Follicular dynamics in synchronized Italian Mediterranean buffalo cows

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ABSTRACT: The aim of this study was to evaluate the length and the characteristics of the oestrous cycle in Italian Mediterranean buffalo cows, undergone synchronization of ovulation. The trial was performed on 32 buffaloes synchronized by the Ovsynch Program, which consists of an injection of GnRH on day 0, $PGF_{2\alpha}$ on day 7 and GnRH on day 9. Starting on day 10 (Day 0 of the new cycle). Buffaloes undergone ultrasound examination of the ovaries on alternate days until the following heat. Follicular growth and corpus luteum formation and dimensions were recorded as well as the number of follicular waves. Statistical analysis was performed by ANOVA. Four animals (12.5%) did not show signs of oestrous and were excluded from the trial. The mean length of the oestrous cycle was 23.7±3.4 days. In particular, 1 animal (3.6%) showed an oestrous cycle characterized by 1 follicular wave with a length of 16 days, 17 subjects (60.7%) showed 2 follicular waves with a cycle length of 22.4±2.3 days and 10 buffaloes (35.7%) showed 3 follicular waves with a cycle of 26.8±2.0 days. These results confirm previous reports performed in buffalo species, although the cycle resulted longer in the 3-waves group.

Key words: Oestrous cycle, Follicular wave, Dominant follicle, Corpus luteum.

INTRODUCTION - Several studies (de Araujo et al., 2002; Neglia et al., 2003) showed low efficiency in Artificial Insemination (AI) and Multiple Ovulation and Embryo Transfer (MOET) programs in buffalo species. Follicular dynamics is the continuous process of growth and regression of a group of antral follicles, one of which develops until it reaches the preovulatory follicular stage (Lucy et al., 1992). Although several studies have been carried out in cattle (Bruke et al., 1994; Roche, 1996), the physiological control of recruitment, selection, growth, dominance and atresia of ovarian follicles is not well understood in water buffaloes. The study of follicular dynamics during the oestrous cycle may provide the basis for improving fertility, for synchronizing oestrus more accurately and for enhancing superovulatory response. Some studies (Baruselli et al., 1997) demonstrated that also in buffalo species follicular growth, after spontaneous oestrus, occurs in waves, as previously demonstrated in bovine (Adams et al., 1994). The aim of this study was to evaluate the length and the characteristics of the oestrous cycle in Italian Mediterranean buffalo cows, undergone synchronization of ovulation.

MATERIAL AND METHODS - The trial was carried out in January on 32 pluriparous Italian Mediterranean buffalo cows at 144±40 days in milk. During the experimental period the animals were maintained in open yards that allowed 15 m^2 for each buffalo and were fed a total mixed ration that consisted of 50-55 % forage and 45-50 % concentrate, containing 0.90 MFU/kg of dry matter (DM) and 15% crude protein /DM. All animals were subjected to clinical examination to establish reproductive soundness. Only buffaloes in good health and withouth any reproductive disorder were included in the study. Buffaloes were synchronized by using the Ovsynch-TAI Program (Pursley et al., 1995; Neglia et al., 2003), which consists of 12 µg buserelin acetate (Receptal®, Intervet, Milan, Italy) administered on Day 0, 0.524 mg cloprostenol (Estrumate®, Schering-Plough Animal Health, Milan, Italy) on Day 7 and 12 µg buserelin acetate on Day 9. Because of the relatively low intensity of oestrous behaviour in buffaloes (Ohashi, 1994) animals were palpated per rectum (the day after the last administration of buserelin acetate) to assess oestrous status. All animals with a follicle > 1.0 cm and a tonic uterus with presence or absence of mucous vaginal discharge were considered to be on heat (Day 0). On alternative days, buffaloes undergone transrectal ultrasound analysis in order to verify ovulation, corpus luteum (CL) formation and follicular dynamics, until the following heat. The dimensions of the dominant follicle and CL, together with the number of medium and small follicles were also recorded. Statistical analysis was carried out by ANOVA.

RESULTS AND CONCLUSIONS - Only 4 animals out of 32 (12.5%) did not show signs of oestrus and were excluded from the trial, confirming the efficacy of the synchronization protocols. On the 28 remaining buffaloes, the mean length of the oestrous cycle was 23.7±3.4 days. In particular, 1 animal (3.6%) showed a cycle characterized by 1 follicular wave with a length of 16 days, 17 subjects (60.7%) showed 2 follicular waves with a cycle length of 22.4±2.3 days and 10 buffaloes (35.7%) showed 3 follicular waves with a cycle of 26.8±2.0 days (Table 1). These results are in accordance with previous reports (Baruselli et al., 1997; Manik et al., 1998), although the length of the cycle in the 3-wave group resulted longer than that reported by Baruselli *et al.* (26.8±2.0 vs. 24.0±2.21 days, respectively). The length of the oestrous cycle was also significantly longer ($P<0.01$) in the 3-wave vs. the 2-wave group. No differences were recorded between the emergence of the first wave between the 2- and 3-wave cycles (1.29±0.46 vs. 1.3±0.48 days, respectively), whereas, in agreement with the results reported by Baruselli *et al.* (1997), the second wave appeared earlier in the 3-wave group vs. the 2-wave group (11.8±2.3 vs. 14.5±2.4 days; $P<0.01$). Hence, also in Mediterranean buffaloes with synchronized oestrus, the length of the cycle is correlated with the number of follicular waves per cycle, as demonstrated in Murrah buffaloes,with spontaneous oestrus (Baruselli *et al.*, 1997).

The maximum diameter of the preovulatory follicles was greater either in the 3- and 2-waves groups vs. the dominant follicles of the other waves (Table 2). This finding is in contrast with the results reported by Baruselli et al. (1997), who recorded no differences in the 2-waves group and a smaller dominant follicle only during the second wave in the 3-waves group. One possible explanation to the phenomenon may be found in the longer duration of the waves recorded in the present study. In fact, it is known that progesterone during

Table 1. Characteristics of the oestrous cycle in Italian Mediterranean buffaloes with 1, 2 or 3 follicular waves.

	N	Cycle length (Day)	I wave	Emergency II wave (Day)	III wave
1 Wave	1	16.0X	1	-	-
2 Waves	17	22.4±2.3Y	1.29±0.46	14.5±2.4X	-
3 Waves	10	26.8±2.0Z	1.30±0.48	11.8±2.3Y	20.4±1.9

Values in the same columns with different superscripts differ (^{X,Y,Z} P<0.01).

the luteal phase affects the growth of the anovulatory dominant follicles, decreasing their diameter, as previously demonstrated in cattle (Bruke et al., 1994). If the wave is longer, it is likely that progesterone may influence the growth of the anovulatory dominant follicles for more time, reducing their diameter.

No differences were observed in terms of number of follicles for the different waves. This result agrees with those reported in cattle (Boni et al., 1993) and buffalo (Baruselli et al., 1997), supporting the hypothesis that the number of follicles recruited depends on the individual animals (Baruselli et al., 1997). Also the maximum diameter of the CL was similar between 3- and 2-waves groups (1.55±0.15 vs. 1.42±0.39). The results recording in this study confirm those obtained by other authors either in cattle and in buffalo. However, further reserches are needed in order to better understand the follicular dynamics and to improve the methods of oestrous synchronization in buffalo species.

Table 2. Characteristics of the dominant follicles during the oestrous cycle in Italian Mediterranean buffaloes with 1, 2 or 3 follicular waves.

		Diameter		
	N	1° dominant follicle	2° dominant follicle (cm)	3° dominant follicle
1 Wave	1	1.6	-	-
2 Waves	17	1.49±0.27^{a}	1.69±0.16^{b}	-
3 Waves	10	1.42±0.35^{a}	1.49±0.25^{a}	1.69±0.17^{b}

Values in the same rows with different superscripts differ (^{a,b} P<0.05).

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