dunning et al., 2007), and down-regulation dampens hyaluronic acid synthesis (Thompson, Lane, & Gilchrist, 2007). Finally, *HDAC2* is expressed in the oocyte MII stage, suggesting its involvement in regulatory pathways affecting oocyte developmental competence (S. Wang et al., 2010; Xu, Chen, Zhang, Wei, & Cao, 2015). In addition, IGF components present in COCs accelerate nuclear maturation and promote oocyte competence (Satrapa, Castilho, et al., 2013). Even down-regulation of the first two genes, which would lead to the impairment of oocyte competition, there was no difference in the rates of cleavage and blastocyst production indicating, indirectly, high meiotic resumption.

Still in cumulus cells, *VNN1* and *CASP3* are respectively up and down-regulated. *VNN1* is a regulator of oxidative stress response, as well as a potential marker for follicular growth and differentiation (Nivet, Vigneault, Blondin, & Sirard, 2013). Its activation may be

209 the result of decreased oocyte competence from oxidative stress (Nivet et al., 2013). The
210 second gene is a pro-apoptotic factor activated only during apoptotic processes; IGF-1 is
211 thought to increase *CASP3* levels (Raile et al., 2003). The increase of an oxidative stress
212 controller and the decrease of a pro-apoptotic factor could suggest a positive effect of IGF-1,
213 mediated by PAPP-A. In the same way, *IMPDH1* was down regulated in cumulus cells. The
214 *IMPDH1* is specifically associated with cGMP regulation, which influences meiosis
215 resumption (Wigglesworth et al., 2013). The *MTIF3* gene is involved in mitochondrial DNA
216 transcription, and its expression decreases during the germinal vesicle (GV) to oocyte MII
217 stages (Mtango, Harvey, Latham, & Brenner, 2008). Taken together, the modulations of both
218 genes were related to normal oocyte maturation and reflect the further *in vitro* embryo
219 development during PAPP-A treatment.

220 Similar to cumulus cells, differential gene expression in blastocysts was related
221 to apoptosis, DNA methylation, gastrulation, energetic metabolism, implantation/placentation,
222 and lipid metabolism. The latter function is unsurprising given the known role of IGFs as
223 regulators of lipogenesis (Ruan & Lai, 2010). Lipid-metabolism genes *ATF4* and *CPT2* were
224 respectively down and up-regulated in PAPP-A blastocysts. Elevated *ATF4* expression is
225 related to high lipid exposure to oocytes and embryos that causes embryonic stress (Wu et al.,
226 2010; X. Yang et al., 2012). Thus, PAPP-A down-regulation of *ATF4* reduced lipid
227 availability to the embryo. Similarly, *CPT2* is associated with ATP production and lipid
228 reduction from fatty acid oxidation (Gentile et al., 2004; Guo et al., 2017). As described
229 above, we observed that post immediate re-expansion and 24 h re-expansion are similar and
230 although the IGF-1 had not been improved on embryo survival after cryopreservation, the
231 PAPP-A treatment increased the expression of genes related to lipid metabolism in both
232 cumulus cells (*AGPAT1*, *AGPAT9*, and *FASN*) and blastocysts (*ATF4* and *CPT2*) and maybe
233 this regulation could impact the lipids accumulation during *in vitro* embryo production,

probably decreasing lipids accumulation. However, future studies should further examine the link between PAPP-A and lipid metabolism in COCs and blastocysts.

Caspases are the regulators of apoptosis both extrinsic and intrinsic pathways, having CASP9 as CASP3 activator, and CASP3 cell death performer (Deb, Dey, Bang, Lee, & Kong, 2012). PAPP-A treatment also affected apoptosis genes in blastocysts, specifically up-regulating *CASP9* and down-regulating *CASP3*.

Other genes up-regulated under PAPP-A treatment were *DNMT3A*, *TFAM*, and *KRT8* in blastocysts. The first gene is directly involved in *de novo* methylation of hemi-methylated and unmethylated DNA as required for the *de novo* methylation, maintaining this process. DNA methylation acts on the repression or activation of genes related to embryonic development, including mechanisms of cell differentiation, tumorigenesis, and aging (Uysal, Akkoyunlu, & Ozturk, 2015). A similar *de novo* methylation gene (*IMPDH1*) was also present in cumulus cells, but was down-regulated.

Next, high *TFAM* expression is necessary for mtDNA maintenance (Rantanen, Jansson, Oldfors, & Larsson, 2001; Silva & Larsson, 2002), as demonstrated in *TFAM* knockout experiments that abolished mtDNA in the respiratory chain (J. Wang, Silva, Gustafsson, Rustin, & Larsson, 2001). The third gene is associated with placenta development and subsequent implantation (Bunel et al., 2015; Machado et al., 2012); *KRT8* appears to be essential for bovine embryonic development, as low transcript levels have been linked to embryonic lethality (Machado et al., 2012). Finally, we also observed down-regulation of *IFITM3* in PAPP-A blastocysts. This gene is a germ cell formation marker (Hu et al., 2015), but its exact role remains poorly understood (Shoubridge & Wai, 2007). In summary, the higher expression of *DNMT3A*, *TFAM*, and *KRT8*, together with down-regulation of *IFITM3* could reflect on blastocyst with remarkable characteristics to increases the possibility of pregnancy probably improving a better oxidative metabolism, coordinating adequately

embryonic DNA methylation to reach a correct implantation and later the maintenance of gestation.

We concluded that PAPP-A addition during IVM elevated IGF-1 without affecting cleavage rate, embryo yield, and post-devitrification embryonic survival. The treatment also positively modulated genes related to COC and blastocyst quality. Specifically, these genes are involved in lipid metabolism, apoptosis, and the development of blastocyst competence (e.g., regulation of implantation and initial embryo differentiation).

4 MATERIAL AND METHODS

Unless otherwise noted, all products were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). The experimental design is shown in Fig. 5.

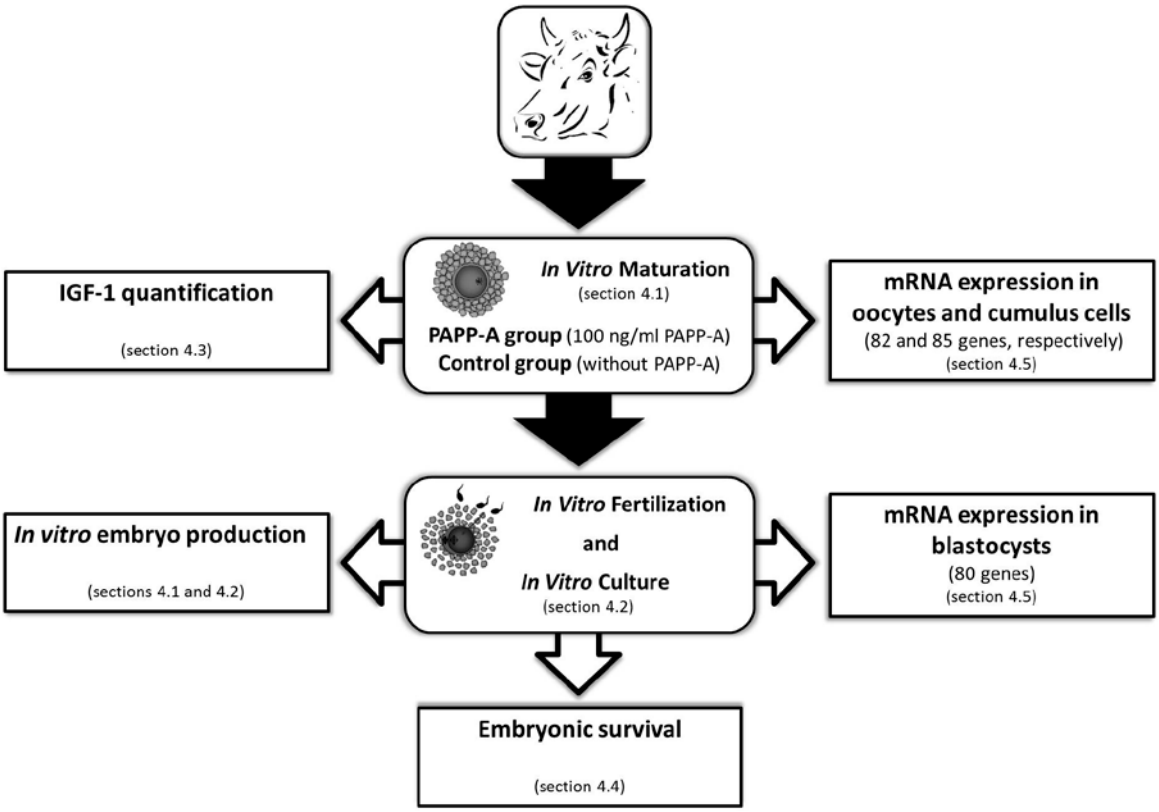

Figure 5. Experimental design to investigate the effects of the PAPP-A addition to the IVM medium of bovine COCs (20 per group). We investigated the effects of the PAPP-A on IGF-1 into IVM medium, gene expression in COCs, *in vitro* embryo production, gene expression in blastocysts and embryonic cryosurvival. PAPP-A: pregnancy-associated plasma protein-A, COC: cumulus-oocyte complex and IGF-1: insulin-like growth factor 1.

4.1 *In vitro* maturation of COCs

Ovaries of predominantly *Bos taurus indicus* were obtained from a local abattoir (Lençóis Paulista, São Paulo, Brazil; 22°35′41.8″S, 48°47′19.4″W) and transported to the laboratory in a thermal container (36°C) containing saline solution (0.9% w/v). Ovarian follicles (diameter 3–8 mm) were aspirated with a syringe and needle (18G). A stereomicroscope was used to select COCs with homogeneous cytoplasm and surrounded by three cumulus-cell layers for the experiment. The time between ovary collection and COC selection did not exceed 3 hours.

Selected COCs (n = 600) were incubated in IVM medium, comprising TCM199 with bicarbonate, bovine serum albumin (BSA, 5 mg/mL), pyruvate (0.22 mg/mL; 100 mM solution), amikacin (75 mg/mL), and follicular stimulating hormone (FSH, 0.1 UI/mL solution; Gonal-f, Merck Serono, Bari, Italy).

The COCs were randomly divided in two groups (20 per group): untreated control and PAPP-A treatment (100 ng/mL rhPappalysin-1; R&D Systems, Minneapolis, MN, USA). Both groups were deposited in 90 µL drops of IVM medium and covered with silicone oil (Quimesp Química, Guarulhos, SP, Brazil) for 24 h in a controlled environment (5.5% CO_2, humidified air, 38.5°C). The final concentration of 100 ng/mL PAPP-A was selected for the main experiment based on a pilot study testing 1, 10, and 100 ng/mL; only the highest concentration caused observable gene expression differences in oocytes, cumulus cells, and blastocysts.

298 At the end of each IVM, the medium was recovered for quantification of IGF-1 (six
299 replicates; section 4.2). Fifteen replicates were performed: six for *in vitro* embryo production
300 (section 4.3), three for embryonic cryosurvival (section 4.4), three for gene expression in
301 oocytes and cumulus cells (section 4.5), and three for gene expression in blastocysts (section
302 4.5).
303
304 **4.2 *In vitro* fertilization and culture**
305 Mature COCs were transferred to TALP drops (Tyrode's medium base) containing BSA (6
306 mg/mL), pyruvate (0.22 mg/mL; 100 mM), amikacin (75 μg/mL), heparin (2 mg/mL), and
307 PHE solution (44 μL/mL; 0.2 nM penicillamine; 0.1 mM hypotaurine; and 1 mM
308 epinephrine). Frozen-thawed semen from a Nelore bull (CRV Lagoa, Sertãozinho, SP, Brazil)
309 was separated with a Percoll gradient (90% and 45%), diluted to 1×10^6 sperm/mL, and
310 added into each drop (6 μL of semen/drop). After *in vitro* fertilization (18–20 h, 5.5% CO_2,
311 humidified air, and 38.5°C), probable zygotes were denuded via vortexing and transferred to
312 culture medium (SOFaaci [Synthetic Oviduct Fluid] with 5 mg/mL BSA , 2.5% fetal calf
313 serum (FCS), and 13 mM pyruvate). Embryos were cultured for 7 d in a humid atmosphere
314 with controlled gases (5% CO_2, 5% O_2, and 90% N_2) at 38.5ºC.
315 Fifty microliters of medium was renewed on day 3 and 5 of culture. Cleavage rate of
316 total fertilized COCs was evaluated (n = 6 replicates) on day 3. On day 7, blastocyst
317 formation rate was determined (n = 6 replicates) before they were collected and stored in
318 RNA-free microtubes at -80ºC, until RT-qPCR.
319 Three replicates of *in vitro* embryo production were also performed to obtain
320 expanded blastocysts for vitrification.
321
322

4.3 Quantification of IGF-1 with ELISA

To measure IGF-1 in IVM medium, in-house ELISA tests were performed. Medium from both treated and control groups were collected (n = 6) after 24 h of maturation, stored in 1.5 mL microtubes, and kept at -80°C until quantification.

Each well of a 96-well microplate (Thermo Fisher Scientific, Waltham, MA, USA) was coated with 100 µL of 0.0522 M carbonate buffer (pH = 9.6), containing 0.25 µg of goat anti-rabbit IgG. After overnight incubation at 4°C, a microplate washer (Thermo Fisher Scientific, Waltham) was used to wash the plate twice with 300 µL/well of 0.05% Tween 80 solution. Next, 300 µL of 1% PBS-BSA (pH = 7.2) was added per well as a blocking agent.

Because PAPP-A was assumed to have disrupted the bond between IGF-1 and IGFBPs, extraction of bound IGF-1 was not performed. Total IGF-1 was quantified with ELISA using the biotin-streptavidin-peroxidase amplification system in a competitive assay. Each well contained 60 µL of control or experimental medium, 100 µL of 1:250.000 diluted anti-IGF-1 antibody (Harbor-UCLA Medical Center, Carson, CA, USA), and 100 µL of biotinylated IGF-1 (0.06 ng/well). After 24 h of incubation at 4°C, two washes were performed. Next, 2.5 mU peroxidase/100 µL was added per well (streptavidin-POD conjugate 500 U/mL; Roche, São Paulo, SP, Brazil).

Microplates were covered to block light, incubated for 30 min at 4°C, and then washed twice before the addition of 100 µL substrate per well, comprising 2 mM 3.3′,5.5′-tetramethylbenzidine (TMB), 100 mM citric acid, 10.63 mM urea peroxide, 126.8 mM Na_2HPO_4, and 4% DMSO.

Covered microplates were incubated again for 40 min at 37°C, followed by the addition of 2 M sulfuric acid (30 µL/well) to stop substrate oxidation. Optical density determination at 450 nm was immediately performed with a Tecan microplate reader.

For dosage calculation, concentrations of standards were first log-transformed. Next, optical densities were converted to percentage of maximum binding (% B/B_0) and then transformed with the logit function. Transformations were performed through interpolation after linear regression.

4.4 Vitrification/warming and subsequent culture

On day 7 of *in vitro* culturing, expanded control (n = 50) and PAPP-A (n = 66) blastocysts were vitrified and warmed (three replicates/group) to evaluate post-warming embryonic survival.

Procedures followed protocol from Vitri-Ingá® (Ingámed, Maringá, PR, Brazil). High-quality expanded blastocysts were selected for vitrification following previous methods (Leão et al., 2015), removed from culture medium, and transferred to a plate containing 20 μL of maintenance solution. They were next transferred to equilibrium solution for 5 min and then to three 20 μL droplets of vitrification solution for 60 s. During the final 20 s, all embryos were removed, along with the last vitrification-solution droplet, and deposited on the end of a 0.7 mm thick polypropylene rod (up to eight embryos/rod), then dipped directly into liquid nitrogen (-196°C). Rods containing vitrified embryos were stored in a cryogenic cylinder at -196°C until warming.

Rods were removed from the cryogenic cylinder and immediately immersed for 1 min in 100 μL of warming solution 1 at 37°C. Subsequently, they were transferred to 50 μL of warming solution 2 for 3 min, and to 50 μL of warming solution 3 for 5 min. *In vitro* survival (i.e., embryonic re-expansion rates) were determined at 3 and 24 h post-reheating, upon placing embryos in four-well plates each containing 500 μL of SOF culture medium (20% FCS). Embryos were considerable survivors when presented blastocoel re-expansion without

presence of vacuoles. The embryonic re-expansion rate was calculated by the number of surviving embryos divided by the total of heated embryos.

4.5 Determining gene expression in cumulus cells, oocytes, and blastocysts through RT-qPCR

After 24 h of IVM, oocyte and cumulus cells (20 each/pool; three pools/experimental or control group) were separated through successive pipetting and vortexing. The oocyte (cumulus free) was stored at -80ºC until RNA extraction. The cumulus cells resulting from the separation (vortexing) was stored at -80ºC until RNA extraction.

Total RNA extraction was performed using Microkit RNeasy® (Qiagen, Valencia, CA, USA), following manufacturer's protocol. Total RNA concentration was measured in a Nanodrop 2000® Spectrophotometer. The 2100 Bioanalyzer® system (Agilent, Santa Clara, CA, USA) was used to analyze RNA quality. Only samples with an RNA Integrity Number (RIN) above seven (scale of 0-10) were used.

After IVC, blastocysts were frozen (-80ºC) as samples for RNA extraction (one replicate = three blastocysts; five pools/group). Total RNA was extracted using the PicoPure® RNA Isolation Kit (Thermo Fisher Scientific, Foster City, CA, USA) following manufacturer's protocol. Concentrations of total blastocyst RNA concentration and quality were determined following the same methods as for oocyte/cumulus cell RNA.

Reverse transcription of RNA was performed following manufacturer protocol in the High Capacity Kit (Applied Biosystem®, Foster City, CA, USA). 100 and 50 ng of RNA per sample was used for cumulus cells and blastocysts, respectively. All oocyte samples (10 µL) were used for reverse transcription.

Gene expression analysis was performed using Applied Biosystems™ TaqMan® Assays, specific for *Bos taurus*. Target genes numbered 82 in oocyte samples (Table S4), 85

396 in cumulus cell (Table S5), and 80 in blastocysts (Table S6). Prior to RT-qPCR, sequence-
397 specific pre-amplification was performed as follows: 1.25 µL assay mix (Taqman® Assay
398 pooled to a final concentration of 0.2× per assay), 2.5 µL TaqMan PreAmp Master Mix
399 (Thermo Fisher Scientific, Foster City), and 1.25 µL cDNA (5 ng/µL). The pre-amplification
400 thermocycling schedule was 95°C for 10 min (reactivation), 95ºC for 15 s (denaturing), and
401 14 cycles of 60ºC for 4 min (annealing and amplification). Amplicons were then diluted six-
402 fold.

403 The RT-qPCR reaction solution consisted of 2.25 µL cDNA (pre-amplified products),
404 2.5 µL of TaqMan Universal PCR 2× Master Mix (Thermo Fisher Scientific, Foster City), and
405 0.25 µL of 20× GE Sample Loading Reagent (Fluidigm, San Francisco, CA, USA). The assay
406 solution contained 2.5 µL of 20× TaqMan Gene Expression Assay (Thermo Fisher Scientific,
407 Foster City) and 2.5 µL of 2× Assay Loading Reagent (Fluidigm). Data collection involved
408 the 96.96 Dynamic Array™ Integrated Fluidic Circuits (Fluidigm) chip. After priming, the
409 chip was loaded with 5 µL each of assay and sample solutions. The Biomark HD System
410 (Fluidigm) and TaqMan GE 96 × 96 Standard was used for RT-qPCR. Thermocycling
411 conditions comprised one Thermal Mix stage (50ºC for 2 min, 70ºC for 20 min, 25ºC for 10
412 min), one Hot Start stage (50ºC for 2 min and 95ºC for 10 min), followed by 40 cycles of
413 denaturation (95ºC for 15 s), annealing, and extension (60ºC for 60 s).

414 Relative expression was calculated using ΔCt (Livak & Schmittgen, 2001) and
415 transformed into fold change ($2^{-\Delta Ct}$). To normalize oocyte and cumulus cell data, geometric
416 means of housekeeping genes *GAPDH*, peptidyl isomerase A (*PPIA*), and β-actin (*ACTB*)
417 genes were used. Blastocyst data normalization involved the additional inclusion of succinate
418 dehydrogenase complex flavoprotein subunit A (*SDHA*).

419

420

4.6 Statistical analysis

Cleavage, blastocyst development, and embryonic survival rates were calculated as percentages and arcsine-transformed. The IGF-1 concentration was expressed as fold change. The IGF-1, cleavage, and blastocysts rate were subjected to t-test. To embryonic survival, chi-square test was applied. For gene expression profile the bioinformatics analysis was performed using R version 3.0.1 (http://www.r-project.org) and Bioconductor (http://www.bioconductor.org/). Gene expression heat maps were generated using the averaged control group data for normalization. Target genes were sorted with hierarchical clustering using the *heatplot* function from the *made4* R package (Culhane, Thioulouse, Perrière, & Higgins, 2005). The analyses were performed using JMP software (SAS Institute, Cary, NC, USA). Differences were considered significant when $p \leq 0.05$.

ACKNOWLEDGEMENTS

The authors would like to thank the Multi-user Laboratory of Phytomedicines, Pharmacology, and Biotechnology (FitoFarmaTec), São Paulo Research Foundation (FAPESP), Coordination for the Improvement of Higher Education Personnel (CAPES), and National Council for Scientific and Technological Development (CNPq).

The authors are also grateful for the technical assistance of Raquiel Bueno Rodrigues and Andressa Carvalho.

REFERENCES

All the references were performed using the APA 6th style from EndNote Web extension to Microsoft Word 2010.

Accorsi, M. F., Leão, B. C., Rocha-Frigoni, N. A., Perri, S. H., & Mingoti, G. Z. (2016). Reduction in cytoplasmic lipid content in bovine embryos cultured in vitro with

linoleic acid in semi-defined medium is correlated with increases in cryotolerance. *Zygote, 24*, 485-494. doi:10.1017/S0967199415000428

Armstrong, D. G., Gutierrez, C. G., Baxter, G., Glazyrin, A. L., Mann, G. E., Woad, K. J., . . . Webb, R. (2000). Expression of mRNA encoding IGF-I, IGF-II and type 1 IGF receptor in bovine ovarian follicles. *J Endocrinol, 165*(1), 101-113.

Bhardwaj, R., Ansari, M. M., Parmar, M. S., Chandra, V., & Sharma, G. T. (2016). Stem Cell Conditioned Media Contains Important Growth Factors and Improves In Vitro Buffalo Embryo Production. *Anim Biotechnol, 27*(2), 118-125. doi:10.1080/10495398.2015.1118383

Block, J., Drost, M., Monson, R. L., Rutledge, J. J., Rivera, R. M., Paula-Lopes, F. F., . . . Hansen, P. J. (2003). Use of insulin-like growth factor-I during embryo culture and treatment of recipients with gonadotropin-releasing hormone to increase pregnancy rates following the transfer of in vitro-produced embryos to heat-stressed, lactating cows. *J Anim Sci, 81*(6), 1590-1602. doi:10.2527/2003.8161590x

Block, J., Wrenzycki, C., Niemann, H., Herrmann, D., & Hansen, P. J. (2008). Effects of insulin-like growth factor-1 on cellular and molecular characteristics of bovine blastocysts produced in vitro. *Mol Reprod Dev, 75*(5), 895-903. doi:10.1002/mrd.20826

Bondareva, A. A., Capecchi, M. R., Iverson, S. V., Li, Y., Lopez, N. I., Lucas, O., . . . Schmidt, E. E. (2007). Effects of thioredoxin reductase-1 deletion on embryogenesis and transcriptome. *Free Radic Biol Med, 43*(6), 911-923. doi:10.1016/j.freeradbiomed.2007.05.026

Bunel, A., Jorssen, E. P., Merckx, E., Leroy, J. L., Bols, P. E., & Sirard, M. A. (2015). Individual bovine in vitro embryo production and cumulus cell transcriptomic analysis to distinguish cumulus-oocyte complexes with high or low developmental potential. *Theriogenology, 83*(2), 228-237. doi:10.1016/j.theriogenology.2014.09.019

Culhane, A. C., Thioulouse, J., Perrière, G., & Higgins, D. G. (2005). MADE4: an R package for multivariate analysis of gene expression data. *Bioinformatics, 21*(11), 2789-2790. doi:10.1093/bioinformatics/bti394

Deb, G. K., Dey, S. R., Bang, J. I., Lee, J. G., & Kong, I. K. (2012). 9-cis Retinoic acid inhibits cumulus cell apoptosis during the maturation of bovine cumulus-oocyte-complexes. *J Anim Sci, 90*(6), 1798-1806. doi:10.2527/jas.2011-4340

Duan, X. X., Ou, J. S., Li, Y., Su, J. J., Ou, C., Yang, C., . . . Ban, K. C. (2005). Dynamic expression of apoptosis-related genes during development of laboratory hepatocellular carcinoma and its relation to apoptosis. *World J Gastroenterol, 11*(30), 4740-4744.

Dunning, K. R., Lane, M., Brown, H. M., Yeo, C., Robker, R. L., & Russell, D. L. (2007). Altered composition of the cumulus-oocyte complex matrix during in vitro maturation of oocytes. *Hum Reprod, 22*(11), 2842-2850. doi:10.1093/humrep/dem277

Gentile, L., Monti, M., Sebastiano, V., Merico, V., Nicolai, R., Calvani, M., . . . Zuccotti, M. (2004). Single-cell quantitative RT-PCR analysis of Cpt1b and Cpt2 gene expression in mouse antral oocytes and in preimplantation embryos. *Cytogenet Genome Res, 105*(2-4), 215-221. doi:10.1159/000078191

Green, C. J., & Day, M. L. (2013). Insulin-like growth factor 1 acts as an autocrine factor to improve early embryogenesis in vitro. *Int J Dev Biol, 57*(11-12), 837-844. doi:10.1387/ijdb.130044md

Guo, J., Lu, W. F., Liang, S., Choi, J. W., Kim, N. H., & Cui, X. S. (2017). Peroxisome

proliferator-activated receptor δ improves porcine blastocyst hatching via the regulation of fatty acid oxidation. *Theriogenology, 90*, 266-275. doi:10.1016/j.theriogenology.2016.11.018

Harvey, A. J., Kind, K. L., & Thompson, J. G. (2007). Regulation of gene expression in bovine blastocysts in response to oxygen and the iron chelator desferrioxamine. *Biol Reprod, 77*(1), 93-101. doi:10.1095/biolreprod.106.058826

Heyner, S., Shi, C. Z., Garside, W. T., & Smith, R. M. (1993). Functions of the IGFs in early mammalian development. *Mol Reprod Dev, 35*(4), 421-425; discussion 425-426. doi:10.1002/mrd.1080350417

Hu, X., Lu, H., Cao, S., Deng, Y. L., Li, Q. J., Wan, Q., & Yie, S. M. (2015). Stem cells derived from human first-trimester umbilical cord have the potential to differentiate into oocyte-like cells in vitro. *Int J Mol Med, 35*(5), 1219-1229. doi:10.3892/ijmm.2015.2132

Jakupoglu, C., Przemeck, G. K., Schneider, M., Moreno, S. G., Mayr, N., Hatzopoulos, A. K., . . . Conrad, M. (2005). Cytoplasmic thioredoxin reductase is essential for embryogenesis but dispensable for cardiac development. *Mol Cell Biol, 25*(5), 1980-1988. doi:10.1128/MCB.25.5.1980-1988.2005

Ji, S., Yang, R., Lu, C., Qiu, Z., Yan, C., & Zhao, Z. (2014). Differential Expression of PPARγ, FASN, and ACADM Genes in Various Adipose Tissues and Longissimus dorsi Muscle from Yanbian Yellow Cattle and Yan Yellow Cattle. *Asian-Australas J Anim Sci, 27*(1), 10-18. doi:10.5713/ajas.2013.13422

Kane, M. T., Morgan, P. M., & Coonan, C. (1997). Peptide growth factors and preimplantation development. *Hum Reprod Update, 3*(2), 137-157.

Kaye, P. L., & Harvey, M. B. (1995). The role of growth factors in preimplantation development. *Prog Growth Factor Res, 6*(1), 1-24.

Körbes, A. P., Kulcheski, F. R., Margis, R., Margis-Pinheiro, M., & Turchetto-Zolet, A. C. (2016). Molecular evolution of the lysophosphatidic acid acyltransferase (LPAAT) gene family. *Mol Phylogenet Evol, 96*, 55-69. doi:10.1016/j.ympev.2015.12.001

Lawrence, J. B., Oxvig, C., Overgaard, M. T., Sottrup-Jensen, L., Gleich, G. J., Hays, L. G., . . . Conover, C. A. (1999). The insulin-like growth factor (IGF)-dependent IGF binding protein-4 protease secreted by human fibroblasts is pregnancy-associated plasma protein-A. *Proc Natl Acad Sci U S A, 96*(6), 3149-3153.

Leão, B. C., Rocha-Frigoni, N. A., Cabral, E. C., Franco, M. F., Ferreira, C. R., Eberlin, M. N., . . . Mingoti, G. Z. (2015). Membrane lipid profile monitored by mass spectrometry detected differences between fresh and vitrified in vitro-produced bovine embryos. *Zygote, 23*(5), 732-741. doi:10.1017/S0967199414000380

Lin, T. M., Galbert, S. P., Kiefer, D., Spellacy, W. N., & Gall, S. (1974). Characterization of four human pregnancy-associated plasma proteins. *Am J Obstet Gynecol, 118*(2), 223-236.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods, 25*(4), 402-408. doi:10.1006/meth.2001.1262

Lonergan, P., Rizos, D., Gutierrez-Adan, A., Fair, T., & Boland, M. P. (2003). Oocyte and embryo quality: effect of origin, culture conditions and gene expression patterns. *Reprod Domest Anim, 38*(4), 259-267.

Lopes, A. C., Palhão, M. P., Fernandes, C., Sudano, M. J., Castilho, A., & Caixeta, E.

S. (2017). Differential expression of insulin-like growth factor family members in immature cumulus-oocyte complexes from dairy cows with different genotypes. *Reprod Domest Anim, 52*(6), 1067-1073. doi:10.1111/rda.13028

Luciano, A. M., Modina, S., Gandolfi, F., Lauria, A., & Armstrong, D. T. (2000). Effect of cell-to-cell contact on in vitro deoxyribonucleic acid synthesis and apoptosis responses of bovine granulosa cells to insulin-like growth factor-I and epidermal growth factor. *Biol Reprod, 63*(6), 1580-1585.

Machado, G. M., Caixeta, E. S., Lucci, C. M., Rumpf, R., Franco, M. M., & Dode, M. A. (2012). Post-hatching development of bovine embryos in vitro: the effects of tunnel preparation and gender. *Zygote, 20*(2), 123-134. doi:10.1017/S0967199411000086

Matsui, M., Takahashi, Y., Hishinuma, M., & Kanagawa, H. (1995). Insulin and insulin-like growth factor-I (IGF-I) stimulate the development of bovine embryos fertilized in vitro. *J Vet Med Sci, 57*(6), 1109-1111.

Mazerbourg, S., Bondy, C. A., Zhou, J., & Monget, P. (2003). The insulin-like growth factor system: a key determinant role in the growth and selection of ovarian follicles? a comparative species study. *Reprod Domest Anim, 38*(4), 247-258.

Mazerbourg, S., & Monget, P. (2018). Insulin-Like Growth Factor Binding Proteins and IGFBP Proteases: A Dynamic System Regulating the Ovarian Folliculogenesis. *Front Endocrinol (Lausanne), 9*, 134. doi:10.3389/fendo.2018.00134

Meiyu, Q., Liu, D., & Roth, Z. (2015). IGF-I slightly improves nuclear maturation and cleavage rate of bovine oocytes exposed to acute heat shock in vitro. *Zygote, 23*(4), 514-524. doi:10.1017/S096719941400015X

Meneghel, M., Dall'Acqua, P. C., Ambrogi, M., Leão, B. C. S., Rocha-Frigoni, N. A. S., & Mingoti, G. Z. (2017). Lipid content and cryotolerance of i*n vitro*-produced bovine embryos treated with forskolin before vitrification. In (Vol. 37, pp. 395-400). Rio de Janeiro: Pesquisa Veterinária Brasileira.

Mtango, N. R., Harvey, A. J., Latham, K. E., & Brenner, C. A. (2008). Molecular control of mitochondrial function in developing rhesus monkey oocytes and preimplantation-stage embryos. *Reprod Fertil Dev, 20*(7), 846-859.

Nagyova, E. (2012). Regulation of cumulus expansion and hyaluronan synthesis in porcine oocyte-cumulus complexes during in vitro maturation. *Endocr Regul, 46*(4), 225-235.

Nivet, A. L., Vigneault, C., Blondin, P., & Sirard, M. A. (2013). Changes in granulosa cells' gene expression associated with increased oocyte competence in bovine. *Reproduction, 145*(6), 555-565. doi:10.1530/REP-13-0032

Nyegaard, M., Overgaard, M. T., Su, Y. Q., Hamilton, A. E., Kwintkiewicz, J., Hsieh, M., . . . Giudice, L. C. (2010). Lack of functional pregnancy-associated plasma protein-A (PAPPA) compromises mouse ovarian steroidogenesis and female fertility. *Biol Reprod, 82*(6), 1129-1138. doi:10.1095/biolreprod.109.079517

Palma, G. A., Müller, M., & Brem, G. (1997). Effect of insulin-like growth factor I (IGF-I) at high concentrations on blastocyst development of bovine embryos produced in vitro. *J Reprod Fertil, 110*(2), 347-353.

Petry, C. J., Ong, K. K., Hughes, I. A., Acerini, C. L., Frystyk, J., & Dunger, D. B. (2017). Early Pregnancy-Associated Plasma Protein A Concentrations Are Associated With Third Trimester Insulin Sensitivity. *J Clin Endocrinol Metab, 102*(6), 2000-2008. doi:10.1210/jc.2017-00272

Raile, K., Hille, R., Laue, S., Schulz, A., Pfeifer, G., Horn, F., & Kiess, W. (2003). Insulin-like growth factor I (IGF-I) stimulates proliferation but also increases

597 caspase-3 activity, Annexin-V binding, and DNA-fragmentation in human
598 MG63 osteosarcoma cells: co-activation of pro- and anti-apoptotic pathways
599 by IGF-I. *Horm Metab Res, 35*(11-12), 786-793. doi:10.1055/s-2004-814140
600 Rantanen, A., Jansson, M., Oldfors, A., & Larsson, N. G. (2001). Downregulation of
601 Tfam and mtDNA copy number during mammalian spermatogenesis. *Mamm*
602 *Genome, 12*(10), 787-792.
603 Rivera, G. M., & Fortune, J. E. (2003). Proteolysis of insulin-like growth factor binding
604 proteins -4 and -5 in bovine follicular fluid: implications for ovarian follicular
605 selection and dominance. *Endocrinology, 144*(7), 2977-2987.
606 doi:10.1210/en.2002-0077
607 Ruan, W., & Lai, M. (2010). Insulin-like growth factor binding protein: a possible
608 marker for the metabolic syndrome? *Acta Diabetol, 47*(1), 5-14.
609 doi:10.1007/s00592-009-0142-3
610 Sakaguchi, M., Dominko, T., Yamauchi, N., Leibfried-Rutledge, M. L., Nagai, T., &
611 First, N. L. (2002). Possible mechanism for acceleration of meiotic progression
612 of bovine follicular oocytes by growth factors in vitro. *Reproduction, 123*(1),
613 135-142.
614 Satrapa, R. A., Castilho, A. S., Razza, E. M., Pegorer, M. F., Puelker, R., & Barros,
615 C. M. (2013). Differential expression of members of the IGF system in OPU-
616 derived oocytes from Nelore (Bos indicus) and Holstein (Bos taurus) cows.
617 *Anim Reprod Sci, 138*(3-4), 155-158. doi:10.1016/j.anireprosci.2013.02.023
618 Satrapa, R. A., Razza, E. M., Castilho, A. C., Simões, R. A., Silva, C. F., Nabhan, T.,
619 . . . Barros, C. M. (2013). Differential expression of IGF family members in
620 heat-stressed embryos produced in vitro from OPU-derived oocytes of Nelore
621 (Bos indicus) and Holstein (Bos taurus) cows. *Reprod Domest Anim, 48*(6),
622 1043-1048. doi:10.1111/rda.12211
623 Sawai, K., Kageyama, S., Moriyasu, S., Hirayama, H., Minamihashi, A., & Onoe, S.
624 (2005). Analysis of mRNA transcripts for insulin-like growth factor receptors
625 and binding proteins in bovine embryos derived from somatic cell nuclear
626 transfer. *Cloning Stem Cells, 7*(3), 189-198. doi:10.1089/clo.2005.7.189
627 Shoubridge, E. A., & Wai, T. (2007). Mitochondrial DNA and the mammalian oocyte.
628 *Curr Top Dev Biol, 77*, 87-111. doi:10.1016/S0070-2153(06)77004-1
629 Silva, J. P., & Larsson, N. G. (2002). Manipulation of mitochondrial DNA gene
630 expression in the mouse. *Biochim Biophys Acta, 1555*(1-3), 106-110.
631 Sirotkin, A. V., Dukesová, J., Makarevich, A. V., Kubek, A., & Bulla, J. (2000).
632 Evidence that growth factors IGF-I, IGF-II and EGF can stimulate nuclear
633 maturation of porcine oocytes via intracellular protein kinase A. *Reprod Nutr*
634 *Dev, 40*(6), 559-569.
635 Thompson, J. G., Lane, M., & Gilchrist, R. B. (2007). Metabolism of the bovine
636 cumulus-oocyte complex and influence on subsequent developmental
637 competence. *Soc Reprod Fertil Suppl, 64*, 179-190.
638 Uysal, F., Akkoyunlu, G., & Ozturk, S. (2015). Dynamic expression of DNA
639 methyltransferases (DNMTs) in oocytes and early embryos. *Biochimie, 116*,
640 103-113. doi:10.1016/j.biochi.2015.06.019
641 Velazquez, M. A., Zaraza, J., Oropeza, A., Webb, R., & Niemann, H. (2009). The role
642 of IGF1 in the in vivo production of bovine embryos from superovulated
643 donors. *Reproduction, 137*(2), 161-180. doi:10.1530/REP-08-0362
644 Wang, J., Silva, J. P., Gustafsson, C. M., Rustin, P., & Larsson, N. G. (2001).
645 Increased in vivo apoptosis in cells lacking mitochondrial DNA gene
646 expression. *Proc Natl Acad Sci U S A, 98*(7), 4038-4043.

doi:10.1073/pnas.061038798

Wang, S., Kou, Z., Jing, Z., Zhang, Y., Guo, X., Dong, M., . . . Gao, S. (2010). Proteome of mouse oocytes at different developmental stages. *Proc Natl Acad Sci U S A, 107*(41), 17639-17644. doi:10.1073/pnas.1013185107

Wigglesworth, K., Lee, K. B., O'Brien, M. J., Peng, J., Matzuk, M. M., & Eppig, J. J. (2013). Bidirectional communication between oocytes and ovarian follicular somatic cells is required for meiotic arrest of mammalian oocytes. *Proc Natl Acad Sci U S A, 110*(39), E3723-3729. doi:10.1073/pnas.1314829110

Wu, L. L., Dunning, K. R., Yang, X., Russell, D. L., Lane, M., Norman, R. J., & Robker, R. L. (2010). High-fat diet causes lipotoxicity responses in cumulus-oocyte complexes and decreased fertilization rates. *Endocrinology, 151*(11), 5438-5445. doi:10.1210/en.2010-0551

Xia, P., Tekpetey, F. R., & Armstrong, D. T. (1994). Effect of IGF-I on pig oocyte maturation, fertilization, and early embryonic development in vitro, and on granulosa and cumulus cell biosynthetic activity. *Mol Reprod Dev, 38*(4), 373-379. doi:10.1002/mrd.1080380404

Xu, X., Chen, D., Zhang, Z., Wei, Z., & Cao, Y. (2015). Molecular signature in human cumulus cells related to embryonic developmental potential. *Reprod Sci, 22*(2), 173-180. doi:10.1177/1933719114536471

Yang, X., Wu, L. L., Chura, L. R., Liang, X., Lane, M., Norman, R. J., & Robker, R. L. (2012). Exposure to lipid-rich follicular fluid is associated with endoplasmic reticulum stress and impaired oocyte maturation in cumulus-oocyte complexes. *Fertil Steril, 97*(6), 1438-1443. doi:10.1016/j.fertnstert.2012.02.034

Yang, Z., Cappello, T., & Wang, L. (2015). Emerging role of microRNAs in lipid metabolism. *Acta Pharm Sin B, 5*(2), 145-150. doi:10.1016/j.apsb.2015.01.002

Yaseen, M. A., Wrenzycki, C., Herrmann, D., Carnwath, J. W., & Niemann, H. (2001). Changes in the relative abundance of mRNA transcripts for insulin-like growth factor (IGF-I and IGF-II) ligands and their receptors (IGF-IR/IGF-IIR) in preimplantation bovine embryos derived from different in vitro systems. *Reproduction, 122*(4), 601-610.

Zeng, Y., Wang, C., Niu, Y., Chi, D., Xu, M., Si, L., . . . Li, J. (2017). The influence of delipidation on triglyceride and LIPIN1 of porcine embryos derived from parthenogenetic activation. *Reprod Domest Anim, 52*(5), 842-850. doi:10.1111/rda.12987

Zhandi, M., Towhidi, A., Nasr-Esfahani, M. H., Eftekhari-Yazdi, P., & Zare-Shahneh, A. (2009). Unexpected detrimental effect of Insulin like growth factor-1 on bovine oocyte developmental competence under heat stress. *J Assist Reprod Genet, 26*(11-12), 605-611. doi:10.1007/s10815-009-9364-0

SUPPLEMENTARY MATERIAL

Table S1. Relative abundance of target genes with similar expression ($p > 0.05$) in oocytes. Data represent the group mean ± S.E.M. from three biological replicates. Gene expressions were generated using the averaged control group data for normalization. Housekeeping genes *GAPDH*, peptidyl isomerase A (*PPIA*), and β-actin (*ACTB*) were used for data normalization.

Gene Symbol	Control	PAPP-A	*P*-value
ACACA	1.032 ± 0.084	1.540 ± 0.354	0.124
ADCY3	1.000 ± 0.247	1.087 ± 0.461	0.793
ADCY6	0.643 ± 0.139	0.755 ± 0.407	0.689
ADCY9	1.015 ± 0.163	0.751 ± 0.122	0.093
AGPAT1	1.002 ± 0.256	0.944 ± 0.168	0.761
AGPAT9	1.021 ± 0.174	1.045 ± 0.212	0.886
AKR1B1	1.067 ± 0.269	1.038 ± 0.137	0.881
ATF4	1.154 ± 0.552	0.536 ± 0.150	0.186
ATP5L	1.044 ± 0.156	1.089 ± 0.318	0.839
BAX	0.986 ± 0.272	0.785 ± 0.020	0.329
BCL2	1.000 ± 0.188	1.768 ± 0.494	0.101
BDNF	0.985 ± 0.187	0.760 ± 0.066	0.163
BID	0.901 ± 0.320	0.994 ± 0.351	0.753
BMP15	1.066 ± 0.221	1.267 ± 0.175	0.287
CASP3	1.099 ± 0.402	1.238 ± 0.215	0.634
CASP9	0.852 ± 0.300	0.661 ± 0.215	0.426
CAT	1.072 ± 0.277	0.902 ± 0.282	0.498
CD36	1.000 ± 0.504	0.845 ± 0.333	0.683
CLIC3	1.000 ± 0.913	1.754 ± 1.228	0.445
CPT1B	0.401 ± 0.089	0.517 ± 0.138	0.298
CPT2	1.128 ± 0.367	1.069 ± 0.113	0.812
DDIT3	1.167 ± 0.121	1.076 ± 0.048	0.324
DGAT1	1.192 ± 0.281	1.107 ± 0.177	0.684
DICER1	0.853 ± 0.184	0.864 ± 0.154	0.945
DNMT1	0.819 ± 0.068	0.739 ± 0.036	0.172
EGFR	1.000 ± 0.219	0.761 ± 0.173	0.216
FASN	1.045 ± 0.209	0.755 ± 0.245	0.195
FDX1	0.950 ± 0.145	0.964 ± 0.194	0.924
FOXO3	0.800 ± 0.117	0.693 ± 0.147	0.381
GATM	1.000 ± 0.951	2.686 ± 1.804	0.247
GDF9	1.046 ± 0184	1.188 ± 0.292	0.523
GFPT2	0.796 ± 0.104	0.816 ± 0.119	0.843
GLRX2	1.000 ± 0.364	1.036 ± 0.395	0.913
GPX1	1.188 ± 0.105	0.980 ± 0.130	0.100
GPX4	0.887 ± 0.384	0.712 ± 0.245	0.549
GREM1	0.999 ± 0.735	0.486 ± 0.117	0.350

GUCY1B3	0.890 ± 0.951	0.568 ± 0.199	0.619
H1FOO	0.972 ± 0.144	1.266 ± 0.235	0.153
H2AFZ	1.000 ± 0.312	0.736 ± 0.646	0.571
H3F3A	0.914 ± 0.317	0.740 ± 0.056	0.442
HAS2	1.150 ± 0.165	1.376 ± 0.125	0.136
HDAC2	1.056 ± 0.287	1.159 ± 0.118	0.610
HMBS	0.941 ± 0.329	1.223 ± 0.202	0.287
HPRT1	1.062 ± 0.118	1.216 ± 0.117	0.183
HSPA1A	1.002 ± 0.551	0.960 ± 0.105	0.907
HSPA5	1.054 ± 0.232	1.105 ± 0.139	0.762
IGF1R	1.178 ± 0.319	1.091 ± 0.198	0.713
IGFBP2	1.134 ± 0.102	1.218 ± 0.295	0.679
IMPDH1	0.489 ± 0.224	0.430 ± 0.078	0.702
IMPDH2	1.046 ± 0.337	1.149 ± 0.283	0.708
KRT8	0.876 ± 0366	0.736 ± 0.278	0.627
LIPE	0.879 ± 0.112	0.971 ± 0.332	0.686
MTIF3	1.157 ± 0.217	0.966 ± 0.013	0.267
NDUFA1	1.064 ± 0.314	1.418 ± 0.599	0.431
NLRP5	0.973 ± 0.224	1.227 ± 0.267	0.277
NOS2	0.766 ± 0.145	0.774 ± 0.182	0.952
NOS3	1.152 ± 0.488	0.940 ± 0.320	0.570
NPR1	1.000 ± 0.358	1.085 ± 0.510	0.827
NPR2	0.799 ± 0.389	0.940 ± 0.076	0.596
OOSP1	1.001 ± 0.251	1.128 ± 0.465	0.706
PDE5A	1.214 ± 0.301	0.980 ± 0.193	0.331
PFKP	0.902 ± 0.197	0.920 ± 0.226	0.923
PGK1	1.240 ± 0.468	1.135 ± 0.133	0.739
PLIN2	0.927 ± 0.121	1.008 ± 0.227	0.626
PLIN3	1.194 ± 0.127	1.292 ± 0.303	0.647
PNPLA2	0.833 ± 0.075	0.995 ± 0.138	0.170
PRDX1	0.902 ± 0.318	0.946 ± 0.188	0.851
PRDX3	0.993 ± 0.240	0.907 ± 0.192	0.651
PTGS2	1.008 ± 0.072	1.181 ± 0.132	0.137
PTX3	1.043 ± 0.788	1.028 ± 0.234	0.978
RPL15	1.043 ± 0.788	1.028 ± 0.234	0.978
RPLPO	1.013 ± 0.126	1.057 ± 0.188	0.752
RPS25	0.933 ± 0.268	1.213 ± 0.779	0.605
SDHA	1.004 ± 0.178	1.131 ± 0.173	0.427
SLC2A1	1.000 ± 0.556	0.828 ± 0.374	0.683
SOD1	1.069 ± 0.242	0.929 ± 0.041	0.424
STAT3	0.942 ± 0.323	1.003 ± 0.221	0.803
TFAM	1.000 ± 0.224	0.681 ± 0.414	0.323
TNFAIP6	1.000 ± 1.434	1.805 ± 1.609	0.553
VCAN	1.009 ± 0.221	1.332 ± 0.042	0.122
XBP1	0.984 ± 0.241	1.218 ± 0.244	0.303

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Table S2. Relative abundance of target genes with similar expression ($p > 0.05$) in cumulus cells. Data represent the group mean ± S.E.M from three biological replicates. Gene expressions were generated using the averaged control group data for normalization. Housekeeping genes *GAPDH*, peptidyl isomerase A (*PPIA*), and β-actin (*ACTB*) were used for data normalization.

Gene Symbol	Control	PAPP-A	*P*-value
ACACA	1.118 ± 0.268	0.670 ± 0.063	0.095
ADCY3	0.791 ± 0.277	1.028 ± 0.470	0.504
ADCY6	0.940 ± 0.079	0.873 ± 0.183	0.599
ADCY9	1.041 ± 0.061	0.740 ± 0.199	0.109
AKR1B1	1.039 ± 0.112	1.074 ± 0.217	0.820
ATF4	1.162 ± 0.097	0.959 ± 0.096	0.063
ATP5L	1.030 ± 0.033	1.243 ± 0.456	0.504
BAX	1.022 ± 0.121	1.121 ± 0.176	0.471
BCL2	0.943 ± 0.226	1.219 ± 0.146	0.163
BDNF	0.837 ± 0.781	0.956 ± 0.166	0.350
BID	0.916 ± 0.133	1.390 ± 0.509	0.245
CASP9	0.943 ± 0.137	1.019 ± 0.211	0.636
CAT	0.847 ± 0.161	0.962 ± 0.226	0.520
CD36	0.870 ± 0.302	0.810 ± 0.298	0.816
CLIC3	0.707 ± 0.298	0.916 ± 0.824	0.714
CPT1B	0.685 ± 0.039	0.985 ± 0.522	0.424
CPT2	1.050 ± 0.102	0.888 ± 0.133	0.173
DDIT3	0.965 ± 0.278	1.051 ± 0.344	0.755
DGAT1	0.988 ± 0.099	0.894 ± 0.255	0.599
DICER1	0.984 ± 0.130	0.733 ± 0.148	0.093
DNMT1	0.976 ± 0.113	0.865 ± 0.193	0.448
FDX1	1.079 ± 0.172	0.942 ± 0.201	0.422
FOXO3	1.015 ± 0.102	0.680 ± 0.314	0.198
GATM	0.962 ± 0.133	0.702 ± 0.134	0.076
GFPT2	1.096 ± 0.061	0.804 ± 0.227	0.147
GLRX2	1.122 ± 0.146	1.205 ± 0.526	0.815
GPX1	1.037 ± 0.072	1.046 ± 0.069	0.890
GPX4	1.180 ± 0.145	0.944 ± 0.194	0.171
GREM1	1.138 ± 0.367	0.522 ± 0.257	0.084
GUCY1B3	1.128 ± 0.291	0.583 ± 0.070	0.068
H2AFZ	1.057 ± 0.108	1.102 ± 0.591	0.907
H3F3A	1.139 ± 0.522	1.072 ± 0.318	0.860
HMBS	1.045 ± 0.123	1.129 ± 0.180	0.544
HPRT1	1.029 ± 0.075	0.873 ± 0.113	0.128
HSPA1A	0.668 ± 0.154	1.082 ± 0.431	0.232
HSPA5	0.827 ± 0.159	0.898 ± 0.099	0.554

IGF1R	1.003 ± 0.008	0.862 ± 0.211	0.368
IGFBP2	0.949 ± 0.384	0.880 ± 0.419	0.843
IGFBP4	1.236 ± 0.459	0.795 ± 0.220	0.235
IMPDH2	1.085 ± 0.108	1.014 ± 0.252	0.685
KRT8	0.556 ± 0.444	1.253 ± 0.439	0.129
LIPE	1.033 ± 0.602	0.685 ± 0.163	0.425
LPL	0.702 ± 0.233	1.169 ± 0.390	0.165
LUM	0.771 ± 0.136	0.976 ± 0.389	0.464
NDUFA1	0.992 ± 0.402	1.057 ± 0.330	0.841
NOS2	0.834 ± 0.406	1.100 ± 0.154	0.378
NOS3	1.104 ± 0.228	1.331 ± 0.607	0.593
NPPC	0.858 ± 0.489	0.792 ± 0.690	0.901
NPR1	1.089 ± 0.428	0.692 ± 0.147	0.245
NPR2	1.069 ± 0.389	1.013 ± 0.568	0.896
NPR3	0.972 ± 0.182	0.742 ± 0.649	0.606
OOSP1	0.741 ± 0.313	1.200 ± 0.322	0.151
PDE5A	1.150 ± 0.391	0.771 ± 0.059	0.233
PFKP	0.929 ± 0.156	1.061 ± 0.165	0.369
PGK1	1.029 ± 0.093	1.181 ± 0.272	0.441
PLIN2	1.074 ± 0.097	0.975 ± 0.040	0.215
PLIN3	1.081 ± 0.078	1.170 ± 0.259	0.622
PNPLA2	1.139 ± 0.140	0.764 ± 0.386	0.229
PRDX1	1.061 ± 0.048	0.988 ± 0.087	0.290
PRDX3	1.106 ± 0.252	0.816 ± 0.105	0.174
PTGS2	0.969 ± 0.089	0.712 ± 0.235	0.190
PTX3	0.844 ± 0.237	1.186 ± 0.5517	0.379
RPL15	0.924 ± 0.475	1.484 ± 0.363	0.184
RPLPO	1.025 ± 0.019	1.493 ± 0.605	0.312
RPS25	1.094 ± 0.120	1.481 ± 0.686	0.433
SDHA	1.101 ± 0.098	0.890 ± 0.186	0.180
SLC2A1	0.809 ± 0.155	0.960 ± 0.237	0.414
SLC2A4	0.738 ± 0.090	1.017 ± 0.264	0.200
SOD1	1.063 ± 0.146	1.035 ± 0.145	0.829
STAT3	1.001 ± 0.238	0.783 ± 0.247	0.334
TFAM	0.971 ± 0.196	0.964 ± 0.032	0.955
TNFAIP6	1.249 ± 0.509	0.686 ± 0.205	0.186
TXNRD1	0.926 ± 0.230	0.807 ± 0.273	0.596
VCAN	0.955 ± 0.088	0.806 ± 0.290	0.472
XBP1	1.086 ± 0.301	0.842 ± 0.230	0.331

Table S3. Relative abundance of target genes with similar expression ($p > 0.05$) in blastocysts. Data represent the group mean ± S.E.M from three biological replicates. Gene expressions were generated using the averaged control group data for normalization. Housekeeping genes *GAPDH*, peptidyl isomerase A (*PPIA*), β-actin (*ACTB*), and Succinate Dehydrogenase Complex Flavoprotein Subunit A (*SDHA)*, were used for data normalization.

Gene Symbol	Control	PAPP-A	*P*-value
ACACA	0.991 ± 0.251	0.957 ± 0.223	0.871
ACSL3	1.041 ± 0.174	1.426 ± 0.245	0.098
ACSL6	0.938 ± 0.233	1.538 ± 0.775	0.311
AKR1B1	0.913 ± 0.516	0.663 ± 0.496	0.577
AQP3	0.964 ± 0.171	1.287 ± 0.700	0.512
BAX	0.895 ± 0.272	1.053 ± 0.161	0.447
BMP15	1.193 ± 1.075	0.326 ± 0.394	0.296
CAT	0.953 ± 0.403	1.116 ± 0.203	0.576
CDH1	1.003 ± 0.059	1.647 ± 0.453	0.131
CDK6	1.044 ± 0.514	1.564 ± 1.095	0.514
CDX2	0.957 ± 0.237	0.983 ± 0.024	0.866
CYP19	1.228 ± 0.660	1.955 ± 0.840	0.307
DDIT3	0.950 ± 0.158	2.257 ± 1.484	0.266
DNMT1	0.939 ± 0.170	1.192 ± 0.334	0.328
DNMT3B	1.101 ± 0.244	0.933 ± 0.162	0.383
DSC2	0.929 ± 0.223	0.549 ± 0.112	0.079
EGFR	1.033 ± 0.379	0.669 ± 0.010	0.238
ELOVL6	1.058 ± 0.025	1.032 ± 0.283	0.890
FADS2	0.955 ± 0.520	0.578 ± 0.342	0.362
FASN	0.976 ± 0.011	0.742 ± 0.135	0.095
FSHR	1.058 ± 0.654	1.154 ± 1.078	0.902
G5K3A	1.052 ± 0.063	0.933 ± 0.108	0.189
G6PD	1.028 ± 0.025	0.986 ± 0.199	0.749
GDF9	1.087 ± 1.290	0.359 ± 0.400	0.436
GPX1	1.011 ± 0.370	1.371 ± 0.220	0.236
GPX4	0.936 ± 0.235	0.649 ± 0.205	0.188
H19	0.807 ± 0.985	1.038 ± 1.094	0.799
H1F1A	0.923 ± 0.606	0.407 ± 0.114	0.277
HAND1	1.166 ± 0.874	0.843 ± 0.362	0.601
HMOX1	1.182 ± 0.295	1.695 ± 0.597	0.276
HP1	1.025 ± 0.203	1.095 ± 0.221	0.707
HSF1	0.990 ± 0.409	0.624 ± 0.201	0.261
HSP90AA1	1.002 ± 0.337	1.554 ± 0.674	0.295
HSPA1A	0.976 ± 0.197	0.619 ± 0.660	0.452
HSPA5	0.901 ± 0.175	1.052 ± 0.127	0.297
HSPD1	1.010 ± 0.244	1.322 ± 0.291	0.231

IFNT2	0.655 ± 0.384	0.363 ± 0.177	0.323
IGF1R	1.020 ± 0.602	1.155 ± 0.172	0.741
IGFBP2	0.993 ± 0.189	1.055 ± 0.047	0.633
IGFBP4	1.024 ± 0.326	1.409 ± 0.181	0.168
KEAP1	0.996 ± 0.293	1.325 ± 0.523	0.409
LIFR	0.540 ± 0.301	0.746 ± 0.939	0.747
LPL	1.000 ± 1.069	1.343 ± 1.192	0.729
LUM	1.124 ± 1.864	0.025 ± 0.029	0.415
MAPK1	1.021 ± 0.237	1.391 ± 0.042	0.110
MORF4L2	1.016 ± 0.484	0.948 ± 0.420	0.863
MTIF3	0.800 ± 0.182	1.454 ± 0.672	0.230
NANOG	1.156 ± 0.549	1.057 ± 0.461	0.823
NFE2L2	0.995 ± 0.217	1.139 ± 0.543	0.702
NFKB2	0.932 ± 0.317	0.998 ± 0.033	0.756
NLPR5	1.000 ± 0.643	0.651 ± 0.656	0.547
NRP2	0.920 ± 0.566	1.327 ± 0.908	0.552
OCLN	1.022 ± 0.158	0.862 ± 0.231	0.385
OTX2	1.112 ± 0.228	1.277 ± 0.234	0.430
PAF1	0.992 ± 0.091	1.640 ± 0.769	0.281
PFKP	0.941 ± 0.742	0.144 ± 0.145	0.200
PGK1	1.000 ± 0.372	1.043 ± 0.710	0.932
PKP2	1.042 ± 0.201	1.477 ± 0.412	0.202
POU5F1	0.979 ± 0.230	1.651 ± 0.697	0.232
PRDX1	1.056 ± 0.320	1.045 ± 0.348	0.969
REST	1.107 ± 0.448	0.898 ± 0.197	0.517
SCD	1.052 ± 0.232	1.247 ± 0.912	0.751
SLC2A1	1.052 ± 0.093	1.308 ± 0.161	0.091
SLC2A3	0.913 ± 0.255	1.275 ± 0.525	0.364
SLC2A5	1.008 ± 0.274	0.783 ± 0226	0.338
SOD1	1.028 ± 0.238	0.857 ± 0.353	0.529
SOD2	1.075 ± 0.681	0.648 ± 0.503	0.435
SOX2	1.067 ± 0.089	0.841 ± 0.256	0.263
SREBF1	1.185 ± 0.657	0.247 ± 0.055	0.131
STAT3	1.121 ± 0.167	1.392 ± 0.333	0.298
VEGFA	0.927 ± 0.486	1.356 ± 0.750	0.459
XBP1	0.981 ± 0.359	1.655 ± 0.594	0.183

716 **Table S4.** Housekeeping and target genes analyzed in oocytes samples by RT-qPCR.

Gene Symbol	Assys ID[†]	Gene Name
ACACA	Bt03213389_m1	Acetyl CoA Carboxylase
ACTB	Bt03279174_g1	Actin, Beta (Housekeeping)
ADCY3	Bt04289077_m1	Adenylate Cyclase 3
ADCY6	Bt03816767_m1	Adenylate Cyclase 6
ADCY9	Bt04287024_m1	Adenylate Cyclase 9
AGPAT1	Bt03224587_g1	1-Acylglycerol-3-Phosphate O-Acyltransferase 1
AGPAT9	Bt04292093_m1	1-Acylglycerol-3-Phosphate O-Acyltransferase 9
AKR1B1	Bt03218049_g1	Aldo-Keto Reductase Family 1, Member B1
ATF4	Bt03221057_m1	Activating Transcription Factor 4
ATP5L	Bt03210836_g1	ATP synthase, H+ transporting, mitochondrial Fo complex subunit E
BAX	Bt03211777_g1	BCL2-Associated X Protein
BCL2	Bt04298952_m1	B-cell CLL/Lymphoma 2
BDNF	Bt03287437_s1	Brain-Derived Neurotrophic Factor
BID	Bt03241255_m1	BH3 Interacting Domain Death Agonist
BMP15	Bt03286494_u1	Bone Morphogenetic Protein 15
CASP3	Bt03250954_g1	Caspase 3, Apoptosis-Related Cysteine Peptidase
CASP9	Bt04282453_m1	Caspase 9, Apoptosis-Related Cysteine Peptidase
CAT	Bt03228713_m1	Catalase
CD36	Bt03212335_mH	CD36 molecule (thrombospondin receptor)
CLIC3	Bt03263038_m1	Chloride Intracellular Channel 3
CPT1B	Bt03244645_m1	Carnitine Palmitoyltransferase 1B
CPT2	Bt03233823_m1	Carnitine Palmitoyltransferase 2
DDIT3	Bt03251320_g1	DNA-Damage-Inducible Transcript 3
DGAT1	Bt03251719_g1	Diacylglycerol O-Acyltransferase 1
DICER1	Bt03217754_m1	Dicer 1, Ribonuclease III
DNMT1	Bt03224737_m1	DNA (Cytosine-5-)-Methyltransferase 1
EGFR	AJT96D7	Epidermal Growth Factor - Receptor
FASN	Bt03210485_m1	Fatty Acid Synthase
FDX1	Bt03217449_m1	Ferrodoxin 1
FOXO3	Bt03649334_s1	Forkhead Box O3
GAPDH	Bt03210912_g1	Glyceraldehyde-3-Phosphate Dehydrogenase (Housekeeping)
GATM	Bt03237896_m1	Glycine Amidinotransferase
GDF9	Bt03223996_m1	Growth Differentiation Factor 9
GFPT2	Bt03250351_m1	Glutamine-Fructose-6-Phosphate Transaminase 2
GLRX2	Bt03229700_m1	Glutaredoxin 2
GPX1	Bt03259217_g1	Glutathione Peroxidase 1
GPX4	Bt03259611_m1	Glutathione peroxidase 4
GREM1	Bt03255355_m1	Gremlin 1
GUCY1B3	Bt03215602_m1	Guanylate Cyclase 1, Soluble, Beta 3
H1FOO	Bt03228652_g1	H1 Histone Family, Member O, Oocyte-Specific
H2AFZ	Bt03216346_g1	H2A Histone Family, Member Z
H3F3A	Bt03278804_g1	H3 Histone, Family 3[a]
HAS2	Bt03212695_g1	Hyaluronan Synthase 2

HDAC2	Bt03244871_m1	Histone Deacetylase 2
HMBS	Bt03234763_m1	Hydroxymethylbilane Synthase
HPRT1	Bt03225311_g1	Hypoxanthine Phosphoribosyltransferase 1
HSPA1A	Bt03292670_g1	Heat Shock 70kDa Protein 1A
HSPA5	Bt03244880_m1	Glucose-Regulated Protein, 78kDa
IGF1R	Bt03649217_m1	Insulin-Like Growth Factor 1 Receptor
IGFBP2	Bt01040719_m1	Insulin-Like Growth Factor Binding Protein 2
IMPDH1	Bt00995384_m1	IMP (inosine 5'-monophosphate) Dehydrogenase 1
IMPDH2	Bt03226238_g1	IMP (inosine 5'-monophosphate) Dehydrogenase 2
KRT8	Bt03225178_g1	Keratin 8
LIPE	Bt03253691_m1	Lipase, Hormone-Sensitive (HSL)
MTIF3	Bt03231844_m1	Mitochondrial Translational Initiation Factor 3
NDUFA1	Bt03216720_g1	NADH: Ubiquinone Oxidoreductase Subunit A1
NLRP5	Bt03218031_m1	NLR Family, Pyrin Domain Containing 5
NOS2	Bt03249597_m1	Nitric Oxide Synthase 2, Inducible
NOS3	Bt03217679_m1	Nitric Oxide Synthase 3
NPR1	Bt04297034_g1	Natriuretic Peptide Receptor 1
NPR2	Bt04316732_m1	Natriuretic Peptide Receptor 2
OOSP1	Bt03233533_g1	Oocyte-Secreted Protein 1
PDE5A	Bt03214261_m1	Phosphodiesterase 5A, cGMP-Specific
PFKP	Bt04316551_m1	Phosphofructokinase
PGK1	Bt03225854_mH	Phosphoglycerate Kinase 1
PLIN2	Bt03212182_m1	Perilipin 2
PLIN3	Bt03230537_m1	Perilipin 3
PNPLA2	Bt03234129_g1	Patatin-Like Phospholipase Domain Containing 2
PPIA	Bt03224617_g1	Peptidylprolyl Isomerase A (Housekeeping)
PRDX1	Bt03223684_m1	Peroxiredoxin-1
PRDX3	Bt03214402_m1	peroxiredoxin 3
PTGS2	Bt03214489_m1	Prostaglandin-Endoperoxide Synthase 2
PTX3	Bt03249011_m1	Pentraxin 3, Long
RPL15	Bt03288449_g1	Ribosomal Protein L15
RPLPO	Bt03218086_m1	Ribosomal Protein, Large, P0
RPS25	Bt03220440_g1	Ribosomal Protein S25
SDHA	Bt04307509_m1	Succinate Dehydrogenase Complex Flavoprotein Subunit A
SLC2A1	Bt03215314_m1	Solute carrier family 2 (facilitated glucose transporter), member 1
SOD1	Bt03215423_g1	Superoxide dismutase 1, soluble
STAT3	Bt03259871_g1	Signal Transducer and Activator of Transcription 3
TFAM	Bt03260078_m1	Transcription Factor A, Mitochondrial
TNFAIP6	Bt03210223_m1	Tumor necrosis factor, alpha-induced protein 6
TXNRD1	Bt03215471_m1	Thioredoxin reductase 1
VCAN	Bt03217633_m1	Versican
XBP1	Bt03227621_g1	X-Box Binding Protein 1

† Thermo Fisher Scientific (Wilmington, DE, USA)

720 **Table S5.** Housekeeping and target genes analyzed in cumulus cells samples by RT-qPCR.

Gene Symbol	Assys ID[†]	Gene Name
ACACA	Bt03213389_m1	Acetyl CoA Carboxylase
ACTB	Bt03279174_g1	Actin, Beta (Housekeeping)
ADCY3	Bt04289077_m1	Adenylate Cyclase 3
ADCY6	Bt03816767_m1	Adenylate Cyclase 6
ADCY9	Bt04287024_m1	Adenylate Cyclase 9
AGPAT1	Bt03224587_g1	1-Acylglycerol-3-Phosphate O-Acyltransferase 1
AGPAT9	Bt04292093_m1	1-Acylglycerol-3-Phosphate O-Acyltransferase 9
AKR1B1	Bt03218049_g1	Aldo-Keto Reductase Family 1, Member B1
ATF4	Bt03221057_m1	Activating Transcription Factor 4
ATP5L	Bt03210836_g1	ATP Synthase, H+ Transporting, Mitochondrial Fo Complex Subunit E
BAX	Bt03211777_g1	BCL2-Associated X Protein
BCL2	Bt04298952_m1	B-cell CLL/Lymphoma 2
BDNF	Bt03287437_s1	Brain-Derived Neurotrophic Factor
BID	Bt03241255_m1	BH3 Interacting Domain Death Agonist
CASP3	Bt03250954_g1	Caspase 3, Apoptosis-Related Cysteine Peptidase
CASP9	Bt04282453_m1	Caspase 9, Apoptosis-Related Cysteine Peptidase
CAT	Bt03228713_m1	Catalase
CD36	Bt03212335_mH	CD36 molecule (thrombospondin receptor)
CLIC3	Bt03263038_m1	Chloride Intracellular Channel 3
CPT1B	Bt03244645_m1	Carnitine Palmitoyltransferase 1B
CPT2	Bt03233823_m1	Carnitine Palmitoyltransferase 2
DDIT3	Bt03251320_g1	DNA-Damage-Inducible Transcript 3
DGAT1	Bt03251719_g1	Diacylglycerol O-Acyltransferase 1
DICER1	Bt03217754_m1	Dicer 1, Ribonuclease III
DNMT1	Bt03224737_m1	DNA (Cytosine-5-)-Methyltransferase 1
EGFR	AJT96D7	Epidermal Growth Factor - Receptor
FASN	Bt03210485_m1	Fatty Acid Synthase
FDX1	Bt03217449_m1	Ferrodoxin 1
FOXO3	Bt03649334_s1	Forkhead Box O3
GAPDH	Bt03210912_g1	Glyceraldehyde-3-Phosphate Dehydrogenase (Housekeeping)
GATM	Bt03237896_m1	Glycine Amidinotransferase
GFPT2	Bt03250351_m1	Glutamine-Fructose-6-Phosphate Transaminase 2
GLRX2	Bt03229700_m1	Glutaredoxin 2
GPX1	Bt03259217_g1	Glutathione Peroxidase 1
GPX4	Bt03259611_m1	Glutathione Peroxidase 4
GREM1	Bt03255355_m1	Gremlin 1
GUCY1B3	Bt03215602_m1	Guanylate Cyclase 1, Soluble, Beta 3
H2AFZ	Bt03216346_g1	H2A Histone Family, Member Z
H3F3A	Bt03278804_g1	H3 Histone, Family 3[a]
HAS2	Bt03212695_g1	Hyaluronan Synthase 2
HDAC2	Bt03244871_m1	Histone Deacetylase 2
HMBS	Bt03234763_m1	Hydroxymethylbilane Synthase
HPRT1	Bt03225311_g1	Hypoxanthine Phosphoribosyl Transferase 1

HSPA1A	Bt03292670_g1	Heat Shock 70kDa Protein 1A
HSPA5	Bt03244880_m1	Glucose-Regulated Protein, 78kDa
IGF1R	Bt03649217_m1	Insulin-Like Growth Factor 1 Receptor
IGFBP2	Bt01040719_m1	Insulin-Like Growth Factor Binding Protein 2
IGFBP4	Bt03259500_m1	Insulin-Like Growth Factor Binding Protein 4
IMPDH1	Bt00995384_m1	IMP (inosine 5'-monophosphate) Dehydrogenase 1
IMPDH2	Bt03226238_g1	IMP (inosine 5'-monophosphate) Dehydrogenase 2
KRT8	Bt03225178_g1	Keratin 8
LIPE	Bt03253691_m1	Lipase, Hormone-Sensitive (HSL)
LPL	Bt03240493_m1	Lipoprotein Lipase
LUM	Bt03211920_m1	Lumican
MTIF3	Bt03231844_m1	Mitochondrial Translational Initiation Factor 3
NDUFA1	Bt03216720_g1	NADH: Ubiquinone Oxidoreductase Subunit A1
NOS2	Bt03249597_m1	Nitric Oxide Synthase 2, Inducible
NOS3	Bt03217679_m1	Nitric Oxide Synthase 3
NPPC	Bt03212844_m1	Natriuretic Peptide C (CNP)
NPR1	Bt04297034_g1	Natriuretic Peptide Receptor 1
NPR2	Bt04316732_m1	Natriuretic Peptide Receptor 2
NPR3	Bt03212867_m1	Natriuretic Peptide Receptor 3
OOSP1	Bt03233533_g1	Oocyte-Secreted Protein 1
PDE5A	Bt03214261_m1	Phosphodiesterase 5A, cGMP-Specific
PFKP	Bt04316551_m1	Phosphofructokinase
PGK1	Bt03225854_mH	Phosphoglycerate Kinase 1
PLIN2	Bt03212182_m1	Perilipin 2
PLIN3	Bt03230537_m1	Perilipin 3
PNPLA2	Bt03234129_g1	Patatin-Like Phospholipase Domain Containing 2
PPIA	Bt03224617_g1	Peptidylprolyl Isomerase A (Housekeeping)
PRDX1	Bt03223684_m1	Peroxiredoxin-1
PRDX3	Bt03214402_m1	Peroxiredoxin 3
PTGS2	Bt03214489_m1	Prostaglandin-Endoperoxide Synthase 2
PTX3	Bt03249011_m1	Pentraxin 3, Long
RPL15	Bt03288449_g1	Ribosomal Protein L15
RPLP0	Bt03218086_m1	Ribosomal Rrotein, Large, P0
RPS25	Bt03220440_g1	Ribosomal Protein S25
SDHA	Bt04307509_m1	Succinate Dehydrogenase Complex Flavoprotein Subunit A
SLC2A1	Bt03215314_m1	Solute Carrier Family 2 (facilitated glucose transporter), Member 1
SLC2A4	Bt03215316_m1	Solute Carrier Family 2 (facilitated glucose transporter), Member 4
SOD1	Bt03215423_g1	Superoxide Dismutase 1, Soluble
STAT3	Bt03259871_g1	Signal Transducer and Activator of Transcription 3
TFAM	Bt03260078_m1	Transcription Factor A, Mitochondrial
TNFAIP6	Bt03210223_m1	Tumor Necrosis Factor, Alpha-Induced Protein 6
TXNRD1	Bt03215471_m1	Thioredoxin Reductase 1
VCAN	Bt03217633_m1	Versican
VNN1	Bt03220248_m1	Vanin 1
XBP1	Bt03227621_g1	X-Box Binding Protein 1

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723 **Table S6.** Housekeeping and target genes analyzed in blastocysts samples by RT-qPCR.

Gene Symbol	Assys ID[†]	Gene Name
ACACA	Bt03213389_m1	Acetyl CoA Carboxylase
ACSL3	Bt04282138_m1	Acyl-CoA Synthetase 3
ACSL6	Bt03231692_m1	Acyl-CoA Synthetase 6
ACTB	Bt03279174_g1	Actin, Beta (Housekeeping)
AKR1B1	Bt03218049_g1	Aldo-Keto Reductase Family 1, Member B1
AQP3	Bt03253663_m1	Aquaporin 3
ATF4	Bt03221057_m1	Activating Transcription Factor 4
BAX	Bt03211777_g1	BCL2-Associated X Protein
BMP15	Bt03286494_u1	Bone Morphogenetic Protein 15
CASP3	Bt03250954_g1	Caspase 3, Apoptosis-Related Cysteine Peptidase
CASP9	Bt04282453_m1	Caspase 9, Apoptosis-Related Cysteine Peptidase
CAT	Bt03228713_m1	Catalase
CDH1	Bt03210093_g1	Cadherin 1
CDK6	Bt04311264_m1	Cyclin-Dependent Kinase 6
CDX2	Bt03649157_m1	Homeobox Protein CDX-2
CPT2	Bt03233823_m1	Carnitine Palmitoyltransferase 2
CYP19A1	Bt03213774_m1	Cytochrome P450 Family 19 Subfamily A Member 1
DDIT3	Bt03251320_g1	DNA-Damage-Inducible Transcript 3
DNMT1	Bt03224737_m1	DNA (Cytosine-5-)-Methyltransferase 1
DNMT3A	Bt01027164_m1	DNA (Cytosine-5-)-Methyltransferase 3A
DNMT3B	Bt03259810_m1	DNA (Cytosine-5-)-Methyltransferase 3B
DSC2	Bt03649202_m1	Desmocollin-II
EGFR	AJT96D7	Epidermal Growth Factor - Receptor
ELOVL6	Bt00907566_m1	ELOVL Fatty Acid Elongase 6
FADS2	Bt03256255_g1	Fatty Acid Desaturase 2
FASN	Bt03210485_m1	Fatty Acid Synthase
FSHR	Bt03212674_m1	Follicle-Stimulating Hormone Receptor
GSK3A	Bt03273695_m1	Glycogen Synthase Kinase 3 Alpha
G6PD	Bt03649181_m1	Glucose 6 Phosphate Desidrogenase
GAPDH	Bt03210912_g1	Glyceraldehyde-3-Phosphate Dehydrogenase (Housekeeping)
GDF9	Bt03223996_m1	Growth Differentiation Factor 9
GPX1	Bt03259217_g1	Glutathione Peroxidase 1
GPX4	Bt03259611_m1	Glutathione Peroxidase 4
H19	*Custom TaqMan*	Imprinted Maternally Expressed Transcript
HIF1A	Bt03259341_m1	Hypoxia Inducible Factor 1 (transcription factor)
HAND1	Bt04318733_g1	Heart and Neural Crest Cell Derivative 1
HMOX1	Bt03218632_m1	Heme Oxygenase (decycling) 1
HP1	Bt03246076_m1	Heterochromatin Protein 1
HSF1	Bt03249686_m1	Heat Shock Transcription Factor 1
HSP90AA1	Bt03218068_g1	Heat Shock Protein 90kDa Alpha
HSPA1A	Bt03292670_g1	Heat Shock 70kDa Protein 1A

HSPA5	Bt03244880_m1	Glucose-Regulated Protein, 78kDa
HSPD1	Bt04301470_g1	Heat Shock 60kDa Protein 1A
IFITM3	Bt03292973_g1	Interferon Induced Transmembrane Protein 3
IFNT2	Bt03210589_g1	Interferon Tau-2
IGF1R	Bt03649217_m1	Insulin-Like Growth Factor 1 Receptor
IGFBP2	Bt01040719_m1	Insulin-Like Growth Factor Binding Protein 2
IGFBP4	Bt03259500_m1	Insulin-Like Growth Factor Binding Protein 4
KEAP1	Bt03817661_m1	Kelch-Like ECH-Associated Protein 1
KRT8	Bt03225178_g1	Keratin 8
LIFR	Bt04310863_m1	Leukaemia Inhibitory Factor Receptor
LPL	Bt03240493_m1	Lipoprotein Lipase
LUM	Bt03211920_m1	Lumican
MAPK1	Bt03216718_g1	Mitogen-Activated Protein Kinase
MORF4L2	Bt03270996_m1	Mortality Factor 4 Like 2
MTIF3	Bt03231844_m1	Mitochondrial Translational Initiation Factor 3
NANOG	Bt03220541_m1	Nanog Homeobox
NFE2L2	Bt03251880_m1	Nuclear Factor (erythroid-derived 2)-Like 2
NFKB2	Bt03272789_g1	Nuclear Factor of Kappa Light Polypeptide Gene Enhancer in B-Cells 2
NLPR5	Bt03218031_m1	NLR Family, Pyrin Domain Containing 5
NRP2	Bt04316732_m1	Natriuretic Peptide Receptor 2
OCLN	Bt03255219_m1	Occludin
OTX2	Bt04316301_g1	Orthodenticle Homeobox 2
PAF1	Bt03239371_g1	RNA Polymerase II Associated Factor
PFKP	Bt04316551_m1	Phosphofructokinase
PGK1	Bt03225854_mH	Phosphoglycerate Kinase 1
PKP2	Bt03257632_m1	Plakophilin
POU5F1	Bt03223846_g1	POU Class 5 homeobox 1 (OCT4)
PPIA	Bt03224617_g1	Peptidylprolyl Isomerase A (Housekeeping)
PRDX1	Bt03223684_m1	Peroxiredoxin-1
REST	Bt03278318_s1	RE1-Silencing Transcription Factor
SCD	Bt04307476_m1	Stearoyl-CoA Desaturase
SDHA	Bt04307509_m1	Succinate Dehydrogenase Complex Flavoprotein Subunit A (Housekeeping)
SLC2A1	Bt03215314_m1	Solute Carrier Family 2 (facilitated glucose transporter), Member 1
SLC2A3	Bt03259514_g1	Solute Carrier Family 2 (facilitated glucose transporter), Member 3
SLC2A5	Bt03258296_m1	Solute Carrier Family 2 (facilitated glucose/fructose transporter), Member 5
SOD1	Bt03215423_g1	Superoxide Dismutase 1, Soluble
SOD2	Bt03244551_m1	Superoxide Dismutase 2, Mitochondrial
SOX2	Bt03278318_s1	SRY (sex determining region Y)-Box 2
SREBF1	Bt03276370_m1	Sterol Regulatory Element Binding Transcription F1
STAT3	Bt03259871_g1	Signal Transducer and Activator of Transcription 3
TFAM	Bt03260078_m1	Transcription Factor A, Mitochondrial
VEGFA	Bt03213282_m1	Vascular Endothelial Growth Factor A
XBP1	Bt03227621_g1	X-Box Binding Protein 1

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ANEXO

Molecular Reproduction & Development

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Authors should kindly note that submission implies that the content has not been published or submitted for publication elsewhere except as a brief abstract in the proceedings of a scientific meeting or symposium.

Once the submission materials have been prepared in accordance with the Author Guidelines, manuscripts should be submitted via the journal's ScholarOne site:https://mc.manuscriptcentral.com/mrd. For more details on how to use ScholarOne, visit www.wileyauthors.com/scholarone. Note, this journal uses iThenticate's CrossCheck software to detect instances of overlapping and similar text in submitted manuscripts.

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Molecular Reproduction and Development takes an integrated, systems-biology approach to understand the dynamic continuum of cellular, reproductive, and developmental processes. This journal fosters dialogue among diverse disciplines through primary research communications and educational forums, with the philosophy that fundamental findings within the life sciences result from a convergence of disciplines.

Increasingly, readers of the Journal need to be informed of diverse, yet integrated, topics impinging on their areas of interest. This requires an expansion in thinking towards non-traditional, interdisciplinary experimental design and data analysis. For example, biologists need to know how nanodevices might be used, while bioengineers need to know how post-translational protein modifications affect developmental mechanisms. The Journal will provide a means for readers to integrate divergent scientific disciplines into their current and future research. Readers will turn to *Molecular Reproduction and Development* not only to learn the latest in research progress, but to become educated in frontier topics of the field of reproductive and developmental biology; students in particular will turn to the Journal to learn about current progress in the diversifying approaches that will become the foundation for the next generation of research.

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DESCRIPTION OF SUBMISSION TYPES:

VISIONS highlight the visual impact inherent in the field of reproduction and development. Researchers are encouraged to submit individual figures of visual scientific interest that convey important concepts or lessons without requiring significant text for this section of the journal. Each image should be submitted through the ScholarOne Manuscripts website. Figure formatting must be at least 22 x 28 cm at a resolution of 600 dpi, and must include a title and a legend of less than 250 words that describes the image sufficiently to be understood by a broad audience of researchers. The Editorial Board will evaluate these submissions for their visual impact and scientific content. There are no color figure in-print charges.

CORRESPONDENCE

CORRESPONDENCE is a section of short, ~one journal page (500 words or less, or about one column length total) communications for the research field. This section is devoted to brief research results, announcements of interest to the community, new databases or software program availability, or other research results not appropriate for full-length Research Articles. A single figure may be included when essential, but these figures will be published at no larger than

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Submissions of REVIEWS and ESSAYS are encouraged. The topics of the manuscript are flexible, but they are intended to reach a broad audience of readers in molecular reproduction and development—from investigators in the field, to students learning the material for the first time. Therefore, it is important that the reviews start generally or with a historical perspective to integrate the topic into a larger context. The bulk of the review should be a critical analysis of the current field and should end with important yet-unresolved questions, speculations, and directions for the field in the future. All other formats for the review are as listed below for manuscripts. ESSAYS follow a similar guideline, but the topics may be more speculative, of historical emphasis, or may integrate more than scientific content. There are no color figure in-print charges.

RESEARCH ARTICLES

Molecular Reproduction and Development is an international journal devoted to an integrated approach towards understanding the dynamic continuum of reproductive and developmental processes. As such, the journal is interested in RESEARCH ARTICLES that advance the field by mechanistic discoveries, and by functional understanding. Manuscripts reporting purely descriptive science must be of particular interest to be considered for the journal. MRD particularly encourages manuscripts with a convergence of disciplines, including systems biology, computational modeling, nanoscience, organic chemistry, bioengineering, evolutionary and synthetic biology - all within the framework that describes or reveals a mechanistic aspect of reproduction and development. These submissions are evaluated by the Editorial Board and by external reviewers in accordance with the Peer Review Policy of MRD (see http://mc.manuscriptcentral.com/mrd).

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4. PREPARING THE SUBMISSION

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The text file should be presented in the following order:

i. The title;
ii. The full names of the authors (do not include academic degrees);
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iv. Grant numbers (as applicable-to ensure proper identification of funders with publication requirements-see note under Author Licensing; below);
v. Abstract and keywords;
vi. Main text;
vii. Acknowledgments;
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ix. Tables (each table complete with title and footnotes);
x. Figure legends;
xi. Appendices (if relevant)

Figures and supplementary/supporting information should be supplied as separate files (see below under "Additional Files"). Figures must be clearly labeled.

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Acknowledgements

Contributions from anyone who does not meet the criteria for authorship should be listed, with permission from the contributor, in an Acknowledgments section. Financial and material support should also be mentioned. Thanks to anonymous reviewers are not appropriate.

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Authors will be asked to provide a conflict of interest statement during the submission process. For details on what to include in this section, see the Conflict of Interest section in the Editorial Policies and Ethical Considerations section below. Submitting authors should ensure they liaise with all co-authors to confirm agreement with the final statement.

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Please provide an abstract of 200 words (Brief Reports only: max 150 words) containing the major keywords summarizing the article.

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Please provide three to five keywords.

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References

The accuracy of references is the responsibility of the authors. Only published papers and those in press may be included in the reference list. Unpublished data and submitted manuscripts must be cited parenthetically within the text. Personal communications should also be cited within the text; permission in writing from the communicator is required.

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According to APA style, in text citations should follow the author-date method whereby the author's last name and the year of publication for the source should appear in the text, for example, (Jones, 1998). The complete reference list should appear alphabetically by name at the end of the paper.

Authors should note that the APA referencing style requires that a Digital Object Identifier (DOI) be provided for all references where available. Also, for journal articles, issue numbers are not included unless each issue in the volume begins with page one.

Reference examples follow:

Journal article

Beers, S. R., & De Bellis, M. D. (2002). Neuropsychological function in children with maltreatment-related posttraumatic stress disorder. *The American Journal of Psychiatry, 159,* 483–486. *doi:*10.1176/appi.ajp.159.3.483

Book

Bradley-Johnson, S. (1994). *Psychoeducational assessment of students who are visually impaired or blind: Infancy through high school* (2nd ed.). Austin, TX: Pro-ed.

Internet Document

Norton, R. (2006, November 4). How to train a cat to operate a light switch [Video file]. Retrieved from http://www.youtube.com/watch?v=Vja83KLQXZs

Footnotes

Footnotes should be placed as a list at the end of the paper only, not at the foot of each page. They should be kept to a minimum. Keep footnotes brief; they should contain only short comments tangential to the main argument of the paper and should not include references. They should be numbered in the list and referred to in the text with consecutive, superscript Arabic numerals.

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Tables should be self-contained and complement, not duplicate, information contained in the text. They should be supplied as editable files, not pasted as images. Legends should be concise but comprehensive – the table, legend, and footnotes must be understandable without reference to the text. All abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶, should be used (in that order) and *, **, *** should be reserved for P-values. Statistical measures such as SD or SEM should be identified in the headings.

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Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement.

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- **Units of measurement**: Measurements should be given in SI or SI-derived units. Visit the Bureau International des Poids et Mesures (BIPM) website for more information about SI units.
- **Numbers**: numbers under 10 should be spelt out, except for: measurements with a unit (8 mmol/L); age (6 weeks old), or lists with other numbers (11 dogs, 9 cats, 4 gerbils).
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Return to Guideline Sections

5. EDITORIAL POLICIES AND ETHICAL CONSIDERATIONS

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The acceptance criteria for all papers are the quality and originality of the research and its significance to journal readership. Papers will only be sent to review if the Editors determine that the paper meets the appropriate quality and relevance requirements.

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For manuscripts reporting medical studies that involve human participants, a statement identifying the ethics committee that approved the study and confirmation that the study conforms to recognized standards is required, for example: Declaration of Helsinki; US Federal Policy for the Protection of Human Subjects; or European Medicines Agency Guidelines for Good Clinical PracticeUS Federal Policy for the Protection of Human Subjects;. It should also state clearly in the text that all persons gave their informed consent prior to their inclusion in the study.

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- Case reports: CARE
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- Quality improvement studies: SQUIRE
- Animal pre-clinical studies: ARRIVE
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- Future of Research Communications and e-Scholarship (FORCE11)
- National Research Council's Institute for Laboratory Animal Research guidelines
- The Gold Standard Publication Checklist from Hooijmans and colleagues
- Minimum Information Guidelines from Diverse Bioscience Communities (MIBBI) website
- FAIRsharing website

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Standard nomenclature format must be used according to the organisms discussed. Reference sites by organism include:

- http://www.genenames.org (humans, non-human primates, domestic species)
- http://www.informatics.jax.org/mgihome//nomen/strains.shtml (mice, rats)
- http://www.xenbase.org (*Xenopus*)
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- EMBL Nucleotide Archive: ebi.ac.uk/ena
- GenBank: www.ncbi.nlm.nih.gov/genbank

Protein sequence data should be submitted to either of the following repositories:

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Página 14, linhas 16 a 19 (**Grants**)

Onde se lê:

São Paulo Research Foundation (FAPESP; grant numbers: 2012/50533-2; 2013/11480-3; 2015/04505-5 and 2016/22812-5).

National Council for Scientific and Technological Development (CNPq; grant number: 403063/2016-7).

Leia-se:

Coordination for the Improvement of Higher Education Personnel (CAPES).

São Paulo Research Foundation (FAPESP; grant numbers: 2013/11480-3 and 2016/22812-5).

National Council for Scientific and Technological Development (CNPq; grant number: 403063/2016-7).