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The effect of ovine oocyte vitrification on expression of subset of genes involved in epigenetic modifications during oocyte maturation and early embryo development

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ABSTRACT

Apart from ultrastructural damages in oocytes subjected to cryopreservation procedures, little is known about the status of epigenetic modification and chromatin remodeling in vitrified oocytes. In present study, the expression patterns of eight genes involved in epigenetic modification (*HAT1*, *HDAC1*, *SUV39H1*, *DNMT1*, and *DNMT3b*), chromatin remodeling (*HMGN3a* and *SMARCAL1*), and transcription (*STAT3*), were investigated in fresh and vitrified germinal vesicle and metaphase II oocytes and their resulting embryos at 2 to 7 cells, 8 to 16 cells, morula, and blastocyst stages. The mRNA relative abundance was quantified by reverse transcriptase real-time polymerase chain reaction, as fold change relative to the value obtained for fresh germinal vesicle oocytes. Vitrified oocytes showed lower cleavage (38.1% vs. 95.5%, $P < 0.001$) and development to blastocyst (8.2% vs. 50.8%, $P < 0.001$) compared with controls. In both fresh and vitrified groups, the genes expressions in oocytes were lower than cleaving embryos, with an exception of *HMGN3a*. Compared with fresh derived embryos, in vitrified groups, the overall expressions of *HMGN3a* and *HDAC1* were decreased, whereas the expressions of *STAT3*, *SMARCAL1*, and *DNMT3B* were increased. Altogether, despite some differences in expression pattern of some genes, the overall pattern of increase and/or decrease in gene expression was almost the same in most of the genes studied between vitrified and fresh groups. Thus, apart from some mismatch in pattern of genes expression in a number of cases, the difference in magnitude and/or primacy and recency in reaching to the maximum expression, in association to embryonic genome activation, between fresh and vitrified groups, might be the reason for the lower developmental competence of vitrified-warmed oocytes compared with fresh ones.

1. Introduction

Oocyte cryopreservation contrasted to the embryo, and sperm freezing is not recognized as a well-established procedure. There are several studies focusing on detrimental alterations in oocyte ultrastructural components after cryopreservation. These alterations depending on species involved and on cryopreservation procedures used, may include a broad spectrum aberrations in plasma membrane selective permeability [1], microtubules and microfilaments organizations, and cytoplasmic organelles distributions [1,2] which can finally impair the subsequent oocyte developmental competence because of improper sperm penetration, aneuploidy, [3] and nuclear fragmentation [4].

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In addition, the study on metabolome and proteome of cryopreserved oocyte has revealed the dramatic effect of cryopreservation on oocyte physiology such as the reduction of maturation promoting factor activity in oocytes after vitrification and warming [5–7].

Regarding to the oocyte developmental stage in which the oocyte is more resistant to cryopreservation, there is no general consensus. Different lines of evidence clearly indicate that surrounding cumulus cells play a fundamental role in the maturation process [8]. In this respect, exposure to the cryoprotectants has been found to disorganize the actin filaments within transzonal projections through which cumulus cells establish physical contact with the oocytes [9]. Therefore, oocytes vitrification at GV and exposure to cryoprotectants can decrease the frequencies of IVM. On the other hand, cryopreserved oocytes at MII stage are more prone to lose spindle fiber integrity [10] which may lead to aneuploidy [11] and premature release of the cortical granules that is likely to lead to zona hardening [12].

Despite numerous studies on structural and morphological damages induced by cryopreservation in oocytes and the resulting embryos, few studies have been done on the impact of vitrification on the expression pattern of genes involved in epigenetic alterations [13–19].

Most of these works concerned mainly the effect of oocytes cryopreservation on the expression of genes involved in oxidative stress, apoptosis, and cell cycle [14,15,17,18]. Nonetheless, there is scarce report (s) on the effect of oocyte cryopreservation on expression of genes involved in epigenetic modification which is essential for the subsequent normal embryonic development [20]. Normally, the major epigenetic reprogramming takes place during gametogenesis and early embryo development; the complex DNA methylation patterns are established and maintained by DNA methyltransferases (DNMTs).

Epigenetic remodeling is also crucial for embryonic genome activation [21]. Embryonic genome activation (EGA) is a critical event for the preimplantation embryo, which is manifested by changes in chromatin structure, transcriptional machinery, expression of embryonic genes, and degradation of maternal transcripts.

In epigenetic modification, numerous components are involved including the products of those genes that can alter histone-DNA interactions within the nucleosome core particle that are thought to influence the expression or repression of transcription [22]. The amino-terminal tails protruding from the core are subject to enzyme-catalyzed and posttranslational modification, such as acetylation, methylation, phosphorylation, and ubiquitination [23–25]. Posttranslational modifications by acetylation of the N-termini portion of nucleosomal histones alter the higher-order chromatin structure to render the DNA accessible to the regulatory and transcriptional machinery.

DNA methylation as another key epigenetic modification is essential for normal embryonic development in which the complex DNA methylation patterns are established and maintained by DNMTs. In sheep, it has been shown that the overall DNA methylation declines from the two-cell stage until the blastocyst stage [26]. In pig, unlike the mouse and sheep, there is no apparent loss of DNA methylation from the two-cell to morula stage [27]. The results in bovine embryo are controversial, while there is a report indicating the widespread demethylation at the 8-cell stage and the subsequent increase in de novo methylation at the 16-cell stage [28], another report has shown the persistence of DNA demethylation in all these steps [29].

Another component of the epigenetic modification is the family of specialized proteins involved in chromatin remodeling. The high levels of structural conservation of these proteins during early mammalian embryonic development highlights the importance of chromatin remodeling in the regulation of gene expression that once more emphasizes the importance of epigenetics on the underlying mechanisms involved in early mammalian development [30].

To our knowledge, very little information is available on gene expression status involved in epigenetic modification in cryopreserved mammalian oocytes [31,32]. The present study was aimed to evaluate the impact of vitrification on the relative mRNA abundance of a panel of developmentally important genes involved in epigenetic modification in vitrified-warmed ovine oocytes and the resulting embryos. Besides, the effect of vitrification on the ability of vitrified-warmed ovine oocytes to proceed through *in vitro* development was assessed.

2. Materials and methods

Except where otherwise indicated all chemicals were obtained from the Sigma (St. Louis, MO, USA).

2.1. Source of oocytes

Ewe ovaries obtained from prepubertal and adult ewes were collected from a local slaughterhouse and transported to the laboratory in Dulbecco's phosphate buffer saline containing antibiotics at 30 °C. At the laboratory, the ovine cumulus oocyte complexes (COCs) were obtained by aspirating follicles with 2- to 6-mm diameter [33].

The follicular content was aspirated in preincubated hepes-TCM, supplemented with 50 IU/mL heparin and antibiotics. For oocyte vitrification, the COCs were selected in sterile Petri dishes containing 20-mM Hepes-buffered TCM 199 supplemented with 10% fetal calf serum (FCS) and antibiotics, on the basis of the presence of several intact cumulus cells layers, evenly granulated oocyte's cytoplasm with homogenous lipid droplets distribution.

2.2. Oocyte vitrification and warming

Cumulus oocyte complexes vitrification was performed in accordance with the method of minimum essential volume using cryotops as device. Briefly, as previously described by Succu et al. (2008) [9], groups of five immature oocytes were initially equilibrated for 1 minute in holding medium (HM) consisted of 20-mM Hepes-buffered TCM-199 supplemented with 20% FCS.

The equilibrated COCs were incubated in 10% ethylene glycol (EG) plus 10% dimethyl sulfoxide in HM for 30 seconds and successively transferred into 20% EG plus 20% dimethyl sulfoxide and 0.5-M sucrose in HM for