Theriogenology 93 (2017) 111–123

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Transcriptomic evaluation of bovine blastocysts obtained from peri-pubertal oocyte donors

Léonie Morin-Doré [a], Patrick Blondin [b], Christian Vigneault [b], François-Xavier Grand [b], Rémi Labrecque [b], Marc-André Sirard [a, *]

[a] Centre de recherche en reproduction, développement et santé intergénérationnelle (CRDSI), Département des Sciences Animales, Faculté des Sciences de l'Agriculture et de l'Alimentation, Université Laval, Québec, Canada
[b] L'Alliance Boviteq Inc, Saint-Hyacinthe, Québec, Canada

ARTICLE INFO

Article history:
Received 21 September 2016
Received in revised form
9 December 2016
Accepted 3 January 2017
Available online 16 January 2017

Keywords:
Transcriptomics
Embryo quality
In vitro blastocyst
Bos taurus
Peri-pubertal heifer
Metabolism

ABSTRACT

Assisted reproduction technologies (ART) and high selection pressure in the dairy industry are leading towards the use of younger females for reproduction, thereby reducing the interval between generations. This situation may have a negative impact on embryo quality, thus reducing the success rate of the procedures. This study aimed to document the effects of oocyte donor age on embryo quality, at the transcriptomic level, in order to characterize the effects of using young females for reproduction purpose.

Young Holstein heifers (n = 10) were used at three different ages for ovarian stimulation protocols and oocyte collections (at 8, 11 and 14 months). All of the oocytes were fertilized *in vitro* with the semen of one adult bull, generating three lots of embryos per animal. Each animal was its own control for the evaluation of the effects of age. The EmbryoGENE platform was used for the assessment of gene expression patterns at the blastocyst stage. Embryos from animals at 8 vs 14 months and at 11 vs 14 months were used for microarray hybridization. Validation was done by performing RT-qPCR on seven candidate genes.

Age-related contrast analysis (8 vs 14 mo and 11 vs 14 mo) identified 242 differentially expressed genes (DEGs) for the first contrast, and 296 for the second. The analysis of the molecular and biological functions of the DEGs suggests a metabolic cause to explain the differences that are observed between embryos from immature and adult subjects. The mTOR and PPAR signaling pathways, as well as the NRF2-mediated oxidative stress response pathways were among the gene expression pathways affected by donor age. In conclusion, the main differences between embryos produced at peri-pubertal ages are related to metabolic conditions resulting in a higher impact of *in vitro* conditions on blastocyts from younger heifers.

1. Introduction

In the dairy industry, the development of ovum pick up (OPU) methods, *in vitro* embryo production (IVM, IVF, IVC), and embryo transfer (ET), combined with the increased efficiency of genomic tools, made the use of young animals for reproduction purpose possible. The use of ultrasound to collect gametes from pre-pubertal and peri-pubertal hormonally stimulated animals is not recent [1–6]. While no deleterious effects are associated with ovarian stimulation or OPU on the health and production potential of young donors [2,3,7], genetic gain is certainly accelerated, as the best animals are identified early in life and the generation interval is considerably reduced [8,9].

Although it is possible to recover more oocytes from the ovaries of young animals [10], it is well documented that calf oocytes are less competent compared to gametes from cows (>14 months of age) [3,11–16]. Despite the fact that healthy embryos can be obtained from pre-pubertal and peri-pubertal donors *in vivo* and *in vitro* [2,17,18], several research groups explored the differences observed between calf and cow oocytes (and also embryos) such that some facts now support the following statements: (1) embryos from pre-pubertal calves transferred into adult recipients produce

* Corresponding author. Centre de recherche en Reproduction, Développement et Santé Intergénérationnelle (CRDSI), Département des Sciences Animales, Université Laval, Québec, Québec G1V 0A6, Canada.
E-mail address: Marc-Andre.Sirard@fsaa.ulaval.ca (M.-A. Sirard).

http://dx.doi.org/10.1016/j.theriogenology.2017.01.005
0093-691X/

live and healthy offspring at a lower rate than embryos from adult donors, suggesting that donor age can affect oocyte and/or embryo quality [1,2,7,13,18]. In a recent study conducted by our team in a commercial setting, we have observed an age effect both on follicle number and on embryo rates [19]; (2) oocytes from young donors have a reduced developmental competence, and a reduced efficiency to undergo nuclear and ooplasmic maturation [20–22], which affects embryo yield; (3) apoptosis is linked to oocyte donor age and is increased in embryos from juvenile donors, contributing to higher rates of embryonic loss after embryo transfer [23]; and (4) protein expression is reduced, even defective, in oocytes from young donors compared to oocytes from adult cows [21,24].

Several studies explored methods to improve oocyte quality, but with variable success rates. In fact, hormonal stimulation of pre-pubertal and peri-pubertal calves may increase oocyte competence and embryo development potential [2,3,13,18,25]. It was also reported, in pre-pubertal animals, that follicles > 8 mm in diameter contained oocytes with a better ability to develop to the blastocyst stage *in vitro* [26]. Oocyte developmental competence is therefore related to the age of the donor and can be improved by hormonal treatment [16,27].

The purpose of this study was to explore why embryos obtained from oocytes from young donors are less competent than those obtained from oocytes from adult cows. The gene expression pathways most significantly affected by age suggest that there is a metabolic cause responsible for the differences observed between embryos obtained from immature and adult subjects.

2. Material and methods

2.1. Chemicals

All reagents and media supplements used in this study were of tissue culture grade and obtained from Sigma-Aldrich Co. unless otherwise specified.

2.2. Animals

Prepubertal *Bos taurus* Holstein heifers (n = 10) were used for this study. Each heifer underwent three ovarian stimulation cycles, resulting in three gamete collections, at the ages of 8, 11 (average 10.8) and 14 (average 13.7) months. The gametes were fertilized *in vitro* with spermatozoa from one adult male, producing three embryo lots per animal. Each heifer was its own control for the measurement of age effect. This animal phase of the study was performed in an industrial IVF setting at Boviteq (Saint-Hyacinthe, QC, Canada), a specialized center for bovine embryo transfer and other assisted reproduction technologies involved in research and development.

The clinical procedures and industrial practices used in Boviteq follow the established cattle reproduction management practices, which have been approved by the College of Veterinary Surgeons of Quebec (OMVQ), the Canadian Embryo Transfer Association (CETA) and the International Embryo Transfer Society (IETS). This company follows the Canadian Council on Animal Care (CCAC) guidelines for farm animals and the research projects do not involve the use of exclusive animals for research purposes, and neither does it involve the implementation of new animal procedures to obtain additional biological samples other than the ones used in our routine commercial activities. The study did not require handling animals on university premises.

2.3. Ovarian stimulation and gamete collection

The protocol for ovarian stimulation and ovum pick-up (OPU) was essentially the same as described by Nivet et al. [28] and Labrecque et al. [29]. Briefly, each heifer was first treated with progesterone (CIDR) during the luteal phase in order to repress dominant follicles to reduce the risk of spontaneous ovulation. The dominant follicle was aspirated 36 h prior to administration of hormones. The ovarian stimulation program consisted of six injections of 30 mg FSH (Folltropin-V, Bioniche Animal Health, Belleville, ON, Canada) administered at 12 h intervals. Ovum pick up was performed 43 h after the last FSH injection, the optimal *coasting* (FSH starvation) period [28]. Using transvaginal ultrasonography, follicular diameters were measured and cumulus-oocyte complexes (COCs) were collected by OPU under epidural (COOK Medical, Bloomington, IN, USA). Granulosa cells and COCs were collected in warm HEPES-buffered Tyrode's medium (TLH) containing Hepalean (10 μL/mL) and transferred to the laboratory for IVM. The gamete collection procedures took place in the time period between October 2014 and May 2015.

2.3.1. In vitro maturation (IVM)

The COCs were placed in HEPES-buffered TLH solution (supplemented with 10% bovine serum, 0.2 mM pyruvate, and 50 mg/mL gentamicin) and washed three times to remove follicular fluid. Healthy COCs were placed in tubes of maturation medium composed of TCM199 (Invitrogen Life Technologies), 10% fetal bovine serum (FBS), 0.2 mM pyruvate, 50 mg/mL gentamicin, 5 mg/mL FSH (Bioniche Animal Health), 0.5 mg/mL LH (Lutropin, Bioniche Animal Health, Belleville, ON, Canada), and 1 mg/mL estradiol. Maturation droplets were incubated for 24 h at 38.5 °C with 5% CO_2 in maximal humidity.

2.3.2. In vitro fertilization (IVF)

After 24 h of IVM, the COCs were collected and washed twice in TLH medium before being transferred in groups of five to 48-μL droplets under mineral oil. The droplets consisted of modified Tyrode's lactate (TL) medium supplemented with 0.6% (w/v) fatty acid-free BSA, 0.2 mM pyruvic acid, 2 μg/mL heparin, and 50 mg/mL gentamicin. Oocytes were transferred 15 min prior to semen addition and 2 μL of a solution containing penicillamine (2 mM), hypotaurine (1 mM), and epinephrine (250 mM) were added to each droplet to stimulate sperm motility. Semen from the same bull (Centre d'Insémination Artificielle du Québec, St-Hyacinthe, QC, Canada) was used for all of the IVF, for all of the heifers, in order to avoid any male-associated bias. The spermatozoa, previously stored in liquid nitrogen, were thawed for 1 min in 35.8 °C water, added to a discontinuous gradient (45 over 90% BoviPure (Nidacon Laboratories AB, Göthenborg, Sweden)), and centrifuged at 600 × g for 5 min. The supernatant containing the cryoprotectant and the dead spermatozoa was discarded, and the pellet was then re-suspended in 1 mL of modified TL and centrifuged at 300 × g for 2 min. The re-suspended spermatozoa were counted on a hemocytometer and diluted with IVF medium to obtain a final concentration of 1×10^6 cells/mL. Finally, 2 μL of the sperm suspension were added to the droplets containing the matured COCs. The fertilization medium was incubated at 38.5 °C for 15–18 h in a humidified atmosphere of 95% air and 5% CO_2.

2.3.3. In vitro culture (IVC)

After fertilization, presumptive zygotes were mechanically denuded, washed three times in TLH supplemented with fatty acid-free BSA and were then placed in groups of 10 in 10-μL droplets of modified synthetic oviduct fluid (mSOF) with non-essential amino acids, 3 mM EDTA, and 0.4% fatty acid-free BSA (ICP bio, Auckland, New Zealand) under embryo-tested mineral oil (#8410, Sigma-Aldrich). The embryo culture dishes were incubated at 38.5 °C with 6.5% CO_2, 5% O_2, and 88.5% N_2 in 100% humidity. Embryos were