Approaches to reduce lipids: a review of its impacts on *in vitro* embryo production

Abordagens para reduzir lipídeos: uma revisão sobre seus impactos na produção de embriões in vitro

José Victor Braga, Eliza Rossi Komninou, Arnaldo Diniz Vieira, Rafael Gianella Mondadori[£]

Universidade Federal de Pelotas, Núcleo de Ensino e Pesquisa em Reprodução Animal (ReproPEL), Campus Capão do Leão, RS, Brasil.

Abstract

In vitro embryo production (IVP) has limitations for better outcomes in blastocyst production, cryopreservation efficiency and pregnancy rates. Among the factors impairing IVP, high lipid content in both oocytes and embryos, and consequent reactive oxygen species (ROS) production, have a negative impact in embryo development and further biotechnologies. The present review aims to address techniques and strategies that collaborate to improve embryo development rates through reduction of lipid content either in the oocyte or in the embryo.

Keywords: delipidation, *in vitro* supplementation, lipid droplets, oxidative stress, early embryo metabolism.

Resumo

A produção in vitro de embriões (PIVE) possui limitações para melhoras na produção de blastocistos, eficiência da criopreservação e taxas de prenhez. Dentre os fatores limitantes à PIVE, o alto conteúdo lipídico tanto em oócitos quanto em embriões e a consequente produção de espécies reativas a oxigênio (ROS), possuem impacto negativo no desenvolvimento embrionário e subsequentes biotecnologias. A presente revisão visa tratar sobre tecnologias e estratégias capazes de colaborar com a melhora nas taxas de desenvolvimento embrionário através da redução do conteúdo lipídico tanto em oócitos quanto em embriões.

***Palavras-chave**: delipidação, suplementação in vitro, gotículas de lipídeo, estresse oxidativo, metabolismo embrionário inicial.*

Introduction

In vitro embryo production (IVP) system is based on *in vitro* maturation (IVM), *in vitro* fertilization (IVF) and *in vitro* culture (IVC) (Somfai and Hirao, 2011). Two important limiting factors in this system, for both oocytes and embryos, are: (1) the high lipid content and accumulation of lipid droplets (LD) that occurs in some domestic species, mainly in pigs (McEvoy et al., 2000; Gajda, 2009) and; (2) the high levels of reactive oxygen species (ROS) produced in response to fatty acids β-oxidation and culturing conditions. Together these factors negatively impacts embryo development rates (Romek et al., 2017), as well as cryopreservation efficiency (Grupen, 2014). Therefore, reduction of lipid content could be beneficial, however the total removal is not indicated, since they have metabolic functions (Tsujii et al., 2001), and LD act as energy source to the cells (Walther and Farese Jr, 2012; Thiele and Penno, 2015; Welte and Gould, 2017) by forming metabolic units together with mitochondria and the endoplasmic reticulum (Warzych et al., 2017).

In oocytes, during follicular growth and development (Paulini et al., 2014) cumulus-oocyte complex (COC) modulates the expression of genes that regulate fatty acid (FA) metabolism (Sanchez-Lazo et al., 2014). As a result, during final development, oocytes already contain LD with triacylglycerol and sterol esters as main stored lipids (Walther and Farese Jr, 2012; Warzych et al., 2017) that requires β-oxidation in order to support the maturation process (Dunning et al., 2014). On the other hand, in embryos, during first cleavage, the energy is obtained preferably via pentose phosphate pathway (PPP), while, for further embryo development, glycolysis and β-oxidation are more prominent (reviewed by Prates et al. 2014).

ROS are by products of the cellular oxidative metabolism, a process where ATP is synthesized by reduction of oxygen in the mitochondria, though proton and electron transfer reactions (Fu et al., 2014). ROS production seems also be related to lipid accumulation (Furukawa et al., 2004). This production, associated with the reduction of antioxidant enzymes, can result in oxidative stress, with serious detrimental effects such as: (1) lipid peroxidation, (2) breaking of DNA double strand, and (3) mitochondrial DNA mutation, which result in decrease in embryo and oocytes viability (Guérin et al., 2001).

IVP embryos had higher lipid content and produce higher ROS levels than their *in vivo* counterparts, ROS production also varies according to the stage of development (Guérin et al., 2001). Culture media composition also has influence over these factors (Romek et al., 2010). Thus, it is necessary to optimize the IVP system aiming to reduce lipid content and to increase protection against ROS.

[£]Correspondência: rgmondadori@gmail.com
Recebido: 20 de dezembro de 2018
Aceito: 22 de maio de 2019

This review aims to gather information about the approaches that have been recently used to reduce lipids levels during IVP. As it will be observed, most of the results use pig as model because it presents oocytes and embryos with a high lipid content when compared to other species (McEvoy et al., 2000).

Strategies to reduce lipids levels

Mechanic delipidation techniques

Mechanical delipidation includes physical methods to remove or disrupt LD from the cytoplasm. One of the first to attempt this approach was Nagashima et al. in 1994, which used centrifugation and micromanipulation as a combined alternative to reduce LD in porcine oocytes. Their results showed improvement on chilling resistance, but no significant differences in embryo development for delipidated or partially delipidated oocytes. More recently, porcine embryos were used as model to test different centrifugation protocols and their benefits in embryo vitrification, with generally better results for the delipidated ones (Kawakami et al., 2008). Delipidation also reduced the triglyceride content and the expression of GPAT1, AGPAT1, AGPAT2, LIPIN1, DGAT, genes related to triacylglycerol synthesis (Zeng et al., 2017), which are essential for LD structure and formation (Walther and Farese Jr, 2012). However, in one study, mechanical delipidation have not improved total blastocyst survival after cryopreservation, but, on the other hand, those that survived and were transferred to recipients resulted in pregnancies and piglets' birth (Men et al., 2011).

Similar behavior was observed in sheep embryos, were Romão et al. (2015) found that centrifugation plus exposure to cytochalasin D or the use of *trans*-10 *cis*-12- conjugated linoleic acid isomer (CLA) resulted in higher blastocyst rate and survival after vitrification and warming, possibly by reduction of the lipid content, although no lipid evaluation was performed. Briefly, besides being a time-consuming practice, mechanical delipidation improves the efficiency of IVP system.

Fatty acids supplementation

FA are a class of lipids that form structural components of membranes and are an energy source as they are stored as triacylglycerol inside LD. They are metabolized by β-oxidation, influencing oocyte development potential (Dunning et al., 2014). Although the addition of lipids may seem controversial, once oocytes and embryo already have a high lipid content, recent studies have shown that supplementation with FA may be beneficial as it forces lipid metabolism and contributes to energy supply. Sanchez-Lazo et al. (2014) described that FA synthesis, lipolytic activity and fatty acid oxidation (FAO) have direct influence in the oocyte maturation process. Likewise, the expression of genes related to FA metabolism is consonant with the modifications of lipid content in cumullus cells, since they comunicate with the oocyte through paracrine signals and tranzonal projections (reviewed by Clarke, 2018).

In bovines, the addition of *trans-10, cis-12 octadeca-dienoic acid* (*t10, c12* CLA), a conjugated isomer of the linoneic acid to the IVC medium, had no effect on cleavage or blastocyst rates, but reduced lipid content and increased survival rate of vitrified-warmed bovine embryos (Pereira et al., 2008). Controversially, addition of high levels of both non-esterified fatty acids (NEFA) or stearic acid (SA) in the maturation media of bovine COC, lead to different effects: (1) the first, significantly reduced blastocyst development and also increased the LD content; (2) and the second, reduced LD accumulation and lowered re-expansion rates after the cryopreservation-warming process (Van Hoeck et al., 2015).

Such as high content of NEFA, an elevated dose of polyunsaturated fatty acids (PUFA) in the maturation media impaired the development of bovine (Oseikria et al., 2016) and porcine (Hoyos-Marulanda et al., 2019) embryos. However, Oseikria et al (2016) found that a lower dose -1μM - of docosahexaenoic acid (DHA), an omega-3 essential fatty acid during IVM, improved cleavage rates of both parthenogenetic or fertilized bovine embryos, although no differences in the expression of FA metabolism related genes such as FA synthase (FASN), diacylglycerol O-acyl-transferase (DGAT1), FA transport (transporters CD36, FA binding protein genes FABP3 and FABP5), lipolysis (phospholipase PNPLA2), lipid storage (perilipin PLIN2), and mitochondrial b-oxidation (CPT1A, CPT2) were found. In our lab, MIV supplementation with eicosapentaenoic acid (EPA), also an omega-3 fatty acid, reduced cleavage rates, while 50μM of DHA improved cleavage rates and reduced blastocysts lipid content (Hoyos-Marulanda et al., 2019).

By modifying maturation media of mice oocytes, Paczkowski et al (2014) found that carnitine, a lipid modulator, alone or in combination with 1μM palmitic acid, common saturated fatty acid, reduced lipid content, while 100μM palmitic acid made the opposite; nevertheless, the use of these agents resulted in alterations of gene expression that directly affected the lipid content (Paczkowski et al., 2014).

Therefore, the use of fatty acids during IVP modulates lipid metabolism in oocytes and embryos. However, they either improve or impair embryo production, depending on the concentration and nature of fatty acid used.

Lipid modulators

Lipid modulators are substances that can reduce and/or modify lipid content within cells through several

mechanisms (Prates et a., 2014). Among them, carnitine, phenazine ethosulfate (PES) and forskolin are examples utilized during IVP procedures.

Carnitine

Carnitine carries FA into the mitochondria for ATP generation via β-oxidation and binds to Acetyl-CoA going back to the cytoplasm, influencing glucose metabolism (Dunning and Robker, 2012) and reducing lipid peroxidation (Somfai et al., 2011). The addition of carnitine during MIV enhanced oocyte maturation, embryo development, and mitochondrial activity, while reducing lipid content and ROS levels in porcine (Somfai et al., 2011; Wu et al., 2011; You et al., 2012), buffalo (Verma et al., 2018) and bovine (Ghanem et al., 2014).

Although being mostly advantageous, Wu et al (2011) found that porcine COC exposed to 2 mg/mL of carnitine presented reduction in maturation and the blastocyst rates, whereas lower (0.5 mg/mL) concentrations were beneficial for parthenote embryo development, reducing ROS levels. On the other hand, You et al. (2012) obtained better blastocyst development when pig oocytes were matured with 1.98 mg/mL of carnitine, increasing glutathione and reducing ROS levels. Interestingly, Somfai et al. (2011), also added lower concentrations of carnitine (1.25 mg/mL) during IVM, resulting in higher maturation rates, although no differences on blastocyst rate were observed. Authors also described reduction on lipid content and ROS production, as also found by Wu et al (2011) and You et al. (2012).

Verma et al (2018) added different doses of carnitine, 48h after buffalo IVF, resulting in lipid droplets reduction, and altered expression of metabolism related genes DGAT1 and DGAT2, as well as blastocyst competence markers GLUT1, OCT4 and INFτ, leading to a higher developmental rates of blastocysts after vitrification. Similarly, Ghanem et al. (2014) related that bovine embryos supplemented with carnitine and/or phenazine ethosulfate (PES) presented reduced lipid content, increased mitochondria density and cryotolerance, presenting also modification in the expression profile of genes related to FA transport (SLC27A1 and SLC22A5), FA oxidation (CPT1B and CPT2), FA synthesis (ACCα), LD formation (DGAT1, DGAT2 and PLIN2) and embryo competency (SOD2, NADH and GLUT8). Therefore, both lipid modulators improved embryo quality and cryotolerance, but carnitine supplementation seems to be more beneficial.

Finally, supplementation of IVP media with lower carnitine concentration is beneficial in reducing lipid content, and increasing cleavage and blastocyst rates. Carnitine is also capable to alter the expression profile of different genes related to energy and lipid metabolism, as well as embryo quality.

Phenazine Ethosulfate (PES)

PES is a metabolic regulator that inhibits fatty acids synthesis by NADH oxidation to NADP, favoring glucose metabolism through phosphate pentose pathway (PPP) (Ghanem et al., 2014). De La Torre-Sanchez et al. (2006) compared metabolic regulators on bovine embryos and found that 0.3μM PES during IVC accelerate glucose metabolism by a higher glucose flux through PPP and reduced the amount of large lipid droplets in blastomere cytoplasm. The same PES concentration during IVC had no detrimental effect on cattle pregnancy and parturition after embryo transfer (Barceló-Fimbres et al., 2009). For pigs, 0.05 μM PES supplementation during IVC had no effect on cleavage rate but increased morula and blastocyst production, also, this concentration was able to reduce lipid content by 23% but the survival rate after vitrification was similar than the control (Gajda et al., 2011).

Even though PES seems promising, few researches demonstrated its potential for lipid reduction. Furthermore, it is interestingly that a lower concentration significantly reduced lipids in the porcine COCs, which has highest lipid content in comparison to bovines (McEvoy et al., 2000) that needed higher concentrations to improve lipid metabolism.

Forskolin

Forskolin is a chemical stimulator of lipolysis through the activation of adenyl cyclase (cAMP) (Prates et al., 2014). This substance is also capable of synchronize cytoplasmic and nuclear maturation of oocytes by arresting meiotic progression (Park et al., 2016).

According to Fu et al. (2011), 10μM of forskolin reduced lipid content and improved survival rate of porcine COC after cryopreservation. However, independently of the period of exposure to forskolin, cleavage rates were lower than the control. Going further, Park et al. (2016) exposed pig COC to 50μM forskolin prior to IVM, resulting in higher blastocyst rates and reduction of lipid levels in parthenogenetically activated embryos, although lower cleavage rates was also observed. However, when compared with cilostamide, another cAMP modulator, forskolin couldn't maintain the same maturation levels for pig COC derived from small antral follicles and seems to inhibit cilostamide effects when both were supplemented (Park et al., 2016). Forskolin was also beneficial to lipid reduction in buffalo and bovine embryos exposed to 10μM during IVC and had progressive lipid reduction from day 2 to day 7 embryos, besides no effect on embryo cleavage or in freezing ability (Panyaboriban et al., 2018).

As can be observed, forskolin is efficient in reducing lipid content, increasing cryotolerance and blastocyst rate, besides these beneficial characteristics, this substance seems to impair cleavage rates.

Conclusion

This review showed alternative ways to deal with high lipid content in oocytes and embryo derived from IVP systems. Significant progress has been made, especially regarding cryoresistance and blastocyst development in different species. On the other hand, there is still no well determined pattern related to the best strategy for lipid reduction, since diverse modulators and concentrations were used in different species. In conclusion, additional studies should be conducted to find the optimal combination of substances and strategies, as well as the concentrations and phases of PIV to be used, to avoid that the accumulation of lipid observed in oocytes and embryos does not become an obstacle to optimize embryonic development and cryotolerance.

References

Barceló-Fimbres, M, Brink Z, Seidel GE. Effects of phenazine ethosulfate during culture of bovine embryos on pregnancy rate, prenatal and postnatal development. Theriogenology, v.71, n.2, p.355-368, 2009.

Clarke HJ. History, origin, and function of transzonal projections: the bridges of communication between the oocyte and its environment. Anim Reprod, v.15, n.3, p.215-223, 2018.

De La Torre-Sanchez JF, Gardner DK, Preis K, Gibbons J, Seidel GE Jr. Metabolic regulation of *in vitro*-produced bovine embryos. II. Effects of phenazine ethosulfate; sodium azide and 2;4- dinitrophenol during post-compaction development on glucose metabolism and lipid accumulation. Reprod Fertil Dev, v.18, p.597-607, 2006.

Dunning KR, Robker RL. Promoting lipid utilization with l-carnitine to improve oocyte quality. Anim Reprod Sci, v.134, n.1-2, p.69-75, 2012.

Dunning KR, Russell DL, Robker RL. Lipids and oocyte developmental competence: The role of fatty acids and β-oxidation. Reproduction, v.148, n.1, 2014.

Fu PP, Xia Q, Hwang HM, Ray PC, Yu H. Mechanisms of nanotoxicity: Generation of reactive oxygen species. J Food Drug Anal, v.22, n.1, p.64-75, 2014.

Fu XW, Wu GQ, Li JJ, Hou YP, Zhou GB, Lun-Suo, Wang YP, Zhu SE. Positive effects of Forskolin (stimulator of lipolysis) treatment on cryosurvival of *in vitro* matured porcine oocytes. Theriogenology, v.75, n.2, p.268–275, 2011.

Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest, v.114, n.12, p.1752-1761, 2004.

Gajda B. Factors and methods of pig oocyte and embryo quality improvement and their application in reproductive biotechnology. Reprod Biol, v.9, n.2, p.97-112, 2009.

Gajda B, Romek M, Grad I, Krzysztofowicz E, Bryla M, Smorag Z. Lipid content and cryotolerance of porcine embryos cultured with phenazine ethosulfate. Cryo-Letters, v.32, n.4, p.349-357, 2011.

Ghanem N, Ha AN, Fakruzzaman M, Bang JI, Lee SC, Kong IK. Differential expression of selected candidate genes in bovine embryos produced invitro and cultured with chemicals modulating lipid metabolism. Theriogenology, v.82, n.2, p.238-250, 2014.

Grupen CG. The evolution of porcine embryo invitro production. Theriogenology, v.81, n.1, p.24-37, 2014.

Guérin P, El Mouatassim S, Ménézo Y. Oxidative stress and protection against reactive oxygen species in the pre-implantation embryo and its surroundings. Hum Reprod Update, v.7, n.2, p.175-189, 2001.

Hoyos-Marulanda V, Alves BS, Rosa PRA, Vieira AD, Gasperin BG, Mondadori RG, Lucia Jr T. Effects of polyunsaturated fatty acids on the development of pig oocytes *in vitro* following parthenogenetic activation and on the lipid content of oocytes and embryos. Animal Reproduction Science, 2019.

Kawakami M, Kato Y, Tsunoda Y. The effects of time of first cleavage, developmental stage, and delipidation of nuclear-transferred porcine blastocysts on survival following vitrification. Anim Reprod Sci, v.106, n.3-4, p.402-411, 2008.

McEvoy TG, Coull GD, Broadbent PJ, Hutchinson JS, Speake BK. Fatty acid composition of lipids in immature cattle, pig and sheep oocytes with intact zona pellucida. J Reprod Fertil, v.118, n.1, p.163-170, 2000.

Men H, Zhao C, Si W, Murphy CN, Spate L, Liu Y, Walters EM, Samuel MS, Prather RS, Critser JK. Birth of piglets from *in vitro*-produced, zona-intact porcine embryos vitrified in a closed system. Theriogenology, v.76, n.2, p.280-289, 2011.

Nagashima H, Kashiwazaki N, Ashman RJ, Grupen CG, Seamark RF, Nottle MB. Removal of Cytoplasmic Lipid Enhances the Tolerance of Porcine Embryos to Chilling. Biol Reprod, v.51, n.4, p.618-622, 1994.

Oseikria M, Elis S, Maillard V, Corbin E, Uzbekova S. N-3 polyunsaturated fatty acid DHA during IVM affected oocyte developmental competence in cattle. Theriogenology, v.85, n.9, p. 1625-1634.e2, 2016.

Paczkowski M, Schoolcraft WB, Krisher RL. Fatty acid metabolism during maturation affects glucose uptake and is essential to oocyte competence. Reproduction, v.148, n.4, p.429-439, 2014.

Panyaboriban S,Tharasanit T, Chankitisakul V, Swangchan-Uthai T, Techakumphu M. Treatment with chemical delipidation forskolin prior to cryopreservation improves the survival rates of swamp buffalo (Bubalus bubalis) and bovine (Bos indicus) *in vitro* produced embryos. Cryobiology, v.84, p.46-51, 2018.

Park B, Lee H, Lee Y, Elahi F, Lee J, Lee ST, Park CK, Hyun SH, Lee E. Cilostamide and forskolin treatment

during pre-IVM improves preimplantation development of cloned embryos by influencing meiotic progression and gap junction communication in pigs. Theriogenology, v.86, n.3, p.757-765, 2016.

Paulini F, Silva RC, Rôlo JL, Lucci CM. Ultrastructural changes in oocytes during folliculogenesis in domestic mammals. J Ovarian Res, v.7, n.1, p.1-12, 2014.

Pereira RM, Carvalhais I, Pimenta J, Baptista MC, Vasques MI, Horta AE, Santos IC, Marques MR, Reis A, Pereira MS, Marques CC. Biopsied and vitrified bovine embryos viability is improved by trans10, cis12 conjugated linoleic acid supplementation during *in vitro* embryo culture. Anim Reprod Sci, v.106, n.3-4, p.322-332, 2008.

Prates EG, Nunes JT, Pereira RM. A role of lipid metabolism during cumulus-oocyte complex maturation: Impact of lipid modulators to improve embryo production. Mediators Inflamm, v.2014, 2014. Doi: 10.1155/2014/692067.

Romão R, Marques CC, Baptista MC, Barbas JP, Horta AE, Carolino N, Bettencourt E, Pereira RM. Cryopreservation of invitro-produced sheep embryos: Effects of different protocols of lipid reduction. Theriogenology, v.84, n.1, p.118-126, 2015.

Romek M, Gajda B, Krzysztofowicz E, Smorag Z. Changes of lipid composition in non-cultured and cultured porcine embryos. Theriogenology, v.74, n.2, p.265-276, 2010.

Romek M, Gajda B, Krzysztofowicz E, Kucia M, Uzarowska A, Smorag Z. Improved quality of porcine embryos cultured with hyaluronan due to the modification of the mitochondrial membrane potential and reactive oxygen species level. Theriogenology, v.102, p.1-9, 2017.

Sanchez-Lazo L, Brisard D, Elis S, Maillard V, Uzbekov R, Labas V, Desmarchais A, Papillier P, Monget P, Uzbekova S. Fatty Acid Synthesis and Oxidation in Cumulus Cells Support Oocyte Maturation in Bovine. Mol Endocrinol, v.28, n.9, p.1502-1521, 2014.

Somfai T, Kaneda M, Akagi S, Watanabe S, Haraguchi S, Mizutani E, Dang-Nguyen TQ, Geshi M, Kikuchi K, Nagai T. Enhancement of lipid metabolism with L-carnitine during *in vitro* maturation improves nuclear maturation and cleavage ability of follicular porcine oocytes. Reprod Fertil Dev, v.23, n.7, p.912-920, 2011.

Somfai T, Hirao Y. Synchronization of *In vitro* Maturation in Porcine Oocytes. In: Banfalvi G. (Ed.). Cell Cycle Synchronization: Methods and Protocols. Totowa, NJ: Humana Press, 2011. p.211-225.

Thiele C, Penno A. Lipid Droplets. Encyclopedia of Cell Biology, v.2, p.273-278, 2015.

Tsujii H, Khandoker M, Hamano K. Lipid in mammalian embryo development. J Mamm. Ova Res, 2001.

Van Hoeck V, Rizos D, Gutierrez-Adan A, Pintelon I, Jorssen E, Dufort I, Sirard MA, Verlaet A, Hermans N, Bols PE, Leroy JL. Interaction between differential gene expression profile and phenotype in bovine blastocysts originating from oocytes exposed to elevated non-esterified fatty acid concentrations. Reprod Fertil Dev, v.27, n.2, p.372-384, 2015.

Verma M, Pandey S, Bhat IA, Mukesh B, Anand J, Chandra V, Sharma GT. Impact of L-carnitine on lipid content and post thaw survivability of buffalo embryos produced *in vitro*. Cryobiology, v.82, p.99-105, 2018.

Walther TC, Farese RV Jr. Lipid Droplets and Cellular Lipid Metabolism. Annu Rev Biochem, v.81, n.2, p.687-714, 2012.

Warzych E, Pawlak P, Pszczola M, Cieslak A, Madeja ZE, Lechniak D. Interactions of bovine oocytes with follicular elements with respect to lipid metabolism. Anim Sci J, v.88, n.10, p.1491-1497, 2017.

Welte MA, Gould AP. Lipid droplet functions beyond energy storage. Biochim Biophys Acta Mol Cell Biol Lipids, v.1862, n.10, p.1260-1272, 2017.

Wu GQ, Jia BY, Li JJ, Fu XW, Zhou GB, Hou YP, Zhu SE. L-carnitine enhances oocyte maturation and development of parthenogenetic embryos in pigs. Theriogenology, v.76, n.5, p.785-793, 2011.

You J, Lee J, Hyun SH, Lee E. L-carnitine treatment during oocyte maturation improves *in vitro* development of cloned pig embryos by influencing intracellular glutathione synthesis and embryonic gene expression. Theriogenology, v.78, n.2, p.235-243, 2012.

Zeng Y, Wang C, Niu Y, Chi D, Xu M, Si L, Qu X, Li J.The influence of delipidation on triglyceride and LIPIN1 of porcine embryos derived from parthenogenetic activation. Reprod Domest Anim, v.52, n.5, p.842–850, 2017.