Italian Journal of Animal Science

ISSN: (Print) 1828-051X (Online) Journal homepage: http://www.tandfonline.com/loi/tjas20

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To cite this article: Marco Russo, Luise Luise, Pasquale Rossi, Filippo Lo Grasso, Natascia Cocchia & Giuseppe Catone (2009) Ultrasonographic appearance of early embryonic mortality in buffalo (Bubalus bubalis), Italian Journal of Animal Science, 8:sup2, 138-140, DOI: 10.4081/ijas.2009.s2.138

To link to this article: https://doi.org/10.4081/ijas.2009.s2.138

Ultrasonographic appearance of early embryonic mortality in buffalo *(Bubalus bubalis)*

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ABSTRACT - Embryonic mortality is one of the main causes responsible of the decline in fertility that occurs in buffaloes during periods of increasing daylight length (out sexual breeding season). Transrectal ultrasonography for pregnancy diagnosis offers some advantages over palpation per rectum: earlier diagnosis of pregnancy/non-pregnancy, determination of embryo/fetus viability, reduction of misdiagnosis, and reduction of .potential. iatrogenic embryo/fetal attrition. Non pregnant buffaloes on Day 25 after AI showed higher Resistive Index (RI) ($P<0.05$) and Pulsatility Index ($P=0.07$) values, registered on CL on Days 10 after AI, compared to pregnant buffaloes. RI values were significantly higher ($P=0.02$) in non pregnant buffaloes also on Day 45 after AI. Colour Doppler sonography could be used to gain specific information relating to the ovarian blood flow in predicting early embryonic loss and to describe the ultrasonographic features of early embryonic death in buffaloes.

Key words: Ultrasound, Buffalo, Corpus luteum, Embryonic mortality.

Introduction - Embryonic mortality is one of the main causes responsible of the decline in fertility that occurs in buffaloes during periods of increasing daylight length (out sexual breeding season). The reasons for the greater embryonic mortality in buffaloes during this period are not fully understood. However, it seems that it may be, at least partially, due to a decrease in progesterone secretion by the *corpus luteum* (CL) during early pregnancy (Campanile *et al*., 2005). Real-time ultrasonography has gained tremendous popularity in recent years as a diagnostic as well as a research tool in veterinary and animal science. In buffalo practice, two methods allow to immediately diagnose pregnant/non-pregnant females: palpation per rectum and transrectal ultrasonography. Palpation per rectum is performed 30 days after breeding/artificial insemination/embryo transfer. Transrectal ultrasonography offers some advantages over palpation per rectum: earlier diagnosis of pregnancy/non-pregnancy, determination of embryo/fetus viability, reduction of misdiagnosis (false negatives and false positives) and reduction of .potential. iatrogenic embryo/fetal attrition (Romano and Magee, 2001). In recent years, local blood flow has been analysed in individual ovarian follicles and the CL in the cow using colour Doppler US. Assessment of the area of blood flow in the CL using this method may offer a useful adjunct in estimating CL function, which could be applied to the diagnosis of non-pregnancy and fetal loss. For the past 10 years, colour Doppler US is being increasingly used for blood flow studies. Bollwein *et al*. (2000, 2002) used this imaging method to demonstrate

changes that occurred in uterine circulation during the oestrous cycle and pregnancy in cows. Doppler sonography has also been used to investigate blood flow to the ovaries (Acosta *et al.*, 2002, 2003, 2005; Miyamoto *et al.*, 2005). The goal of our research was to investigate whether transrectal colour Doppler sonography could be used to gain specific information relating to the ovarian blood flow in predicting early embryonic loss and to describe the ultrasonographic features of early embryonic death in buffaloes.

Material and methods - The trial was carried out on 122 multiparous Italian Mediterranean buffaloes during a transitional period. After passing a voluntary waiting period of 35 days postpartum, buffaloes were included in the estrous synchronization program for artificial insemination. Animals were synchronized by the Ovsynch/TAI protocol and artificial insemination was performed 16 and 40 h after the second injection of GnRH. All ultrasonographic examinations were performed by the same operator on Day 10 to the CL and Day 25 and 45 to check the pregnancy status. A portable SonoAce Pico with a 10 MHZ linear transducer specially adapted for transrectal examination in large domestic animals was used. Once visualized each ovary, the colour-Doppler mode was activated in order to display signals for blood flow in vessels of the CL and the spectral mode was applied for calculating the resistive index (RI) and pulsatily index (PI). Differences in RI and PI between pregnant, non pregnant, and late embryonic mortality buffaloes were tested by ANOVA (SPSS 12.0). All Doppler scans were performed at a constant colour-gain setting, velocity setting, and a colour-flow filter setting. The entire CL was scanned in a slow continuous motion. Real-time B-mode/colour-Doppler images of the continuous scans were captured with a digital video-recording system. At 20, 25, and 45 days b-mode was used in order to visualize pregnancy.

Results and conclusions - Pregnancy rate on Day 25 after AI was 53.3% (65/122) but declined to 41.0% (50/122), with an embryonic mortality of 21.5% (14/65). Non pregnant buffaloes on Day 25 after AI showed higher RI ($P<0.05$) and PI ($P=0.07$) values, registered on CL on Days 10 after AI, compared to the pregnant buffaloes (Table 1). RI values were significantly higher ($P=0.02$) in non pregnant buffaloes also on Day 45 after AI, and this index is not different in EM buffaloes than pregnant and non pregnant subjects (Table 1). No differences were found between different reproductive status registered on Day 45 after AI in PI values (Table 1) and in embryo size on Day 25 after AI between pregnant and EM buffaloes.

Table 1. Resistive index (RI) and pulsatily index (PI) in Non pregnant (NP), Pregnant (P) and in buffaloes that have undergone embryonic mortality (EM) on Days 25 and 45 after AI.

Days after AI		NP	(n)	P	(n)	EM	(n)
25	RI	0.61±0.05[a]	(57)	0.46±0.05[b]	(65)		-
	PI	0.99±0.11*	(57)	0.71±0.1**	(65)		-
45	RI	0.61±0.05[a]	(57)	0.44±0.05[b]	(50)	0.55±0.09	(15)
	PI	0.99±0.11	(57)	0.73±0.12	(50)	0.63±0.22	(15)

[a,b]= *P<0.05; *,** = P=0.07.*

On Day 25 after AI all pregnant animals showed the gestational sac as an oblong structure with an echogenic wall and anechoic fluid. The forming embryo appeared as a c-shaped measuring 0.8 cm and the cardiac activity was present. In the abnormal pregnancy, we observed a chance in the embryonic fluid from anechoic to echogenic due to the presence of ecogenic foci, a reduction of em-

bryo size and absence of heart beat. In some cases we noticed free floating extra-embryonic membranes and the CL showed an elevated RI probably due to the lower level of progesterone (Acosta *et al.*, 2003). The importance of progesterone concentration during the first weeks of pregnancy for reducing embryonic mortality has been previously demonstrated in buffalo (Campanile *et al.*, 2008a, 2008b; Neglia *et al.*, 2008). According to some reports, the presence of an early Progesterone peak (within 5 days after mating or AI) facilitates the elongation of the conceptus and, consequently, the secretion of adequate interferon-tau (Starbuck *et al.*, 1999; Mann, 2002). In artificially mated buffaloes the early embryonic mortality (17-22 days of pregnancy) was found in 16% of NP buffaloes (Vecchio *et al.*, 2008). With improvements in portability and cost-effectiveness, the evaluation of ovarian blood flow by colour Doppler US is likely to become widely used as a diagnostic tool for monitoring ovarian function in buffaloes.

The Authors thank the Schering-plough for technical and financial support.
Research supported by by FISR – Fondo Integrativo speciale ricerca – DM 17/12/02.

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