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Effect of recombinant bovine somatotropin (bST) on follicular population and on *in vitro* buffalo embryo production

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ABSTRACT

The objective of this study was to evaluate the effect of bovine somatotropin (bST) on ovarian follicular population in buffalo heifers and its influence on oocyte quality, recovery rates and *in vitro* embryo production. We tested the hypothesis that bST treatment in buffalo females submitted to an ovum pick-up (OPU) program would improve the number of follicles recruited, oocyte quality and *in vitro* embryo production. A total of 10 heifers were assigned into two treatment groups: group bST ($n = 5$; receiving 500 mg of bST in regular intervals) and control group ($n = 5$; without additional treatment). Both groups were subjected to OPU sessions twice a week (every 3 or 4 days), for a total of 10 sessions per female, although due to procedural problems, only the first five OPU sessions produced embryos. The number of follicles and the diameters were recorded at all OPU sessions. The harvested oocytes were counted and classified according to their quality as either A, B, C, D or E, with A and B considered good quality. Cleavage and blastocyst production rates were evaluated 2 and 7 days after *in vitro* fertilization, respectively. The bST treatment increased the total number of antral follicles (>3 mm in diameter; 12.2 compared with 8.7; $p < 0.05$) and of small antral follicles (<5 mm; 9.1 compared with 6.5; $p < 0.05$) per OPU session. The bST also tended to increase the number of

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oocytes recovered per session (5.2 compared with 4.1; $p = 0.07$), and enhanced the percentage of good quality oocytes (48.8% compared with 40.6%; $p = 0.07$). bST showed no effect on cleavage and blastocyst production rates ($p > 0.05$). The significant effects of performing repeated OPU sessions were decreasing the follicular population ($p < 0.001$) as well as the number of follicles aspirated ($p < 0.001$), and oocytes recovered ($p < 0.02$). In conclusion, bST treatment improves the follicular population, demonstrating its possible application in buffalo donors submitted to OPU programs.

1. Introduction

Buffalo respond less to multiple ovulation and embryo transfer procedures in comparison to cattle. Buffalo produce slightly poorer ova and have fewer embryos recovered than cattle (Drost et al., 1983; Baruselli et al., 2000; Carvalho et al., 2002). Pregnancy in buffalo has been achieved through the transfer of fresh (Madan et al., 1991; Boni et al., 1997; Sá Filho et al., 2005), frozen (Galli et al., 1998) and vitrified (Neglia et al., 2004; Sá Filho et al., 2005) buffalo embryos produced *in vitro*. Given each of these considerations, utilizing ovum pick-up (OPU) technology in conjunction with *in vitro* embryo production (IVP) presents the possibility of enhancing genetic progression through the female lineage (Galli et al., 2001; Gasparrini, 2002; Neglia et al., 2003).

Although there has been interest in applying OPU and IVP techniques in buffalo females, certain factors have limited the commercial use of OPU–IVP in buffalo. These include the lesser number of primordial follicles present in the ovaries (Danell, 1987; Baruselli et al., 1997), lesser number of oocytes recovered per OPU session (Gasparrini, 2002; Gasparrini et al., 2006), greater incidence of atretic follicles (Le Van Ty et al., 1989) and oocytes, and lesser cleavage rates (Gasparrini, 2002; Gasparrini et al., 2006). Therefore, the development of strategies that increase the ovarian follicular population, improve the proportion of non-atretic follicles, and enhance oocyte quality is very important for improving the OPU–IVP efficiency in buffalo.

In cattle, bovine somatotropin (bST) treatment has been applied during OPU–IVP programs to improve follicular population prior to OPU. Positive results include enhancing oocyte quality and embryo development capacity, improving *in vitro* oocyte maturation, and increasing fertilization (Pavlok et al., 1996; Bols et al., 1998; Tripp et al., 2000; Roth et al., 2002). However, the precise effects of the bST treatment in such programs are still not clearly defined.

The objective of the present study was to evaluate the effect of bST on follicular population in buffalo heifers and its influence on oocyte quality, recovery rate and *in vitro* embryo production. The hypothesis was that bST treatment in buffalo females submitted to ovum pick-up (OPU) programs would increase the number of follicles susceptible to puncture, enhance oocyte recovery both in quality and quantity, and consequently improve the *in vitro* embryo production.

2. Materials and methods

2.1. Animals

Ten buffalo heifers (*Bubalus bubalis*), ages 20–30 months and weighing 418.1 ± 35.2 kg, were selected from a commercial dairy farm (Santa Eliza Farm, Dourado-SP, Brazil). All females were previously examined by ultrasonography and selected according to the following criteria: (1) had or had not initiated onset of estrous cycle (all heifers should have a corpus luteum—CL), (2) ovarian diameter (each ovary should have ≥2 cm of diameter) and (3) follicular population (all heifers should have ≥8 follicles per ovary).

The females were maintained at UNESP-Jaboticabal Campus (Jaboticabal-SP, Brazil) under grazing conditions with mineral salt and water *ad libitum*, from July to August of 2004.