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# Follicular development, plasma Inhibin-A and Estradiol-17-beta concentrations in Buffalo cows during different treatment schedules for MOET programs

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**ABSTRACT:** Buffalo cows were submitted to three superovulatory treatments. T1 (n = 7): PRID for 10 days (d0-d9) plus decreasing doses of 500 IU FSH/LH (12 h-intervals d7-d10); T2 (n = 8): PRID for 11 d (d0-d10) plus 2000 IU PMSG at d7; T3 (n = 9): PRID for 11 d plus 2000 IU PMSG at d7 and decreasing doses of 175 IU FSH/LH (12 h-intervals d10- d11). Overall plasma inhibin-A (In-A) concentrations correlated with large follicles (LF, diameter >6mm, R=0.83, P<0.01) and small follicles (SF, <6mm, R=-0.21, P<0.01). Plasma E2 correlated weakly with LF (R=0.21, P<0.05). Within treatment, there was always a positive correlation between In-A and LF (T1 R=0.83; T2 R=0.81; T3 R=0.83; P<0.01), but only in T3 there were correlations between In-A and E2 (R=0.67, P<0.01) and SF (R=-0.42, P<0.01), between E2 and LF (R=0.39, P<0.01) and SF (R=-0.35, P<0.05). T3 was followed by a higher number of follicles with diameter >10 mm at d12-13 (T1=5.0+/-1.4, T2=1.2+/-0.9, T3=8.3+/-2.3). In-A concentrations significantly rised at d11-13 of T1 and T3. In-A seems a good indicator of the follicular development during superovulation in buffalo cows, while E2 is not. Furthermore T3 was followed by better ovarian follicular responses.

**Key words**: Buffalo cows, Inhibin-A, Estradiol 17β, Superovulation.

**INTRODUCTION -** Inhibins are gonadal peptides that selectively and potently inhibit FSH secretion from the pituitary gland (De Jong, 1988; Ying, 1988). They are composed of an α-subunit and one of two β-subunits (βA or βB), with α-βA and α-βB dimers forming inhibin-A and inhibin-B, respectively. The importance of inhibins in the control of the reproductive function has been reported (de Kretser et al. 2002, Medan et al. 2007) and it appears that βB-subunit might be produced in small developing follicles, which is replaced by the inhibin βA-subunit as the follicles approach the preovulatory stage (Medan et al. 2007). In cattle (Kaneko et al. 2002) and goats (Medan et al. 2005) an inverse relationship between FSH and inhibin-A was demonstrated, suggesting the key role of inhibin-A produced by dominant follicle(s) in terminating the transient peaks of FSH secretion (Medan et al.

2007). Superovulation is a prerequisite for embryo transfer programs, and was suggested that inhibin-A may provide an index of follicular development (Lockwood et al., 1996), but studies exploring the relationship between controlled ovarian stimulation, inhibin levels and ovarian follicular development in ruminants are limited (Gonzalez-Bulnes et al., 2002, Gonzalez-Bulnes et al., 2004), furthermore very few data are available on the inhibin plasma concentrations in buffaloes during treatment for superovulation (Palta et al., 1997). This our preliminary paper reports results on the relationships between inhibin-A, estradiol 17β and the follicular development in three different treatment to obtain superovulatory response in adult buffalo cows.

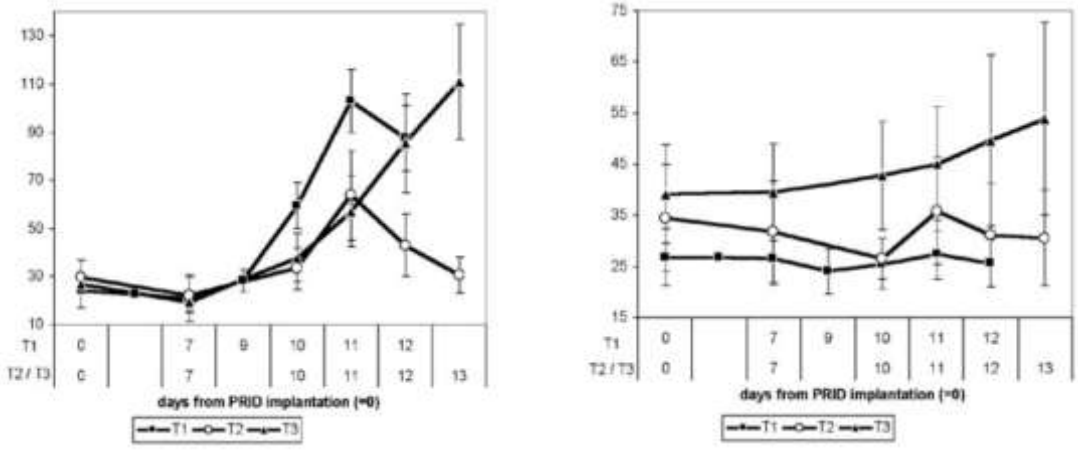
**MATERIAL AND METHODS -** In an experimental farm 24 adult buffalo cows were submitted to three different treatments for MOET (Multiple Ovulations and Embryo Transfer) programs during autumn (favourable reproductive period in Italy). In the group 1 (T1, n = 7) a progesterone releasing intravaginal device (PRID) was kept *in situ* for 9 days (d0-d9). Starting day 7 to day 10 post-implantation, were administered 2 i.m. injections per day, 12 h apart, i.m. decreasing dosage (87.5, 87,5, 75, 75, 50, 50, 37.5, 37.5 IU) of an equal mixture of 500 IU of FSH and LH (Pluset; Serono, Veterinary, Italy); In a second group (T2, n = 8) PRID was kept *in situ* 10 d (d0-d10) and a single i.m. injection of 2000 IU of pregnant mare serum gonadotrophin (PMSG, Folligon; Intervet,Italy) was administered on d7. In a third group (T3, n = 9) PRID was kept *in situ* 10 d as in T2, a single i.m. injection of 2000 IU of PMSG was administered on d7 and decreasing doses of 175 IU FSH/LH, by i.m. injections at 12 h intervals on d10 (50 IU) and d11 (37.5 IU). All the cows were treated with Prostaglandin F2α at the PRID removal. All the animals were submitted to blood sampling and to ultrasound to check follicles number and size on day 0, 7, 9, 10, 11, 12 (T1) or 0, 7, 10, 11, 12, 13 (T2 and T3). Following their diameter, follicles were classified as small (<6 mm, SF) or large (>6 mm, LF) .The number of follicles >10 mm in diameter was counted (VLF). The blood samples were immediately centrifuged and plasmas were stored at -20°C until assays. Hormones assays were performed by ELISA kits for human serum or plasma validated for buffalo species (Todini et al., 2007). The data were subjected to ANOVA considering as factors sample and treatment. Where differences were observed, B-Tukey post hoc test was performed. Correlations between data were calculated using Pearson bivariate analysis (SPSS 12.0).

Table 1. Correlation coefficients between the hormonal concentrations and the follicles number. In-A = inhibin-A, E2 = Estradiol 17β, LF = follicles > 6 mm diameter, SF = small follicles.
Bold character means P < 0.01, otherwise P < 0.05.

| | E2 | | | LF | | | SF | | |
|---|---|---|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T1 | T2 | T3 | T1 | T2 | T3 |
| In-A | n.s. | n.s. | 0,67 | 0,83 | 0,81 | 0,83 | n.s. | n.s. | -0,41 |
| E2 | - | - | - | n.s. | n.s. | 0,39 | n.s. | n.s. | -0,35 |
| LF | | | | - | - | - | n.s. | n.s. | -0,43 |

**RESULTS AND CONCLUSIONS -** The overall mean plasma concentrations of inhibin-A (In-A) and Estradiol 17β (E2) were respectively 20.86+/-3.81 (s.e.) pg/mL and 33.10+/-5.01 pg/mL at day 7 and 77.28+/-12.12 and 37.83+/-7.99 at day 12 (T1) or 13 (T2 and T3). The mean hormone concentration profiles during the trial are reported in figure 1. Plasma In-A concentrations significantly rose at d11-13 of T1 and T3, but non in T2 group. E2 concentrations did not changed in any group. Follicles numbers on day 7, 12 (T1) or 13 (T2 and T3) are reported in figure 2. Mean plasma In-A concentrations by all the animals were positively correlated with plasma E2 levels (R=0.43, P<0.01) and with LF number (R=0.83, P<0.01), negatively with SF number (R=-0.21, P<0.01). Plasma E2 values correlated weakly with LF number (R=0.21, P<0.05) and non significantly with SF number (R = 0.15). Within treatment (table 1), there was always a positive correlation between In-A and LF number, but only in T3 there were correlations between all the considered data. No correlation was found between the In-A or E2 values at the start of the gonadotropin treatment and the number of the LF or VLF at the end of the observation period. T3 treatment was followed by a higher number of follicles with diameter more than 10 mm at d12-13 (T1=5.0+/-1.4, T2=1.2+/-0.9, T3=8.3+/-2.3). The In-A plasma mean concentrations resulted well correlated with the large follicles number in the overall of the animals subjected to superovulatory treatments and in each of the groups, well following the dynamics of the follicle growth in each group. The treatment combining PMSG and FSH/LH in decreasing doses (T3) had the better results as follicular growth and In-A raised regularly from the start of the overstimulation to the end of the trial. In the T3 group also the E2 concentrations gradually increased during the period, but the very high standard errors indicates a strong variability within the data. Therefore it is concluded that plasma In-A can be a good indicator of the follicular development during MOET treatments in buffalo cows, and that to evaluate the In-A levels can be an useful data to comprehend the ovarian stimulated dynamics, while E2 is not. In-A nor E2 plasma values at the start of the stimulating treatment seem could be considered a criteria for selection of buffalo donors. Furthermore T3 treatment was followed by better ovarian follicular responses, especially if it is considered the number of very large follicles (>10 mm diameter) counted at the end of the trial.

Figure 1. Mean plasma concentrations and s.e. of In-A and E2 in the treatment groups.



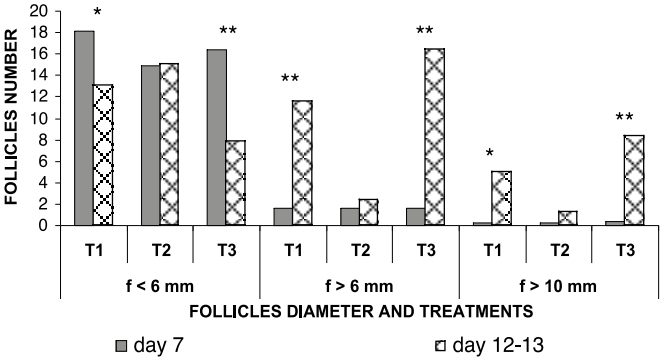



Figure 2.
Mean number of follicles in the tretment groups at day 7 (start of gonadotropin administration) and at day 12 (T1) or 13 (T2, T3).
f= diameter follicles,
*= P<0.05, **= P<0.01.

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