Animal Reproduction Science 144 (2014) 95–101

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Influence of oocyte donor on *in vitro* embryo production in buffalo

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ARTICLE INFO

Article history:
Received 7 June 2013
Received in revised form
14 November 2013
Accepted 22 November 2013
Available online 12 December 2013

Keywords:
Buffalo
Ovum pick-up
Blastocyst yield
Repeatability

ABSTRACT

The aim of this research was to estimate the variability between buffalo as oocyte donors. In Experiment 1, reproductive variables were retrospectively analyzed in buffalo ($n = 40$) that underwent repeated ovum pick up (OPU), over 16 puncture sessions (PS). The follicular recruitment among individuals and the relationship between follicular population and oocyte production were evaluated. In Experiment 2, eight buffalo underwent OPU for 28 PS and the oocytes were processed separately to correlate follicular and oocyte population at the first PS to blastocyst (BL) production. In Experiment 1, the average number of total follicles (TFL), small follicles (SFL), cumulus-oocyte complexes (COC) and Grade A + B COC recorded in each 4-PS period had great repeatability ($r = 0.52$, 0.54, 0.60 and 0.57, respectively). The average number of Grade A + B COC recovered during the subsequent 15 PS was positively correlated with the first PS number of TFL ($r = 0.60$; $P < 0.001$), SFL ($r = 0.68$; $P < 0.001$), COC ($r = 0.48$; $P < 0.01$) and Grade A + B COC ($r = 0.40$; $P < 0.05$). In Experiment 2, a large variability among animals was observed in blastocyst yields. When animals were grouped according to the BL yield, the greatest BL yield group had a greater ($P < 0.05$) number of TFL (8.3 ± 0.9 compared with 5.6 ± 0.7) and SFL (7.3 ± 0.3 compared with 3.8 ± 0.7) at the first PS than the lesser BL yield group. The average number of BL produced over the subsequent sessions was correlated with the number of TFL ($r = 0.80$; $P < 0.05$) and COC ($r = 0.76$; $P < 0.05$) observed at the first PS. These results demonstrated a donor influence on the oocyte and BL production, suggesting a preliminary screening to select the donors with greater potential.

1. Introduction

The profitability of buffalo breeding highly depends on genetic improvement, and hence on the utilization of reproductive biotechnologies. *In vitro* embryo production (IVEP) is the most desirable procedure to increase the maternal contribution to genetic improvement of buffalo, due to poor and inconsistent responses to multiple ovulation and embryo transfer (MOET) programs (Carvalho et al., 2002; Misra and Tyagi, 2007; Neglia et al., 2010). In fact, the ovum pick-up (OPU) technique combined with IVEP has greater potential than MOET, allowing a greater production of embryos on a long term basis.

Due to the improvements of the *in vitro* system, the IVEP efficiency has considerably increased throughout the years, leading to greater blastocyst yields (Neglia et al., 2003; Gasparrini et al., 2006) and to the production of offspring (Hufana-Duran et al., 2004, Neglia et al., 2004; Saliba et al., 2011; Galli et al., 2000). However, the major constraint to apply OPU and IVEP technologies in this species is the lesser recovery of viable oocytes (<2.0 oocytes/donor) (Gupta et al., 2006; Manjunatha et al., 2008, 2009; Di

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http://dx.doi.org/10.1016/j.anireprosci.2013.11.010

Francesco et al., 2012; Neglia et al., 2011), arising from physiological peculiarities, such as the greater incidence of atresia (Ocampo et al., 1994; Palta et al., 1998) and lesser number of primordial (Samad and Nasseri, 1979; Danell, 1987) and antral follicles (Kumar et al., 1997) in buffalo ovaries. Nevertheless, a great individual variability in follicular recruitment and oocyte recovery, similar to cattle (Tamassia et al., 2003), was reported in buffalo (Neglia et al., 2003, 2011; Gasparrini, 2002). Furthermore, in cattle the donor influence on the production of blastocysts was demonstrated (Tamassia et al., 2003) and a model to predict follicular recruitment from donor cows based on the results of earlier OPU sessions was developed (Boni et al., 1997). The aim of the present research was to assess the donor influence on the yield and development potential of buffalo oocytes.

2. Materials and methods

All chemicals and reagents, if not otherwise stated, were purchased from Sigma (Sigma–Aldrich, Milan, Italy).

2.1. Experimental design

A retrospective study was conducted (Experiment 1), with the aim to analyze the reproductive variables indicating the folliculogenic and oogenic potentials in buffalo that underwent OPU at different farms ($n = 5$) located in the province of Caserta. For this purpose, results were utilized from a previous OPU study conducted on 40 buffalo cows that were aspirated twice per week for at least 16 puncture sessions (PS). In this trial, the oocytes, after an appropriate morphological selection, were pooled for *in vitro* maturation (IVM), fertilization (IVF) and culture (IVC), as the benefits of culturing gametes (Brum et al., 2005) and embryos (O'Doherty et al., 1997; Gopichandran and Leese, 2006) in groups rather than individually on IVEP efficiency are well known.

Results of Experiment 1 laid the basis for a subsequent experiment aimed to assess whether a preliminary screening of donors based on the initial follicular population could predict, in addition to the oocyte yield, the oocyte competence to develop into blastocysts after IVM, IVF and IVC.

Therefore, in Experiment 2, that was developed on a limited number of buffalo ($n = 8$), OPU was conducted twice weekly for 28 PS. In this case, the oocytes collected from each donor were separately processed through IVM, IVF and IVC, to provide individual data on blastocyst production. To reduce the paternal effect, sperm from the same bull, that was previously proven suitable for IVF, were used in both experiments.

2.2. Ovum pick-up and COC processing

In both experiments, the donors were under controlled nutrition, housed inside barns, and restrained in a chute at the time of the oocyte retrieval session. Ovum pick up consisted of the use of a portable ultrasonic unit (Aloka SSD-500, Aloka Co., Tokyo) with a 5 MHz sector scanner mounted on a properly designed vaginal support (WTA Ltda., Cravinhos/SP, Brazil) equipped with a guide for 18 gauge needles. A vacuum pressure of 40 mm-Hg was constantly maintained by using a suction unit (K-MAR-5100, Cook IVF Co., Australia) and the aspiration line was continuously rinsed with 25 mM Hepes-buffered TCM 199 supplemented with 100 USP units/mL of heparin, 10% fetal calf serum (FCS) and 1% penicillin and streptomycin complex (20000 IU and 20,000 μg/mL, respectively, Lonza, Milan, Italy) during follicular aspiration. The 15 mL Falcon tubes (Becton & Dickinson Co., Lincoln Park, NJ, USA) for oocyte collection were constantly maintained at 37 °C. All visible antral follicles (total follicles, TFL) were punctured and classified into: small (SFL, diameter < 0.5 cm), medium (MFL, diameter between 0.5 and 1 cm) and large (LFL, diameter > 1 cm).

The COC were searched immediately after follicular aspiration by using proper filters (Emcon Technologies, Columbus, IN, USA) and classified according to their morphology as previously described (Di Francesco et al., 2011; Fig. 1). With the exception of degenerated oocytes, all the categories were further processed for IVEP. For each session, the number of total COC and that of superior quality oocytes (Grade A + B COC) were recorded. The COC were washed twice in Hepes-buffered TCM 199 (H 199) with 10% FCS, then placed in the same medium supplemented with 50 μM cysteamine, 0.5 μg/mL FSH, 5 μg/mL LH, 1 μg/mL 17-β-estradiol and transported to the laboratory in 15 mL Falcon tubes in a portable incubator at 38.5 °C within 4–6 h.

2.3. In vitro embryo production

For IVM, COC were individually transferred into 50 μL droplets (10 COC/droplet) of the final maturation medium under mineral oil. Final IVM medium had the same composition as the transportation medium described above but was buffered with 25 mM of sodium bicarbonate. The droplets were incubated at 38.5 °C for 22 h under controlled gas atmosphere of 5% CO_2 in humidified air.

In vitro fertilization was conducted according to the method previously described by Parrish et al. (1986). Frozen-thawed sperm were prepared by Percoll density gradient (Nidacon, Mölndal, Sweden). The pellet obtained after centrifugation was re-suspended to a final concentration of 2×10^6 mL in the IVF medium with the following composition: modified TALP supplemented with 0.2 mM/mL penicillamine, 0.1 mM mL^{-1} hypotaurine, and 0.01 mM/mL heparin. The oocytes were removed from the IVM drops, thoroughly washed in IVF medium and allocated in 50 μL fertilizing droplets (five COC/droplet) covered by mineral oil were incubated under the same gas atmosphere as for IVM.

After 20–22 h of co-incubation with spermatozoa, presumptive zygotes were cultured (IVC) in 20 μL droplets (10 COC/droplet) of synthetic oviduct fluid (SOF) medium (Tervit et al., 1972) supplemented with essential and non-essential amino acids and bovine serum albumin (BSA) for 7 d in a modular chamber with a gas atmosphere of 5% CO_2, 7% O_2, and 88% N_2. At Day 5 (Day 0 = IVF day) cleavage rate was assessed and embryos were transferred into fresh droplets of the same medium for further 2 d of