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Embryonic and foetal mortality in buffalo species

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ABSTRACT - The aim of this study was to verify the incidence of late embryonic mortality (between 25 and 45 days post-insemination; LEM) and foetal mortality (between 45 and 70 days post-insemination; FM) in buffaloes synchronized and mated by AI during the transitional period. The trial was performed on 288 multiparous Mediterranean Buffaloes, synchronized and inseminated by AI. Trans-rectal ultrasonography was performed 25, 45, and 70 days post-insemination to assess embryonic development. Milk samples were collected on Days 10, 20, 25, 30, and 45 post-insemination to determine progesterone concentration in whey. Pregnancy rate on Day 25 after AI was 48.6% but declined to 35.4% and to 30.6% by Day 45 and 70 respectively, representing a LEM of 27.1% and a FM of 13.7%. Progesterone concentration was higher ($P < 0.01$) in pregnant compared to LEM buffaloes after 20 days post-insemination. Differences ($P < 0.05$) were found between FM and LEM buffaloes on Days 25 and 30. Furthermore, progesterone concentration in pregnant buffaloes was higher ($P = 0.09$) than that of FM buffaloes on Day 30 and 45. In conclusion, the success of application of reproductive biotechnologies in the transitional period depends from the incidence of embryonic and foetal mortality.

Key words: Buffalo cows, Late embryonic mortality, Foetal mortality, Progesterone.

Introduction - Reproductive seasonality may be a main cause of poor fertility in buffalo cows: delayed puberty, silent oestrus, and long post-partum ovarian inactivity (Singh, 1988; Singla *et al.*, 1996) are mainly affected by daylight length. In buffalo, the incidence of reduced *corpus luteum* (CL) activity is between 5 and 50% during the daylight length period (Campanile *et al.*, 1992). This condition does not cause always ovarian inactivity, but oestrus behaviour may be present with ovulation, followed by an inadequate luteal phase (Zicarelli, 1992). During a period of increasing daylight length (transitional period) a lower function of the *corpus luteum* reduces progesterone (P4) levels and increases embryonic mortality in buffalo cows (Campanile and Neglia 2007). In fact, it has been observed that embryonic loss in animals mated by artificial insemination (AI) is 20-40% during seasons characterized by high number of light hours (Campanile *et al.*, 2005, 2007a, 2007b). In buffaloes naturally mated (Vecchio *et al.*, 2007), independently from the conception period, 8.8% and 13.4% of animals show embryonic or foetal mortality between 28-45 days and 46-90 days of pregnancy, respectively. The aim of this study was to verify the incidence of late embryonic and fetal mortality in buffaloes synchronized and mated by AI during the transitional period.

Material and methods - The trial was performed on 288 multiparous Italian Mediterranean Buffaloes between January and March. The animals were synchronized by the Ovsynch/TAI protocol (Neglia *et al.*, 2003) and artificial insemination was performed 16 and 40 h after the second injection of

GnRH. Milk samples were collected on Days 10, 20, 25, 30, and 45 after AI in all buffaloes to determine progesterone concentration in whey by RIA method (Campanile *et al.*, 2007a). Twenty-five days after AI buffaloes underwent trans-rectal ultrasonography to assess embryonic development. Ultrasonography was conducted with an Aloka SSD-500 unit equipped with a 5.0 MHz linear array probe (Aloka Co., Tokyo, Japan) and was carried out by the same experienced operator. Pregnancy diagnosis was confirmed on Day 45 and Day 70 after AI using ultrasonography. Buffaloes that were pregnant on Day 25 but not on Day 45 were considered to have undergone late embryonic mortality (LEM) and those pregnant on Day 45 but not on Day 70 were considered to have undergone foetal mortality (FM).

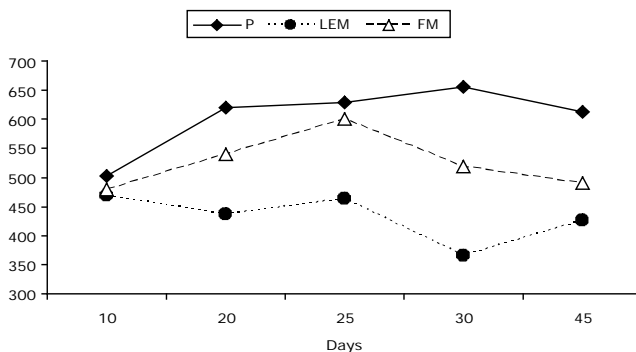
Differences in P4 milk whey concentrations on Days 10, 20, 25, 30, and 45 after AI between pregnant and buffaloes that had embryonic and foetal mortality were tested by repeated measures ANOVA (SPSS 12.0).

Results and conclusions - Pregnancy rate on Day 25 after AI was 48.6% (140/288) but declined to 35.4% (102/288) and to 30.6% (88/288) by Day 45 and 70, respectively. Late embryonic and foetal mortality were respectively 27.1% (38/140) and 13.7% (14/102). The pattern of P4 whey concentrations in pregnant and in buffaloes that showed embryonic or foetal mortality are shown in Figure 1. Pregnant buffaloes had higher ($P<0.01$) concentrations of P4 after Day 20 than buffaloes which showed late embryonic mortality, whilst P4 in FM buffaloes did not differ from the other two groups on Day 10 and 20 (Figure 1). Differences were found between FM and LEM buffaloes in whey P4 levels ($P<0.05$) on Days 25 and Days 30. P4 concentration in pregnant buffaloes was higher ($P=0.09$) than FM buffaloes on Day 30 and Days 45.

In this study the incidence of late embryonic mortality was similar to that observed in a previous study which was performed in the same period of the year (Campanile *et al.*, 2007a). Buffaloes that underwent embryonic mortality showed significantly lower whey concentrations of progesterone than pregnant cows and the function of *corpus luteum* is compromised by the 20th Day after AI. In fact P4 concentration decreases after 10th Day post-insemination in LEM buffaloes, while it increases in pregnant buffaloes. It is known that embryonic mortality in buffalo species is primarily due to a reduced secretion of progesterone by *corpus luteum*. A close cross talk between the conceptus and the mother is on the basis of the implantation process. As previously mentioned, an adequate luteal activity, and consequently an adequate progesterone concentration, induces an appropriate uterine environment

together with a sufficient elongated embryo, that are the essential counterparts in this phenomenon. The synchronization of the embryo with the status of the uterus is critical for a successful implantation (Dey, 1996; Paria *et al.*, 1993).

Figure 1. Progesterone concentrations (pg/ml) in milk for pregnant (P) buffaloes, buffaloes that underwent late embryonic mortality (LEM) and buffaloes that underwent Foetal mortality (FM) on day 10, 20, 25, 30, and 45 after AI.



In FM buffaloes, a reduction in *corpus luteum* activity after 25 Days of pregnancy was observed. The lack on P4 in this phase of pregnancy may compromise placentation and cause fetal loss. No differences were found in foetal mortality between this study, in which buffaloes were mated by AI, and the previous study (Vecchio *et al.*, 2007) carried out in buffaloes naturally mated. In conclusion, the

success of application of reproductive biotechnologies in the transitional period depends from the incidence of embryonic and foetal mortality, which represent the major causes of fertility losses.

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