Theriogenology 86 (2016) 868–878

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Effects of oocyte vitrification on epigenetic status in early bovine embryos

Huanhuan Chen, Lei Zhang, Tengfei Deng, Pengda Zou, Yongsheng Wang, Fusheng Quan**, Yong Zhang*

Key Laboratory of Animal Biotechnology of the Ministry of Agriculture, College of Veterinary Medicine, Northwest A&F University, Yangling, Shaanxi, China

A R T I C L E I N F O

Article history:
Received 5 June 2015
Received in revised form 3 March 2016
Accepted 5 March 2016

Keywords:
Oocyte vitrification
Embryonic development
Epigenetics
Bovine

A B S T R A C T

Oocyte cryopreservation has a great impact on subsequent embryonic development. Currently, several studies have primarily focused on the consequences of vitrification and the development potential of cellular structures. This study determined whether oocyte vitrification caused epigenetic instabilities of bovine embryos. The effects of oocyte vitrification on DNA methylation, histone modifications, and putative imprinted genes' expression in early embryos derived by intracytoplasmic sperm injection were examined. Results showed that oocyte vitrification did not affect zygote cleavage rates (67.0% vs. 73.8% control, $P > 0.05$) but reduced the blastocyst rate (9.6% vs. 23.0%, $P < 0.05$). The levels of DNA methylation and H3K9me3 in oocytes and early cleavage embryos were lower ($P < 0.05$) than those in control group, but the level of acH3K9 increased ($P < 0.05$) in the vitrification group during the early cleavage phases. No differences were observed for DNA methylation, H3K9me3, and acH3K9 in the inner cell mass of blastocysts, whereas decreased levels of DNA methylation and acH3K9 ($P < 0.05$) existed in TE cells after vitrification. The expression of putative-imprinted genes *PEG10*, *XIST*, and *KCNQ1O1T* was upregulated in blastocysts. These epigenetic abnormalities may be partially explained by altered expression of genes associated with epigenetic regulations. DNA methylation and H3K9 modification suggest that oocyte vitrification may excessively relax the chromosomes of oocytes and early cleavage embryos. In conclusion, these epigenetic indexes could be used as damage markers of oocyte vitrification during early embryonic development, which offers a new insight to assess oocyte vitrification.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Vitrification has been widely employed to cryopreserve animal and human oocytes and embryos. It can not only promote oocyte donation program but also preserve fertility in cancer patients who have to receive frequent radiation therapy [1]. At present, oocyte vitrification has been successfully introduced in humans [2] and many other species, such as cattle [3] and porcine [4]. However, oocyte vitrification still faces some challenge with relatively lower embryonic development potential.

Epigenetics refers to certain heritable modifications primarily occurring in the DNA or chromosome, such as DNA methylation, histone modification, and genomic imprinting, without changing the DNA sequences [5,6]. The erasure and remodeling of these epigenetic markers during mammalian development is called epigenetic reprogramming. It occurs primarily during gamete formation and early embryonic development before preimplantation [7]. The epigenome must be reprogrammed accurately during the early

* Corresponding author. Tel./fax: +86 29 87080085.
** Corresponding author. Tel.: +86 29 87080092; fax: +86 29 87080085.
E-mail addresses: quanfusheng@nwsuaf.edu.cn (F. Quan), zhangyong1956@nwsuaf.edu.cn (Y. Zhang).

0093-691X/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2016.03.008

embryonic development because it is closely related to the activation of zygote genomes and cell fate determination. In fact, DNA methylation has key functions in transcriptional regulation, such as X chromosome inactivation, genome imprinting, and transcriptional silencing of specific genes and repetitive elements [8,9]. Reprogramming of DNA methylation is necessary for the normal development of early embryos. Aberrant methylation changes would impair embryonic development and cleavage [10]. Recently, histone methylation was shown to be essential for early embryonic development and pluripotency maintenance of embryonic stem cells [11]. Histone acetylation is closely associated with several cell functions, such as chromosome decondensation, DNA double-strand break repairing, and transcription [12–14]. Genome imprinting often leads to single allele expression of genes, which regulates embryonic development, placenta function, and neurobehavior [15,16]. The establishment and maintenance of imprinting depends mostly on DNA methylation in differential methylated regions of genes and some other epigenetic mechanisms. In cattle, the proportion of cloned embryos with correct epigenotype was similar to that of embryos developing to the blastocyst stage [17].

Epigenetic reprogramming could be sensitive to an external environmental stimulus. For example, the normal pattern of imprinted gene expression was affected by certain assisted reproduction technologies, such as ovary stimulation, IVF, IVC of embryos and embryo transfer [18–20]. Levels of DNA and histone methylation can be altered by components in culture media [21,22]. In addition, birth weight, and the pattern of gene expression could be changed by cryopreservation methods [23,24]. Aberrant or incomplete epigenetic reprogramming before implantation may result in delayed or even lethal embryonic development. Here we infer that oocyte vitrification, as a very strong stress factor, may cause enormous effects on epigenetic reprogramming during early embryonic development, which finally results in a decreased development potential. Thus, the objective of this study was to investigate the effects of oocyte vitrification on DNA methylation, histone H3K9 modifications, and putative-imprinted genes' expression during the early development of bovine embryos.

2. Material and methods

All reagents and chemicals were purchased from Sigma–Aldrich except those specifically indicated otherwise.

2.1. Oocyte collection and IVM

Bovine ovaries were derived from a nearby slaughterhouse and transported to our laboratory at 20°C to 25 °C within 4 hours. The cumulus–oocyte complexes were aspirated from antral follicles (diameter 2–8 mm). Cumulus–oocyte complexes with evenly granulated cytoplasm and compact cumulus were selected for IVM. Before maturation, the cumulus–oocyte complexes were carefully cleaned three times with PBS containing 3% fetal bovine serum (FBS) and then cultured for 20 hours for maturation in saturation humidity with 5% CO_2 at 38.5 °C in the maturation medium. The maturation medium was prepared with bicarbonate-buffered tissue culture medium 199 (TCM 199, Gibco) containing 10% FBS, 1 μg/mL 17β-estradiol, and 0.075 IU/mL human menopausal gonadotropin (HMG).

2.2. Oocyte vitrification and thawing

The Cryotop method was employed for oocyte vitrification and thawing. The base medium was prepared with 25 mM HEPES-buffered TCM 199 supplemented with 20% FBS. Two primary steps—equilibrium and vitrification—were included during the vitrification process. Each time, eight to 12 secondly meiotic metaphase (MII) oocytes were first placed onto the middle surface of the equilibrium solution, which was prepared by addition of 7.5% (v:v) ethylene glycol (EG) and 7.5% (v:v) dimethyl sulfoxide (DMSO) to the base medium. After 5 minutes of equilibrium, the initially shrunken oocytes would dilate to the original volume, which indicated the time for vitrification. One to two oocytes were transferred into the vitrification solution, which was prepared using 15% (v:v) EG, 15% (v:v) DMSO, and 0.5-M sucrose dissolved in the base medium. Within 1 minute, the oocytes have to be completely rinsed with vitrification solution, loaded on the Cryotop straw in a volume of less than 0.1 μL drop, and finally plunged into liquid nitrogen. The maximum time for which the oocytes could be kept in the equilibrium solution should be less than 12 minutes. The oocytes were stored in liquid nitrogen for at least 2 weeks before the next thawing step. For thawing, three kinds of solutions were prepared: the thawing solution (TS), diluting solution, and washing solution using 1, 0.5, and 0-M sucrose, respectively, dissolved in the base medium. The vitrified oocytes were removed from liquid nitrogen and plunged into TS at 37 °C for 1 minute. Next, the oocytes were transferred into diluting solution for 3 minutes and cleaned twice for 5 minutes in washing solution. Oocytes with an intact zona pellucida and membrane were assumed live. Except for TS incubated at 37 °C for 1 hour, all other solutions involved in vitrification and warming were warmed at the room temperature for 30 minutes before use.

2.3. Intracytoplasmic sperm injection and embryo culture

Immediately before intracytoplasmic sperm injection (ICSI), 2 μL of sperm suspension was mixed with 8 μL of HEPES-buffered TCM 199 under paraffin oil, in which 10% (v:v) polyvinylpyrrolidone was included to reduce sperm motility and avoid spermatozoa being stuck to the micropipette. Intracytoplasmic sperm injection was performed on an inverted microscope (Olympus, Tokyo, Japan) equipped with a hydraulic micromanipulator (Narishige, Tokyo, Japan) in 40-μL droplets of TCM 199 under mineral oil in a 35-mm cell culture dish (Nunc Thermo Scientific, Shanghai, China). Oocytes were held at the 12 o'clock position of the first polar body by the holding pipette. During injection, the pipette having the sperm was inserted into the oocyte at the 3 o'clock position of the first polar body. A small volume of ooplasm was aspirated into the injection pipette to rupture the cytoplasmic membrane of the

Download English Version:

https://daneshyari.com/en/article/2094972

Download Persian Version:

https://daneshyari.com/article/2094972

Daneshyari.com