

Embryonic mortality in buffalo cows

G. Campanile, G. Neglia

Department of Scienze Zootecniche ed Ispezione degli Alimenti, Faculty of Veterinary Medicine,
Via F. Delpino 1, 80137 Napoli, Italy

Corresponding author: G. Campanile, Department of Scienze Zootecniche ed Ispezione degli Alimenti, Faculty of Veterinary Medicine, Via F. Delpino 1, 80137 Napoli, Italy - Tel. 081 2536069 - Fax: 081 292981 - Email: giucampa@unina.it

ABSTRACT: In buffalo species embryonic mortality is considered one of the major causes of fertility loss, especially in the animals that are not mated during their reproductive period. Embryonic loss in animals mated by artificial insemination (AI) is 20-40% during seasons characterized by high number of light hours. Also in buffalo naturally mated the incidence of embryonic mortality is about 20% and a higher incidence is observed between 28-60 days of gestation in buffaloes that conceive during increasing daylight length. A reduced capacity to secrete progesterone seems to explain in part this embryonic mortality but other as yet unidentified factors contribute between 40-50% to the embryonic losses. Treatments with hCG, GnRH agonist or progesterone on Days 5 after AI not always reduce embryonic mortality in buffalo species. Embryonic mortality in buffaloes appears to occur later (Day 25-40) than in cattle and P₄ treatments should perhaps be applied later in buffaloes.

Key words: Embryonic mortality, Buffalo, Progesterone, GnRH, Hcg.

BUFFALO REPRODUCTIVE CHARACTERISTICS - Buffalo is an animal species that lives in regions found between 31°N parallel and 2°S. Currently, the distribution of the buffalo population covers the major climatic regions of the lower latitudes (Tropical zone) and middle latitudes (Temperate zone). This geographic origin and distribution logically suggests that buffaloes are adapted to hot, humid macro or microclimates (Shafie, 1985). Buffalo is a photoperiodic species. Like sheep, buffaloes have to be considered a "short day" species. They have heats throughout the year but are more fertile when daylight hours decrease. According to Zicarelli (1995), this characteristic is due to their tropical origins; in fact, in these areas the availability of forage coincides with the period in which dark hours increase. Therefore, it has been supposed that animals which calve in the most suitable period for survival of the offspring were selected. It seems that they have retained this characteristic even when transferred to places where forage is always available (Zicarelli, 1995). In countries like Italy, where market demand requires a concentration of deliveries in the spring-summer period (not corresponding to buffalo reproductive activity) the out-of-season technique is widely applied. As a result, buffaloes which are less sensitive to photoperiodic effects have been selected. When the out-of-season technique has been applied for long periods a lower loss of fertility was observed (15% vs. 30%) compared to the farms in which it has been adopted for shorter periods (Campanile, 1997). These results are also due to the renewal of the herd that is very

frequent on farms that apply the out-of-breeding-season mating technique (Campanile, 1997). Moreover, the season-dependent reproduction phenomenon is more frequent in older buffaloes (Zicarelli *et al.*, 1988a) that are more sensitive to the bull effect and show seasonal acycilia more easily.

Buffalo reproduction is characterized by delayed puberty, silent oestrus, long post-partum ovarian inactivity, and, on the whole, poor fertility (Singh, 1988; Madan *et al.*, 1994; Singla *et al.*, 1996). Most of these problems result from the use of the “out of breeding season mating” technique (Zicarelli, 1997; Gasparrini B., 2002). In fact, if buffalo are bred without modification of their natural seasonality and without controlled breeding, an inter-calving period of less than 400 days and a culling rate of less than 12% has been observed in Italy, Brazil, Venezuela, and Argentina (Zicarelli *et al.*, 1993). Poor fertility has also been observed when biotechnologies are applied to reproduction.

Immediately after parturition buffaloes show several physiological modifications, which are fundamental to sustain the new pregnancy. The first step is the resumption of ovarian cycle. This is blocked during pregnancy by progesterone, which avoids other ovulations and maintains hypotonic the uterus. In buffalo species, the resumption of ovarian activity is affected by the calving season. In fact, buffaloes that delivered during the spring period, showed an intercalving period on average longer by 30 or 70 days, respectively if they are pluriparous or primiparous (Zicarelli, 1994). This phenomenon is observed until the delivery happens in July-August period. Usually, after 90 days of lactation during the spring period, 44% of pluriparous and 80% of primiparous are acyclic (Zicarelli, 1994). The reproductive activity of Italian buffalo cows is also influenced by climatic variation. As regards spontaneous heats, temperatures lower than 8°C and continuous light for more than 11 hours cause a delay in ovulation, starting from the end of heats. This is probably a delay in the pituitary response to ovarian steroid secretion (Zicarelli *et al.*, 1988a). Thermal excursions higher than 7°C and 9°C appreciably increase the incidence of double ovulation in both spontaneous oestrus and prostaglandin-induced oestrus (Zicarelli *et al.*, 1988b). Such conditions limit the adoption of A.I. during specific periods of the year. Moreover, Sastry and Georgie (1978) found a correlation between conceptions and temperature, relative humidity or rainfall. Specifically, a lower temperature and increased rainfall improve the conception rate. Obviously, rainfall during the three months previous to the conceptions improves the availability of herbage, meeting productive requirements. This represents an indirect effect of climate on buffalo reproduction; a hot climate, in fact, affects living and reproductive behaviour directly by its effect on their systemic functions and indirectly by governing the availability of nutrients.

EMBRYO DEVELOPMENT - A fundamental condition for the maintenance of a new gestation, is the presence of a suitable uterine environment, which is able to guarantee an adequate embryo development (Campanile, 2006). In bovine the first cleavage division is at around 30 hours after insemination and the second at around 48 hours. During the following 4-5 days, each blastomere undergoes subsequent divisions in the oviduct, yielding 4, 8, 16, 32 cells, tight morula and, finally, blastocyst (Senger, 2003). On day 6.5-7 the new embryo reaches the uterus and during the second week of gestation it begins to elongate and to send signals to the mother, in order to prevent luteolysis and maintain adequate progesterone circulating levels. Progesterone is important for allowing uterine secretions

and reducing myometrial tone, favouring embryo development and attachment. In buffalo species Karaivanov *et al.* (1987) observed that from the flushing of oviducts and uterine horns of slaughtered superovulated donors between 74 and 100 h, eggs were recovered only from the oviducts, while flushing conducted between 102 and 108 yielded eggs from both the oviducts and uterine horns. This observation suggests that embryo development is faster in buffalo than in bovine, as confirmed by several trials carried out *in vitro*, during which tight morulae and blastocysts were observed already on day 5-6 (Neglia, *et al.*, 2001). Therefore, the maintenance of pregnancy is due to either the embryo capacity of signalling its presence and the mother capacity of recognizing these signals and maintaining an adequate uterine environment.

EMBRYONIC MORTALITY - Embryonic loss is increased when physiological regulation of oviductal and uterine function is inadequate or when the mother is exposed to one or more of the many stresses that can compromise embryonic survival (Hansen, 2002). Embryonic mortality usually happens during the first phases of gestation in various species: in cattle, for example, it is evident within 40 days of pregnancy. In particular, 30-40% of embryonic losses in bovine occurs between 7 and 17 days post fertilization and, in some cases, it can take place before embryo becomes foetus (Thatcher *et al.*, 1995). Vasconcelos *et al.* (1997) recorded that during the subsequent phases of pregnancy (after 42 days), embryonic loss is a remote eventuality (around 10%).

In buffalo species embryonic mortality is considered one of the major causes of fertility loss, especially in the animals that are not mated during their reproductive period. In Italy, in fact, the application of the out of breeding season mating technique guarantees milk production in accordance with market requirements, but it forces the breeders to mate buffaloes during the less favourable periods. It was 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; Campanile *et al.*, 2007a; Campanile *et al.*, 2007b), whereas values of around 7% were recorded in Brazil during decreasing light days (Baruselli *et al.*, 1997). In contrast to the previous work, an embryonic mortality rate of 20% was reported for buffaloes close to the equator (Vale *et al.*, 1989). In any case, embryo mortality in buffalo occurs later than in bovine, usually between 25 and 40 days from AI (Campanile *et al.*, 2005). In buffaloes naturally mated (Vecchio *et al.*, 2007), independently from the conception period, 8.8% and 13.4% showed respectively embryonic mortality between 28-45 days (embryonic mortality-EM) days and between 46-90 days (foetal mortality – FM) of pregnancy. In this work no differences were found between the incidence of EM in relation to the conception period (Table 1), while a high incidence ($P < 0.01$) of FM was found (Table 1) during a period of increasing daylight length (transitional period: December-March) compared to the April-July period. It is hypothesized that this condition is due to the presence, in the transitional period, of subjects that become pregnant, even if they have a lower function of the corpus luteum because they are going into anoestrus. In the subsequent months (April-July) an increased incidence of acyclic buffaloes is observed and, hence, only the subjects that are not sensitive to the photoperiod are cyclic and become pregnant. In fact, the incidence of FM is similar to that observed in the decreasing daylight length period (August-November), that is the favourable period for reproductive activity.

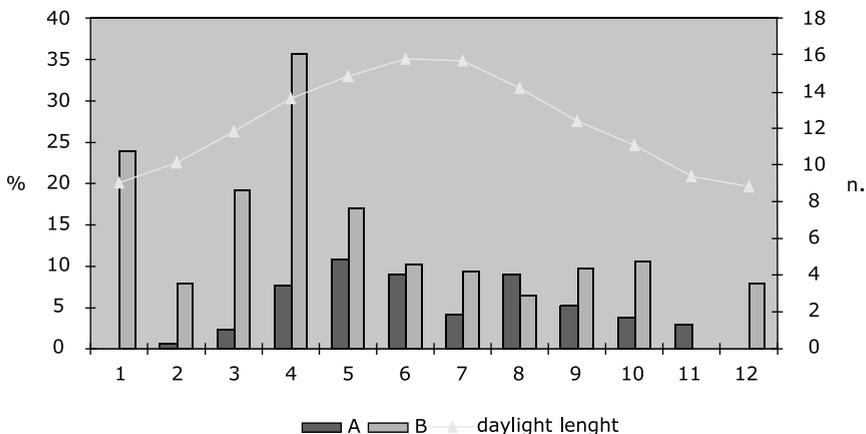
Table 1. Incidence of embryo (EM) and fetal (FM) mortality during the periods.

	EM %	FM %
December-March	9.1	16.6 a
April-July	7.0	10.9 b
August-November	10.8	11.2 ab
Total	8.8	13.4

a vs. b = P<0.05

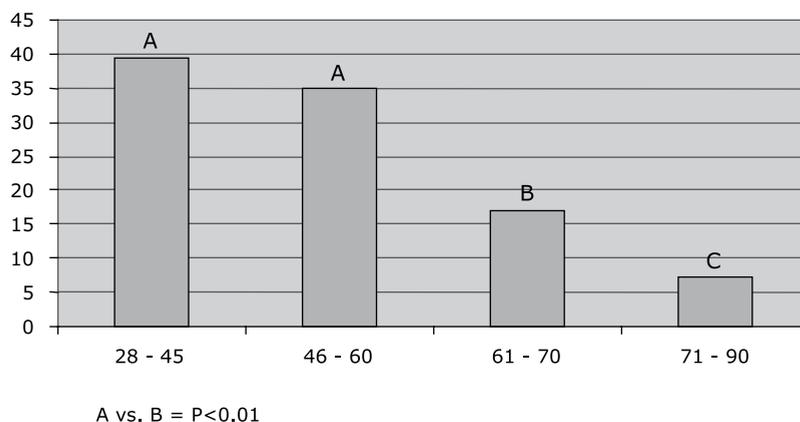
These data are in accordance with Baruselli (personal communication), that found an embryonic mortality (after 30 days from AI) of 13.2% and 7.0% respectively in decreasing and increasing daylight length period. In 1994, Zicarelli (1994) found an embryonic mortality of 21.8% in buffaloes naturally mated. The phenomenon was not correlated with the breeding season (spring vs. summer), but with the farm and the ovarian resumption after calving. In another trial (Zicarelli, unpublished data) performed on 3000 conceptions, a higher incidence of embryonic mortality was reported between 30 and 90 days in buffaloes that conceived during increasing daylight length (Figure 1). However, also in this case the phenomenon was affected by farm, management and environment. The incidence of embryonic mortality in Farm A was 5% vs. 14% observed in Farm B, but either these values were lower than those recorded in 1994.

Figure 1. Incidence of embryonic mortality in relation to month of conception and daylight length in two farms.



The incidence of embryonic mortality found in Italy was higher between 28-60 days of gestation and lower after 71 days (Figure 2). This result is different from that reported in cattle (Silke *et al.*, 2002), in which the embryonic loss from 28-87 days of gestation was similar.

Figure 2. Embryonic loss rate during stage of gestation.



Embryonic mortality, in buffalo species, was not affected by age, parity or lactation stage and infectious agents explained only about 2-8% of the cases (Campanile *et al.*, 2005; Campanile *et al.*, 2007a). Campanile *et al.* (2005) found a higher P₄ plasma levels in pregnant buffaloes than in buffaloes which showed embryonic mortality since day 10 after AI, whilst P₄ in non-pregnant buffaloes was intermediate. Pregnant buffaloes had also higher plasma P₄ on day 20 than both non-pregnant buffaloes and buffaloes that showed embryonic mortality. P₄ plasma concentration significantly decreased only in non-pregnant buffaloes between day 10 and 20. In a further trial, it was observed that pregnant buffaloes showed higher concentrations of P₄ milk whey than both animals showing embryonic mortality and non-pregnant buffaloes on day 20 and day 25 but only than non-pregnant buffaloes on Day 10 (Campanile *et al.*, 2007b).

It may be hypothesised that embryonic mortality in buffalo species is primarily due to a reduced secretion of P₄ by corpus luteum. This conclusion would be consistent with several findings in cattle and sheep, where early embryonic mortality was associated with reduced circulating concentrations of P₄ (Garret *et al.*, 1988; Mann and Lamming, 1999; Mann and Lamming, 2001). During a trial carried out in Italy in a period of increasing daylight length it was proposed that the relatively high incidence of buffaloes with low circulating concentrations of P₄ after oestrus synchronisation was reflective of a reduced activity of the reproductive endocrine system. As previously specified, buffaloes are seasonal animals, showing increased reproductive activity in response to decreasing daylight length (Zicarelli, 1997). Impaired P₄ secretion has been linked with a reduced capacity of the developing embryo to secrete interferon-tau (IFN τ) at threshold amounts necessary to prevent luteolysis (Wathes *et al.*, 1998). In fact, as above mentioned, the maintenance of pregnancy is due either to the maternal recognition of pregnancy and to the embryo capability of blocking luteolysis since day 16 post-AI (Mann and Lamming, 1999). This process occurs by the production of bovine trophoblastic protein-1 (bTP-1), also called IFN τ (Roberts *et al.*, 1992). This protein is able to avoid corpus luteum regression by two mechanisms: i) by inhibiting oxytocin receptors (OTR) development on endometrium (Robinson *et al.*, 1997); ii) by activating a prostaglandin inhibitor (Thatcher *et al.*, 1995).

It has been supposed that oestradiol is another factor involved in the luteolytic process, either by promoting OTR development and by stimulating prostaglandin secretion (Wathes *et al.*, 1998). In fact, it has been demonstrated in ovine that the number of oestradiol receptors on endometrium is significantly lower in pregnant vs. not pregnant animals (Lamming *et al.*, 1995; Spencer *et al.*, 1995). However, in buffalo species oestradiol plasma levels do not differ between pregnant, not pregnant and buffaloes undergone embryonic mortality on day 0, 10, 20 and 25 after A.I. (Spagnuolo *et al.*, 2007).

Gametes quality is one of the main factors involved in the phenomenon of embryonic mortality in domestic animals. Oocyte quality is able to affect embryo development and interfere with the following gestation. In buffalo species this phenomenon may be more frequent during the seasonal anoestrus, which coincides with day length increase (Campanile *et al.*, 2005) and, consequently, with the resumption of sexual promiscuity in the farms in which the out of breeding season mating technique is applied. Campanile *et al.* (2005) demonstrated that 51% of buffaloes which showed embryonic mortality had P₄ concentrations on days 10 and 20 similar to those of animals which maintained pregnancy. Therefore, it is possible that other factors, rather than reduced circulating P₄ concentrations, also contributed to embryonic mortality. With this regard, it was reported that oocyte quality, judged as the capacity to result in embryonic development and pregnancy, is worse in buffaloes during the anoestrous period (Abdoon *et al.*, 2001), occurring when daylight length increases (Zicarelli, 1997). Furthermore, the incidence of embryonic mortality between 40th and 60th day post AI is three times higher in buffaloes that are acyclic 70 days post partum (Zicarelli, 1994), compared to those that are cyclic. It is known that in buffalo species high incidence of atresia is present and the mean recovery of good quality oocytes per ovary is low? (Gasparrini, 2002). The maturation and the quality of oocyte depend on the function of the granulosa cells that are sensitive to oxidative stress (Dharmarajan *et al.*, 1999). It is worth mentioning that the antioxidant defence system plays a key role in preventing apoptosis and atresia, thus preserving steroidogenic function of granulosa cells (Cassano *et al.*, 1999). Spagnuolo *et al.* (2007) found no significant differences in redox status between pregnant, not pregnant and cows with embryonic mortality.

TREATMENTS FOR PREVENTING EMBRYONIC MORTALITY IN BUFFALO SPECIES - The importance of progesterone (P₄) concentration during the first weeks of pregnancy for reducing embryonic mortality has been demonstrated in cattle (Mann and Lamming; 1999 and 2001). According to some reports the presence of an early P₄ 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 cattle, interferon-tau extends the lifespan of the corpus luteum (Plante *et al.*, 1989) by suppressing estradiol receptor and oxytocin receptor genes (Spencer and Bazer; 1996) and by attenuating the endometrial secretion of PGF₂ (Helmer *et al.*, 1989a). It has also been shown that interferon-tau reduces PGF₂ secretion by bovine endometrial explants (Helmer *et al.*, 1989b) and endometrial epithelial cells (Danet-Desnoyers *et al.*, 1994). Several approaches have been used to increase P₄ concentration in blood in order to reduce the occurrence of embryonic mortality. Increased plasma P₄ concentrations were achieved either by inducing increased endogenous secretion or by administering exogenous P₄ (Mann and Lamming; 1999). Studies have shown that administration of natural sequence GnRH,

GnRH agonists or hCG after AI can stimulate corpus luteum function, induce accessory corpus luteum formation, increase P_4 , and reduce estradiol production, with a consequent positive effect on embryonic survival (Kerbler *et al.*, 1997; Thatcher *et al.*, 2003; Bartolome *et al.*, 2005). In buffalo species there are some controversial results, regarding the best moment for hormonal treatment. Campanile *et al.* (2007a) reported that treatment with exogenous P_4 (PRID[®], Vetem) on day 5 after A.I gave the lowest pregnancy rate and highest incidence of embryonic mortality, suggesting that exogenous P_4 can have had a detrimental effect on conception. It is possible that exogenous P_4 may contribute to the regulation of LH and reduce the capacity of the preformed corpus luteum to increase P_4 synthesis and release. After removal of the exogenous source of P_4 the corpus luteum may not be able to secrete P_4 in the amount required to maintain pregnancy. Furthermore, the injection of 12.6 μ g GnRH agonist (buserelin) or 1500 I.U. of hCG on Day 5 after A.I. increased P_4 concentrations without reducing the incidence of embryonic mortality. It should be noted, however, that P_4 in buffaloes treated with buserelin and hCG was significantly different to control buffaloes only on Day 15 after AI. It is therefore possible that P_4 was not elevated for a sufficient time in the period after AI to have a major effect on uterine function and embryo-maternal interactions (Campanile *et al.*, 2007a). The present findings are in contrast with Kumar *et al.* (2003) who reported an increase in conception rate in buffaloes treated with 125 mg of 17- α hydroxyprogesterone caproate s.c. on Day 4 after AI. It is possible that the type and mode of exogenous P_4 treatment may influence the response in buffaloes. With this regard, 341 mg of 17- α hydroxyprogesterone caproate administered i.m. 3 times, at 4-day intervals, starting on Day 25 after AI, reduced the incidence of embryonic mortality in a buffalo herd characterised by a high incidence of embryonic mortality (Campanile *et al.*, 2007b). Treatment with buserelin or hCG on Day 25 after A.I. in pregnant buffaloes also reduced the incidence of embryonic mortality in buffaloes bred in a farm characterized by high incidence of embryonic mortality (Campanile *et al.* 2007b).

In cattle, treatment with hCG on Day 5 or Day 7 after AI increases P_4 concentrations by enhancing secretion from the existing corpus luteum and also by inducing ovulation and formation of an accessory corpus luteum (Kerbler *et al.*, 1997; Schmitt *et al.*, 1996; Santos *et al.*, 2001). In buffalo species, P_4 increased on Day 10 after injection of 1.500 I.U. of hCG. It is speculated that in buffaloes hCG may not increase the P_4 secreting capacity of the existing corpus luteum, but can induce ovulation and formation of an accessory corpus luteum which leads to increased P_4 some time later. It is known that hCG administered at Day 25 after AI induces ovulation in around 57% of buffaloes (Campanile *et al.*, 2007c) and that there is a similar response to GnRH agonists, using which ovulation rates of 62% (Campanile *et al.*, 2007d) and 68.6% (Campanile *et al.*, 2007c) are observed, respectively after administration on day 5 or 25 post AI. The mean follicular diameter which resulted sensitive to the hormonal treatment was about 8.9 mm in both treatments, varying between 4.2 and 13.0 mm (Campanile *et al.*, 2007c; Campanile *et al.*, 2007d). It is worth pointing out that the dimensions of the follicles recorded in buffaloes responsive to the treatments were similar to those of buffaloes in which ovulation did not occur. These data are in accordance with those reported in bibliography in cattle (Martinez *et al.*, 1999), regarding the incidence of subjects responsive to the treatment with GnRH and the dimensions of responsive follicles. Buffaloes that ovulated in response to the treatment with a GnRH agonist showed a progressive increase in milk whey progesterone concentrations on Days 10, 15 and 20, while progester-

one levels remained relatively constant for buffaloes that did not ovulate. The injection of a GnRH agonist on Day 5 after AI increased milk whey progesterone concentrations in 97% of buffaloes subsequently pregnant on Day 40, compared to 68% in the non-pregnant buffaloes ($P<0.01$). A greater ($P<0.05$) proportion of the buffaloes that ovulated (96.7%), compared to buffalo that did not ovulate (68.4%) recorded a gestational chamber on Day 40 after AI and were judged to be pregnant (Campanile *et al.*, 2007d). Ovulation also increased milk whey progesterone levels and reduced embryonic mortality in buffalo cows treated with 1500 I.U. of hCG or 12.6 µg of GnRH agonist on Day 25 after A.I. (Campanile *et al.* 2007c).

CONCLUSIONS - In buffalo species embryonic mortality is one of the major causes of fertility loss, above all in those animals that are not mated during their reproductive period. Buffaloes that undergo oestrus synchronisation and AI during a period of increasing day length have a relatively high occurrence of embryonic mortality. In naturally mated buffalo embryonic loss is about 20% and a higher incidence is reported between 28-60 days of gestation in buffaloes that conceive during increasing daylight length. This phenomenon is probably affected by farm, management and environment. A reduced capacity to secrete progesterone seems to explain in part this embryonic mortality but other as yet unidentified factors contribute between 40-50% to the embryonic losses. It will be important to further elucidate the factors that can contribute to embryonic mortality in buffaloes so that strategies can be developed to optimise fertility after synchronisation and AI during periods of reduced reproductive activity.

The treatments to increase P_4 early in pregnancy, that have proven successful in increasing pregnancy rates in cattle, are not necessarily applicable to buffaloes. It is however acknowledged that whilst P_4 tended to be elevated on Day 10 in buffaloes treated with buserelin and hCG, concentrations were significantly different to controls only on Day 15. As noted above, this finding was interpreted to suggest that hCG and buserelin did not stimulate increased P_4 secretion from the existing corpus luteum but rather induced ovulation and formation of an accessory corpus luteum, at least in a proportion of buffaloes. Embryonic mortality in buffaloes mated by AI in mid-winter appears to occur later than in cattle and hence P_4 treatments applied on day 25 after A.I. reduce embryo mortality in farms in which embryo loss is highly present.

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REFERENCES - **Abdoon**, A.S.S., Kandil, O.M., 2001. Factors affecting number of surface ovarian follicles and oocytes yield and quality in Egyptian buffaloes. *Reprod Nutr Dev* 41: 71-77. **Bartolome**, J.A., Melendez, P., Kelbert, D., Swift, K., McHale, J., Hernandez, J., Silvestre, F., Risco, C.A., Arteché, A.C.M., Thatcher, W.W., Archibald, L.F., 2005. Strategic use of gonadotrophin-releasing hormone (GnRH) to increase pregnancy rate and reduce pregnancy loss in lactating dairy cows subjected to synchronization of ovulation and timed insemination. *Theriogenology*. 63: 1026–1037. **Baruselli**, P.S., Visintin, J.A., Barnabe, V.H., Barnabe, R.C., Amaral, R., Souza, A.C., 1997. Early pregnancy ultrasonography and embryonic mortality occurrence in buffalo. *Proc. V World Buffalo Congress*, 776-778. **Campanile**, G., Vecchio, D., Rendina, M., Grassi, C., Balestrieri, A., Di Palo, R., 2007c. Ovary response

and embryonic mortality in buffaloes treated with GnRH or hCG. Proc. VIII Buffalo Congress. In press. **Campanile, G.**, Vecchio, D., Neglia, G., Di Palo, R., Prandi, A., D'Occhio, M.J., 2007d. Progesterone and pregnancy status in buffaloes treated with a GnRH agonist. Livestock Science. In press. **Campanile, G.**, 1997. Relationship between nutrition and reproduction. Third course on biotechnology of reproduction in buffaloes. Suppl. *Bubalus bubalis*. II/98: 217-235. **Campanile, G.**, 2006. Gestione riproduttiva e redditività dell'azienda bufalina. Proc. 8° Congresso Nazionale Multisala SIVAR: 25. **Campanile, G.**, Di Palo, R., Neglia, G., Vecchio, D., Gasparri, B., Prandi, A., Galiero, G., D'Occhio, M.J., 2007a. Corpus luteum function and embryonic mortality in buffaloes treated with a GnRH agonist, hCG and progesterone. *Theriogenology*. 67: 1393-1398. **Campanile, G.**, Neglia, G., Gasparri, B., Galiero, G., Prandi, A., Di Palo, R., D'Occhio, M.J., Zicarelli, L., 2005. Embryonic mortality in buffaloes synchronized and mated by AI during the seasonal decline in reproductive function. *Theriogenology*. 63: 2334-2340. **Campanile, G.**, Vecchio, D., Zicarelli, L., Neglia, G., Di Palo, R., Balestrieri, A., D'Occhio, M.J., 2007b. Strategies to reduce embryonic mortality in buffalo cows. Proc. VIII Buffalo Congress. In press. **Cassano, E.**, Tosto, L., Balestrieri, M., Zicarelli, L., Abrescia, P., 1999. Antioxidant defense in the follicular fluid of water buffalo. *Cellular Physiology and Biochemistry*. 9: 106-116. **Danet-Desnoyers, G.**, Wetzels, C., Thatcher, W.W., 1994. Natural and recombinant bovine interferon 7 regulate basal and oxytocin-induced secretion of prostaglandins F_h and & by epithelial cells and stromal cells in the endometrium. *Reprod. Fertil. Dev.* 6:193-202. **Dharmarajan, A.M.**, Hisheh, S., Singh, B., Parkinson, S., Tilly, K.I., Tilly, J.L., 1999. Antioxidants mimic the ability of chorionic gonadotropin to suppress apoptosis in the rabbit corpus luteum *in vitro*: a novel role of superoxide dismutase in regulating *bax* expression. *Endocrinology*. 140: 2555-2560. **Garrett, J. E.**, Geisert, R.D., Zavy, M.T., Morgan, G.L., 1988. Evidence for maternal regulation of early conceptus growth and development in beef cattle. *J. Reprod. Fertil.* 84: 437-46. Gasparri, B., 2002. In vitro embryo production in buffalo species: state of the art. *Theriogenology* 57: 237-256. **Hansen, P.J.**, 2002. Embryonic mortality in cattle from the embryo's perspective. *J. Anim. Sci.*, 80: E33-E44. **Helmer, S.D.**, Hansen, P.J., Thatcher, W.W., Johnson, J.W., Bazer, F.W., 1989a. Intrauterine infusion of highly enriched bovine trophoblast protein-1 complex exerts an antiluteolytic effect to extend corpus luteum lifespan in cyclic cattle. *J. Reprod. Fertil.* 87:89-101. **Helmer, S.D.**, Gross, T.S., Newton, G.R., Hansen, P.J., Thatcher, W.W., 1989b. Bovine trophoblast protein-I complex alters endometrial protein and prostaglandin secretion and induces an intracellular inhibitor of prostaglandin synthesis *in vitro*. *J. Reprod. Fertil.* 87: 421-430. **Karaivanov, C.**, Vlahov, K., Petrov, M., Kacheva, D., Stojanova, M., Alexiev, A., Polihronov, O., Danev, A., 1978. Studies on preimplantation development of buffalo embryos. *Theriogenology*. 28: 747 - 753. **Kerbler, T.L.**, Buhr, M.M., Jordan, L.T., Leslie, K.E., Walton, J.S., 1997. Relationship between maternal plasma progesterone concentration and interferon-tau synthesis by the conceptus in cattle. *Theriogenology*. 47: 703-714. **Kumar, R.**, Phogat, J.B., Singh, I., Kumar, V., Singh, U., Sethi, R.K., 2003. Efficacy of post-insemination progesterone supplementation for enhancement of fertility in buffaloes. *Bubalus bubalis*. 3: 76-82. **Lamming, G.E.**, Wathes, D.C., Flint, A.P.F., Payne, J.H., Stevenson, K.R., Vallet, J.L., 1995. Local action of trophoblast interferons in suppression of the development of oxytocin and oestradiol receptors in ovine endometrium. *J. Reprod. Fertil.* 105: 165-175. **Madan, M.L.**, Chauhan, M.S., Singla, S.K., Manik, R.S., 1994. Pregnancies established from water buffaloes (*Bubalus bubalis*) blastocysts derived from *in vitro*

matured, in vitro fertilized oocytes and co-cultured with cumulus and oviductal cells. The riogenology. 42: 591-600. **Mann, G.E.** 2002. Corpus luteum function and early embryonic death in the bovine. XXII World Buiatrics Congress, Hannover, Germany, August 18-23: 300-306. **Mann, G.E., Lamming, G.E.** 1999. The influence of progesterone during early pregnancy in cattle. *Reprod Dom Anim.* 34: 269-274. **Mann, G.E., Lamming, G.E.** 2001. Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. *Reproduction.* 121: 175-180. **Martinez, M.F., Adams, G.P., Bergfelt, C., Kastelic, J.P., Mapletoft, R.J.** 1999. Effect of LH or GnRH on the dominant follicle of first follicular wave in beef heifers. *Anim. Reprod. Sci.* 57: 23-33. **Neglia, G., Gasparini, B., Caracciolo di Brienza, V., Presicce, G.A., Zicarelli, L.** 2001. Buffalo and Bovine in vitro embryo production from ovum pick up and abattoir derived oocytes. Proceedings of the A.S.P.A. XIV Congress: 624-626. **Plante, C., Hansen, P.J., Martinod, S., Siegenthaler, B., Thatcher, W.W., Pollard, J.W., Leslie, M.V.** 1989. Effect of intrauterine and intramuscular administration of recombinant bovine interferon- alpha 1 on luteal lifespan in cattle. *J. Dairy Sci.* 72: 1859-1865. **Roberts, R.M., Cross, J.C., Leaman, D.W.** 1992. Interferons as hormones of pregnancy. *Endocrine Reviews.* 13: 432-452. **Robinson, R.S., Mann, G.E., Wathes, D.C., Lamming, G.E.** 1997. The effect of pregnancy on oxytocin and oestrogen receptor expression in the bovine uterus. *J. Reprod. Fertil. Abstr. Ser.* 19: 122. **Santos, J.E.P., Thatcher, W.W., Pool, L., Overton, M.W.** 2001. Effect of human chorionic gonadotropin on luteal function and reproductive performance of high producing lactating Holstein dairy cows. *J. Anim. Sci.* 79: 2881-2894. **Sastry, N.S.R., Georgie, G.C.** 1978. An appraisal of factors contributing to a seasonal breeding tendency in Indian water buffaloes. *Proc. Indo-Soviet Symp. Buff. Breed:* 137-142. **Schmitt, E.J.P., Drost, M., Diaz, T., Roomes, C., Thatcher, W.W.** 1996. Effect of a gonadotropin-releasing hormone agonist on follicle recruitment and pregnancy rate in cattle. *J. Anim. Sci.* 74: 154-161. **Senger, P.L.** 2003. Chapter 13. Early Embryogenesis and Maternal Recognition of Pregnancy. In: Pathways to pregnancy and parturition: 284- 303. **Shafie, M.M.** 1985. Physiological responses and adaptation of water buffalo. In: Mohamed K. Yousef (Ed.). *Stress Physiology in livestock, vol. II, Ungulates*, CRC, Florida: 67-80. **Silke, V., Diskin, M.G., Kenny, D.A., Boland, M.P., Dillon, P., Mee, J.F., Sreenan, J.M.** 2002. Extent, pattern and factors associated with late embryonic loss in dairy cows. *Animal Reproduction Science.* 71: 1-12. **Singh, G.** 1988 Seasonal trend of calving and subsequent service-period in rural buffaloes in Punjab (India). *Acta Vet. Stand. Suppl* 83: 80-84. **Singla, SK, Manik, RS, Madan, ML.** 1996. Embryo biotechnologies in buffaloes: A review. *Bubalus bubalis.* 1: 53-63. **Spagnuolo, M.S., Vecchio, D., De Rosa, R., Polimero, F., Balestrieri, A., Zicarelli, G., Ferrara, L., Campanile, G.** 2007. Effect of different housing conditions on several indices of blood redox status and on reproductive performance in buffalo cows. *Proc. VIII Buffalo Congress.* In press. **Spencer, T.E., Ing, N.H., Ott, T.L., Mayes, J.S., Becker, W.C., Watson, G.H., Mirando, M.A., Bazer, F.W.** 1995. Intra-uterine injection of interferon-tau alters oestrogen receptor and oxytocin receptor expression in the endometrium of cyclic ewes. *J. Mol. Endocrinol.* 15: 203-220. **Spencer, TE, Bazer, FW.** 1996. Ovine interferon tau suppresses transcription of the estrogen receptor and oxytocin receptor genes in the ovine endometrium. *Endocrinology.* 137: 1144-1147. **Starbuck, G.R., Darwash, A.O., Mann, G.E., Lamming, G.E.** 1999. The detection and treatment of post insemination progesterone insufficiency in dairy cows. In Diskin MG (editor), *Fertility in the High-Producing Dairy Cow.* Occasional Publication N°26, British Society of Animal Science. 2: 447-450. **Thatcher,**

W.W., Guzeloglu, A., Meikle, A., Kamimura, S., Bilby, T., Kowalski, A.A. 2003. Regulation of embryo survival in cattle. *Reproduction*. 61: 253–266. **Thatcher**, W.W., Meyer, M.D., Danet-Desnoyers, G. 1995. Maternal recognition of pregnancy. *J. Reprod. Fertil. Suppl.* 49: 15-28. **Vale**, W.G., Ohashi, O.M., Sousa, J.S., Ribeiro, H.F.L., Silva, A.O.A., Nanba, S.Y. 1989. Morte embrionária e fetal em búfalos, *Bubalus bubalis* Lin. *Revista Brasileira de Reprodução Animal*. 13: 157–165. **Vasconcelos**, J.L.M., Silcox, R.L., Lacerda, J.A., Pursley, J.R., Wiltbank, M.C. 1997. Pregnancy rate, pregnancy loss and response to heat stress after AI at 2 different times from ovulation in dairy cows. *Biol. Reprod.* 56: 140. **Vecchio**, D., Di Palo, R., Zicarelli, L., Grassi, C., Cammarano, A., D’Occhio, M.J., Campanile, G. 2007. Embryonic mortality in buffalo naturally mated. *Proc. VIII Buffalo Congress*. In press. **Wathes**, D.C., Robinson, R.S., Mann, G.E., Lamming, G.E. 1998. The establishment of early pregnancy in cows. *Reprod Dom Anim*. 33: 279–284. **Zicarelli**, L. 1994. Anaestro e induzione dell’estro in bufale acicliche. *Agricoltura Ricerca*. 153: 25-40. **Zicarelli**, L. 1995. Management in different environmental conditions. *Buffalo J.* 2: 17-38. **Zicarelli**, L. 1997. Reproductive seasonality in buffalo. *Proc. Third Course on Biotechnology of Reproduction in Buffaloes (Issue I)*: 29–52. **Zicarelli**, L., Campanile, G., Infascelli, F., Esposito, L., Ferrari, G. 1988b. Incidence and fertility of heats with double ovulations in the Mediterranean buffalo cows of Italy. *Proc. II World Buffalo Congress*: 56-62. **Zicarelli**, L., Di Palo, R., Palladino, M., Campanile, G., Esposito, L. 1993. Embryo transfer in Mediterranean *bubalus bubalis*. *Proc Int Symp Prospects of Buffalo Production in the Mediterranean and the Middle East, Cairo, Egypt*, 9-12 Nov: 73-75. **Zicarelli**, L., Infascelli, F., Esposito, L., Consalvo, F., de Franciscis, G. 1988a. Influence of climate on spontaneous and alfaprostol-induced heats in Mediterranean buffalo cows bred in Italy. *Proc. II World Buffalo Congress*: 47-56.