Reproductive Performance of Water Buffalo Cows: A Review of Affecting Factors

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Abstract: This article aims to review both the economic impact of reproductive failures on the profitability of water buffalo systems and the effect of different factors on the reproductive performance of water buffaloes. Besides, an overview of various non-hormonal alternatives to improve reproductive performance is made. The optimal reproductive efficiency in water buffaloes implies calving to conception interval around 90 days to reach a calving interval of 400 days, with longer calving intervals having a negative impact on profitability. Reproductive efficiency is the consequence of the interaction of genetic and non-genetic factors, and the recognition of these factors by analyzing the reproductive information must be a priority. Although each factor's impact can be of greater or lesser magnitude depending on the conditions of each herd, some factors like nutrition, milk yield, body condition score, negative energy balance, parity, bull presence, low estrus intensity, and season can be considered high-impact factors. Not all factors are common among farms; therefore each farm must implement a program for the identification, control, and prevention of reproductive problems, especially during early lactation, to prevent a long anestrus; and when artificial insemination is used, so that it is done at the correct time with respect to the beginning of estrus to enhance fertility.

Keywords: Parity, season, BCS, energy balance, milk yield, silent estrus, anestrus.

1. INTRODUCTION

The world population of buffaloes (Bubalus bubalis) has increased steadily, and there is currently more than 200 million head [1,2]. In the American continent, the water buffalo population has increased by 26.4% between 2005 and 2016 [1], exceeding 5 million head [3]. This dynamic may be due to the better adaptation of the species to the hot and humid climate of the tropical areas at the north of the Equator compared to cattle of European origin and to the higher market price of their milk, which contributes to higher profitability of buffalo milk production [4]. In Venezuela, buffalo milk's price may be 40% higher than that of cow's milk, while in Italy, it can be between two and three times greater [5,6]. However, to maximize this profitability, it is necessary to achieve good reproductive performance by controlling the duration of the calving interval (CI) [7,8], whose economic optimum is around 400 days [7,9,10].

The CI depends on the calving to conception interval and has a correlation of 0.90-0.99 with this parameter [11,12]. In turn, the calving to conception interval is regulated by the voluntary waiting period, the calving to first service interval, and fertility, which will be the factors to control to reach the optimal CI [13]. A longer interval to first estrus or mount and a high incidence of anestrus [14-17] could be the leading causes of long CI observed in different studies [10,11,18,19]. However, despite this situation, in buffalo farms managed under natural service, the use of other parameters in addition to the CI remains scarce. This implies a limitation to make an accurate diagnosis of the herd's reproductive situation, compromising the identification of risk factors to reproductive failures and limiting alternative solutions. The CI is not a very useful

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parameter and does not show the herd's current reproductive situation, since it can only be calculated in the females calving again, excluding the nulliparous and first calving; and those to which it is decided to not service [13]. The CI is a historical parameter that identifies the reproductive problem late and therefore hinders its prevention and early correction.

Obtaining an interval to the conception of 90 days and consequently, a CI close to 400 days must be a priority for farmers, and this depends on management conditions and reproductive practices [20,21]. Several factors influence the achievement of these reproductive goals. Therefore, the objectives of this article are to review the impact of a long calving interval on the profitability of buffalo production systems and how different factors affect the reproductive performance of water buffaloes. Additionally, a review of non-hormonal alternatives to improve the reproductive performance of water buffaloes is presented.

2. ECONOMIC IMPACT OF A LONG CALVING INTERVAL

Reproductive performance has the most significant impact on system profitability [8]. Optimum CI is around 400 days [7,9,10], and long CIs involve losses due to decreased milk production per year, an increase of non-productive days, feeding costs, delayed replacement of animals, sale of potentially productive animals, and a delay in the genetic progress of herds. Economic losses generated by anestrus were calculated at 5.53 USD/day/buffalo [22]. Khan *et al.* [10] reported a decrease in economic return between 24 and 27% for water buffaloes with longer calving to conception interval; while the increase in the CI caused a decline in milk production of 2.1 kg per day when it increased from 365 to 635 days [9] and a negative correlation between CI and milk production per day of CI has been reported [18]. More recently, it was determined that the cost of each additional day of CI after 365 days, was 6.07 USD [23] and that each additional day over the average CI implies a decrease in earnings of 1.87 USD [24]. However, it is necessary to evaluate how reproductive strategies, like out-of-breeding mating strategy, a practice to change the calving calendar, and increases milk offer during months with long photoperiod when water buffaloes have low reproductive activity [69]**,** but this reproductive strategy produces a decrease of fertility and an increase of calving interval, affecting the economic performance of water buffalo systems.

3. FACTORS AFFECTING THE REPRODUCTIVE PERFORMANCE OF WATER BUFFALO COWS

3.1. Genotype

The genotype has an important effect on the reproductive performance of buffaloes, contributing 77.61% in the total variation of CI [25], which coincides with Kumar *et al.* [26], who suggest that, although more research is needed, genetic factors could affect the variation of postpartum anestrus period. Sanker *et al.* [25] observed that Murrah buffaloes had a shorter CI (424.32±2.60 days, $P < 0.05$) compared to Diara buffaloes (464.21±2.57) or those genetically not described (462.19±2.55 days). Christa Charlini and Sinniah [27] observed significant differences in the CI between Nili-Ravi and Surti buffaloes. In Bhutan, Murrah crossbred buffaloes reached the first calving younger and achieved a shorter first CI than local buffaloes of genotype not described [28]. In Venezuela, there was no effect of the breed on the calving to first service interval. However, it was observed in the calving to conception interval and CI, with buffaloes of undetermined genotype presenting a better performance (102.68±3.32 days and 427.18±13.57 days, respectively) compared to those predominantly Murrah (132.91±2.35 days and 445.08±8.09 days, respectively) or the Mediterranean (137.72±11.65 days and 452.01±8.38 days, respectively) [29]. Recently, it has been observed that F1 Italian x Egyptian or ¾ Egyptian ¼ Italian buffaloes, had a lower incidence of calvings with difficulty and needing veterinary assistance and lower incidence of stillbirths compared to those of pure Egyptian, this could be related to the higher birthweight of pure Egyptian calves or the absence of genetic selection to improve the easy calving; in addition, it was observed than pure Egyptian buffaloes had lower first service fertility than ¾ Egyptian ¼ Italian buffaloes [30]. The breed affected the age at the first calving, with Mediterranean buffaloes being younger (37.81±0.35 months, $P < 0.05$) than Murrah buffaloes (39.16±0.26 months) or those with undetermined genotype (40.35±0.72 months) [29]. Differences in reproductive efficiency between genotypes could be due to the difference in adaptation capacities to different environments and the different genetic merit to milk yield [30,31]. F1 Italian/Egyptian, which produced more milk at 305 days than the ¾ Egyptian ¼ Italian or pure Egyptian buffaloes, had a longer interval to conception and more services per conception [30].

Some genes have been associated with the reproductive efficiency of buffaloes. The CC, AA and

GG genotypes of the CYP191A gene, which encode the cytochrome P450 aromatase enzyme that regulates estrogen synthesis, and the C, A and G alleles, are associated with a higher risk of anestrus [32]. Moreover, haplotypes containing two or three of these alleles are related to low levels of estrogen and antioxidant enzymes in blood and at a low expression of the CYP191A, alpha estrogen receptor, and glutathione peroxidase 3 enzyme genes in ovarian tissue [32]. Additionally, the GG genotype of the leptin gene was associated with a higher number of services per conception (3.67, $P = 0.03$) compared to the AA (2.19) and AG (2.65) genotypes, and this could be related to the fact of that buffalo with the GG genotype gave birth to heavier calves [33]. Also, de Camargo *et al.* [34] identified genes related to the age at first calving, calving interval, services per conception, and open days, while Li *et al.* [35] identified 25 genes associated with follicle development.

3.2. Age at First Calving

Age at first calving has an important impact on the profitability of farms and is a good indicator of the reproductive performance of the herd [36]. The costs related to the management of the females can amount to up to 20% of the total costs of the farm [37]. Therefore, getting females to reach their first calving younger should be a priority given that it allowed for reduced costs related to feeding and increased the income from milk and calf production [38]. In Venezuela, 38.9 ± 0.51 months of age at first calving was recently reported [29]. However, there is little information in the scientific literature on the effect of age at first calving on the reproductive performance of water buffaloes. Zicarelli [39] observed that the older buffaloes at first calving had a longer CI. Verma *et al*. [36] observed that the youngest buffaloes at first calving (34.65 months) had a 31.85 days shorter service period and 44.2 days less in CI, than the older buffaloes at the first calving (54.21 months), although, these differences were not statistically significant. Reduced age at first calving depends on genetic selection and diet that decreases the age at puberty and allows reaching the weight for incorporation into the mating program at a younger age. It was observed in Italy that the age at first calving decreases one month every five years [39]. At first calving, age was 28-32 months when buffalo heifers received a diet with 1700 kcal NEL and 12-13.5% of crude protein and [39]. Sabia *et al.* [40] reported a negative correlation between daily weight gain and age at puberty ($r = -0.299$, $P < 0.01$). Hussein and Abdel-Raheem [41] subjected a group of heifers to two diets and observed that those with the highest feeding level reached the first service younger (30.8±1.6 months), heavier (340.1±3.5 kg) and had higher fertility (87.8%) than those managed in a low feeding plane (35.3±1.1 months; 312.4±4.2 kg and 10%, respectively).

3.3. Calf Sex

Christa Charlini and Sinniah [27] did not observe a significant effect of calf sex on the CI; however, in Venezuela, Montiel Urdaneta [42] reported longer calving to conception interval and CI for buffaloes whose calves were male (71.84 days and 388.22 days respectively, $P < 0.01$) compared to those with female calves (54.13 days and 370.04 days respectively). Khan *et al.* [43] observed that both cows and buffaloes with male calves had longer calving to conception interval, and this coincides with Kantharaja *et al.* [44] who observed that buffaloes with male calves had longer calving to first service interval and calving to conception interval. Amjad *et al.* [45] observed that buffaloes with male calves had a higher incidence of reproductive disorders (dystocia, fetal membrane retention, and uterine prolapse), and this was related to the higher weight of the males.

3.4. Suckling

In several species, suckling delays the onset of postpartum reproductive activity. El-Fouly *et al*. [46] reported that buffaloes who nursed their calves had the first postpartum ovulation at 87 days, while those weaned ovulated for the first time at 51.6 days, the delay being longer in primiparous buffaloes (66 days) than in multiparous (45 days). Suckling caused a decrease in the percentage of buffaloes that showed estrus in the first 60 days postpartum, with calving to first estrus interval of 131.5 days for buffaloes suckling and 77.9 for weaned, with a higher negative impact on primiparous. Qureshi and Ahmad [14] observed a positive correlation between the days of duration suckling period and the interval at the first ovulation ($r = 0.19$, $P < 0.01$) and the first estrus ($r = 0.23$, $P < 0.01$), with buffaloes suckling for a maximum of 30 days having a shorter interval to the first ovulation and estrus than those with longer suckling period. Rijasnaz *et al.* [47] observed that suckling reduced cyclicity and pregnancy in the first 150 days postpartum, causing a longer interval to estrus and conception (128 ± 9.94 days and 143.50 ± 5.50 days, respectively) compared to buffaloes that did not suckle their calves (53.29 ± 7.52 days and 75.67 ± 10.16 days, respectively, $P < 0.05$).

Furthermore, Kantharaja *et al.* [44] reported that weaned buffaloes had a shorter interval to first estrus (62.50 ± 1.50 days) compared to those that suckled their calves (99.25 ± 9.15 days, $P < 0.05$). The mechanism by which suckling delays the onset of reproductive activity is complex and implies decreased LH secretion and, consequently, a delayed follicular development [48]. These adverse effects could be mediated by cortisol and can occur even with the offspring's mere presence, without the need for physical contact [49].

3.5. Parity

Parity has a determining effect on reproductive efficiency [11,12,17-19,25,26,50-54]. Sosa *et al.* [16] observed that the incidence of anestrus was higher in first calving water buffaloes (21.59%) compared with those of two (11.63%) and those of three or more calvings (9.97%) and this implies a lengthening of the calving to first estrus and calving to conception intervals. These results coincide with those observed in Venezuela, where the primiparous water buffalo had an interval at first mount 30 days longer than multiparous. They were also less likely to be mounted for the first time at both 60 and 100 days postpartum [17]. First-calving buffaloes had a higher rate of stillbirths [55] and lower pregnancy rates than multiparous [54], and the latter agrees with the longer CI observed in primiparous. Primiparous water buffaloes had a CI of 43 and 79 days [18] and 35.3 and 55.5 days [19] longer than those with two and three or more calvings, respectively ($P < 0.05$). The lengthening of the CI in primiparous buffaloes could be a consequence of a longer anestrus period, a higher number of open days and services per conception [56,57]. This could be related to the level of milk yield [19], and probably with the fact that primiparous water buffaloes have a higher negative energy balance and higher levels of stress, which can affect the onset of postpartum reproductive activity, without ruling out inappropriate management [58-60]. Additionally, parity affects the pregnancy rate after the Ovsynch protocol and fixed-time artificial insemination. Primiparous buffaloes had lower pregnancy rate (20.8% and 30.8%) than multiparous (61.7 and 72.4%, $P < 0.05$) [61].

3.6. Season of Calving

Water buffaloes are a short-day species, and season of calving affects their reproductive performance [17,19,52,62-66]. Buffalo cows calving in months of short photoperiod has an early onset of reproductive activity and shorter calving to conception interval and, therefore, a shorter CI than those that calving during long photoperiod season [53,63]. Buffalo cows calving during the long photoperiod season (March-August) have a more extended period of anestrus and are less likely to have their first mount before 60 and 100 days postpartum (0.134 and 0.312 respectively, $P < 0.05$) compared to those calving during the short photoperiod season (September-February; 0.354 and 0.536, respectively), when mounts were more frequent (70.6%) [17]. Rossi *et al.* [67] observed that buffaloes calving during October-December, when daylight decreases, had a higher probability of pregnancy after an Ovsynch protocol and fixed-time artificial insemination than those calving during the rest of the year. Buffalo cows ovulating during the long photoperiod season, have a higher rate of silent estrus, smaller follicles, lower estrogen levels, and irregular cycles, as well as lower levels of progesterone, lower embryo quality, higher early and late embryo death, a higher rate of repeat breeding and lower fertility [68-71]. In Venezuela, buffaloes calving between December and March or April and July had a longer CI (452.2 and 450.6 days, respectively) than those calving between August and November (435.6 days, $P < 0.05$) [19]. Recently, lower pregnancy rates have been reported during spring/summer than during autumn/winter [54,72,73]. Some of these consequences are related to the increase in prolactin levels observed during the long photoperiod season, which are related to a decrease in gonadotropin secretion, a decrease in the follicular steroidogenic capacity, generating anestrus and infertility [74,75]. Additionally, during the long photoperiod season, the corpus luteum receives lower blood flow, which compromises its ability to produce progesterone, which is related to embryos of smaller size, which are more prone to embryonic death [69,76-80]. The season did not affect buffalo heifer's fertility, probably because they have higher levels of melatonin during the hours of light in comparison with buffaloes, which have previously had a calf [64, 202]. The better reproductive efficiency observed in the short photoperiod season could be related to the decrease in temperature, relative humidity, and temperature-humidity index (THI) [53,80]. Dash *et al.* [81] observed a negative relationship between THI and pregnancy rate in Murrah buffaloes, with a significant decrease in this parameter when THI was higher than 75. A THI > 80, caused a significant reduction in conception rate at first service compared to a THI < 80, and consequently this reduction in fertility led to a considerable increase in the calving interval [31,54]. However, the effect of high THI on reproductive performance seems to depend on the breed [31]. Nevertheless, the effect of high THI is questionable because, in Italy, fertility improves during July and September, when temperature and THI are higher, and this highlights the role of photoperiod as the main regulator of seasonality [64].

3.7. Presence and Health of Bulls

As part of the buffalo management system, the continuous presence of a bull improves reproductive efficiency [26,82]. The presence of vasectomized bulls increased the rate of buffalo in estrus, estrus intensity, the rate of ovulatory estrus, and the pregnancy rate in buffaloes in both natural and induced estrus; additionally, these effects were higher during the season of low reproductive activity [83]. The bull's presence between days 40 and 90 postpartum shortened the interval to estrus and ovulation, reduced the percentage of silent estrus, increased progesterone levels, increased fertility at first service, and the percentage of pregnancy at 60 days postpartum [84]. The continuous presence of bulls from day five postpartum, shortened the interval to the first estrus (55.2 ± 0.78 days, $P < 0.05$) compared to buffaloes in which the bull presence was intermittent (66.71 ± 0.93 days) or to those buffalo cows exposed to bull urine and feces (68.25±0.87 days) or control (68.57±0.93 days) [85]. During the season of low reproductive activity in Pakistan, the continuous or partial presence of the bull increased the percentage of buffalo in estrus (60% and 40% respectively) and the rate of pregnancy at 60 days post-service (40% and 20%, respectively) in comparison with those unexposed (5% and 0%) [86]. However, to observe a shortening of the interval to estrus, a bull's continuous presence was necessary [86]. Recently it was determined that the presence of a bull at less than 20 feet shortened the onset of postpartum ovarian activity [26]. Biostimulation's effects could be due to the action of bull pheromones acting on the hypothalamic-pituitary axis to promote the secretion of LH and consequently follicular activity and ovulation and the formation of a competent corpus luteum that secretes higher levels of progesterone favoring the establishment of pregnancy [84].

Different hereditary, congenital, and infectious conditions affect the reproductive potential of buffalo bulls and, consequently, the reproductive performance of the herds [87-89]. Positive correlations were observed between body weight and testosterone levels ($r = 0.58$; $P < 0.0001$) and between testosterone and scrotal circumference ($r = 0.16$; $P < 0.02$) [90], and low testosterone and nitric oxide levels were associated with poor sexual behavior [91]. Age and season can affect sexual behavior and sperm quality. Adult bulls (4-8 years) had higher body weight, scrotal circumference, and libido than those younger or older bulls; and theses parameters were higher during the peak breeding season (September-November) [92], and similarly, quality of thawed semen was higher in adult buffalo bulls than young or old bulls, and during the peak than the low breeding season [93]. Old bulls (13.6±1.0 years) had worse sperm quality (lower ejaculate volume, lower viability, and lower DNA integrity) than younger bulls (3.4±0.3 years) [94]. Scrotal circumference and sperm quality are related to nutrition. Bodyweight correlated positively with scrotal circumference, and this with sperm concentration, while intake of crude protein was correlated positively with sperm motility and lipid intake with sperm motility and sperm membrane integrity [95]. Kumar and Srivastava [96] observed a positive correlation between body weight and scrotal circumference, testicular volume, sperm concentration, sperm motility, viability, while a negative correlation with sperm abnormalities was observed.

Nevertheless, Yadav *et al.* [97] observed than a higher rump fat thickness was associated with a high percent of sperm abnormalities. In addition, it was observed that rump fat thickness was higher in low libido bulls (7.29±0.93 mm) than in bulls with a high libido (5.29±0.62 mm, $P < 0.05$) [98]. Also, the reproductive potential of younger and submissive bulls could be affected by the aggressive behavior of dominant bulls [99]. Moreover, aggressive behavior is the main reason to cull bulls [100]. Scientific literature available about these subjects is scarce, and more research is warranted.

3.8. Milk Yield

Few studies have evaluated the relationship between milk yield and reproductive efficiency in water buffalo. El-Belely *et al.* [101] suggest that milk yield could have a negative effect on fertility. Qureshi and Ahmad [14] observed a positive correlation between milk yield and the calving to ovulation interval ($r = 0.31$, $P < 0.01$) and that although buffaloes with higher yield reached a higher conception rate at first service, they needed more days to uterine involution and to first ovulation postpartum. Buffaloes that produced more than 8 kg of milk per day had a more extended postpartum anestrus period (El-Fadaly [102]: 107±36 days; El-Azab *et al.* [103]: 76±25 days) than those producing less than 8 kg of milk per day (77±30 days and 56±24 days, respectively). Valsalan *et al*. [104] reported a 0.9% decrease in pregnancy for each increase of 100 kg of milk at 305 days, and El-Tarabany [54] observed that buffaloes with a daily production higher than 9 kg of milk, had a lower probability of pregnancy than those producing less than 7 kg of milk per day. Nava-Trujillo *et al.* [18], observed a positive correlation between the CI and milk yield ($r = 0.34983$, $P < 0.0001$) and with lactation length ($r = 0.67408$, $P < 0.0001$); and this coincides with Seno *et al.* [105], who reported a positive correlation between milk yield and the length of first CI. Moreover, de Camargo *et al.* [34] observed a positive genetic and phenotypic correlation between milk yield, kg of fat, kg of protein; % of fat, % of protein and somatic cell score with age at first calving, services per conception, open days and calving interval.

Some studies did not identify relationships between milk production and the reproductive performance of buffaloes [106-109]. Nevertheless, Vilela *et al.* [110] reported a positive correlation between milk yield and age at first calving and the CI. These results, together with previous reports [19,54,103], highlight the importance of the proper design of genetic programs to improve milk yield in buffaloes to avoid negative consequences on reproductive efficiency, as previously suggested [67].

The use of oxytocin is common to induce the letdown of milk with mechanical milking [111]. The use of oxytocin at the time of milking increased the calving to the first ovulation interval [14], and more recently a positive correlation has been observed between the use of oxytocin and the postpartum anestrus period [26], and this could be related to its effect on milk production [112] and oxidative stress [113]. Additionally, the use of oxytocin increases the incidence of reproductive problems such as follicular and luteal cysts, retention of placenta, anestrus, and repetition of services [114]. However, given the frequent use of oxytocin at milking and the few studies reporting a negative relationship between oxytocin use and reproductive performance of buffalo cows, more research is warranted to have a greater understanding of the possible effects of this hormone on the different reproductive events in buffaloes.

3.9. Body Condition Score and Negative Energy Balance

Body condition is a determining factor in the reproductive performance of buffaloes [115,116]. Buffaloes with an intermediate body condition at calving, had a shorter conception interval (128.3 days) than those over-conditioned (144.1 days) or very thin (165.5 days) [117] and this has been recently corroborated [118]. Buffalos with ovarian inactivity had lower body condition score than those with active ovaries (2.08±0.11 *vs*. 2.88±0.28) [119]; and a higher body condition during the first 90 days postpartum was related to a short interval to first estrus [120] and a higher body condition score at insemination was related with a larger ovulatory follicle, lower progesterone at estrus and a higher estrus intensity [121]. Water buffaloes with a BCS < 2.5 submitted to 7-CIDR Cosynch protocol had lower pregnancy rate in comparison with those with a BCS > 2.5 [122], and similar results were observed in water buffaloes treated with PGF2α, GnRH or a mix of vitamins, pregnancy rate was higher in buffaloes with a BCS between 2.5 and 3.5 [123]. A lower body condition score is more frequent in older buffalo cows, which could be associated with their lower reproductive performance [228]

After calving, buffalo cows enter in a period of negative energy balance characterized by a decrease in dry matter intake, with a corresponding reduction in weight, body condition score, glucose and insulin levels, and an increase in non-esterified fat acid (NEFAs) and beta-hydroxybutyrate (BHB) levels [124-130]. Deka *et al.* [126] observed a loss in dry matter intake of 22.7% on the day of calving with respect to pre-calving intake (9.91 kg *vs*. 12.83 kg, $P < 0.001$). Bhalaru *et al.* [131] observed a loss of weight during the first five months of lactation that varied between 1.13% and 7.65% of the calving weight, and this loss was related to the weight of the buffalo at calving, with those heaviest losing more weight, it was also observed that the higher intensity of loss occurred during the first postpartum month. Infascelli *et al*. [132] observed that the percentage of weight loss during the first 60 days postpartum was related to parity, being higher in multiparous (3.8%) than in primiparous (1.55%). Abayawansa *et al.* [125] reported a loss of weight during the first nine weeks postpartum of 14.7 and 14.5% for buffalo calving in winter and summer respectively; and more recently Reddy *et al.* [127] observed a decrease in body weight from 625.7 ± 3.67 kg at calving to 551.55 ± 4.18 kg at day 90 postpartum, which represents a decrease of 11.85%. Loss of weight and body condition as a result of the decrease in dry matter intake and the increase in energy expenditure for milk synthesis results from the mobilization of fat reserves accumulated during the dry period, with the corresponding increase of NEFAs and BHB [126,127,133-139].

The intensity of negative energy balance is related to the level of milk yield [127,128,132,139,140], and this affects the reproductive performance of buffaloes [125,141]. In both, primiparous and multiparous buffaloes with a milk yield > 5000 kg, body weight loss was higher (4.2±4.6 and 5.6±5.1% respectively) in comparison with those producing < 5000 kg (+3.06±7.6% and 2.0±4.0% respectively) [140]. Buffaloes losing more than 10% of weight immediately after calving, needed more services per conception (3.38 ± 0.7) and had a longer conception interval (297±32.54 days) than those losing less than 10% of their weight (2.40 ± 0.81 services and 207.6±46.46 days, respectively) [141]. Huseein *et al*. [142] observed that buffaloes that became pregnant in the first 75 days postpartum had a higher weight and body condition score during that period and a shortest first service interval (65.71±11.31, P = 0.03) that buffaloes that were not pregnant (93.20±9.81), the latter also presented lower body condition and lower weight from day 24 postpartum. Banu *et al.* [143] observed that buffaloes with lower body condition had a longer interval to the first estrus; while Senosy and Hussein [144] observed that both weight, during weeks 4 and 9 postpartum, and body condition, during weeks 4 and 5 postpartum, were higher in pregnant buffaloes than in non-pregnant ones.

The mechanism through which negative energy balance affects reproductive performance is complex and has not been well studied in water buffaloes. Elsayed *et al.* [145] observed that buffaloes with delayed postpartum ovarian activity (> 45 days postpartum) and consequently with a longer conception interval (170.50±16.20), had lower body condition and lower levels of IGF-I, glucose, albumin, SOD and GSH, as well as higher levels of NEFAs and BHB during postpartum than those with an early restart of ovarian activity (< 45 days postpartum) and a shorter interval to conception (121.60±15.00). In addition, the levels of NEFAs and BHB were positively correlated with the interval to first ovulation postpartum, while the body condition score and levels of IGF-I, albumin, SOD, and GSH were negatively correlated [145]. These findings indicate that restriction of dry matter intake and the increase of energy demand of milk synthesis during the postpartum period leads to low glucose levels and consequently insulin, that affects the expression of receptors for growth hormone (GH) in the liver, this implies a low hepatic synthesis of IGF-I and an increase in GH levels and excessive lipolysis. This scenario can affect gonadotropin secretion, follicular growth, and expression of estrus and progesterone levels, as observed in cows [146,147]. Moreover, the negative energy balance can compromise the health of buffaloes. A low body condition was a predisposing factor for the occurrence of endometritis [144], while buffaloes with metritis, endometritis, and mastitis had higher levels of NEFAs and BHB and lower levels of glucose and calcium than healthy buffaloes [148]. Moreover, the predisposition of water buffaloes to reproductive disorders during the early postpartum period could be related to the immunosuppressive state-observed [149]. At least in dairy cows, postpartum immunosuppression is related to negative energy balance [150].

In addition to energy, protein status and mineral deficiencies affect the reproductive performance of water buffaloes. The level of crude protein in the diet was positively correlated with urea in serum (r = 0.22, P < 0.01), and both were correlated with a longer interval to the first estrus and ovulation postpartum [151]. Campanile *et al*. [233] did not observe any adverse effect of high urea levels in the blood and vaginal mucus on the reproductive performance of Mediterranean buffaloes, but high blood ammonia could affect the recovery of postpartum body condition score. Nevertheless, it was observed than a level of urea ≥ 6.83 mmol/L was associated with a reduction in the probability of pregnancy, and buffaloes with lower blood urea had 2.6 times more probability of becoming pregnant [234]. Hypocuprosis is related to a lower level of progesterone, and 21% of hypocupremic buffaloes had ovarian inactivity, and this could be associated with the lower body condition score, higher level of oxidative stress and lower levels of ceruloplasmin observed in hypocupremic animals [152]. Hafez [153] observed that anestrus buffaloes had lower phosphorus levels and zinc, and a lower ratio of P:Ca and Zn:Cu than cyclic buffaloes.

3.10. Diseases

Several pathologies occurring during the periparturient period generate a reproductive efficiency decrease in water buffaloes. The occurrence of abortion, dystocia, retention of fetal membranes, metritis, endometritis, pyometra, anestrus, repeated breeder, prolapse, and stillborn decrease reproductive efficiency [55,68,154-156]. In addition, different infectious [157-166] and non-infectious [167] agents associated with abortions in cows also generate them in buffaloes; nevertheless, there are few reports about the reproductive consequences of these pathogens other than abortion in buffaloes. Leptospirosis has been associated with repeated service [168] and neosporosis with the occurrence of repeated service, early losses, and retention of fetal membranes in addition to abortions [166,169]. Water buffaloes infected with *Toxoplasma gondii* had longer calving to conception interval, while *Neospora caninum* and *Toxoplasma gonddi* co-infection was associated with embryo mortality and abortion [166]. Bovine viral diarrhea has been detected in 61.60% in buffaloes having reproductive problems compared to 8% in animals without difficulties [170], and their incidence varied according to the pathology: 62.86% in buffaloes with ovarian inactivity; 51.85% in buffaloes with endometritis; 60% in buffaloes with delayed puberty; 58.33% in repeated breeding buffaloes; 62.50% in those with retention of fetal membranes, and 66.67% in buffaloes that aborted [170].

Some traditionally non-reproductive infectious diseases affect the reproductive performance of water buffaloes. Clinical mastitis occurring before the first service increased the conception interval (148.79±12.66 *vs*. 76.1±2.89 days), being longer when mastitis occurred between the first service and pregnancy diagnosis (232.47±17.96 days, $P < 0.05$) [171]. More recently, it has been observed that both clinical and subclinical mastitis affect follicular function, reduce estradiol production and conception rate, being as low as 18.18% in the case of buffaloes with clinical mastitis [172]. The conception rate after estrus synchronization was lower at day 25 and 45 after insemination in buffaloes in clinical (28% and 16% respectively) and subclinical mastitis (55.56% and 44.45% respectively) compared to buffaloes without mastitis (69.57% and 60.87% respectively, $P < 0.05$). The impact was more significant when mastitis occurred between 15 days before and 30 days after insemination, and this could be a consequence of the reduction in the diameter of the corpus luteum and the production of progesterone observed in buffaloes with mastitis [173]. In addition, parasitic gastrointestinal infection (coccidia and nematodes) reduced pregnancy rate in buffaloes treated with PGF2α or with a mix of vitamins and minerals ($P < 0.05$) [123]. *Haematopinus tuberculatus* (pediculosis) caused a lengthening of calving to conception interval [174]. A previous report observed that in water buffaloes with pediculosis, ovarian inactivity was 50.25%, while in cyclic animals with pediculosis, progesterone was lower (1.63±0.27 *vs*. 2.89±0.17, $P < 0.01$). Additionally, water buffaloes with pediculosis had lower body condition score, higher oxidative stress, anemia, lymphopenia, eosinophilia, neutrophilia and monocytosis, and lower levels of Zn, Fe, and Se [175]. The prevalence of *Fasciola hepatica* detected by fecal examination and ELISA was higher in buffaloes with ovarian inactivity compared to cyclic buffaloes (28.9% and 38.9% *vs*. 4.7% and 6%, respectively). *Fasciola* positive buffaloes had higher progesterone levels in the follicular phase of the estrus cycle and different hematological alterations, lower antioxidant capacity, lower levels of serum copper, iron, and selenium [176].

3.11. Estrus Behavior, Ovarian Structures, and Hormonal Profiles: Relationship with Fertility

Water buffaloes have low-intensity estrus signs and a high incidence of estrus during the night and silent estrus [14,143,177,178]. Estrus has a duration that varies between 5 and 27 hours [179], although during the season of low reproductive activity, the variation is between 2 and 72 hours [180]. Frequent urination, redness, and vulvar edema, and decreased milk production are the most obvious signs [181,182]. The presence of mucus is inconstant, and 52.3% of the females did not present discharge [183], while the frequency of homosexual behavior is low (3.4%) [184], so that the best indicator of estrus is the bull's mount [185]. Additionally, between 10 and 63% of estrus were silent [83,186-189]. Both low intensity and silent estrus make it difficult to detect buffalo cows in estrus and compromise the success of artificial insemination programs under natural estrus. Banu *et al.* [143] observed a low rate of estrus detection (28%) with a higher number of estrus missed during the first 70 days postpartum and an increase of the interval to the first associated with the number of missed estrus. Buffaloes with intense signs of estrus had a higher conception rate (81.16%) in comparison with those with intermediate (60.47%) and weak signs of estrus (44.11%) [190]. Artificial insemination early (0 and 12 hours) or late (36 hours) according to the beginning of the estrus reduced the pregnancy rate in comparison to artificial insemination that was carried out 24 after estrus beginning (26%, 37%, 13%, and 53%, respectively; $P < 0.05$) [191]. The phenomenon of silent estrus seems to be related to a lower follicular production of estradiol, which depends on the follicular diameter. Awasthi *et al*. [187] observed that buffaloes with silent estrus had ovulatory follicles of smaller diameter (7.7±0.4 mm, $P < 0.05$) and with a lower growth rate (0.7 ± 0.02 mm/day, $P < 0.05$) than buffaloes with obvious estrus behavior (11.0±0.7 mm and 1.1±0.1 mm/day, respectively).

The preovulatory follicle diameter and estradiol levels and the corpus luteum diameter and progesterone levels, are related to fertility in water buffaloes. Pandey *et al*. [192,193] reported that buffaloes pregnant on day 40 post-ovulation had a preovulatory follicle of larger diameter and higher levels of estradiol at estrus than non-pregnant buffaloes. Furthermore, the conception rate was higher in buffaloes with higher preovulatory follicles [192], and this could be explained by the positive relationship between preovulatory follicle diameter, corpus luteum diameter, and progesterone levels [192,193]. Mansour *et al*. [173] observed positive correlations between pregnancy rate with corpus luteum diameter and progesterone concentrations at 2, 9, 12, 16, 21, and 25 days post-insemination.

Higher progesterone levels at day five [192,193], ten [78,80] and 12, 16, or 21 [192,193] post-service have been reported in pregnant buffaloes in comparison with non-pregnant; and this highlights the importance of an early increase in progesterone concentration on embryo development and the establishment of pregnancy, and this seems to be related to the diameter of the preovulatory follicle. Pregnant buffaloes whose ovulatory follicles were less than 12 mm had smaller corpus luteum and produced less progesterone at days 5, 12, and 16 post-ovulation compared to pregnant buffaloes with preovulatory follicles between 12 and 14 mm or 14 and 16 mm [193]. Progesterone's importance in the establishment and maintenance of pregnancy is evident during the long photoperiod season when the incidence of embryonic death is higher. In Mediterranean water buffaloes inseminated during the long photoperiod season, those who remained pregnant had higher progesterone concentrations between days 10 and 20 than buffaloes with embryo mortality at day 40 post-service [76]. Low progesterone levels retard embryo growth, and smaller embryos are more likely to suffer embryo mortality. Buffaloes that on day 25 had an embryo with a width < 2.7 mm, which also were shorter compared to embryos with a width > 2.7 mm (5.6 ± 0.4 mm *vs*. 9.0 ± 0.5 mm, $P < 0.01$), had lower progesterone levels at day 20 [78]. Besides, buffaloes that suffered embryonic mortality on day 45 had smaller embryos on day 25 compared to those that remained pregnant [78,79]

4. ALTERNATIVES TO IMPROVE THE REPRODUCTIVE PERFORMANCE OF WATER BUFFALOES

There are several alternatives to improve the reproductive performance of water buffaloes and to achieve a conception interval around 90 days; because experimental articles and literature reviews have been published especially regarding the hormonal control of the estrus cycle and ovulation to fixed-time artificial insemination [194-198], in this section some non-hormonal alternatives to improve the reproductive performance of water buffaloes are reviewed.

Feeding management during prepuberal development is critical. Achieving appropriate weight gain is essential for the buffalo to reach puberty, and first calving younger. This, in turn, has been related to a shorter first calving interval [39]. Recently, it was observed that buffalo calves feeding with milk ad libitum by four months has a lower age at first calving (20-26 months); while those receiving more reconstituted milk (150 kg vs. 105 kg) had the first calving six months earlier (28.5 vs. 34) [232]. The postweaning daily weight gain has a negative correlation with age at puberty ($r = -0.299$, $P < 0.01$) and a positive correlation with weight at puberty ($r = 0.548$, $P < 0.01$) [40]. Buffaloes with a diet formulated to gain 450 gr/day reached puberty at 24 months, while those with a diet formulated to gain 650 gr/day reached puberty at 21 months ($P < 0.05$) [199]. Hussein and Abdel-Raheem [41] observed that heifers kept with a high energy diet (8.63 kg/dry matter/day, 19.2 Mcal ME/day, 1.15 kg CP/day) initiated the reproductive activity youngest and heaviest and were more fertile than those maintained under a 50% dietary restriction (4.32 kg/dry matter/day, 9.51 Mcal ME/day and 0.58 kg CP/day). Incorporation of 100 gr/day of mineral mix to the diet of anestrus buffalo heifers (age 42.05±1.18 months) for four weeks increased the percentage of heifer showing estrus behavior (65% *vs*. 33%) [200]. Furthermore, because buffalo heifers are less sensitive to long photoperiod and exhibit less seasonal reproductive behavior [66,201-203], if proper nutritional management is implemented, it may be possible to incorporate heifers to calve during the start of the long photoperiod and increase milk supply during this season. However, exceptional reproductive management is necessary to avoid a long period of postpartum anestrus and infertility.

In postpartum buffaloes, it is necessary to improve the dry matter and energy intake to reduce the weight and body condition score loss to improve reproductive performance and diminish the incidence of diseases, emphasizing primiparous and buffaloes with high milk yield [19]. Proper grazing management is necessary since a high density of animals per hectare can diminish the supply of grass and compromise pregnancy [204]. Supplementation of primiparous water buffaloes with propylene glycol or calcium propionate during the pre and postpartum periods reduced the negative energy balance. It shortened the interval to first estrus (53.50 ± 3.69 and 48.50 ± 2.79 days respectively) and conception (63.33 ± 6.66 and 52.17 ± 2.70 days respectively) in comparison with control (82.33 ± 6.70 and 128.50 ± 15.39 days respectively) [205]. In addition, the inclusion of *S. cerevisiae* in the diet reduced fat mobilization [206], and in multiparous water buffaloes, it was observed by reduced days to the first estrus, improve fertility and reduce open days as a consequence of an improvement in the energy status [207]. Increased energy density in the diet using protected fat reduced the calving to conception interval [208], while supplementation with flaxseed reduced PFG2α secretion, increased progesterone level, and improved pregnancy rate after estrus synchronization [207]. Vitamin E, selenium and niacin has been observed to reduce postpartum anestrus [210,211] and oral administration of vitamins A, D3, and E from four weeks prepartum until the seventh week postpartum decreased the level of NEFAs at week 5 and 7 postpartum, improved the quality of vaginal mucus, decreased the incidence of subclinical endometritis and decreased the open days in comparison with the control group (97.50±15.75 *vs*. 152.1±24.45), all this was accompanied by decreases in oxidative stress of treated buffaloes [137]. The shortening of the dry period to < 60 days increased the capacity of adaptation of water buffaloes to postpartum metabolic and hormonal adjustments, decreasing the negative energy balance, meaning less of weight and BCS and lower levels of NEFAs and higher levels of glucose, and in consequence, a shortened interval to conception [138,212].

When natural service is used, adult bulls with similar age, size, and body weight (to minimize dominance/subordination), with high scrotal circumference, and in positive energy balance and with a positive qualification after the breeding soundness evaluation, must be selected. Balanced energy, protein, and mineral nutrition are necessary to improve the bulls reproductive performance in short photoperiod when estrus activity is higher, and during the long photoperiod season if bulls are introduced to reduce the seasonal anestrus by biostimulation. In young buffalo bulls, a specific mineral formulation designed for buffaloes increased scrotal circumference and sperm quality compared to those receiving a mineral mix specific to dairy and beef cattle [213]. Palm kernel cake increased testosterone secretion [90], while parental application of tonophosphan, oral zinc, or ascorbic acid increased scrotal circumference, testicular volume, reduced reaction time, improved sperm quality, and conception rate [214]. These could serve as supportive therapy to bulls with low libido or before the beginning of the reproductive season.

A preventive medicine program is also recommended to reduce the incidence of different bacterial and viral diseases, including those that have not been traditionally considered to impact the reproductive performance of water buffaloes (mastitis, hemoparasites, ectoparasites, and gastrointestinal parasites). Treatment of pediculosis with alphacypermethrin, especially during early lactation, improved milk yield, BCS, and shortened the calving to conception interval [174]. In addition, controlling the suckling period duration could reduce the duration of postpartum anestrus period and the incidence of this pathology and improve fertility. Weaning immediately after calving [215] or at day three postpartum [47] or the substitution of calves with a synthetic dummy calf [215] reduced postpartum anestrus, the calving to conception interval, and improved fertility and pregnancy rate. Given the low number of studies about the effect of weaning on the reproductive performance of buffalo cows and health of calves; the age at weaning and weaning system must be considered carefully because weaning could have a negative impact on calf health [216].

In those systems, whose main objective is milk production, the use of sexed semen to produce female calves, because their lower birth weight, could contribute to improving reproductive efficiency. A program of genetic selection for fertility must be implemented. Calving interval has a low heritability [12,105,217], and it is not a parameter to be considered a selection criterion. However, some authors suggest that despite having a low heritability, it should be a criterion to include in genetic programs due to its impact on buffalo production systems [8,24]. In addition, new phenotypes for better fertility could be used for selection. In dairy cows, selection based on estrus cyclicity, estrus expression, and absence of silent ovulation has been proposed to improve fertility

[218]. Considering that anestrus, low estrus intensity, and silent estrus are a significant problem in water buffaloes, selection by these phenotypes could be useful. In seasonal dairy cows, it was observed that selection for a shorter gestation length increased cow's chances to be mated early in the season and calve early [219], and heritability of gestation length in cattle range from 0.33 to 0.62 [235,236]. Given that gestation is longer than dairy cows in water buffaloes, selection by a negative breeding value to gestation length could be evaluated. Recently in two studies, a negative relationship between anogenital distance and pregnancy rate in dairy cows was observed [220,221]. This could be considered a new phenotype for genetic selection for higher fertility, and in dairy cows, the heritability of anogenital distance was 0.37±0.08 [222]. The selection of dairy cows for a higher body condition score improved health and fertility [223,224,225], and dairy cows with positive genetic merit to fertility had a higher dry matter intake and kept a higher body condition score [226]. These phenotypes are associated with a better adaptation to lactation, and their inclusion in the genetic program is important, especially when a higher milk yield is the central goal. This recent information about potential phenotypes, in addition to the identification of new candidate genes to improve reproductive performance warrants more research in water buffaloes.

Other strategies to improve reproduction could include better management of heat stress and estrus detection. Calving to conception interval was higher, and conception rate lower during months with higher THI [81]. Incorporation of swimming pools and other strategies to mitigate heat stress could improve reproductive performance [227,228]. In order to improve the detection of buffaloes in estrus and to carry out the insemination at the right moment to improve the fertility, more time for observation especially during the cooler part of the day and night hours [143, 229,230], the use of teaser bulls [83], or different devices for automatic detection of estrus [231] could be useful. However, if the farm's purpose is to keep a uniform milk supply throughout the year, it is necessary to incorporate technologies such as the synchronization of estrus and ovulation and fixed-time artificial insemination to change the calving calendar [64]

5. CONCLUSIONS

Optimal reproductive efficiency in water buffaloes implies calving to conception interval near 90 days to maximize herds profitability by decreasing the losses caused by reproductive failures and decreased milk yield. Achieving these goals should be based on an improvement of the information analyzed. That should include more than the dates of calving and dry-off to improve the evaluation of reproductive efficiency and the identification of risk factors for both failure and reproductive success. While, the factors that affect the achievement of reproductive goals are numerous and some factors act concomitantly; primiparity, calving in the long photoperiod season, high milk yield, low body condition and a negative energy balance, estrus detection, and presence of a healthy bull, should receive more attention. Additionally, a special management program must be designed to reduce delay in the onset of postpartum ovarian activity and the lengthening of calving to conception interval. Each farm should implement a reproductive epidemiology program that facilitates the identification, control, and prevention of reproductive problems. Different non-hormonal alternatives are available to reach an improvement in reproductive performance. Moreover, a genetic improvement program, including the selection by phenotypes and genes associated with better reproductive performance, could be implemented.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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