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LH peak and ovulation in buffalo cows treated for oestrus synchronisation using two different hormonal schedule

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RIASSUNTO – Picco di LH ed ovulazione in bufale trattate per la sincronizzazione dell'estro con due diversi protocolli ormonali. *Scopo del lavoro è stato quello di valutare il picco di LH e l'ovulazione in bufale sottoposte a due diversi protocolli ormonali di sincronizzazione dei calori al fine della applicazione della inseminazione artificiale (IA). Gli animali sono stati assegnati a due gruppi: gruppo A (n=12), trattato con una spirale intravaginale contenente progesterone (PRID), associata alla somministrazione di PMSG e di un analogo della PGF2 α ; gruppo B (n=12) trattato con GnRH + PGF2 μ + GnRH (Ovsynch). La determinazione dell'LH è stata fatta mediante ELISA. Il picco di LH e l'ovulazione sono avvenuti rispettivamente a 51,30 \pm 13,94 e a 85,14 \pm 13,63 ore dalla fine del trattamento, mentre l'intervallo medio tra picco LH e ovulazione è stato di 33,71 \pm 4,30 ore. Non si sono registrate differenze significative né tra i gruppi né tra i mesi in cui è stato effettuato il trattamento. Una migliore efficienza nella sincronizzazione del picco di LH e dell'ovulazione è stato ottenuto col trattamento Ovsynch (gruppo B).*

Key words: LH peak, ovulation, oestrus synchronisation, fixed-time A.I.

INTRODUCTION - Since oestrus behaviour in buffalo is frequently scarcely clear and the oestrus signs, even when showed, are not reliable, ovulation can not be predicted. Furthermore high individual variability of the oestrus signs – LH peak interval was observed both in spontaneous and hormonal induced oestrus (Moioli *et al.*, 1998; Barile *et al.*, 1998). To obtain satisfactory conception rates, the insemination should be performed at the correct time relatively to ovulation, that in turn is depending from the LH peak. Therefore to promote the use of artificial insemination (A.I.) different hormonal treatments have been scheduled to obtain oestrus synchronisation and, in particular, LH peak and ovulation synchronisation, aimed to perform fixed time A.I. (Barile, 2005). In buffaloes two prominent methods was investigated in the last years, utilising GnRH + prostaglandin after 7 days + GnRH after 36-48 hours (Ovsynch protocol), or progesterone-containing devices (PRID) along with estradiol, PMSG and prostaglandins (Barile *et al.*, 2004; Baruselli *et al.*, 1999; de Araujo Berber *et al.*, 2002; Neglia *et al.*, 2003). Both methods resulted more effective during the season when the cows are cyclic, but the farmer interest is also to increase the conception rate during the low breeding season, to shorten the calving interval and to have a milk production more distributed along the year.

In the present work we compare the efficacy of the two above mentioned hormonal treatment in synchronise the LH peaks and ovulation in buffalo cows in the period of transition to seasonal anoestrus.

MATERIALS AND METHODS - The experiment was carried out on 24 pluriparous lactating Italian Mediterranean buffalo cows, in two trials during the months of February and March, corresponding in the Italian conditions to the ending of the high fertility season and to the beginning of the low fertility season. In each of the two trials 12 cows were randomly divided in two groups of 6 animals. The treatments schedule were: - PRID group: a progesterone releasing intravaginal device (PRID) containing 1.55 g natural progesterone and a capsule with 10 mg oestradiol benzoate were kept for 10 days, and 1000 IU PMSG and 0.15 mg cloprostenol (PGF2 α analogue) were injected on the 7th day after PRID insertion; - GnRH group: 150 μ g GnRH on day 0 plus 0.15 mg cloprostenol (PGF2 α analogue) on the 7th day plus 150 μ g GnRH on the 9th day (Ovsynch protocol). Blood samples were taken from the jugular vein in vacutainer with EDTA at 4 hours intervals, starting at the 24th hour after PRID removal (PRID group) or at the 12th hour from PGF2 α injection (GnRH group), and ending at the 108th hour. Plasma was immediately separated and stored at -20°C until analysed. The LH determination was performed by an ELISA test (LH detect, INRA, France) suitable for the species (Maurel *et al.*, 1995), assaying all the sample from each cow in duplicates in the same plate; in this preliminary note we report the qualitative evaluation of the assay, the peak time corresponding at the wells of the plate having the higher pronounced colour. The ovarian status, the number and development of the follicles, and the ovulation time, were assessed by ultrasound monitoring effected twice a day during all the period of blood sampling. Data were analysed by ANOVA using the GLM procedure (SAS, SAS Institute, Inc., Cary, NC) considering as factors the treatment scheme and the month of treatment.

RESULTS AND CONCLUSIONS - After the treatments 23 of the 24 cows (96 %) had a well distinguishable LH peak. In PRID group only one buffaloes of six treated in March did not show LH peak and did not ovulate, while in GnRH group these result were observed for one buffalo treated in February and one in March. Consequently, the overall rate of ovulating animals resulted of 87.5 %. The mean time of the LH peaks, of the ovulations and of the intervals LH peak-ovulation are showed in table 1. The results are similar to those reported in other works (Seren *et al.*, 1994; Barile *et al.*, 1998; Malfatti *et al.*, 2003), and the mean values are not different between the treatment groups nor between the months.

Table 1. Time (h) of LH peaks, of ovulations and of the LH peak-ovulation intervals in buffalo cows treated with PRID+PMSG+PGF2 α (PRID group) or GnRH+PGF2 α +GnRH (GnRH group) in late February or mid March.

	n	mean	s.d.	c.v. %
LH peak				
PRID group	11	53.45	20.10	37.60 ^A
GnRH group	12	49.33	3.55	3.55 ^B
February	12	48.67	12.40	25.47
March	11	54.18	15.53	28.66
Total	23	51.30	13.94	27.18
Ovulation				
PRID group	11	86.18	18.45	21.41 ^A
GnRH group	10	84.00	5.66	6.73 ^B
February	11	81.82	11.78	14.40
March	10	88.80	15.18	17.09
Total	21	85.14	13.63	16.01
LH peak-ovulation interval				
PRID group	11	32.73	4.67	14.27
GnRH group	10	34.80	3.79	10.90
February	11	33.09	4.04	12.20
March	10	34.40	4.70	13.65
Total	21	33.71	4.30	12.76

Different superscripts means different values (P<0.01).

In the PRID group the higher s.d. of the LH peak and of the ovulation times leads to a lesser degree of synchronization respect to the Ovsynch treated cows: the coefficients of variation are higher in the PRID group and significantly different from the values of the GnRH group, whereas no difference was observed between the treatment periods.

The presence of a well synchronized LH peak in the GnRH treated animals indicates that in this group the LH release was probably caused by the second GnRH administration. The occurrence of the ovulation in these animals at the same interval after the LH release than in that of the PRID group and in buffaloes during spontaneous heats (Seren *et al.*, 1994) suggests that the GnRH injection was effected at the proper time in respect to the follicular development.

From this trial can be concluded that both the PRID and GnRH based treatments are effective in inducing follicular development, LH peak and ovulation in buffalo cows during late winter, when the reproductive activity in the Italian conditions is decreasing. The Ovsynch protocol can induce a better synchronization of LH peaks and ovulations and thus only one AI at 84 h from PG injection could be used. The variability in the release time of LH peak, in the PRID protocol, exerts influence on ovulation time reducing the ovulation synchronization efficiency; in this case, 72 and 96 h after PRID removal could be more appropriate time for AI.

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