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Plasma Inhibin A determination at periovulatory period could be predictive for buffalo fertility

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Abstract

To test whether Inhibin-A assays can be used to predict the fertility in buffalo cows, 15 buffaloes were assigned to 3 synchronization treatments: group A (n=5) treated with PRID for 10 days+1000 IU PMSG and PGF2α (0.15mg cloprostenol) on the 7th day; group B (n=5) treated as group A but PMSG and PGF2α were administered on the 10$^{th.}$day; group C (n=5) received GnRH (150μg gonadorelin) on day 0 + PGF2α on the 7th day + GnRH on the 9th day. Buffaloes were artificially inseminated at 72 and 96h from PRID removal in group A and B and at 40h from the 2nd GnRH injection in group C. Starting 2 days (-2d) before the 1st insemination the number and size of all follicles>2mm were assessed for 4 days by ultrasound and plasma Inhibin-A levels were measured. The conception rates were 80%, 40% and 20% in groups A, B and C, respectively. In all groups there was a positive correlation between number of follicles>6mm and Inhibin-A ($r=0.92$, $P<0.0001$)assayed two days before the 1st insemination; a positive correlation between the same parameters ($r=0.97$,$P<0.0003$) assayed the same day (-2d) was found only in pregnant buffaloes. In the same day (-2d) the Inhibin-A levels were 29.6, 9.9 and 6.5 pg/ml ($P<0.05$) in groups A, B and C, respectively and 21.5 and 9.9 pg/ml in pregnant and non pregnant buffaloes. These results suggest that follicles>6 mm are the main source of Inhibin-A and this latter could be useful to predict the outcome of artificial insemination.

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). Following the isolation of Inhibin-A and Inhibin-B, a large number of studies have defined a variety of physiological roles for these substances that range well beyond their ability to suppress or stimulate follicle-stimulating hormone (FSH) secretion. Using an ELISA assay that have now been shown to detect not only dimeric Inhibin but products of the α-subunit, changes in Inhibin-A secretion have been identified in a variety of patho-physiological states related to reproductive functions. Evidence is emerging that monitoring the stimulation phase of assisted reproductive technologies with Inhibin-A as well as ultrasound scans give a good indication of follicular recruitment and development. Very few data are available on the Inhibin-A plasma concentrations in buffaloes during reproductive treatments (Palta et al., 1997), so this our preliminary paper reports results on the relationship between Inhibin-A levels, follicular development and conception rate in three different synchronization treatments in adult buffalo cows.

Material and Methods

The trial was carried out during the non-breeding season on 15 buffalo cows divided in three homogeneous groups: Group A (n=5) treated with a progesterone releasing intravaginal device

(PRID) inserted for 10 days + an i.m. injection of 1000 IU of PMSG and 0.15 mg of cloprostenol ($PGF_{2\alpha}$ analogue) on day 7; Group B(n=5) treated as in Group A but received the PMSG and $PGF_{2\alpha}$ on day 10 (day of PRID removal) instead on day 7 (Day 0= day of PRID insertion); Group C (n= 5) treated with Ovsynch protocol and received i.m. injection of GnRH on day 0 + $PGF_{2\alpha}$ on the 7^{th} day + GnRH on the 9^{th} day (GnRH=μg150 gonadorelin; $PGF_{2\alpha}$=0.15mg cloprostenol). Buffaloes were artificially inseminated with frozen/thawed semen of progeny testing bulls at 72 and 96 h from removal in the PRID groups (A and B) and at 40 h from the 2^{nd} GnRH injection in the Ovsynch group (C). The pregnancy diagnosis was performed on day 26 after the first artificial insemination (AI) by an ultrasound sector scanner with a 7.5 Mhz rectal linear probe and confirmed by rectal palpation on day 45.

On day -2, -1, 0 (day 0 = day of 1th AI in group A and B; day of a single AI in group C) all buffaloes undergone an ultrasound examination of ovaries by using a portable ultrasound unit (Aloka SSD-500, Aloka CO. Ltd., Tokyo, Japan) together with a 7.5 MHz linear rectal probe. Follicles were classified in four categories according to their size: small (diameter < 3 mm), medium (3 mm< diameter <6 mm), large (6 mm< diameter <9 mm) and very large (diameter >9 mm). The number of all follicles and the number and the diameter of medium and large follicles were recorded each day.

At the same time of ultrasound examination blood samples were collected by jugular venipuncture with evacuated tubes containing K3 EDTA (Venoject, Terumo Europe NV, Leuven, Belgium), immediately centrifuged (2500 G for 15 min) and the plasma stored at −20°C until assayed. Inhibin-A concentrations were determined in the plasma samples by an enzimatically amplified "two-step" sandwich type immunoassay kit for human serum or plasma (Inhibin A DSL-10-28100, Diagnostic Systems Laboratories Inc, Webster, Texas, USA).

Data were analyzed by a standard statistical analysis (SAS, GLM Procedures).

Results

On day -2 all animals had one large and very large follicle, except for one animal of group B that had only medium follicles; although the number of large and very large follicles was higher in group A than in group B and C, the differences were not significant. In group A (P< 0.05), B and C a reduction in the number of very large follicles was observed on day 0 (Table 1).

Table 1. Follicles number among treatment groups within the categories observed: small (<3mm), medium (3<diameter<6), large (6<diameter<9), very large (>9mm).

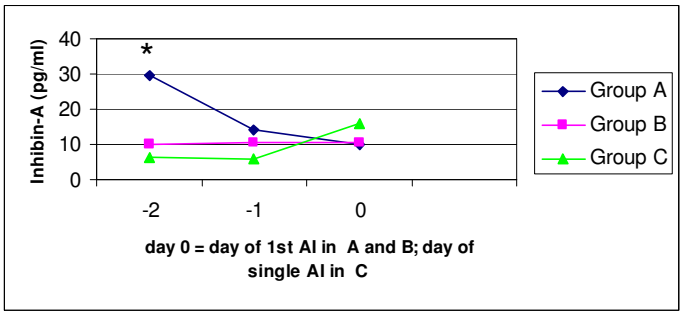
Day	Group	N° animals	Follicles					
			Small	Medium	Large	Very large	Large + Very large	Total
Day -2	Group A	5	10.4a	1.7	2.0	1.6	2.4	13.8a
Day -2	Group B	5	6.8b	2.3	1.3	1.0	1.5	9.8b
Day -2	Group C	5	6.8b	2.3	1.5	1.0	1.2	9.8b
Day -1	Group A	5	10.2	1.0	2.0	1.4	2.2	12.8
Day -1	Group B	5	7.2	3.0	1.3	1.0	1.4	11.0
Day -1	Group C	5	7.4	2.0	1.0	1.0	1.0	9.2
Day 0	Group A	5	13.2a	3.0	2.0	0.4	0.8	15.8
Day 0	Group B	5	6.3b	4.0	1.5	0.5	1.4	9.6
Day 0	Group C	5	8.6ab	5.5	1.0	0.5	0.6	12.8

Day 0 = day of 1th AI in group A and B; day of a single AI in group C.
Values within columns (day per day) with different letters differ for P<0.05.

Plasma concentration of Inhibin-A was significantly different (P<0.05, figure 1) among the three treatment groups on day -2 when the Inhibin-A levels were 29.6, 9.9 and 6.5 pg/ml in groups A, B and C, respectively. In buffalo cows resulting pregnant and non pregnant the Inhibin-A levels were 21.5 and 9.9 pg/ml, respectively.

In all groups there was a positive correlation between number of follicles>6mm and Inhibin-A (r=0.92, P<0.0001) assayed two days before (-2d) the AI; a positive correlation between the same parameters (r=0.97, P<0.0003) assayed the same day (-2d) was found only in pregnant buffaloes. Time to ovulation tended to be shorter in association with higher Inhibin-A assayed two days before (-2d) the AI (r=-0.44; P<0.09) (Welt et al., 1999).

Figure 1- Mean peripheral plasma Inhibin-A concentrations relative to the artificial insemination



Means marked with an asterisk (*) are significantly different (P<0.05)

Time of ovulation was influenced by treatments: better results have been observed at the time of AI in group A where the overall ovulation rate was 100% (80% of animals was observed ovulated at the 1th AI and 20% at the 2nd AI); in group B the overall ovulation rate was 40% (20% of animals was observed ovulated at the 1th AI and 20% at the 2nd AI); in group C 60% of animals was observed ovulated at the time of AI. The overall conception rates were 80%, 20% and 40% in group A, B and C, respectively (Table 2).

Table 2. Ovulation rate and conception rate among treatment groups.

Group	**N° Animals**	**Ovulation rate % (n)**		**Conception rate % (n)**
		At 1st A.I*	At 2nd A.I**	on day 26 after the first AI
Group A	5	80 (4/5)	20 (1/5)	80 (4/5)
Group B	5	20 (1/5)	20 (1/5)	20 (1/5)
Group C	5	60 (3/5)	--	40 (2/5)

* 1st AI = 72h from PRID removal in group A and B; single insemination at 40 h from 2nd GnRH injection in group C
** 2nd AI = 96h from PRID removal in group A and B

Conclusion

This is the first report to describe the dynamic changes in plasma concentration of Inhibin-A in buffalo cows subjected to different synchronization protocols. The results suggest that follicles > 6 mm are the main source of Inhibin-A and day-2 peripheral Inhibin-A levels measured during different synchronization protocols could be a good marker for follicular development and pregnancy rate. A large prospective study is needed to confirm our findings.

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