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Strategies to reduce embryonic mortality in buffalo cows

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ABSTRACT: The aim of the present study was to examine whether treatment with a GnRH agonist, hCG or P_4 on Day 25 after AI increased P_4 concentrations and reduced the incidence of embryonic mortality (EM) in pregnant buffaloes mated in mid-winter in a Mediterranean environment. The trial was carried out in two farms characterized, in previous years, by low (LEM Group), 153 buffaloes (DIM=150±7 days), and high (HEM Group), 284 buffaloes (DIM=163±5 days), incidence of embryo mortality. Animals were synchronized by Ovsynch-TAI Program and artificially inseminated. On day 25, pregnant buffaloes were randomly assigned to four groups: Control (no treatment), GnRH agonist (buserelin acetate, 12.6 μ g), hCG (1500 IU) and P_4 (341 mg of P4 i.m. every 4 days for three times). Progesterone (pg/ml) was determined in milk whey on Days 10, 20 and 25 after AI in all buffaloes and in Days 30 and 45 only in buffaloes pregnant on day 25 and assigned to four groups of treatment. Pregnancy diagnosis was undertaken on Day 45 by ultrasound. All treatments increased P_4 milk whey and reduced embryonic mortality in buffalo cows bred in the farm characterized by high EM.

Key words: Embryonic mortality, Buserelin, hCG, Progesterone.

INTRODUCTION - It has been demonstrated that an early P_4 peak reduces embryonic mortality in cows (Mann, 2002). In buffaloes treatment with buserelin, hCG and progesterone, on day 5 after A.I., increase P4 on Day 15, but did not reduce the incidence of embryonic mortality (Campanile et al., 2007a). Embryonic mortality in buffaloes (Campanile et al. 2005) mated by AI in mid-winter appears to occur later, Day 25-40, than in cattle (Mann, 2002), Day 14-17. The aim of the present study was to examine whether treatment with a GnRH agonist, hCG or P_4 in pregnant buffaloes on Day 25 after AI reduced the incidence of embryonic mortality in buffaloes mated in mid-winter in a Mediterranean environment.

MATERIAL AND METHODS - The trial was carried out between January and March on 437 pluriparous buffaloes (DIM=153±6 days) bred in two farms characterized, in previous years, by low (LEM Group), 153 buffaloes (DIM=150±7 days), and high (HEM Group), 284 buffaloes (DIM=163±5 days), incidence of embryo mortality. All animals were synchronized by the Ovsynch-TAI Program and each buffalo was inseminated twice, 16 h and 40 h after the second injection of GnRH agonist. On day 25 after AI, buffaloes were monitored by ultrasound to verify the presence of a pregnancy. In both farms, pregnant buffaloes on day 25 were equally divided on the basis of DIM and underwent to 4 pharmacological treatments: 1) 12 µg of Buserelin acetate i.m.- 12.6 µg; 2) 1500 IU of hCG i.m.; 3) 341 mg of P4 i.m. every 4 days for three times; 4) no treatments (control). Milk samples were collected on Days 10, 20 and 25 from each animal and, only in buffaloes pregnant on day 25 and assigned to four groups, on Days 30 and 45 after AI, to assess P_4 concentrations in the milk whey. The milk samples were clotted and skimmed and the concentration of P_4 was determined by RIA as previously described (Campanile et al., 2007a). In order to verify the effects of the treatments on embryo mortality (EM), buffaloes undergone ultrasound 45 days after AI. Data of milk whey P4 concentrations were analyzed by ANOVA with repeated measures (SPSS 15.0, 2007) using the treatment group and the reproductive status as main factors. The percentages of animals with different reproductive status between treatment groups were analyzed by Chi square test (SPSS 15.0, 2007).

EM (n) % 0.5 ^{A x}	LEM (n) % 31.3 ^y	Total (n) % 16.7
		16.7
(
(4)	(5)	(9)
16.2ª	5.6	12.7
(6)	(1)	(7)
13.2ª	18.8	14.8
(5)	(3)	(8)
2.8 B ^{bx}	11.1 ^y	28.3
(13)	(2)	(15)
	(6) 13.2 ^a (5) 2.8 B ^{bx} (13) = P < 0.05	(6) (1) 13.2 ^a 18.8 (5) (3) 2.8 B ^{bx} 11.1 ^y

within columns x, y = P < 0.01.

RESULTS AND CONCLUSIONS -The incidence of pregnant buffaloes on day 25 and 45 was similar in the two farms (25 days: HEM= 54.2 vs. LEM= 48.4; 45 days: HEM = 43.0 vs. LEM = 37.9). All treatments significantly reduced the incidence of EM in HEM Group, improving the reproductive efficiency. In contrast, treatment with buserelin negatively (P<0.05) influence EM in LEM Group (Table 1). If only control groups are considered, the incidence of EM was significantly (P<0.05) lower in LEM Group (Table 1). Pregnant buffaloes had higher (P<0.01) concentrations of P_4 milk whey on Day 20 and Day 25 than embryonic mortality and non-pregnant buffaloes and Day 5 (P<0.05) only than non-pregnant buffaloes (Table 2). The overall analysis of

milk whey progesterone indicated no differences between two experimental groups (HEM vs. LEM), before (Days 25) and after treatment (Day 30 and 40). The differences (P<0.01) on P₄ concentrations in milk whey were found on Day 25 (377.6 pg/ml vs. 215.5 pg/ml), 30 (332.9 pg/ml vs. 203.8 pg/ml) and 45 (374.6 pg/ml vs. 217.5 pg/ml) between pregnant and embryonic mortality buffaloes on the total of animals (Table 3). In the present study, treatment with buserelin, hCG and progesterone on Day 25 after A.I. in pregnant buffaloes reduce the incidence of embryonic mortality in HEM group. Campanile et al. (2007b) found that treatment of buserelin induce acute increase in circulating concentrations of LH, FSH and oestradiol-17 β and in progesterone levels after 10 days. The induction of a new corpus luteum (CL) reduces embryonic mortality in cattle after induced regression of the

original CL when this induced CL was on the ovary adjacent to the pregnant uterine horn (Lulai et al. 1994). In another work, maintenance of pregnancy was examined after induction of a new CL between the days 27 and 54 after breeding (Bridges et al., 2000) in cows in which original CL had either regressed or been removed earlier and pregnancy had been maintained with an exogenous progestogen. In LEM group any treatment modified the incidence of embryonic mortality, in fact the progesterone levels of pregnant, non-pregnant and embryo-mortality buffaloes did not differ before the treatment. Therefore, it is hypothizable that other factors, together with the reduced circulating concentrations of P_4 , also

-	10	20	25
)	282.4±10.2ª	299.6±9.8 ^A	367.6±15 ^A
	(161)	(161)	(161)
Р	243.6±29.1 ^b	195.3±10.6 ^B	234.9±14.3 ^B
	(181)	(181)	(181)
M	291.5±26.5 ^{ab}	227.4±23.1 ^B	214±20 ^B
	(43)	(43)	(43)

Table 2.	Milk whey progesterone (pg/ml) in P, NP and EM buffaloes on different
	days after AI.

Table 3.	Milk whey progesterone (pg/ml) in P and EM buffaloes for each treatment
	groups on different days after AI.

	Day after AI							
	25		30		45			
-	Р	EM	Р	EM	Р	EM		
GnRH	350. 5±24.9	224. 8±52.0	312.4±20.68	167. 5±37. 2	371. 3±23.9	240. 5±36. 8		
	(39)	(7)	(39)	(7)	(39)	(7)		
hCG	369. 5±31.2	240.4±73.3	372.4±27.47	251. 6±52	395 .8±30.49	167. 8±76.3		
	(36)	(5)	(36)	(5)	(36)	(5)		
progesteron	401. 2±39.7	168±52.7	302. 0±19.34	102. 6±27.3	363. 8±27.78	174. 3±75.7		
	(35)	(6)	(35)	(6)	(35)	(6)		
control	401±42.2	227. 5±65.1	354. 0±40.65	281. 6±812	363 ±22.29	260. 6±41.1		
	(22)	(8)	(22)	(8)	(22)	(8)		
total	377. 5±16.8 ^A (132)	215. 5±29.3 ^B (26)	332. 9±12.97 ^A (132)	203. 8±31.2 ^B (26)		217. 4±27. 2 ^B (26)		

contributed to embryonic mortality in this group. A reduced capacity to secrete progesterone would seem to explain some of this embryonic mortality but other as yet unidentified factors contribute between 40-50% to the embryonic losses (Campanile et al., 2005). It is concluded that buffaloes require exogenous hormone treatments that induce elevated P_4 throughout the period from initial development to embryonic attachment. In fact, the use of pharmacological treatments in order to increase P4 blood levels between 25 and 40 days post AI, period characterized by the 45% of embryo mortality in buffalo, play a determinant role only in those farms, where the low efficiency of AI is due to the high incidence of EM.

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