

Factors affecting the reproductive capacity of water buffalo (*Bubalus bubalis*)

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Abstract

The water buffalo has historically been one of the most important agriculture animals of Asia and today herds are now raised across the Mediterranean regions as well as the Americas. Of great economic value for the milk's high fat content and lean meat, buffalo production lags behind other domestic cattle species in terms of reproductive efficiency. Water buffalo have a reputation as being poor breeders due to a propensity for long calving intervals, high incidence of postpartum anestrous and late age of maturity. The objectives of this investigation were to examine the environmental and genetic factors that affect male and female reproductive potential. When implemented, simple management practices such as proper nutrition year round, prevention of heat stress and the housing of male and females together have the potential to overcome the water buffalo's apparent reproductive shortcomings. The maintenance of production and progeny testing records are essential for genetic improvement programs, which are slowing being more prominent at institutional farms across the world.

Keywords: Water buffalo, reproductive capacity, reproduction

Introduction

While water buffalo (*Bubalus bubalis*) are not commonly raised in the United States, it is fast becoming the most important agricultural animal of Asia, serving as both a dairy and meat animal as well as a beast of burden. Measures of production capacity and reproductive potential of the buffalo are often compared to the domestic cow *Bos taurus* and *Bos indicus*, to which buffalo exhibit a comparatively excellent feed conversion rate and an innate ability to adapt and thrive in hot humid conditions of the tropics. The main issue affecting the farmer is the buffalo's relatively poor reproductive potential which is most commonly attributed to a late age at first calving, high incidence of postpartum anestrous and seasonal depression of reproduction. By discussing the genetic and environmental factors effecting male and female reproductive potential with the global buffalo community, management solutions may be shared and adapted to promote water buffalo production in the various diverse region in which they thrive.

Water buffalo reproduction: the bull

Breeding soundness examinations for buffalo bulls have yet to be standardized and long term progeny testing records are scarce to non-existent in most parts of the world. Italy has the most advanced genetic improvement program with milking records kept on 27.8% of the population.¹ Natural mating continues to be the main mode of breeding in buffalo herds, largely due to weak estrus expression and subsequent difficulties associated with the timing of artificial insemination (AI). In Italy and Pakistan, only 5-10% of buffalo farmers utilize AI.¹ Small herd size, long generational intervals and scarcity of production records are major factors limiting the use of progeny testing of buffalo herds for genetic improvement.²

Factors affecting male reproductive capacity

Genetic factors. The male buffalo typically reaches sexual maturity between 16 and 30 months of age, depending on breed and management system.³ Peak reproductive potential is reached at five years of age after which a decline in sperm output per gram of testis per ejaculate may be observed.^{2,5} As in cattle, scrotal circumference (SC) is strongly correlated with semen quality and quantity in Murrah buffalo bulls.²⁴ Scrotal circumference in Murrah bulls aged 37 to 48 months is on average 29.2 cm; nearly 40% lower than corresponding mean value of 40.0 cm observed in Holstein bulls aged 34-42 months. The maximum SC (32.1cm +/- 1.5) is typically reached by five years of age in buffalo. Ejaculate volume ranges from 2.5 to 3.6 ml and sperm concentration ranges from 1475 to 1666 x 10⁶/ml. In the 2009 study

by Ahmad et al, bulls were collected with an artificial vagina twice a day for five weeks. Average daily output was 2.18×10^9 to 3.37×10^9 sperm/day, significantly lower than that observed in Holstein bulls. There was no significant difference between Holstein and Murrah buffalo bulls when sperm output per gram of testis per ejaculate (15.3×10^6 vs. 14.8×10^6) was compared. This information highlights the potential for buffalo breeders to increase bull fertility by selecting individual bulls with greater SC and maintaining long term progeny testing records.

Table 1. Characteristics of Murrah buffalo bull testis size and semen output collected as compared to age.⁵

Attributes	25-36 Months	37-48 Months	49-60 Months	>60 months
Scrotal Circumference (cm)	26.9	29.2	30.4	32.4
Estimated Testis weight (g)	261	427	498	652
Sperm Volume (ml)	2.5	3.1	3.5	3.6
Sperm Concentration ($\times 10^6$ /ml)	1,677	1,493	1,475	1,666

Breed variability. The Murrah bull has been shown to reach sexual maturity by two to three years of age while Murrah x Mediterranean crossbred buffalos raised in intensive management systems in Brazil may reach sexual maturity between 16 to 24 months.^{5,6} The average age at sexual maturity of Nili-Ravi bulls found in one study was 22.8 months.² Several studies document onset of puberty earlier in crossbred buffalos (Mediterranean X Jaffarabadi) at 10-14 months and (Murrah X Jaffarabadi) 13 months.⁶ In most swamp buffalo bulls, puberty is attained by 24 months of age, when SC is approximately 16 cm while sexual maturity, defined as the attainment of adult levels of daily sperm production per gram of testis parenchyma (14×10^6) occurs at 30 to 33 months of age, when SC is in the 17-to 20-cm range.⁷

The two subspecies of agricultural interest; the river buffalo (*Bubalus bubalis bubalis*) and the swamp buffalo (*Bubalus bubalis carbanesis*) have different karyotypes with $2N=50$ and 48 chromosomes for the river and swamp buffalo, respectively. Cross breeding between the two subspecies produced subfertile F1 hybrids.^{8,9} Histological examination of hybrid F1 (Murrah X swamp; $2N=49$) testis revealed a large proportion of degenerating spermatocytes and abnormal spermatids in the process of spermatogenesis, presumably a result of abnormal pairing configurations F1, F2 and backcrosses with $2N=48, 49$ or 50 had a significantly higher frequency of morphologically abnormal spermatid (48-72%) when compared to purebred river (29%) and swamp (16%) buffalo.^{8,10} Prevalence of interbreeding between subspecies in rural areas could be a contributing factor to decreased male fertility and further studies may be warranted.

Environmental factors. Buffalo bulls are capable of breeding all year round but seasonal fluctuations in reproductive output are consistently observed in most countries. Not only is the male libido decreased during the warmer months, examination of semen showed significant differences in the post-thaw sperm motility of semen frozen according to season, with motility being significantly lower during summer months compared to winter and/or monsoon season.^{11,12,13} In a 2007 study examining post-thaw plasma membrane stability in Thai swamp water buffalo spermatozoa, Koonjaenak et al¹⁴ found the percentage of live sperm to be significantly higher (54.6%) when collected during the winter months than the rainy season (43.5%) or summer season (46.7%). The authors recommended that semen only be collected and processed during spring and winter.¹⁴

A bull's access to females has been correlated with a positive effect on blood testosterone levels. In the study by Malfatti et al,¹⁵ bulls were divided into two groups and blood testosterone levels were measured monthly for one year. Bulls in group A were housed with females, while bulls in group B were held separate from the cows during the winter months, October to February. Blood testosterone levels of group A showed no significant difference between winter and summer months while mean serum

testosterone concentrations were significantly lower in the bulls during the separation period (October-February) than during the rest of the year. It may be concluded that the contact with females has a stimulating effect on testicular androgen secretion in buffalo bulls even when considering seasonal factors affecting reproduction.¹⁵

In tropical countries such as Pakistan, male libido has been shown to significantly decrease during the hot dry season.¹⁶ Bulls housed in intensive management systems of Italy, do not show a significant difference in blood testosterone levels or libido during winter vs. summer months. The seasonal depression in libido and reproduction in general can most likely be attributed to environmental factors such as heat stress, lack of green fodder and decreased food availability as apposed to being influenced by an increased photoperiod.¹⁵ It may be concluded that access to adequate feed, wallowing ponds, and cooling misters as well as presence of females during the hot dry season may help to overcome problems associated with buffalo reproductive seasonality.^{15,16}

Water buffalo reproduction: the buffalo cow

Buffalos are commonly known as “poor breeders” when compared to other bovine species.¹⁷ This is often attributed to late onset of puberty and sexual maturity, weak or silent estrus expression, prolonged calving interval, postpartum anestrus, seasonality of reproductive cycles and obstetrical problems such as uterine torsions.¹⁸ On the other hand, current research has shown that fertility parameters such as conception rate, pregnancy rate and calving interval under intensive management systems are comparable to the rates achieved in cattle.¹⁹ While water buffalo, as compared to *Bos taurus*, may be relatively late in reaching sexual maturity, they are renowned for their longevity. In Italy, it is common to find highly productive buffalo cows with three, four, or more daughters with registered lactations.²⁰

Factors affecting female reproductive capacity

Genetic factors. The productive life of a buffalo, while often lasting seven to ten lactations, is often considered reduced due to the late age at onset of puberty.²¹ Buffalos typically reach sexual maturity at 55-60% of their final body weight.¹⁸ Age at onset of puberty in the buffalo shows a wide range — between 13 to 40 months depending on breed and management system (Table 3). Under favorable conditions puberty typically occurs at 15 to 18 months in river buffalo and 21 to 24 months in swamp buffalo.²²

Table 2. Effect of farming system on reproductive parameters of buffalo cows. Adapted from Pasha et al²³, Borghese et al²⁸, Marai IFM et al²⁴, Zicarelli L et al²⁰

Reproductive Parameters	Pakistan: Rural subsistence	Pakistan: Rural Market Oriented	Pakistan: Peri-urban	Pakistan: Comercial Urban	Commercial: Italy	Intensive: Egypt
Age at Puberty	34.2 mo	35.6mo	33.7mo	27.6mo	17-20mo	n/a
Calving Interval	18.4mo	17.2mo	16.9mo	15.2mo	13.3mo (400) days	13.5mo
Use of AI	15%	14.70%	33.40%	23.10%	5%	n/a

In female buffalos, estrus behaviors such as homosexual activity are rare compared to *Bos taurus* species making heat detection significantly more challenging.^{25,26} While subtle signs of heat may include clear mucus, restlessness, frequent voiding of small amounts of urine, bellowing and swelling of the vulvar lips, 100% of females in estrus will exhibit standing heat. When attempting to use natural estrus for artificial insemination (AI), the use of a teaser animal (buffalo male with penile deviation and vasectomy or androgenized female) with a chin-ball marker device is necessary to identify females in estrus. The ideal time to inseminate is six to 18 hours after the start of estrus as ovulation takes place ten to 16 hours after the end of heat.⁴⁰

Estrus duration can range between 10 and 20 hours in normally cycling buffalos during the breeding season, although the period of courtship or interest showed by the male has been reported to range anywhere from nine to 68 hours.²⁰ The luteinizing hormone (LH) surge typically occurs between one and 12 hours after the start of estrus and ovulation occurs between 26 and 33 hours after the LH surge.²⁹ Due to the absence of consistent signs of behavioral estrus and variability in time of ovulation in relation to estrus, AI at observed natural heats is of debatable value in buffalo.

Post partum anestrous and prolonged calving interval. Gestation length in river buffalo averages 310 days while swamp buffalo may gestate up to 330 days; relatively long when compared to the Holstein cows.¹⁸ Buffalo embryos enter the uterus at four to five days after ovulation and may hatch as early as day 5; much earlier than in *Bos taurus*.²⁸ Compact morulae are observed five days after estrus with blastocysts typically developing by day 6.²⁹ Circulating progesterone levels ≥ 1.5 ng/ml, an indication of a functional corpus luteum (CL), occur at day 6 of the estrous cycle. Embryonic development and CL formation in the buffalo are thus more tightly coupled than in cattle. Research suggests a positive correlation between pregnancy rates and circulating progesterone levels on day 5 after ovulation. The use of exogenous hormonal protocols to support early embryonic development is currently being investigated.²⁹

Long calving intervals and postpartum anestrous are major causes of economic loss to buffalo breeders. Based on anatomical features, the uterus of the buffalo is completely involuted by 45 days postpartum.³⁰ Studies in Egypt, India, and Pakistan demonstrated that only 34-49% of buffaloes showed estrus during the first 90 days after calving and 31-42% remained anestrous for more than 150 days.³⁰ Several factors inherent to traditional farming practices negatively affect return to cyclicity. There is sufficient evidence to conclude that the presence of a suckling calf significantly prolongs the period of postpartum anestrous.³⁰ On the intensively managed dairy farms of Italy, it is common practice to remove calves from their dam at one day of age. This early weaning results in a calving interval of 400-430 days as compared to an average range of 480-600 days found in many areas of rural India.^{12,20}

Studies carried out in postpartum anestrous cows have demonstrated that progesterone treatment stimulates an increase in LH pulse frequency resulting in greater follicular fluid and circulating concentrations of estradiol and increased numbers of receptors for LH in granulosa and theca cells as compared to untreated animals.¹⁷ Baruselli et al¹⁷ found the addition of a progesterone-releasing implant (CIDR) combined with a fixed time AI protocol was successful in combating the depressive effects of seasonality and could also be used in the postpartum anestrous period to hasten a return to cyclicity.¹⁷ The following protocol was used in postpartum females with a 50% pregnancy rate at first insemination:

- Day 0: CIDR plus 2.0 mg estradiol benzoate im
- Day 9: CIDR is removed and prostaglandin F₂ α and 500 IU equine chorionic gonadotropin (eCG) im
- Day 11: 1500 IU eCG with timed AI 16 hours later

Complications of pregnancy. Prepartum cervico-vaginal prolapse is hereditary and eradication may be obtained by removing predisposed females from breeding stock. Uterine torsion is the most common cause of dystocia (70%) in the buffalo. Use of Sharma's detorsion method (rolling of the dam with external fixation of the uterus) and anti-stress measures increase survival rates in cases presented within 36 hours.³¹ In a recent study, most cases of uterine torsion were postcervical torsion (85.7%; the fold palpated caudal to the cervix) and only a few cases were precervical torsion (14.3%; the fold palpated cranial to the cervix).³² Traditionally, many farmers associate uterine torsion with wallowing behavior during late gestation.

Environmental factors

Seasonality. Buffalos are polyestrous animals capable of breeding throughout the year but a distinct seasonal reproductive pattern has been noted in several countries.¹⁸ When buffalo are bred in non-equatorial regions, they demonstrate reproductive behavior that is positively influenced by a decreasing photoperiod and is mediated by melatonin secretions.^{17,18} The endocrine profile of a buffalo

during summer anestrus is characterized by low plasma concentrations of gonadotropic and gonadal hormones and high levels of prolactin leading to reduced LH pulse frequency, poor follicle maturation and decreased estradiol production.^{12,33} Heat, humidity and availability of forage are the main contributing factors to lack of cyclicity and poor fertility in equatorial regions.¹³

Heat stress. Buffalos are adapted to hot, humid, muddy terrains but exhibit fewer physiological adaptations to extreme heat from direct solar radiation than other cattle breeds.²⁴ The black color of buffalo skin combined with only one-sixth the density of sweat glands in cattle skin, contribute to the buffalo's poor ability to dissipate heat through sweating.¹² Under natural conditions, buffalos prefer to wallow in water rather than seeking shade during periods of intense heat. Management systems to prevent the deleterious effects of heat stress such as access to wallowing ponds or sprinkler systems have been shown to have a positive effect on dry matter intake, milk production and fertility.²⁴

Bull effect. The presence of bulls in a herd has been shown to have a biostimulatory effect on ovarian activity of buffalo cows.²⁰ In order to combat the seasonal depression on cyclicity, Italian farmers have developed a management practice technique termed out-of-breeding-season-mating (OBSM), which takes advantage of the bull's biostimulatory effect. This is especially important in Italy where demand for milk is highest during a period of the year when calving is less frequent.³⁴ In this technique (Figure), bulls are removed from the herd in October (fall) and returned between March and the end of September (spring-summer) so that most calvings occur between the end of January and the beginning of August (spring-summer).

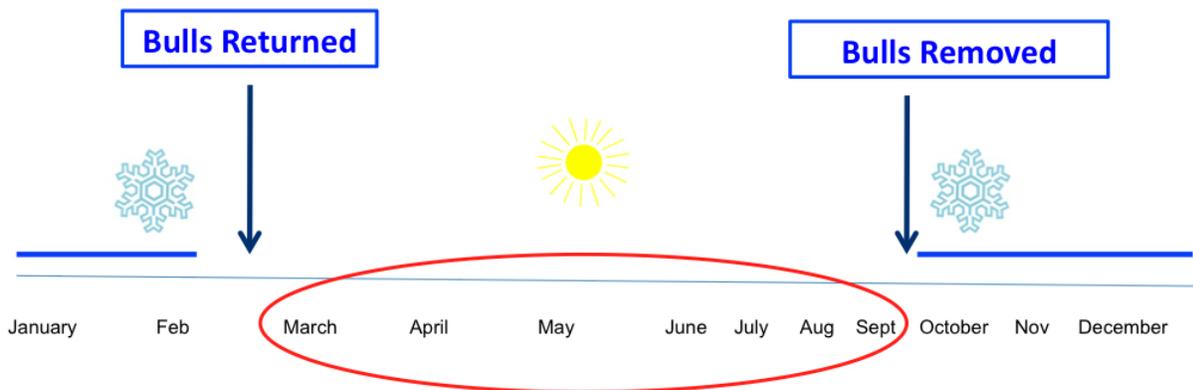


Figure: Out of breeding season mating technique implemented in Italy to combat the seasonal depression on reproduction.

In a 2010 study by Gokuldas et al.³⁵ the authors were able to show that bull exposure during the postpartum period accelerates resumption of ovarian cyclicity, reduces incidence of silent ovulation and enhances first service AI conception rates (Table 3).

Table 3. Comparison of reproductive parameters of female buffalos when exposed to vasectomized bull vs. when isolated from the bull between 40 and 90 days postpartum.³⁵

Reproductive parameter	Exposure to vasectomised bull from 40 th to 90 th day post-partum	Isolation from the bull
Resumption of ovarian cyclicity	47 ± 2.58 days	56 ± 2.37 days
Observation of behavioral estrus	57 ± 3.61 days	71 ± 5.13 days
Conception by 60 days post-partum (AI)	54%	15%
First service AI conception rate	100%	37.50%

Nutrition. When heifers are exposed to intensive management and feeding systems, the age at puberty can be drastically reduced from those recorded at the rural farm level. When 30 heifers in Italy received 4.5-5.5 milk forage units (MFU)/day, all achieved cyclicity before 20 months of age at a body weight of 421 kg or less. On the other hand, when heifers received 3.7-4.4 MFU/day, only 24% reached puberty by 20 months.³⁶ These trials highlight the importance of a proper feeding system and early weight gain to hasten puberty and thus increase the productive life span of a buffalo.

Body condition score (BCS) and overall plane of nutrition are extremely important factors affecting reproductive efficiency. Baruselli et al¹⁷ reported earlier first postpartum estrus in buffaloes (49±1 day) with BCS of 3.5 or higher than in those with a BCS of 3.5 or lower (77±1 day). Adequate nutrition is essential for not only the onset of sexual maturity and cyclicity but also a timely return to normal cyclicity after parturition.³⁰

In a recent study, buffalo heifers undergoing regular estrous cycles were randomly assigned to high energy diet group (HE; 5.8 MFU/day) or a low energy diet group (LE; 3.6 MFU/day). Metabolic substrates and reproductive hormones were monitored weekly over a period of 19 weeks. Results showed no significant difference in levels of circulating reproductive hormones between the two groups. Heifers fed the low energy diet had a negative calculated daily energy provision yet were able to maintain body weight and reproductive activity. It may be concluded that buffalos have the capacity to undergo metabolic adjustments and reduce their energy requirements when dietary energy is scarce which may explain why buffalos have the ability to stay productive in environments unsuitable for other ruminants.³⁷

Infertility and repeat breeding. Clinical features of repeat breeding vary across the world and production systems. Kishore et al established several infertility camps to examine features of repeat breeding in the Chitwan District of Nepal. Of 85 buffalo presented to the camp, 51 (60%) were heifers with 29% being in poor body condition; 46% of the animals presented had a functioning CL; and 51% had a dominant follicle, indicating that about 50% of the animals had normal ovarian function. Twenty percent of the animals were diagnosed with cervicitis, while only a few cases of endometritis were observed. Animals were given three weeks of vitamin/mineral supplement and those with cervicitis were treated by washing the vagina with Lugol's solution. Animals with a CL were injected with prostaglandin F2α (PGF) and those with a dominant follicle were given a dose of gonadotropin releasing hormone (GnRH). Females responded well to hormonal treatment and vitamin supplementation; at the end of 6

months, 100% of the females initially presented to the infertility camp were pregnant.³⁸ One might infer that rural farmers could benefit greatly from education on proper nutrition of heifers, maintenance of body condition score and proper heat detection methods in order to decrease incidence of cervicitis.

Conclusions

The reputation of water buffalos as having a low reproductive potential appears to be highly dependent on inefficient management practices and environmental factors rather than on their natural physiology. Puberty can be hastened with highly managed feeding programs to encourage rapid weight gain. Systems used for estrus detection in cows cannot be directly applied to buffalos but the use of a teaser bull can accurately identify females in standing heat as well as impart a biostimulatory effect on cyclicity. By ensuring animals are kept in good body condition and by removing sucking calves at one day of age, a return to cyclicity by 60 to 90 days postpartum can be reliably achieved. OvSynch and CIDRsynch protocols developed for use in cattle can be applied to the buffalo with pregnancy rates comparable to or exceeding those in cattle despite the depressive effects of seasonality on buffalo fertility.

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