IDEAPUZZLE
DESIGN AND DEFEND YOUR PHD

Development of vitrification cryotolerance in bovine oocytes through cold stress

Author: Jaume Gardela Santacruz
Date: 2017-12-14 - 19:42:53 (GMT)
Self-evaluation: 74%

Key words

1/21. Theoretical question: which are the two main keywords of your research?

The main objective of my research is to develop vitrification cryotolerance in bovine oocytes by applying cold stress.

Vitrification cryotolerance refers to the capability to resist the osmotic damage, toxicity and cryoinjury produced during vitrification (Huang et al. 2006), a method of gamete cryopreservation consisting in storage of cells at an ultra-low temperature (normally in liquid nitrogen, at-196 °C) in to an amorphous, noncrystalline solid, such that it remains capable of survival upon thawing (FAO 2012). Cold stress, a specific sub-lethal stressor, refers to apply hypothermic temperatures in order to obtain a cell adaptive response (Neutelings et al. 2013).

References:
FAO. 2012. Cryoconservation of Animal Genetic Resources.
Huang J, J Y, Chen HY, Tan SL, Chian RC. 2006. Effects of Osmotic Stress and Cryoprotectant Toxicity on Mouse Oocyte Fertilization and Subsequent Embryonic Development In Vitro. Cell Preserv. Technol. 4:149.
Neutelings T, Lambert CA, Nusgens B V., Colige AC. 2013. Effects of Mild Cold Shock (25°C) Followed by Warming Up at 37°C on the Cellular Stress Response. PLoS One 8:1.

Self-evaluation: 50%

Streams of thought

2/21. Theoretical question: which are the two main streams of thought of your literature review?

The two main streams of though of my literature review are the role of cold-shock proteins in mammalian cells as well as the induction of this

proteins in oocytes and embryos.

The exposure to mild hypothermic cold-shock (32 ºC) induces the expression of cold-shock proteins (CSP). The only two characterized CSP in mammals are the cold-inducible RNA-binding protein (CIRBP) and the RNA-binding motif protein 3 (Liao et al. 2017). CIRBP, also called CIRP and A18 hnRNP, is a constitutively expressed CSP highly conserved among different species which its expression is present in a large variety of tissues and cells, including the ovaries among others (Zhong and Huang 2017). CIRBP expression is increased in response to cold-stress, but it should be noted that variations exist in the response of CIRBP homologs between species (Zhong and Huang 2017). CIRBP is involved in several cellular processes such as cellular proliferation and cell survival (Liao et al. 2017; Zhong and Huang 2017). Although it is mainly located in the nucleus, CIRBP can be subjected to cytoplasmic translocation upon certain stress conditions such as osmotic pressure or heat shock (De Leeuw et al. 2007). Nevertheless, an isoform of CIRBP called xCIRP2 was mainly localized in the cytoplasm of Xenopus oocytes (Matsumoto et al. 2000). Moreover, CIRBP was localized in mouse spermatids cytoplasm in contrast with the nuclear location of CIRBP in spermatocytes (Nishiyama et al. 1998) and spermatogonial cells (Masuda et al. 2012). Taken together, these findings have been suggested that CIRBP might be nuclear in somatic cells while the distribution in haploid cells might be cytoplasmatic.
Although Matsumoto et al. (2000) did not observe an increase of the xCIRP2 protein expression in Xenopus oocytes treated with cold, some studies reported an increase of Cirbp gene expression after mouse oocyte and embryo vitrification and slow freezing (Shin et al. 2011; Wen et al. 2014). In somatic cells, Neutelings et al. (2013) reported some re-programming gene expression induced in somatic cultured cells caused by return to normothermia after hypothermic conditions.

References:
De Leeuw F, Zhang T, Wauquier C, Huez G, Kruys V, Gueydan C. 2007. The cold-inducible RNA-binding protein migrates from the nucleus to cytoplasmic stress granules by a methylation-dependent mechanism and acts as a translational repressor. Exp. Cell Res. 313:4130.
Liao Y, Tong L, Tang L, Wu S. 2017. The role of cold-inducible RNA binding protein in cell stress response. Int. J. Cancer 141:2164.
Masuda T, Itoh K, Higashitsuji H, Higashitsuji H, Nakazawa N, Sakurai T, Liu Y, Tokuchi H, Fujita T, Zhao Y, et al. 2012. Cold-inducible RNA-binding protein (Cirp) interacts with Dyrk1b/Mirk and promotes proliferation of immature male germ cells in mice. Proc. Natl. Acad. Sci. 109:10885.
Matsumoto K, Aoki K, Dohmae N, Takio K, Tsujimoto M. 2000. CIRP2, a major cytoplasmic RNA-binding protein in Xenopus oocytes. Nucleic Acids Res. 28:4689.
Neutelings T, Lambert CA, Nusgens B V., Colige AC. 2013. Effects of Mild Cold Shock (25ºC) Followed by Warming Up at 37ºC on the Cellular Stress Response. PLoS One 8:1.
Nishiyama H, Danno S, Kaneko Y, Itoh K, Yokoi H, Fukumoto M, Okuno H, Millán JL, Matsuda T, Yoshida O, et al. 1998. Decreased expression of cold-inducible RNA-binding protein (CIRP) in male germ cells at elevated temperature. Am. J. Pathol. 152:289.
Shin MR, Choi HW, Kim MK, Lee SH, Lee HS, Lim CK. 2011. In vitro development and gene expression of frozen-thawed 8-cell stage mouse embryos following slow freezing or vitrification. Clin. Exp. Reprod. Med. 38:203.
Wen Y, Zhao S, Chao L, Yu H, Song C, Shen Y, Chen H, Deng X. 2014. The protective role of antifreeze protein 3 on the structure and function of mature mouse oocytes in vitrification. Cryobiology 69:394.
Zhong P, Huang H. 2017. Recent progress in the research of cold-inducible RNA-binding protein. Furture Sci. OA 3:FSO246.

Self-evaluation: 50%

Research gap

3/21. Theoretical question: which is the main gap that your research addresses?

Cryopreserved animal germplasm (sperm, oocytes and embryos) has multiple functions and objectives, such as recuperation of breeds or breeding lines in case of catastrophe, serve as a support of "in vivo" conservation or as a substitute if a genetic problem occurs (FAO 2012). Human medicine has also used oocyte cryopreservation as a preventative medical treatment to protect women against premature loss of ovarian function, age-related fertility decline, or cytotoxic therapy (Baldwin et al. 2015). However, there are some difficulties regarding to the application of cryopreservation methods in oocytes due to the large size of these cells and their marked sensibility to cooling injuries (Sprícigo et al. 2012).

Cryopreservation of oocytes has been performed by controlled slow freezing and vitrification in human, mouse and cow models (Rienzi et al. 2016). Better results have been reported in vitrified oocytes compared with slow freezing (Gualtieri et al. 2011; Martínez-Burgos et al. 2011). The use of high concentration of cryoprotectants and the cooling effect of liquid nitrogen during vitrification produce osmotic damage, toxicity and cryoinjury (Huang et al. 2006). Further investigations are needed in order to find the optimal protocol for oocyte vitrification.

References:
Baldwin K, Culley L, Hudson N, Mitchell H, Lavery S. 2015. Oocyte cryopreservation for social reasons: Demographic profile and disposal intentions of UK users. Reprod. Biomed. Online 31:239.
FAO. 2012. Cryoconservation of Animal Genetic Resources.
Gualtieri R, Mollo V, Barbato V, Fiorentino I, Iaccarino M, Talevi R. 2011. Ultrastructure and intracellular calcium response during activation in vitrified and slow-frozen human oocytes. Hum. Reprod. 26:2452.
Huang J, J Y, Chen HY, Tan SL, Chian RC. 2006. Effects of Osmotic Stress and Cryoprotectant Toxicity on Mouse Oocyte Fertilization and Subsequent Embryonic Development In Vitro. Cell Preserv. Technol. 4:149.
Martínez-Burgos M, Herrero L, Megías D, Salvanes R, Montoya MC, Cobo AC, Garcia-Velasco JA. 2011. Vitrification versus slow freezing of oocytes: Effects on morphologic appearance, meiotic spindle configuration, and DNA damage. Fertil. Steril. 95:374.
Rienzi L, Gracia C, Maggiulli R, LaBarbera AR, Kaser DJ, Ubaldi FM, Vanderpoel S, Racowsky C. 2016. Oocyte, embryo and blastocyst cryopreservation in ART: systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance. Hum. Reprod. Update 23:139.
Sprícigo JFW, Morais KS, Yang BS, Dode MAN. 2012. Effect of the exposure to methyl-B-cyclodextrin prior to chilling or vitrification on the viability of bovine immature oocytes. Cryobiology 65:319.

Self-evaluation: 100%

Research question or hypothesis

4/21. Theoretical question: which is the main question or hypothesis of your research?

The main question of my research is whether the "in vitro" maturation of oocytes in conditions of hypotermia increases CIRBP activity, and whether this increase in expression of CIRBP could have a beneficial effect on vitrification cryotolerance and developmental competence in oocytes.

Self-evaluation: 100%

State of the science

5/21. Theoretical question: which is the current answer to your research question or hypothesis?

There is no current answer for my research question.
Different strategies have been assessed to improve cryotolerance in mammalian embryology through a temporary increase of general adaptation induced by sub-lethal stressors (Pribenszky et al. 2010) such as high hydrostatic pressure (Gu et al. 2017) and heat stress (Vendrell-Flotats et al. 2017). Cells react against these stress impacts with temporary tolerance of damage thus counteracting the initial stressor (Pribenszky et al. 2010). However, cold stress has not been studied in order to improve cryotolerance in mammalian germ cells and embryos.

References:
Gu R, Feng Y, Guo S, Zhao S, Lu X, Fu J, Sun X, Sun Y. 2017. Improved cryotolerance and developmental competence of human oocytes matured in vitro by transient hydrostatic pressure treatment prior to vitrification. Cryobiology 75:144.
Pribenszky C, Vajta G, Molnar M, Du Y, Lin L, Bolund L, Yovich J. 2010. Stress for stress tolerance? A fundamentally new approach in mammalian embryology. Biol. Reprod. 83:690.
Vendrell-Flotats M, Arcarons N, Barau E, López-Béjar M, Mogas T. 2017. Effect of heat stress during in vitro maturation on developmental competence of vitrified bovine oocytes. Reprod. Domest. Anim. 52:48.

Self-evaluation: 50%

Philosophical stance

6/21. Methodological question: which is the philosophical stance of your research?

The specific term for my research's philosophical stance is quantitative objectivism.

Self-evaluation: 50%

Research strategy

7/21. Methodological question: which is the qualitative, quantitative or mixed method of your research?

The method of my research is quantitative.

Self-evaluation: 50%

Collection techniques

8/21. Methodological question: which are the data collection techniques of your research?

The data collection techniques adopted in my research are based on evaluation of embryological development of young heifer oocytes after being or not vitrified.

Self-evaluation: 100%

Analysis techniques

9/21. Methodological question: which are the data analysis techniques of your research?

The data analysis techniques adopted of my research is based on interpretation of frequencies for oocytes that are capable of reaching an embryonic development up to the blastocyst stage.

On data from each oocyte, a logistic regression will be performed using blastocyst yield as the dependent variable (0 or 1) and replicate and group as independent factors. All variables above will be considered as class variables. Logistic regressions analyses will be performed using RStudio (R version 3.4.4.).
According to Hosmer & Lemeshow (2000), logistic regression involves five steps as follows: 1) preliminary screening of all variables for univariate associations; 2) construction of a full model using all the variables found to be significant in the unvariate analysis; 3) stepwise removal of non-significant variables from the full model and comparison of the reduced model with the previous model for model fit and confounding; 4) evaluation of plausible two ways interactions among variables; 5) assessment o model fit using Hosmer-Lemeshow statistics. Variables with univariate associations showing $p<0.25$ will be included in the initial model. We will continue modelling until all the main effects or interaction

terms will be significant according to the Wald statistic at $p<0.05$.

References:
Hosmer, D.W. & Lemeshow, S. (2000). Applied Logistic Regression. Second Edition. Wiley. New York.

Self-evaluation: 100%

Quality criteria

10/21. Methodological question: which are the tactics of your research to ensure scientific quality criteria?

In order to ensure scientific quality criteria diverse different approaches will be used including external validity, internal validity, convergent validity and reliability.
A large sample of bovine oocytes will be used for inferential statistic generalisation. Embryo development to the final stage of blastocyst in vitrified and no vitrified oocytes will be used to guarantee internal validity. The triangulation of streams of thought, collection techniques and the origin of data will be assure convergent validity. In our research reliability will be assessed by the replicates of the experiment.

Self-evaluation: 0%

Unit of analysis

11/21. Empirical question: which is the unit of analysis of your research?

The unit of analysis of my research that I compare in order to operationalize the relationship between vitrification cryotolerance and cold stress are bovine oocytes from young heifers.
Each oocyte will be monitorized at day 2, day 7 and day 8 post "in vitro" fertilization for the propose to assess cleavage (day 2) and blastocyst yield (day 7 and 8).

Self-evaluation: 50%

Level of analysis

12/21. Empirical question: which is the level of analysis of your research?

The level of analysis of my research is individual.

Self-evaluation: 50%

Nature of data

13/21. Empirical question: which is the nature of the data of your research?

The nature of the data of my research is quantitative. It is based on percentages of cleavage and embryo achievement in control and cold stressed bovine oocytes after being or not vitrified.

Self-evaluation: 100%

Origin of data

14/21. Empirical question: which is the origin of the data of your research?

The data of my research is primary obtained from the evaluation of cleavage and embryo development.

Self-evaluation: 100%

Sample

15/21. Empirical question: which is the sample of your research?

The sample of my research are bovine oocytes obtained from bovine ovaries that will be collected at slaughter from a local abattoir (Escorxador de Mercabarna, Barcelona) and transported to the laboratory to perform the "in vitro" procedures. In order to obtain enough data for statistical analysis, I will replicate the experiment 8 times. For each experiment I will need at least 50 oocytes per group (4 groups: control fresh, control vitrified, cold-shock fresh, cold-shock vitrified = 200 oocytes for experiment, 1 600 oocytes in total). Normally, for one ovary we select 3-4 suitable oocytes for "in vitro" process. For that reason, we expected to use between 540 and 400 young heifer ovaries.

Self-evaluation: 100%

Pathos

16/21. Rhetorical question: which are the positive and negative emotions of your research?

The development of new strategies for the cryopreservation of bovine oocytes will provide future tools for assisted reproductive technology, not simply in animal husbandry, but also in human medicine. In addition, scientific advances in germplasm cryopreservation could be used for

conservation biology purposes. Moreover, it will increase the knowledge about the role of the cold-shock proteins in mammalian reproductive cells that could be involved in more signalling pathways hitherto known.
On the negative site, my research could not necessarily be seen as a progress process by the more conservative sectors of society. Also, the fact that the samples are obtained from sacrified animals could have an impact on some animal defence groups and environmentalists despite the fact that the local abattoir follows the European regulations for animal sacrifice.

Self-evaluation: 100%

Logos

17/21. Rhetorical question: which is the scientific logic of your research?

The scientific logic of my research is hypothetic-deductive, founded on deduction of the hypothesis that cold-shock proteins could improve the results obtained from oocyte vitrification.

Self-evaluation: 100%

Ethos

18/21. Rhetorical question: which are the limitations of your research?

The principal theoretical limitation of my research is the lack of knowledge of how cold-shock proteins are involved in stress tolerance in reproductive mammalian cells.
The main methodological limitation of my research is the use of young heifers instead of cows may result in more variability within experiments.
The main empirical limitation of my research is the absence of information about the effect of cold stress in mammalian oocytes.

Self-evaluation: 50%

Wisdom

19/21. Authorial question: which is your education and experience related with your research?

My previous education is based on Veterinary Medicine. I have graduated from the Universitat Autònoma de Barcelona, where I completed a 5-year Bachelor's Degree in Veterinary Medicine obtaining a special award for academic qualifications. During the last academic course I participated in an Exchange Programme of two months at the Faculty of Veterinary Science of the University of Pretoria (South Africa) where I completed a Clinical Rotation Service Programme including Animal Reproduction and Production Animal services among others. Currently, I'm in a PhD programme in Animal Medicine and Health of Universitat Autònoma de Barcelona where I have joined the ERPAW Research Group (Endocrinology, Reproductive Physiology and Animal Welfare Research Group).

Self-evaluation: 100%

Trust

20/21. Authorial question: who are the partners of your research?

Our Research Group has large experience in Reproductive Physiology having already developed many other research projects in bovine reproduction that contribute to the practicability of the thesis project. Besides, my supervisors had been involved in many publications in the highest factor impact journals in the field of Animal Reproduction.
Moreover, the Research Group has also made international collaborations with other universities that might allow me to do international stays thus enriching my thesis project with regard to methodology and applicability of different research strategies.

Self-evaluation: 50%

Time

21/21. Authorial question: which is your availability of time and resources for your research?

I have a predoctoral grant for the recruitment of early-stage research staff from the Government of Catalonia co-financed with the European Social Fund (Agència de Gestió d'Ajuts Universitaris i de Recerca, Generalitat de Catalunya, 2018 FI_B 00236) that devote me full-time to complete the research project in three years. Also, this thesis project is part of a Spanish financed project called "Development of optimal strategies to improve bovine oocyte and embryo cryopreservation" (Proyectos Excelencia, AGL2016-79802-P), in which I am a team member.

Self-evaluation: 100%