# Effect of OPU interval and bST treatment on embryo production in buffalo

M.L. Ferraz[1], L.U. Gimenes[1], M.F. Sá Filho[1], Y.F. Watanabe[2], D.C. Joaquim[2], M.F. Accorsi[2], F.V. Meirelles[3], P.S. Baruselli[1]

[1] Departamento de Reprodução Animal, FMVZ – USP, São Paulo – SP, Brasil

[2] Vitrogen® Pesquisa e Desenvolvimento em Biotecnologia da Reprodução, Cravinhos – SP, Brasil

[3] Departamento de Ciências Básicas, FZEA – USP, Pirassununga – SP, Brasil

*Corresponding author:* M.L. Ferraz. Departamento de Reprodução Animal, FMVZ – USP, São Paulo - SP, Brasil - Tel. +55-11-3091-7674 - Fax:+55-11-3091-7412 - Email: marcioleao@usp.br or barusell@usp.br

**ABSTRACT:** The objective of present study was to evaluate the effect of OPU interval and the effect of bST treatment on embryo production in buffalo. Sixteen females were assigned in 4 groups, in a 2x2 experimental design, with main effects of bST (0 or 500mg) and interval of OPU session (every 7 or 14 days), as follows: G-CONT7: OPU once a week; G-CONT14: OPU each 14 days; G-bST7: OPU once a week + 500mg of bST and G-bST14: OPU each 14 days + 500mg of bST. Animals of G-CONT7 and G-bST7 were submitted to 8 OPU sessions, and those of G-CONT14 and G-bST14 to 4 OPU sessions. Overall, OPU once a week (without bST) reduced the number of aspirated follicles, and the total and viable oocytes. Despite of this, OPU every 14 days + bST increased the number of degenerated oocytes, and reduced the number of blastocysts produced on days 6 and 7.

**Key words:** OPU, bST, Buffalo, Embryo production.

**INTRODUCTION -** In buffaloes, OPU-IVP programs are promising biotechnologies, since superovulation and embryo transfer are not yet sucessful in this species. However, there are two biological problems related to OPU-IVP in buffaloes: low recovery rate and poor oocyte and embryo quality.

In cattle, interval of OPU session and bST treatment can influence the follicular population, quantity and quality of oocytes and embryo production rate (Buratini Jr *et al*., 2000; Merton *et al*., 2003; Tripp *et al*., 2000). In buffaloes, the bST treatment improved the follicular population in superstimulated females (Baruselli *et al*., 2003) and in heifers submmited to OPU (Sá Filho, 2006). Additionaly, bST tended to increase the number of oocyte recovered per session (5.2 *vs* 4.1; p=0.07) and the percentage of good quality oocytes (48.8% *vs* 40.6%; p=0.07; Sá Filho, 2006). Nevertheless, in this species there are no reports about interval of follicular puncture.

The objectives of the present work were to evaluate the effects of bST and of OPU interval on follicular population in buffalo heifers, its influence on oocyte quality, recovery rate and on *in vitro* embryo production.

**MATERIAL AND METHODS -** The present study was conducted in Santa Eliza Farm (Dourado - São Paulo - Brazil). Sixteen multiparous Murrah x Mediterranean, aging between 3 and 7 years and weighing above 550kg, were maintained at pasture and supplemented with concentrate and hay.

All animals were assigned in 4 groups, in a 2x2 experimental design: G-CONT7: OPU once a week; G-bST7: OPU once a week + 500mg of bST (Boostin®, Schering-Coopers, Brasil); G-CONT14: OPU each 14 days and G-bST14: OPU each 14 days + 500mg of bST. Animals of G-CONT7 and G-bST7 were submitted to 8 OPU sessions, and those of G-CONT14 and G-bST14 to 4 OPU sessions.

Follicular puncture was performed using a 7.5 MHz micro-convex transducer (SSD-500, Aloka, Japan) with a 40mm long and 19-g needle at a 60-65 mmHg of negative vacuum pressure. Prior to each OPU session, follicles > 3mm were recorded for analysis of the ovarian follicular population.

All recovered oocytes were graded in 1, 2, 3, degenerated, atretic and expanded cumulus cells. The first three grades were considered suitable for *in vitro* production.

Oocytes were washed in Hepes TCM-199 with 10% fetal calf serum and then allocated in a bicarbonate TCM-199 supplemented with FSH, hCG, estradiol, sodium piruvate, amikacine, cystine and cysteamine. The COCs were placed for *in vitro* maturation (IVM) into 90µl droplets of TCM-199 medium under mineral oil and incubated under 5% of $CO_2$ in air, at 38.5 ºC and high humidity, for 22-24 h. After IVM, the oocytes were transfered to fertilizing 90µl droplets, containing TALP supplemented with heparin, PHE and piruvate. Semen of a tested bull was used in a final concentration of $2x10^6$ sptz/mL. IVF droplets were incubated under the same gas atmosphere for IVM, during 18-20 hours. After the IVF period, the putative zygotes were removed from the fertilization medium, stripped of cumulus cells by gentle pipetting and washed in CR4 medium (Vitrogen®, Brazil). Presumptive zygotes were allocated into 90µl droplets and the culture was carried out under the same atmosphere as for IVM and IVF. On Day 2 of embryo development (Day 0 = day of insemination) cleavage rate was evaluated and "feeding" was performed. On day 6 and 7 embryo production evaluation was performed.

**Statistical analysis -** The number of punctured follicles, total of oocytes, viable oocytes, grade of oocytes, number of cleaved embryos and number of blastocists on D6 and D7 were analyzed by ANOVA for repeated measures using the Mixed Procedure in SAS program. Recovery, cleavage and embryo production rates were analyzed by $\chi^2$.

**RESULTS AND CONCLUSIONS -** There were no treatment neither interval effects for groups G-CONT7, G-bST7, G-CONT14 and G-bST14, respectively, for the following variable: number of grade 1 oocytes (0.6±0.1, 0.5±0.1, 0.8±0.1 and 0.6±0.2), number of grade 3 oocytes (4.4±0.3, 5.4±0.4, 5.4±0.5 and 5.3±0.5), number of oocytes with expanded cumulus cells (0.1±0.0, 0.2±0.1, 0.1±0.1 and 0.3±0.1), number of atretic follicles (1.2±0.1, 1.2±0.1, 1.1±0.2 and 1.3±0.2), number of cleaved embryos (2.0±0.2, 2.2±0.2, 2.4±0.4 and 1.9±0.3) and cleavage rate (33.4%, 35.1%, 32.7% and 26.0%). There was treatment effect for G-CONT and G-bST, respectively, for number of punctured follicles ($12.1±0.4^b$ and $15.3±0.5^a$; P<0.0001), number of degenerated oocytes ($0.9±0.1^b$ and $1.3±0.1^a$; P=0.008), number of blastocysts on day 6 ($1.1±0.1^a$ and $0.5±0.1^b$; P=0.0007) and on day 7 ($1.4±0.2^a$ and $0.8±0.1^b$; P=0.003), percentage of blastocysts on day 6 (13.7%$^a$ and 6.8%$^b$; P=0.003) and on day 7 (18.9%$^a$ and

10.9%[b]; P=0.009). There was interval effect for G-7 and G-14, respectively, for number of punctured follicles (12.8[b] and 15.6[a]; P<0.0001), number of total oocytes (8.5[b] and 10.0[a]; P=0.004) and number of degenerated oocytes (1.0[b] and 1.4[a]; P=0.04). There was interaction between treatment and interval for groups G-CONT7, G-bST7, G-CONT14 and G-bST14, respectively, for recovery rate (69.3%[a], 67.4%[a], 73.6%[a] and 58.5%[b]; P=0.03), number of viable oocytes (5.5±0.4[b], 6.8±0.4[a], 7.6±0.5[a] and 6.8±0.6[a]; P=0.01) and number of grade 2 oocytes (0.4±0.1[b], 0.9±0.1[b], 1.4±0.2[a] and 0.8±0.2[b]; P=0.001).

Although in the present study it was observed a positive effect of bST in the number of punctured follicles, the number of total oocytes recovered was more related to a day effect (a higher number of total oocytes were recovered in intervals of 14 days). There are no reports in the literature about 14 days intervals between OPU sessions. Even though, data about recovery rate and number of oocytes are controversial. Viana *et al*. (2004) recovered a higher number of oocytes per session when cows were submitted to once week OPU instead twice a week. Inversely, other authors did not observed differences related to OPU interval (Garcia; Salaheddine 1998; Gibbons *et al*., 1994).

Oocyte quality was not affected by OPU interval or bST treatment in the most of variables. However, the number of degenerated oocytes was higher in the 14 days intervals and bST groups. This was not expected, once Merton *et al*. (2003) verified better oocyte quality when 3 to 4 days intervals of OPU sessions were performed, relative to 7 days intervals. Additionally, Pavlok *et al*. (1996) observed higher number of good quality oocytes in animals treated with bST. In the present study bST treatment reduced the number and the percentage of blastocysts yielded on days 6 and 7. It is possible that high bST doses can increase ovarian IGF-1 levels, leading to damages in the follicles and oocytes (Armstrong *et al*., 2003).

In conclusion, a 14 days intervals between OPU sessions provided a higher number of punctured follicles and total oocytes. Blastocysts yields decreased when 500mg of bST was used, maybe due this dose be inadequate for buffaloes.

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