Theriogenology 81 (2014) 982–987

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

# Effect of temporary meiosis block during prematuration of bovine cumulus–oocyte complexes on pregnancy rates in a commercial setting for *in vitro* embryo production

Samuel Guemra [a,b], Eriko da Silva Santo [a], Renato Zanin [a], Paulo Sergio Monzani [a,b], Tobias Canan Sovernigo [b], Otávio Mitio Ohashi [c], Cláudia Lima Verde Leal [d], Paulo Roberto Adona [a,b,*]

[a] *Central Avançada de Biotecnologia da Reprodução Animal (CENABRA), Agropecuária Laffranchi, Tamarana, Paraná, Brazil*
[b] *Escola de Medicina Veterinária, Universidade Norte do Paraná, Arapongas, Paraná, Brazil*
[c] *Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, Pará, Brazil*
[d] *Departamento de Ciências Básicas, Universidade de São Paulo, Pirassununga, São Paulo, Brazil*

ARTICLE INFO

*Article history:*
Received 16 July 2013
Received in revised form 12 December 2013
Accepted 21 January 2014

*Keywords:*
Butyrolactone I
Maturation
Transport
Ovum pick up and oocytes

ABSTRACT

Ovum pick up (OPU) associated with *in vitro* production (IVP) of embryos has been shown as an important tool in cattle breeding to increase the number of descendants from animals of high genetic value. In herds maintained distant from the laboratory, collecting cumulus–oocyte complexes (COCs) and transporting them to the laboratory may take several hours and decrease COCs viability, representing a challenge for commercial settings. In this study, a prematuration culture to induce temporary meiosis block was evaluated in a commercial scale IVP setting as a strategy to transport bovine OPU-derived COCs from Nelore and Brangus donors. Effects on embryo yield and pregnancy rates were assessed. Viable COCs from each donor were destined to one of the experimental groups (control, blocks 1 and 2). Control group COCs were placed in cryotubes with 1 mL TCM199–HEPES. In block groups (1 and 2), COCs were placed in cryotubes with 300 μL TCM 199 + 12 μM butyrolactone I (block medium). All groups were gassed and kept in a thermos bottle for 4 hours at 36 °C. Next, COCs in the control group were transferred to IVM medium and block 1 group to block medium, and cultured for 22 hours and 15 hours, respectively, at 38.5 °C and 5% $CO_2$ in air. Block 2 COCs were kept in the cryotubes and in the thermos bottle for another 15 hours at 36 °C to simulate long-term transport conditions. After meiosis block in prematuration culture, blocks 1 and 2 COCs were matured *in vitro* for 22 hours as for the control group. After IVM, COCs in all groups were submitted to IVF and IVC, and blastocyst rates were evaluated on day 7. Embryos were transferred and pregnancy rates evaluated at 60 days of gestation. The mean total number of COCs retrieved by OPU did not differ between Nelore and Brangus donors (16.8 and 17.2, respectively, $P > 0.05$), but Nelore donors produced more viable COCs than Brangus (10.1 and 7.6, respectively, $P < 0.05$) and more embryos/cow (3.8 and 2.7, respectively, $P < 0.05$). Blastocyst rates were similar for control (40.2% and 36.7%), block 1 (37.3% and 34.5%), and block 2 groups (34.7% and 33.6%) for Nelore and Brangus cattle, respectively ($P > 0.05$). Pregnancy rates did not differ regardless of breed or treatment (36.7%, $P > 0.05$). In conclusion, temporary meiosis block during prematuration culture did not affect embryo development or pregnancy rates; therefore, this strategy may be used to transport bovine COCs in a commercial IVP setting.



* Corresponding author. Tel.: +55 43 33994700; fax: +55 43 33717835.
*E-mail address:* paulo_adona@yahoo.com.br (P.R. Adona).

0093-691X/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2014.01.026

## 1. Introduction

In cattle, oogenesis begins during fetal life and many oocytes present in the ovaries do not develop to ovulation [1,2]. Cows have, at puberty, about 70,000 oocytes in their ovaries [3] that can generate a number close to 10 descendants throughout their reproductive life. Transvaginal follicular aspiration biotechnology using ultrasound-guided ovum pick up (OPU), associated with *in vitro* production (IVP) of bovine embryos, allows immature oocytes to generate viable embryos that can be transferred to previously synchronized recipients [4,5], thus allowing to increase the number of offspring of high genetic value in a shorter period of time, maximizing the reproductive capacity of animals.

The use of IVP of embryo biotechnology has increased significantly in recent years, especially because of the improvement of this technology on a commercial scale, both in beef and dairy cattle [6,7]. Despite the advances obtained in embryo IVP, the use of this technique in commercial scale still has certain limitations, especially when oocyte donor animals are distant from the embryo production laboratory, requiring several hours of oocyte transportation. Time and transport conditions are essential to maintain oocyte viability and quality, which are the determining factors in early embryonic development [8–11]. Immature bovine oocytes, removed from the follicular environment, lose their natural meiotic block mechanism, and spontaneously resume meiosis [12], so soon after OPU, oocytes need to be subjected to IVM to avoid compromising their viability and, consequently, embryonic development. So when immediate IVM is not possible and the site of oocyte collection is distant from the laboratory, oocytes need to be transported in conditions to maintain viability before they are placed in IVM culture. Information on the maintenance and transportation of oocytes for long periods in a commercial setting for production and transfer of embryos has been limited. Although there are some studies with different strategies for prematuration of oocytes [12–15] and improvements on prematuration culture conditions with a variety of drugs [15–19], such studies have focused mainly on meiosis block rates and subsequent embryo development rates but have not been assessed for maintaining and transporting oocytes for long periods in commercial IVP settings. In this context, it is evident that there is still the need for new alternatives for transporting bovine oocytes, especially for long periods, to maintain the viability of oocytes for embryonic development.

Temporarily blocking meiosis during the transportation period could be an alternative when immediate culture is not possible or desirable. Induction of meiosis block during a prematuration period would allow oocytes to remain immature for an additional period of time before being subjected to IVM itself. This strategy would allow not only transportation of oocytes for long periods (i.e., herds distant from the laboratory) but would also enable oocyte collection to occur on two consecutive days for a single routine of IVF and embryo transfer, optimizing time, laboratory work, and costs. This possibility, however, has not been fully evaluated on a commercial scale.

Butyrolactone I (BLI) is described as a substance capable of promoting a reversible meiosis block *in vitro*, by preventing oocytes to resume meiosis (germinal vesicle breakdown) when removed from the ovarian follicle. Oocytes are held in germinal vesicle stage in the presence of BLI and when the drug is removed and oocytes placed in maturation medium, they proceed normally through meiosis to the metaphase II stage [13–15]. Butyrolactone I has been found to effectively block germinal vesicle breakdown in bovine oocytes for a period of up to 24 hours without significantly compromising embryonic development [14–16].

The present study aimed to evaluate the effect of inducing a temporary meiosis block in bovine oocytes, as a strategy for long-term transportation of oocytes in a commercial scale OPU–IVP setting using two different cattle breeds (Nelore and Brangus).

## 2. Materials and methods

Chemicals used were purchased from Sigma Chemical Company (St Louis, MO, USA) unless otherwise specified.

### 2.1. Donors and recipients

The embryo recipients were half-blood heifers (Nelore/Brown Swiss) cycling normally and in good body condition. Synchronization occurred on random days of estrous cycle [20]. Oocyte donor cows were selected as having high genetic value and according to the history of oocyte yield after follicle aspiration (12–25 oocytes) and were of Nelore (*Bos indicus*, n = 18) and Brangus breeds (standard breed 3 of 8 *B indicus* and 5 of 8 *Bos taurus*, n = 24) aged between 4 and 9 years, cycling normally and in good body condition. The animals were maintained on *Brachiaria decumbens* pasture with access to water and mineral salt ad libitum. During the experiment, the donors received no exogenous hormones to stimulate the production of follicles or synchronization of the follicular waves. All procedures were performed at random stages of the estrous cycle, and the interval between aspirations was of approximately 15 days.

### 2.2. Follicular aspiration

Follicles (≥3 mm diameter) were aspirated using ultrasound equipment (Aloka SSD-500) fitted with a 5-MHz convex transducer (UST 974-5) and a transvaginal device [21]. During aspiration, oocytes were placed in phosphate-buffered saline (Nutricell, Campinas, SP, Brazil) supplemented with 1% fetal bovine serum (FBS) and 10 IU/mL heparin (Liquemine-Roche, Rio de Janeiro, RJ, Brazil).

### 2.3. Oocyte selection

After each follicular aspiration, the material obtained was immediately filtered (ECE 051—Bioniche) and washed with phosphate-buffered saline supplemented with 1% FBS. Oocytes were collected from the filter sediment and classified as viable oocytes when presenting two or more layers of compact *cumulus* cells or with little expansion and with homogeneous cytoplasm or slight granulation. The oocytes selected from each donor were washed in TCM199-HEPES