Theriogenology 86 (2016) 1240–1246

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Effect of cow age on the *in vitro* developmental competence of oocytes obtained after FSH stimulation and coasting treatments

David A. Landry [a], Anne-Marie Bellefleur [b], Rémi Labrecque [b], François-Xavier Grand [b], Christian Vigneault [b], Patrick Blondin [b], Marc-André Sirard [a,*]

[a] Centre de Recherche en Biologie de la Reproduction, 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 18 February 2016
Received in revised form 11 April 2016
Accepted 15 April 2016

Keywords:
Bos taurus
Oocyte donor age
Ovarian stimulation
Follicle population
Developmental competence

ABSTRACT

The use of oocytes obtained from younger donors for IVF followed by embryo transfer represents an opportunity to accelerate genetic gain by reducing generation time. In this study, we investigated the relationship between donor age and the *in vitro* developmental competence of oocytes obtained from Holstein females (aged 5–18 months) after FSH stimulation and coasting. The follicle size patterns showed a significantly higher total number of small follicles (5–6 mm) from donors aged 5 to 10 months and a higher total number of medium-sized follicles (7–10 mm) in donors aged 6 to 7 months. Our analysis also revealed that the total number of follicles was significantly higher ($P < 0.05$) in donors aged 5 to 8 months and tended to be higher ($P = 0.053$) in nine-month-old donors. However, oocytes obtained from donors aged 5 to 10 months yielded fewer embryos reaching the morula and blastocyst stages. In summary, our results demonstrate that a higher number of oocytes can be obtained from younger animals but lower developmental competence negates this gain.

1. Introduction

Over the past decade, the development of *in vitro* production of embryos from young donors has become a valuable tool for enhancing genetic gain in dairy cattle. Combined with genomic improvement of bulls, the progressive use of oocyte donor genomic analysis at an early age has changed the approach to improve the next generation of dairy cows. The use of peripubertal donors for IVF and embryo transfer (IVF-ET) represents a significant opportunity to accelerate gains in genetic merit and to compress the time interval between generations. It is well-established that peripubertal cattle can provide oocytes that yield healthy embryos *in vitro* and *in vivo* [1–3].

Ovarian stimulation, ET, and pregnancy can be achieved without detrimental physiological effects on the prepubertal donor [4–6]. Oocytes can be collected by repeated ovum pick-up (OPU) on calves without affecting subsequent reproductive or lactation performances [1,7]. Normal oocyte development up to the transferable blastocyst stage and beyond can be achieved in peripubertal animals using protocols already established for adult oocytes by combining ovarian stimulation (OS), OPU, IVM, IVF, or IVC, and ET [1–7].

Many studies show that bovine embryos can be produced successfully *in vitro* using oocytes from

* Corresponding author. Tel.: +1 418 656 2131 ext. 7359; fax: +1 418 656 3766.
E-mail address: marc-andre.sirard@fsaa.ulaval.ca (M.-A. Sirard).

0093-691X/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2016.04.064

sexually immature calves as young as 2 to 4-months old [1,3,8]. A number of studies have investigated developmental differences between embryos obtained from calf and adult oocytes with or without OS. No significant differences in percent maturation, fertilization, and early cleavage after IVF have been shown, but reduced blastocyst yield, and significantly greater embryo loss after ET has been associated with younger donors [4,6,8–10]. Ax et al. [7] showed that 10-month-old animals produced oocytes with similar developmental competence and provided transferable embryos in yields comparable to postpubertal cyclic animals (16–18 month old), whereas younger animals (6–9 month old) produced fewer transferable and total embryos. However, embryos originating from younger donors and developing to Day 7 were of similar quality grade and had gene transcription levels similar to those of sexually mature donors [11]. Studies of *Bos indicus* have also shown a higher number of embryos from 10-month-old animals in comparison with younger donors with or without OS [12,13].

Although oocytes from young donors have lower ability to produce good quality blastocysts, superovulation in calves has been shown to increase oocyte developmental competence almost to the same level as in mature animals [1]. Different studies comparing oocyte competence in nonstimulated calves versus hormonally stimulated calves suggest that stimulation increases the percentage of usable oocytes (cumulus-oocyte complex or COC), the cleavage rate, and the percentage of embryos that reach the morula and blastocyst stages [6,9,14].

In cattle, OS followed by a gonadotropin-free resting period called “coasting” has been shown to constitute an efficient regimen for generating oocytes of higher quality and for increasing the yield of blastocysts. The use of coasting in OS protocols has produced blastocyst yields as high as 80% in mature cows [15,16]. The coasting period creates a progressive follicular hypoxia associated with an increase in follicular apoptosis and inflammation [17]. This early follicular atresia mimics several preovulatory changes in the dominant follicle and is associated with improved oocyte competence [18].

The objective of this present study is to determine whether donor age affects the developmental potential of oocytes obtained from calves and heifers following a commercial protocol of OS and coasting.

2. Material and methods

2.1. Experimental design

This retrospective study was performed on data gathered for 1031 *Bos taurus* calves or heifers (Holstein breed) aged 5 to 18 months. All ovarian stimulations, *in vitro* fertilizations, and *in vitro* culture were performed from January 2012 to July 2015 in a controlled commercial environment. The number of follicles, the follicle size, and oocyte developmental competence after IVF were investigated to identify any link between donor age and oocyte quality. All animals were grouped according to nominal age: 5 months (n = 22); 6 months (n = 57); 7 months (n = 102); 8 months (n = 131); 9 months (n = 189); 10 months (n = 190); 11 months (n = 131); and 12 months (n = 103). Each age group contained animals aged up to 1 day less than the next age group (1 month = 30 days). The control (sexually mature) group contained animals aged 16 to 18 months (n = 106).

2.2. Chemicals

All reagents and media supplements used in these experiments were obtained from Sigma–Aldrich Co. unless otherwise indicated.

2.3. Ovarian stimulation treatment and oocyte recovery

The dominant follicle was aspirated 36 to 48 hours before hormone administration. Animals were stimulated for 3 days with FSH. According to the body condition score, FSH was administered in 5 × 30 mg, 6 × 30 mg, or 6 × 40 mg doses of NIH Folltropin-V (Bioniche Animal Health, Belleville, Ontario, Canada), followed by a coasting (no FSH) period of 19, 30, or 43 hours. Using transvaginal ultrasonography, follicle diameters were measured, and COCs were collected by transvaginal puncture under epidural anesthesia, using an 18G needle and cook aspiration unit (COOK Medical, Bloomington, IN, USA). Cumulus-oocyte complexes and granulosa cells were collected in warm HEPES-buffered Tyrode’s medium (TLH) containing heparin (10 IU/mL) and transferred to the laboratory for IVM.

2.4. In vitro maturation

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 droplets (50 μL) of maturation medium under mineral oil. Maturation medium was composed of TCM199 (Gibco 11150059, Invitrogen Life Technologies), 10% fetal bovine serum (Wisent Bioproducts), 0.2-mM pyruvate, 50-mg/mL gentamycin, 0.5-mg/mL FSH (Folltropin-V, Bioniche Animal Health, Belleville, Ontario, Canada), 5-mg/mL luteinizing hormone (Lutropin, Bioniche), and 1-mg/mL prostaglandin E_2. Maturation droplets were incubated for 24 hours at 38.5 °C with 5% CO2 in maximal humidity.

2.5. In vitro fertilization

After 24 hours of IVM, COCs were collected and washed twice in TLH medium before being transferred in groups of five to droplets (48 μL) under mineral oil. The droplets consisted of modified Tyrode’s lactate medium supplemented with fatty acid-free BSA (0.6% w:v), pyruvic acid (0.2 mM), heparin (2 μg/mL), and gentamycin (50 mg/mL). Oocytes were transferred under mineral oil 15 minutes before adding semen. To stimulate sperm motility, penicillamine, hypotaurine, and epinephrine (2 μL, 1 mM, and 250 mM, respectively) were added to each droplet. Selected spermatozoa (Semex, Canada) stored in liquid nitrogen were thawed for 1 minute in water at 35.8 °C, laid on a