

Enhancing reproductive performance in domestic dairy water buffalo (*Bubalus bubalis*)

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The purpose of the review is to describe the factors that affect fertility in domestic water buffalo (*Bubalus bubalis*) and the techniques that enable an improvement in reproductive performance. On Italian and Latin American farms where natural mating is practiced and bulls are always present in the herd, the inter-calving interval is approximately 400 days and the culling rate is lower than 15%. The buffalo has a tendency for seasonal reproductive activity. Reproduction is favoured when there is a decrease in day length. Ovarian activity stops if conception does not occur within 3 to 5 ovarian cycles. It is important, therefore, that appropriate management of the transition period is practiced, particularly with respect to the hygienic conditions of the uterus. In tropical countries located north of the equator, feed deficiencies and heat stress are considered the main factors that lead to poor fertility in the summer. In Pakistan, for example, the increase in body condition score during the autumn was associated with the commencement of the breeding season in buffaloes. Anoestrus is observed also in Italy, however, where the average daily temperature during the same period is 13.5 to 23.5°C and feeding is constant throughout the year. The only common element between the two areas is the progressive increase in daylight hours between April and June and the day length greater than 12 hours up to September. In Italian herds that apply an out-of-season breeding strategy, an improvement in fertility (measured as the percentage of corpora lutea corresponding to subsequent pregnancy) is observed when water pools are present on the farm. This demonstrates that an improvement in environmental conditions reduces the incidence of embryonic mortality and/or abnormal cycles. To summarize, in the absence of serious nutritional problems, an improvement in environmental conditions increases fertility in buffalo.

Introduction

Domestic water buffalo (*Bubalus bubalis*) represent a fundamental and irreplaceable resource for tropical countries. Buffalo populations are increasing at a greater rate than those of bovine species. Between 1961 and 2008, the bovine and buffalo populations increased by 43% and 105% and between 1995 and 2007 increased by 2% and 13%, respectively (Faostat.fao.org/faostat). The population data refer to the population present in 43 of 143 countries that currently breed buffalo (Misra & Tyagi 2007).

The majority of the authors believe that the main reproductive characteristics of buffalo are delayed puberty, prolonged postpartum ovarian inactivity, long inter-calving intervals and a tendency for seasonality (Madan 1988; Misra & Tyagi 2007). The reproductive problems depend on the region of the world. In tropical countries north of the equator (TCNE), for example, the majority of the authors assert that the summer anoestrus is caused by heat stress and forage scarcity. In Italy, however, anoestrus is observed in the same period of the year as TCNE (as demonstrated by the calving distribution; Fig. 1) although the diet in Italy is constant throughout the year and the ambient temperatures are milder. The aim of this review is to examine the main factors affecting fertility in buffalo and the strategies that may be adopted to enhance the reproductive performance of buffalo.

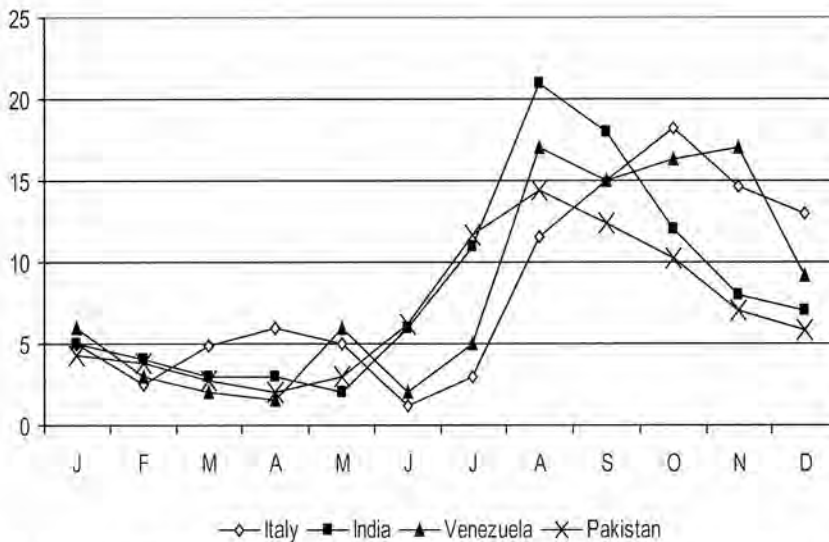


Fig. 1. Monthly calving percentage for Italy (Zicarelli *et al.* 1977), India (Singh, 1988), Venezuela (Zicarelli, 1994), and Pakistan (Hassan *et al.* 2007). Calendar months (single letter abbreviations) are indicated on the x-axis.

Reproductive seasonality

The place of origin and the duration of gestation have undoubtedly influenced the way in which reproductive seasonality occurs. The natural necessity to coincide calving and weaning with the most-suitable time of year represents one of the causes of this 'adaptation' process (Zicarelli 1994). This time of year should satisfy the nutritional requirements of the offspring through a period in which etiologic agents (infectious and parasites) are less aggressive and/or present. Those born under the most-favourable conditions have brought about the natural selection of individuals endowed with a more ideal reproductive seasonality that promotes the survival of the species (Zicarelli 1997). Spring calving (March to May), which guarantees good availability of forage to offspring in temperate zones north of the equator, occurs when reproduction takes place in autumn in the case of five month gestations (sheep and goats) or in the previous spring in the case of 11 and 12 month gestations (horses and donkeys). The same calving period, therefore, is conditioned by the neuroendocrine system. The reactivation of the reproductive cycle with regard to the length of gestation (short day breeder [negative photoperiod] or long breeder [positive photoperiod]) is therefore controlled.

In tropical zones where the domestic buffalo developed (between 31°N and 2°S) forage availability is usually adequate after the rainy season (July through September). In species like the domestic buffalo, therefore, whose pregnancy lasts 310 to 316 days, the sensitivity to decreasing light stimulus and the reproductive season seems to take place between September and January. Around the equatorial belt, where the light/dark ratio varies little throughout the year, the reproductive season is highly conditioned by forage availability (Vale *et al.* 1990; 1996). There is a tendency towards seasonality in buffalo because buffalo evolved far away from the equator. Moving buffalo to other regions of the world has not modified the sensitivity of the hypothalamic hypophyseal axis to a decreasing light/dark ratio.

In Italy, the seasonality makes it necessary to change the calving calendar in order to meet the milk market demand. This is accomplished by using an out-of-breeding mating strategy (OBMS) that entails the interruption of natural mating or the use of artificial insemination (AI) between October and late January in adult females and between September and late March in heifers. These months are the most-favourable periods for reproductive activity (Zicarelli 1994; Zicarelli 1997; Campanile *et al.* 2009).

In research carried out in Italy (Parmeggiani & Di Palo 1994), it was demonstrated that buffaloes with a tendency towards seasonality showed high plasma melatonin concentrations two hours after sunset even when they were moved to another farm where other females showed low plasma melatonin concentrations and less sensitivity towards light stimulation (Di Palo *et al.* 1997). The plasma melatonin concentrations had a repeatability of 0.733 (Di Palo *et al.* 1993). If the heredity of plasma melatonin turns out to be high, as we expect based on the high repeatability, the plasma melatonin could be incorporated into genetic selection programmes for buffalo (Zicarelli 1994). In an effort to validate this theory, we reported that Romanov 58°N, Karakul 41°N and White-faced 51°N sheep (Lincoln 1992) showed continuous cyclic activity throughout the year even if living at latitudes where other genotypes were sensitive to the light/dark ratio.

In heifers and adult female buffaloes, the differences between night and day concentrations of plasma melatonin in March were less in heifers (5.0 times) compared with adult buffaloes (28.3 times) (Borghese *et al.* 1995). Both buffaloes that calve in spring (Di Palo *et al.* 1993; more adaptable to out-of-breeding-mating-strategy) and the heifers (Borghese *et al.* 1995; less sensitive to the photoperiod; Zicarelli 1994) show the same behavioural pattern.

Heifer fertility is not compromised by the season (Campanile *et al.* 1991; Avallone *et al.* 1994; Zicarelli 1994). During the summer and when daylight hours are more than dark hours, there is an increase in prolactin in the blood but, contrary to assertions by Madan (1988), buffalo regularly conceive. We believe that hyperprolactinemia is a consequence of the hypothyroidism (Campanile *et al.* 1994) during the warm months. The hypothyroidism exerts a positive feedback on thyroid stimulating hormone and hence on thyrotropin releasing hormone. Thyrotropin releasing hormone promotes an increase in prolactin (Zicarelli 1994; 1997).

The majority of authors attribute reproductive seasonality to nutritional factors. The breeding period in areas where 97% of the buffalo population are bred takes place in the months of greater forage availability (Roy *et al.* 1968; Singh *et al.* 1988; Shah 1990; Singh & Lal 1992; Qureshi *et al.* 1999a). Greater forage availability is found during the months of July through November characterized by decreasing daylight length in TCNE.

Recently Hassan *et al.* (2007) reported the different seasonality of Nili-Ravi buffaloes, purebred Sahiwal and cross-bred cattle in Pakistan. The difference between Nili-Ravi buffaloes and purebred Sahiwal was characterized by negative and positive photoperiod sensitivity, respectively. Data similar to those recorded in buffaloes have been observed in cross-bred cattle (Holstein or Jersey x Sahiwal). It cannot be ruled out, however, that the high temperatures (35 to 41°C) and milder temperatures (22 to 28°C) may influence negatively and positively (respectively)

the ovarian activity. This finding suggests, moreover, that crossbreeding with *Bos taurus* may modify the seasonality of native bovines, which have the higher incidence of deliveries during the first 5 months of the year and, hence, conceive in the hottest months (April to August).

If heat stress is the main cause of the anoestrus then it should adversely affect the reproductive activity of buffalo compared with *Bos indicus* cattle. In Italy exactly the opposite pattern is observed. For out-of-season mated buffaloes, the conception rate increases between July and September, a period during which Holstein cows shows a low conception rate.

In Italian herds where the OBMS technique is not used (Zicarelli *et al.* 1977) as well as 30 to 40 years ago on the majority of farms (Ferrara 1957), the resumption of the reproductive cycle (RRC) took place from September (decreasing light period) until January (light increasing period but predominantly dark hours) (Fig. 1). Sensitivity to the negative photoperiod is also found on farms where a constant balanced diet is provided year-round (Zicarelli 1994). This type of seasonality, where reproductive events are not synchronised with forage availability, indicates that the buffalo bred in Italy are not autochthonous in the sense that they will sometimes calve during periods of forage scarceness and low temperature which in turn hinders the survival of the calf. Italian findings should be sufficient to define the buffalo as a short day breeder. Indeed, a similar seasonality to that found in Italy and Asian tropical areas is also found in Venezuela (Montiel 2000) and Egypt (Zoheir *et al.* 2007) (Fig. 1).

In southern Brazil (Da Silva & Grodzki 1991; Baruselli *et al.* 2001) and Argentina (L Zicarelli, unpublished observations), the wet season (and consequently, increased pasture availability) starts in October to November and continues until March to April. Pasture scarcity goes from May to June until October to November. The buffalo calving period under these conditions is mainly concentrated from February to May (Fig. 2). The breeding period is from April to July and the calf weaning calf period under free range and suckling calf conditions is from September to December. These events permit the coincidence of forage availability within the first 2 to 4 months of lactation and most of the dry period (October to April). The breeding period, however, is mainly concentrated during the pasture scarcity period (May to July).

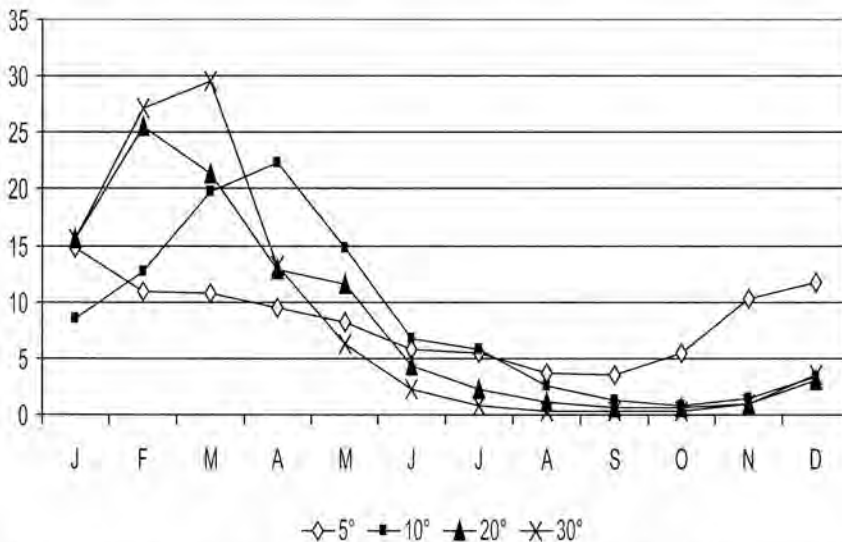


Fig. 2. Monthly calving percentage in Brazil as a function of latitude ($^{\circ}$ S). Calendar months (single letter abbreviations) are indicated on the x-axis.

Baruselli *et al.* (2001), who evaluated data from the Brazilian Breeders Association, observed that the seasonality is more accentuated from the north (0 to 8 degrees latitude) to the south (24 to 32 degrees latitude) of Brazil (Fig. 2) and hence the calving season is influenced by latitude. From the findings shown here, it can be unequivocally stated that, although the domestic water buffalo shows reproductive activity throughout the year, there is a greater tendency to concentrate reproductive activity in months of decreasing daylight or if increasing, when dark hours prevail.

Reproductive efficiency

Many scientists affirm that the buffalo shows delayed puberty and a long inter-calving interval. These characteristics are affected by several factors, such as year of calving, season of delivery, genotype and heat stress (Zicarelli *et al.* 2007). The delayed puberty and the consequent older age at first calving have been addressed by many authors. In a study performed on 86 farms (30,735 primiparous buffaloes, which calved between 1975 and 2005), it was observed that the mean age at first calving decreased by 1 month every 5 years (Zicarelli 2007). The hormonal pattern in cyclic buffalo is similar to that described in a cyclic *Bos taurus* cow (Seren *et al.* 1994). The main difference between the two species is the percentage of cyclic individuals in the different seasons.

The different reproductive efficiency of buffalo compared with *Bos taurus* is due to several features. These features must be considered because there is a need to modify the calving calendar in the buffalo so that the milk market demand is met. There are fewer primordial and antral follicles (Le Van *et al.* 1989) as well as a lesser ovarian weight and lesser ovarian volume in the buffalo compared with the bovine (Vittoria 1997). The number of oocytes in a buffalo calf is one-fifth that recorded in a bovine calf (Le Van *et al.* 1989; Gasparini 2002). After calving, there are a low number of ovarian follicles and follicular waves and few cycles occur. If conception does not take place, therefore, an anoestrus of variable length begins (Zicarelli *et al.* 1994). With regard to this topic, the transition period and the postpartum period have a major importance for fertility in buffalo. It should be pointed out that in the farms adopting semi-free housing, the presence of mycotoxins in the roughages, *Clostridia*, *Coxiella burnetii*, and the incorrect input of Ca, P and crude protein in the last two months of pregnancy often lead to the occurrence of vaginal or uterine prolapse which impairs the RRC (Zicarelli 2000). It is not clear whether the seasonality of the species depends on the reduced follicular population or if this latter effect is the cause of buffalo seasonality.

If the calving calendar is not modified, the delayed RRC after calving can be due to the lack of the "bull effect" (Zicarelli *et al.* 1997) and/or to poor nutritional conditions. In small farms in TCNE where the bull is not present, the dry period of the animals (March to June) coincides with the scarcity of forage. South of the equator over the 20th parallel, the dry period (November to March) coincides with the abundance of forage and the bull is always present in the herd. In the first case, prolonged inter-calving intervals are observed (Shah 1990) whereas in the second case inter-calving intervals less than 400 days are recorded (Zicarelli 1994).

These observations suggest that in those areas, the meeting of nutritional requirements and the absence/presence of the bull are the most important factors (Zicarelli *et al.* 1997), especially taking into account that the protein content of the pastures is very low (6 to 10% of DM) and leguminosae are found in irrigated areas or during the rainy season. In Pakistan, Qureshi *et al.* (1999a, 1999b) reported that the seasonality is influenced by nutritional and non-nutritional factors. Unless the feed deficiencies are serious, however, nutritional factors in general do not play a significant role (Zicarelli 1999; Paul & Lal 2010). The buffalo cannot yet be considered

exactly as having a “*lactiferous habitus*” and therefore a “*catabolicus habitus*” such as that found in the high milk producing bovine cow which uses its reserves to compensate energy and protein deficiency during early lactation. As a matter of fact, within reasonable limits, the buffalo uses its reserves in its aim to reproduce and to the detriment of her own milk production.

The effects of the tendency toward seasonal reproduction in the buffalo are particularly evident. Studies conducted in Italy may be useful for countries wishing to increase the consistency of their production of buffalo milk during the year. When primiparous buffaloes are excluded from the survey (heifers are less sensitive to photoperiod), a decrease in calving rate between March and June for farms that are adopting the OBMS technique is observed in Italy whether OBMS is applied or not (Fig. 3). This confirms that the increase in daylight hours (April to June) or number of daylight hours > 12 h, negatively interfere with reproductive activity. The buffalo that undergo OBMS can show anoestrus and this phenomenon is worsened by unfavourable climatic conditions.

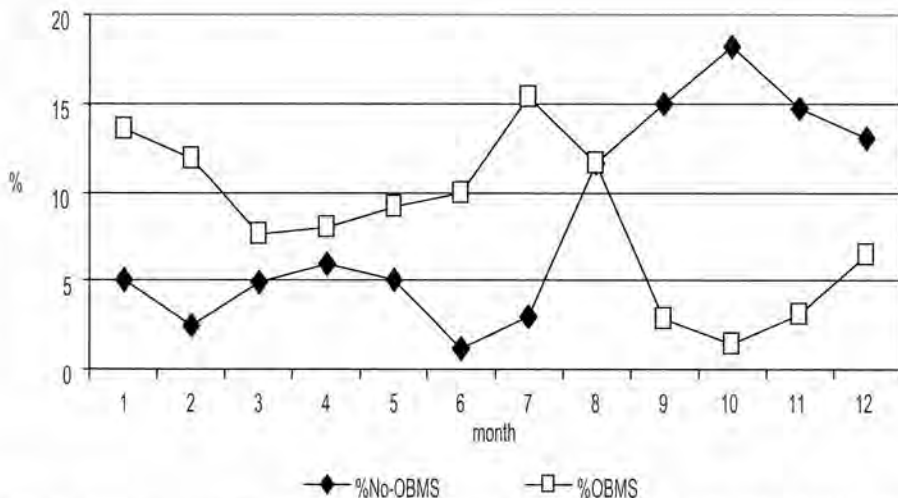


Fig. 3. Calving percentage in farms that use (%OBMS) or do not use (%No-OBMS) the out-of-breeding mating strategy (OBMS) technique.

It is possible to define a “temporary anoestrus” (<150 days) and a “deep anoestrus” (>150 days) based on the number of days open. It is also possible to distinguish between buffalo that come into oestrus within 70 days from calving, and conceive within or after 90 days, from those that come into oestrus after 70 days and conceive within or after 150 days (Zicarelli *et al.* 1994). Approximately 70% of buffaloes that deliver out of the breeding season showed RRC within 70 days from calving. Fifty-three percent of these buffalo were able to conceive within 3 months (around 75% of the animals that resumed the ovarian activity within 70 days) whereas 17.3% later than 3 months. The remaining 30% of animals had RRC after 70 days from calving and 10.6% and 18.8% conceived within and after 5 months, respectively.

Anoestrus can be identified as a non RRC after parturition or as an interruption of cyclic activity for varying reasons. Environmental conditions that are responsible for the anoestrus are accompanied by changes in blood hormones (Jain 1988) such as prolactin (Razdan *et al.* 1981), follicle stimulating hormone (Janakiraman *et al.* 1980), luteinizing hormone (Batra & Pandey 1982; Aboul-Ela *et al.* 1983), progesterone (Kaur *et al.* 1983; Qureshi *et al.* 2000), estradiol (Sheth *et al.* 1978; Heranjal *et al.* 1979; Razdan *et al.* 1981), thyroid hormones (Khurana & Madan 1985; Gupta & Dhoble 1988), and corticosteroids (Madan *et al.* 1983).

Some of these hormones represent the response to stressful factors rather than the cause of the arrest in reproductive activity. Buffalo that deliver out of the breeding season may need several months to conceive. During this period, they are not always acyclic, but are able to conceive and then undergo embryonic mortality, in particular between April and May, months characterized by progressively increasing day length. During the year, the embryonic mortality rate is 10% of the pregnancies diagnosed at 40 days and is 22% for pregnancies that take place in the month of April (Zicarelli *et al.* 1994). The incidence of this event is affected by the "farm" factor and ranges between 10 and 45% if calculated on the pregnancies after AI diagnosed by ultrasonography at day 26 (Campanile *et al.* 2005).

A pregnancy is not always detected after the observation of a corpus luteum. It can be assumed that an early embryonic mortality or an anoestrus condition has occurred. The anoestrus condition may have been preceded by an ovarian cycle which forms a corpus luteum with inadequate function because of a short luteal phase or normal luteal phase with low progesterone (Zicarelli 1997; Zicarelli *et al.* 1997). This phenomenon has been found at the onset of anoestrus season in sheep, after first ovulation postpartum in bovine and during the prepubertal phase in both species (Garverick *et al.* 1992).

Some recent papers report an incidence of double ovulations of 15.5% in dairy cows (Lopez-Gautius *et al.* 2005). A similar value has been previously reported in buffalo (Zicarelli *et al.* 1988). In the latter, however, only 0.06% of double ovulation led to a twin pregnancy. The double ovulations reduce the efficiency of AI in the case of spontaneous oestruses, but not in the case of induced oestruses (Zicarelli *et al.* 1997).

In Italy, the OBMS technique leads to lesser fertility. In fact, when the OBMS technique was not applied, calving intervals of 400 to 445 days were recorded (Zicarelli *et al.* 2007). Recently, a mean inter-calving interval of 487 ± 133 days was reported for 6,052 inter-calving intervals over a period of 5 to 10 years on 5 farms with an annual culling rate lower than 10% (Zicarelli *et al.* 2007). The OBMS technique was adopted and a constant rationing was given throughout the year. It was demonstrated that the shortest inter-calving periods were recorded in buffaloes that deliver between April and June, and that, consequently, conceived in the warmest months (between June and August). Short inter-calving intervals were also found in buffalo that delivered between July and September; the period of the year during which the highest temperatures are recorded in Italy. The longest inter-calving periods were recorded for buffaloes calving between October and December, because of the OBMS technique, which delays mating until February. Long inter-calving intervals were also found in buffaloes that calved between January and March, the coldest period of the year (Zicarelli *et al.* 1994; 1997; 2007). These buffalo would conceive in spring, the mildest period of the year, characterized by temperatures between 15 and 22°C. Conception is typically delayed until September, however, except for the 40% of animals that conceive within 90 days from calving. We can conclude, therefore, that in Italy nutrition and the warmest months, especially if a swimming-pool is present on the farm (Di Palo *et al.* 2009; Neglia *et al.* 2009), do not affect the inter-calving period. The main factor that has to be taken into account in Italy is the light stimulus. Buffaloes that deliver between January and March delay their conception until August to September, after three months of decreasing day length. Similarly, buffaloes that deliver in the period April to September show the shortest inter-calving period because after 58 days decreasing day length begins.

The majority of Indian and Egyptian authors assert that the lower concentration of calving observed between January and May depends on the reduced conception rate between March and July. This phenomenon is influenced by the hot and dry climate of this period of the year. In Italy, in farms that do not use the OBMS, a drop of calving is also observed between January and June (Fig. 1); a period of the year in which, unlike India, the climate is either cold or mild and moderately rainy. The fertility markedly improves between July and September; a period

that in Italy coincides with the highest temperature (Fig. 4) and temperature humidity index of the year. This observation makes buffalo very different from cattle, that in the hottest months of the year (July to September), show a marked decrease in fertility.

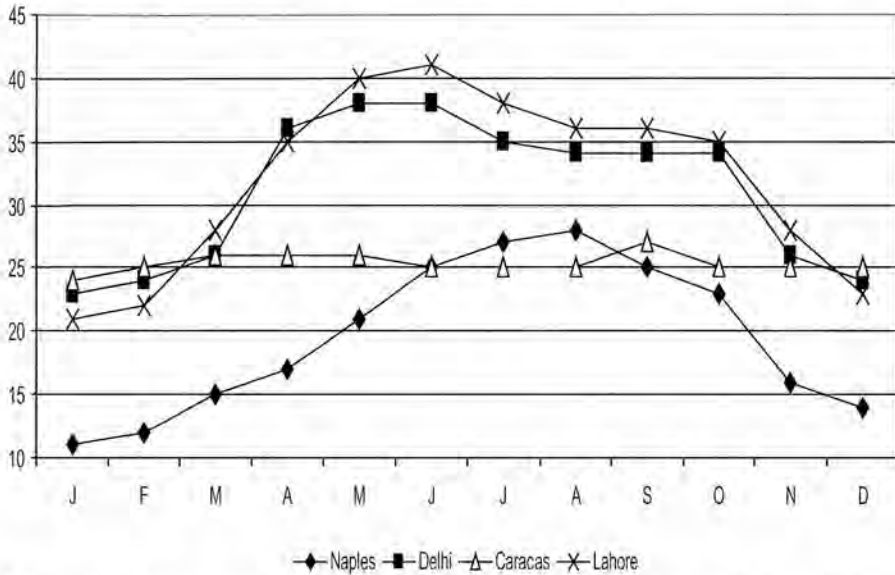


Fig. 4. Monthly maximum temperature (°C) in Naples, Delhi, Caracas, and Lahore. Calendar months (single letter abbreviations) are indicated on the x-axis.

We have already highlighted that in the areas located north of the equator a drop in the number of calvings is observed between January and June. The authors from countries located north of the equator attribute the anoestrus of buffalo to heat stress (India, Pakistan) or to the low environmental humidity (Venezuela). In our opinion, the decrease in calving rate between January and June (Fig. 1) depends on the reduced reproductive activity between March and September. In the latter period, the maximum daily temperature (Fig. 4) ranges between 15°C (March) and 27°C (August) in Naples, Italy; between 25°C and 38°C in Delhi, India; between 27°C (March) and 41°C (June) in Lahore, Pakistan; and between 25°C (March) and 26°C (August) in Caracas, Venezuela. The maximum daily temperatures that are recorded in Italy and in Venezuela rule out a direct action of environmental temperature on anoestrus. If only India and Pakistan are considered, it is not possible to exclude that heat stress, even if it is not the main factor, contributes to summer anoestrus. Furthermore, the RRC (August to September) coincides with a monthly maximum temperature of 28°C (August) and 25°C (September) in Italy, 25°C (August) and 27°C (September) in Venezuela, 34°C (September) in India and 36°C in Pakistan.

The monthly rainfall recorded in Italy and Venezuela between September and October is not different from that observed between March and May (Italy) and between June and August (Venezuela) when the lower conception rate is observed (Fig. 5). On the contrary, in India and in Pakistan the rainy season takes place between July and September. The cessation of reproductive activity in Italy and Venezuela, therefore, cannot be attributed to the rainfall. In India and Pakistan, the reproductive activity is good in October and November (calving August and September), when the temperature is lower and the rainfall is already minimal. The trend of daylight hours, although with different daily values, is shared by all the areas of breeding situated north of the equator (Fig. 6). Interestingly, a 4-year retrospective analysis of data (B Gasparini,

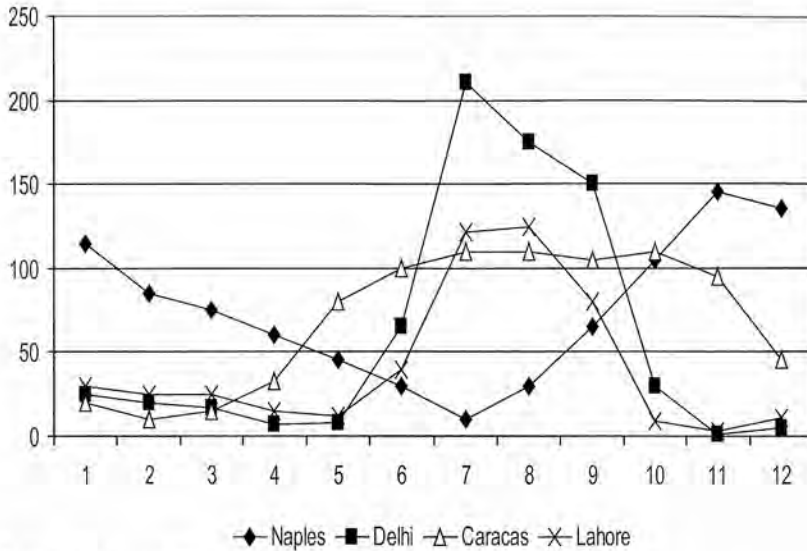


Fig. 5. Monthly rainfall (mm) in Naples, Delhi, Caracas, and Lahore.

unpublished observations) obtained in an in vitro embryo production laboratory showed that a significant decrease in blastocyst rate was observed between April to June compared with October to December. Intermediate values were recorded between July and September and between January and March. The drop in oocyte developmental competence coincides with the spring months that at our latitudes are characterized by mild environmental temperatures. This pattern confirms that the light stimulus plays the most critical role in determining seasonality.

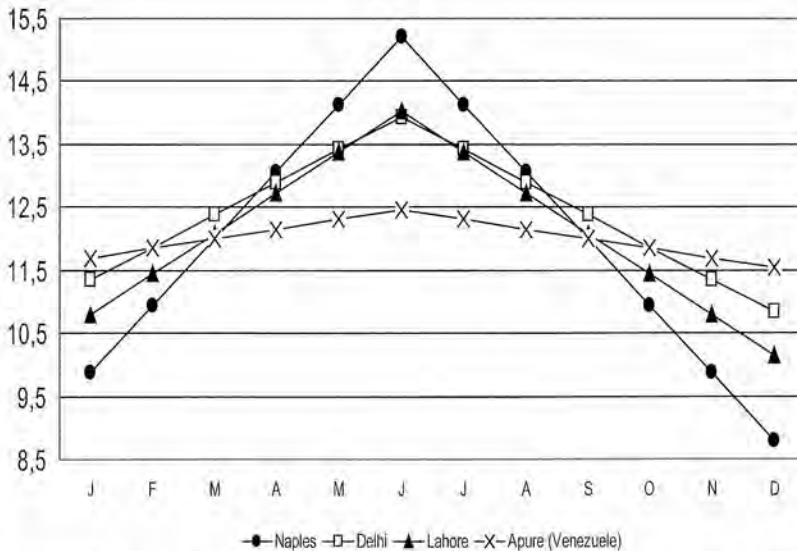


Fig. 6. Daylight hours (h) in Naples, Delhi, Appure (Venezuela), and Lahore. Calendar months (single letter abbreviations) are indicated on the x-axis.

South of the equator, Baruselli *et al.* (2001) demonstrated that the concentration of calving increases proportionally with increasing distance from the equator (Fig. 2). It is not possible to

show any relationship between daily maximum temperature, rainfall and the calving calendar (Fig. 2) whereas an evident relationship exists between the calving calendar and the daylight hours at different latitudes (Fig. 7).

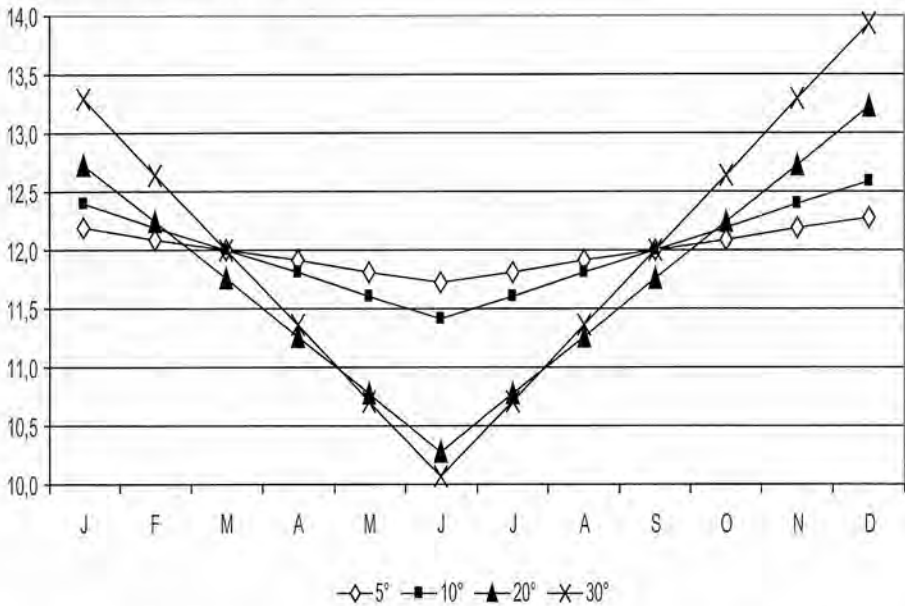


Fig. 7. Daylight hours (h) in Brazil as a function of latitude (°S). Calendar months (single letter abbreviations) are indicated on the x-axis.

Throughout this paper, the seasonality of the females has been considered. The phenomenon may affect the males as well. The effects are clear when the animals are maintained in free mounting condition. Recently, we observed in the same farm a higher pregnancy rate between January and April in buffaloes inseminated by AI compared with buffalo that were bred by using the bull. This perhaps occurred because the AI avoided the negative effect of the bull. We have recently verified that in April, only 23%, 31% and 29% of the bulls showed values higher than the average value for testosterone, dihydrotestosterone and androstenedione, respectively (Pelagalli *et al.* 2009).

Strategies to enhance reproductive performance

The delayed puberty and the consequent older age at first calving depend on both inappropriate weaning techniques and inadequate feeding during growth. The age at first calving was 28 to 32 months when feeding a diet characterized by an energy density of 0.8 MUF (MUF = Milk unit forages = 1700 kcal NE_l) and 12.5 to 13% protein content on a dry matter basis and a forage to concentrate ratio of 50 to 60%, (Zicarelli 2007). Embryonic mortality in buffalo is primarily due to a reduced secretion of progesterone by the corpus luteum. The importance of progesterone concentration during the first weeks of pregnancy for reducing embryonic mortality has been demonstrated in both cattle (Mann 2002) and buffalo (Campanile *et al.* 2005; 2007; 2008; 2009). In cattle, several treatments have been used typically within 5 days after conception to increase progesterone secretion by the existing corpus luteum or to induce ovulation and formation of an accessory corpus luteum (Mann 2002). Treatments on day 5 after

insemination do not have any effect on reducing embryonic mortality in buffalo (Campanile *et al.* 2007). Treatment of buffaloes with a GnRH agonist, hCG or progesterone on day 25 after AI reduced embryonic mortality (Campanile *et al.* 2008).

Although the light/dark ratio is the main factor affecting reproductive efficiency, another important factor is the satisfaction of the buffalo's physiological need of water for bathing. The presence of a swimming-pool reduced the not pregnant buffaloes per corpora lutea ratio (NP/CL) found at rectal examination in buffaloes that calved between April and August (Di Palo *et al.* 2009; Neglia *et al.* 2009). The swimming pool apparently acted to reduce heat stress. The NP/CL ratio, as an indicator of an anomalous oestrous cycle or embryonic mortality, may be proposed as a specific tool for evaluating buffalo welfare (Di Palo *et al.* 2009).

The treatments for anoestrus are based on the use of progesterone devices combined with PMSG. The response is influenced by the effects of year and farm and are, therefore, variable. The above treatments in natural mating conditions do not have an immediate impact but in primiparous females they have a beneficial effect on the RRC. Unsatisfactory responses are perhaps more useful because they lead to the assessment of the environmental causes that underlie the reproductive failures (Zicarelli *et al.* 1994). For instance, we demonstrated that more space and better welfare conditions improved fertility in Italy. On 21 farms in which the OBMS is performed we observed that 38.1%, 52.4% and 4.8% of the farms increased fertility rate respectively in June, July to August and September (L. Zicarelli, unpublished observations). Out of the 8 farms that resumed fertility in June, 6 (75%) had at their disposal either covered sheds, that shorten the day length on average by 2 h during the year, or wide open spaces in which buffalo cows can move for at least 6 h/day or swimming pools. Out of the 11 farms that resumed fertility in July only 2 (18.2%) had swimming pools available (6/8 versus 2/11). In a farm with a variable number of breedings over 10 years, an increase in reproductive activity in June and July was recorded. Between January and June 77% and 68% of calving were observed, respectively, for 352 and 451 buffaloes. These findings suggest that the effects of season can in part be attenuated by improving the welfare status of the animals.

Conclusions

The seasonality in buffalo is influenced by the light/dark ratio throughout the year. In some countries the seasonality is influenced by nutritional factors. Furthermore, an improvement in the welfare of buffalo (swimming pool, space, brightness of the stalls, etc.) can increase the percentage of calvings between March and June. Delayed treatment of buffaloes with GnRH agonist, hCG or progesterone on day 25 after AI can reduce embryonic mortality in the months in which daylight hours increase. A significant improvement in reproductive efficiency can be achieved by increasing the culling rate from 10-14% to 25-30%, and hence eliminating older buffalo and those with reproductive problems.

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