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Ovum pick-up interval in buffalo (*Bubalus bubalis*) managed under wetland conditions in Argentina: Effect on follicular population, oocyte recovery, and in vitro embryo development

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ABSTRACT

The excellent adaptation of water buffalo (*Bubalis bubalis*) to swampy environments means that animals are frequently managed in areas with restricted access for reproductive procedures. The objective of this study was to evaluate the effect of the ovum pick-up (OPU) interval on follicular population, oocyte recovery, oocyte quality and in vitro embryo production. Twelve Murrah buffaloes were subjected to two consecutive dominant follicle reductions, and randomly assigned to either 7-day (n = 6) or 14-day (n = 6) OPU interval groups. Although there was no significant difference in the average number of small (< 3 mm) and large (> 8 mm) diameter follicles available per OPU, a higher proportion of medium-sized follicles (3–8 mm) were observed in the 14-day interval group (5.129 vs 3.267; $p < 0.05$). The number of recovered oocytes per donor was also significantly higher (4.51 vs. 2.8; $p < 0.05$) in the 14-day interval group, although this was attributed to an increase in the proportion of lower quality oocytes (grades III and IV). After in vitro fertilization, embryo developmental competence from grade I and II oocytes was superior to that from grade III and IV oocytes, irrespective of OPU interval group. There was no significant difference in the proportion of grade I and II oocytes cleaved after sperm co-incubation; however, there was a higher proportion of blastocysts produced in 14-day interval group (28 vs. 6%, $p < 0.05$). No blastocysts were produced from grade III and IV oocytes. This study indicates it is possible to use a 14-day interval for oocyte collection in water buffalo; this approach could be considered as an alternative when access to animals is restricted.

1. Introduction

In domestic farm animal species, the technique of repeated ovum pick-up (OPU) allows for the continuous collection of competent oocytes, which in turn can be used for the production of in vitro-derived embryos. In 1988, Pietrese et al. reported the first repeated ultrasound-guided follicular puncture in cattle, and concluded that consecutive procedures could be conducted without detrimental effects to donor health. Boni et al. reported the first OPU in water buffalo in 1994. In that initial report, donors in deep anestrus were subjected to FSH priming followed by weekly oocyte aspirations; the average recovery rate was 31.9% for untreated controls and 44% for FSH-stimulated donors. Since then, this technique has become an integral part of assisted reproductive technologies for the

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production of in vitro-derived embryos, which has become a competitive alternative to superovulation for in vivo embryo recovery and transfer (Lambert et al., 1983; Gasparrini 2002; Presicce, 2007).

Optimal OPU interval is still a matter of debate in buffalo. OPU interval and its relation with follicular wave emergence, phase of follicular wave at the time of collection and oocyte developmental competence are key interacting elements that are not completely understood. Although consecutive OPU procedures have been reported using twice-weekly (Boni and Zicarelli, 1996; Sá Filho et al., 2009; Neglia et al., 2011; Di Francesco et al., 2012; Gasparrini et al., 2014), weekly (Gupta et al., 2006; Ferraz et al., 2007, 2015; Neglia et al., 2011) and bi-weekly (Gimenes et al., 2015; Ferraz et al., 2007, 2015) intervals, results are not consistent among studies and are sometimes difficult to extrapolate due to confounding variables such as breed, hormonal stimulation treatments and seasonal effects.

Neglia et al. (2011) indicated that a short, twice-weekly OPU interval was associated with a progressive decline in follicular population. In that study, which was the longest continuous OPU reported for buffalo to date, 18 OPU procedures were conducted every 3–4 days, followed by 16 procedures conducted every 7 days. Although no difference in oocyte recovery rate was observed, weekly OPU was associated with a reduction in the proportion of lower quality oocytes, higher cleavage and blastocyst rates and overall improved oocyte competence. This study contradicted earlier results from Boni et al. (1997) showing that an extension of the OPU interval resulted in larger diameter follicles and reduced oocyte quality.

The first OPU conducted at a 14-day interval was reported by Ferraz et al. (2007, 2015). The effect of weekly (7-day) versus bi-weekly (14-day) intervals, with or without bovine somatotropin (bST) treatment was evaluated in lactating Murrah x Mediterranean crossbreeds; follicular population, oocyte recovery and embryo production were analyzed. Bi-weekly interval resulted in an increase in the number of follicles available for aspiration (15.6 vs. 12.8; $p < 0.0001$) and the number of oocytes recovered (10 vs. 8.5; $p < 0.004$). An interaction between the 14-day OPU interval and bST treatment was reported, which resulted in an increased rate of oocyte degeneration and reduced embryo production. Noteworthy, experiments were conducted in lactating animals managed under twice-daily milking regimes, intensive management and high nutritional planes; results may not necessarily apply to animals managed under more extensive conditions.

The rusticity and excellent adaptation of water buffalo to swampy environments means that in Argentina, as in many other regions of the world, animals are often managed in wetland areas with restricted access. This presents a challenge for routine assisted reproductive procedures such as super ovulatory treatments, ultrasound screenings and ovum pick-up for in vitro embryo production. For this reason, there is a growing interest among local producers and practitioners to evaluate the efficacy of protocols that require less frequent access to animals. Therefore, the objective of this study was to evaluate the effect of 7- versus 14-day OPU intervals on follicular population, oocyte recovery, oocyte quality and in vitro embryo production.

2. Materials and methods

2.1. Animals and experimental conditions

The study was conducted during the buffalo reproductive season in the Southern Hemisphere (March to May). All experimental animals were part of a commercial herd managed under wetland conditions in the province of Corrientes, Argentina (-27.742859 latitude, −57.773611 longitude).

A total of twelve (n = 12) non-pregnant, 4- to 12- year old Murrah buffalo cows (*Bubalus bubalis*), with good body condition scores (2.97 ± 0.40 on 1–5 scale; Baruselli et al., 2001) and proven fertility of at least one live offspring recorded were available for this study (Fig. 1). All experimental animals were considered to be cyclic based on ultrasound detection of corpus luteum prior to initiation of treatments and consistent ovarian activity with the presence of multiple follicles per ovary.

Ultrasound-guided follicular ablation has been reported as a non-hormonal physical method to synchronize follicular wave emergence in water buffalo (Honparkhe et al., 2014). All animals in the present study were subjected to double, consecutive dominant follicle reduction (DFR) procedures 1 week apart in order to initiate a new follicular wave; they were then randomly assigned to OPU conducted at either weekly (7-day) or bi-weekly (14-day) intervals. The initial OPU was conducted either 7 or

Fig. 1. a. Swampy, extensive management conditions of experimental animals, Little Punjab Ranch, Corrientes, Argentina. b. Animals available for this experiment.