INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

IJPAES

Volume-6, Issue-2, April-June-2016 Coden: IJPAJX-CAS-USA, Copyrights@2016 ISSN-2231-4490

Received: 8th Feb-2016 *Revised: 20th Feb 2016* *Accepted: 20th Feb 2016*

Review article

SEASONALITY IN BUFFALOES REPRODUCTION

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ABSTRACT: The buffalo is considered as sluggish breeder as the reproductive efficiency of buffalo is adversely affected by certain constraints such as late maturity, poor expression of the estrous signs particularly during summer, irregular estrous cycle, silent heat, seasonality in breeding, poor conception rate/early embryonic mortality and prolonged inter-calving interval. Buffalo reproduction in tropical and subtropical part of the world is considered as short day breeder and its reproductive efficiency is greatly and adversely influenced by biometeorological factors. However, in equatorial zone buffalo can show estrous cycle throughout the year provided adequate nutrition is provided to maintain the reproductive efficiency and become seasonally polyestrous with distance from the equator attributed to the environmental factors and photoperiod. Season of calving has a profound effect on service period. As reported in India, buffaloes calving in late winter and early summer have lower reproductive efficiency compared to those calving during other periods and calving during rainy or monsoon season had shorter anoestrus period than other season calvers.

Key words: Buffalo, calving, estrous cycle, season.

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INTRODUCTION

Buffalo plays a significant role in the rural agricultural economy of south and south-east Asian countries, Mediterranean region of Europe and Latin America due to its unique quality as a multipurpose species contributing toward milk, meat production and draft purpose [1,2]. The buffalo (*Bubalus bubalis*) can be divided in to subspecies i.e. riverine and swamp buffaloes; both have qualities like adaptation to harsh environment, maintenance on poor quality roughages and resistance to tropical diseases. The world buffalo population is estimated roughly 177 million and approximately 97% are reared in Asian countries [3]. The riverine buffalo in addition to source as draft and meat is considered more important as high milk producer and found in South Asian countries, Egypt and Italy while swamp buffaloes are smaller and less productive in milk and mainly reared in Southeast Asia and China, and predominantly used as draft power and meat source [4]. Good feed conversion efficiency and low maintenance requirements of buffalo also endeared it to the farmers [1]. Like other farm animals buffalo production is also mainly dependent upon successful reproduction. The buffalo is considered as sluggish breeder as the reproductive efficiency of buffalo is adversely affected by certain constraints such as late maturity, poor expression of the estrous signs particularly during summer, irregular estrous cycle, silent heat, seasonality in breeding, poor conception rate/early embryonic mortality and prolonged inter-calving interval [5]. All these factors directly or indirectly affect the reproductive efficiency of buffaloes through modulation of sex hormones. The main emphasis in this review is given on seasonality of breeding and associated factors responsible for poor reproductive efficiency of this virtuous species.

Seasonal reproductive behavior

There is ample evidence that buffalo reproduction in tropical and subtropical part of the world is considered as short day breeder and its reproductive efficiency is greatly and adversely influenced by biometeorological factors i.e. day length, ambient temperature, relative humidity etc [4,6]. However, in equatorial zone buffalo can show estrous cycle through out the year provided adequate nutrition is provided to maintain the reproductive efficiency and become seasonally polyestrous with distance from the equator attributed to the environmental factors and photoperiod [1,7,8]. Photoperiod is perceived by the neuroendocrine system which subsequently modulates the circadian and short day related melatonin secretion from pineal gland [9,10]. However, role of melatonin in altering the reproductive neurohormones/hormones and mechanisms involved therein needs to be established.

Although buffaloes are polyestrous, they exhibit a distinct seasonal variation in display of estrus, conception rate, and calving rate in Indian buffaloes having better reproductive efficiency during winter compared to summer months attributed to environmental factors [11,12]. Similarly, the breeding frequency in Pakistani buffaloes was highest during the winter, decreased in autumn and spring, and was lowest in the summer [13]. Likewise, environmental factors play a significant role in seasonality of reproduction in Italian buffalo [1]. The stress and adverse environmental factors have direct effect on neoroendocrine set up resulting in hyper-prolactinemia, reduced pulsatile gonadotrophin secretion, poor follicular maturation and poor oestradiol production culminating in poor heat expression and anoestrus condition [14,15,16]. During summer, under nutrition coupled with high ambient temperature has been implicated with anoestrus condition in buffaloes [13], however, poor availability of green fodder is not the sole factor responsible in seasonality of poor breeding during summer as reported in Pakistani buffaloes [13]. However, non-availability of good quality nutritious food along with reduction in voluntary feed intake during thermal stress is also attributed towards poor reproductive efficiency [17].

Season of calving has a profound effect on service period [11,18]. As reported in India, buffaloes calving in late winter and early summer have lower reproductive efficiency compared to those calving during other periods [4,19] and calving during rainy or monsoon season had shorter anoestrus period than other season calvers [20]. The resumption of ovarian activity after calving was significantly delayed in buffaloes that calved from February to May (116–148 days) compared to the rest of the year (38–64 days) [19]. The mean service period of buffaloes calving from December to June was more than 140 days which was significantly higher than calving between July to November (< 110 days), the high service period during former group was associated with more silent estruses [21]. Furthermore, conception rates were lower between February and August [11], and number of services per conception was higher in summer calvers than those calving in other seasons. Similary, in Italy, buffaloes calving during the non-breeding season have an extended period of post-partum anoestrus and resume cyclicity only during the following breeding period [22]. The port-partum anoestrus has been differentiated into a temporary (les than 150 days) and deep anoestrus (more than 150 days) according to the time elapsed between calving and conception [7] and superficial and deep anoestrus based upon presence or absence of follicular turn over [23]. However, in Egyptian buffaloes, reports concerning effect of season of calving on post-partum acyclicity are conflicting [24] without any seasonal effect [25] whereas, a profound effect of season resulting in longer interval of calving during hot months compared to colder months have been reported [26].

The normal oestrus cycle in buffaloes can vary from 16 to 28 days [27] with duration of oestrus 10-20 h during breeding season [28]. The interval between onset of oestrus and LH surge is 1-12 hours in buffaloes and ovulation occurs between 18-40 h after the LH surge [21]. The interval from end to oestrus to ovulation has been reported to be between 12-24 hours in different Indian breeds and swamp buffaloes [29] where as from onset of estrus to ovulation time in Murrah buffalo is 28-60 h [21]. Seasonal suppression (summer versus winter) of reproductive function has been documented by a shorter duration of estrus (8–10 versus 18 h) [30], apparent prolongation of the interval from estrus to ovulation (15.8±0.4 versus 14.9±0.4 h in Nagpuri buffaloes; 29 Raut and Kadu, 1988), and fewer ovulatory cycles (71% versus 92%) [30] during the summer months. Incidence of summer anoestrus has been reported to be between 36-60% [31. In more recent study, an incidence of summer anestrus 63 and 83% has been reported in nomadic and housed rural buffaloes, respectively [32]. Further, the incidence of true anestrus (defined as the absence of large follicles and CL in the ovaries) was 78% in July and 14% in November [33]. The summer anoestrus has been found to be associated with smooth/inactive ovaries without any follicle or CL [34,35] may be due to lower follicular reserve in acyclic buffaloes than cyclic buffaloes [36], however, others have reported a follicular growth followed by atresia [37,38]. Recently, ultrasonic studies by Rohilla *et al*. [39] revealed presence of CL in some summer anestrus buffaloes suggesting possibility of silent/unobserved oestrus but in majority of the cases they encountered smaller population of follicles followed by anovulation of the preovulatory follicle. This retarded follicular growth, atresia and anovulation is perhaps attributed to lower gonadotropin support during summer [14]. Furthermore, poor quality of oocyte during summer season is also responsible poor reproductive efficiency in buffaloes [40,41].

The buffaloes are weak in oestrus expression [42] and which is further exacerbated during hot season [43] which are less intense with short duration [44] and majority show silent oestrus during summer [45]. Season has also got adverse effect on expression of oestrus symptoms. Unobserved (silent) estrus and abbreviated duration of estrus are common in swamp buffalo during summer [46]. Maximum occurrence of various heat symptoms is seen during the breeding season (September to February) where about 59% estruses are exhibited between 10 PM to 6 AM and manifestation of these symptoms becomes weaker during summers [47]. Looking at the effect of environmental temperature on silent heat, Prakash *et al*. [21] observed that out of 292 estrus detected by milk progesterone, 37% went unobserved through out the year where incidence of silent heat was minimum during December (10.5%) and maximum was recorded during April (70%) which gradually decreased May onward. This high incidence of silent heat was responsible for longer service period (139 days). The reduced intensity of heat symptoms are perhaps associated with lower oestradiol levels coming from smaller size pre-ovulatory follicle [27] as compare to cattle and this mechanism is further exaggerated by low progesterone during lueal phase, reduced pulsatile LH secretion, slow follicular growth of ovulatory follicle and low oestradiol production during summer months. [48,14,49]. The adverse effect of summer season on expression of estrus signs, duration, and service period may be attributed to high ambient temperature and/or photoperiod which needs to be elucidated with conducting experiments uncoupling photoperiod from high air temperature.

Endocrine alterations during different seasons

The concentrations progesterone are at nadir during estrus (0.1-0.3 ng/ml) and remain basal for another 3-4 days (close to 1 ng/ml) which experience a significant increase about 7 days after oestrus and reach to peak concentrations 4-5 ng/ml about days 15 post-oestrus [50,38]. Different views have been put forwarded by different workers regarding the functional status of the corpus luteum during different season and low reproductive efficiency during summer has been attributed to low luteal activity [5]. The luteal function is adversely affected during the summer season; maximum circulating concentrations of progesterone were 2.0±1.2 ng/ml and 2.27 ng/ml during the summer versus 3.1±0.2 ng/ml and 3.96 ng/ml during the winter as reported by Rao and pandey [51] and Harjit and Arora [52], respectively, which may therefore be responsible for poor expression of estrus and low conception rate. Even in normally fed animals progesterone concentrations were lower during summer than in winter [48]. Others report that higher concentrations of progesterone were recorded during summer than during fall and winter [38]. Still others detected similar progesterone concentrations during the summer and winter months (1.3±0.4 and 1.3±0.2 ng/ml, respectively) [53].

During normal oestrus cycle, circulating concentrations of oestradiol are at low level during luteal phase (10-20 pg/ml) with minor fluctuations around day 4 and day 10 of the cycle which reach at peak (30-35 pg/ml) either a day before or on day of estrus in buffalo plasma 54]. These peak concentrations during oestrus are responsible for expressions of heat symptoms by its action of central nervous symptoms are found to be lower during summer compared to cooler months may be responsible for higher incidence of silent estrus during summer [55]. The minor fluctuations during luteal phase may be associated with follicular wave. In rural anoestrus buffaloes low concentrations of oestradiol have been reported [56,57]. As proposed in cattle, the both adequate level and duration of progesterone during luteal phase which is responsible for regulation ostradiol receptors in brain and sufficient amount of oestradiol at the time of heat are responsible for onset of LH surge and oestrus expression, the high incidence of silent or anestrus or anovulation during summer may be attributed lower concentrations of these two ovarian steroids.

Inhibin which regulates FSH secretion from pituitary and plays significant role in folliculogenesis during late luteal to periestrus phase has been found to be significantly higher during winter compared to summer [15] may be due to poor ovarian activity during summer [58]. However, further studies are required to understand the role of Inhibin in buffaloes during different seasons of the year.

Lower circulating concentrations of FSH (measured during estrus and during the luteal phase) were detected during summer than winter months in Surti and Murrah buffaloes [59,60] which may be associated with lower follicular activity during summer. The pre-ovulatory FHS peak has also been found missing during summer season [61]. In addition, levels of FSH were recorded lower in anestrus buffaloes compared to cyclic buffaloes during summer season [62,11,28].

The luteinizing hormone which is directly under control of brain/hypothalamic neurohormones and also regulated by ovarian steroids play a significant role in reproduction [16]. Thus, adverse seasonal effect on its secretory pattern should affect reproductive efficiency adversely. Similarly, Rao and Pandey [55] found that LH peak level on the day of estrous was higher during cooler months compared to summer but others Sheth *et al*., 1978; 64 Kaker *et al*., 1980) [63,64] did not found any difference between the seasons. No optimum LH peak was detected in anestrus versus cycling buffaloes during summer [65,66] may be attributed to stressful condition [67]. Razdan *et al*. [34] suggested that lack of LH surge during summer is the main cause of ovarian inactivity as during the study from May-June they observed fluctuating basal levels of LH between 1.0 and 3.0 ng/ml.

The amplitude and frequency of LH secretion during the follicular phase were lower during the summer (2.8 pulses/ 8 hour with amplitude of 2.5 ng/ml) than during the winter (3.6 pulses per 8 hour with amplitude of 3.7ng/ml) and a lower LH pulse frequency even during the luteal phase was observed in the summer (2.1/8 h) than winter (3.2/8 h) [14]. This lower pulsatile and peak LH secretion during summer may account for poor follicular growth, estradiol concentrations and subsequent expression of heat symptoms/silent estrus and anovulation. Thus, during summer season role of photoperiod, harsh and stressful environmental conditions which are directly involved in reduced pulsatile and oestradiol-induced LH secretion [68] involved therein needs to be elucidated in this species.

Photoperiodic information is processed through a complex nervous and endocrine pathway to modulate reproductive activity. Light information perceived at the level of the retina is transformed through neural processing into an endocrine signal by the pineal gland in terms of melatonin release. Studies in species showing seasonal reproductive pattern strongly suggest that melatonin has a hypothalamic target to modulate the reproductive neuroendocrine axis and modulate prolactin secretion by acting on pituitary. The role of melatonin as a key hormone controlling the reproductive process in seasonal breeders is well established but information related to its role in buffalo seasonality comes from circannual secretion with high secretion during shorter days [9,10] and a circadian secretion of melatonin with a significant level during dark hours with great variability among the animals [9]. The sensitive buffaloes easily enter in to state of seasonal anestrus with increasing day light [69]. Thus, based upon the information related to melatonin secretion reflecting the variable sensitivity of individual animals or farms to photoperiod, out-of-breeding-season-mating (OBSM) technique was established in Italy and found to be successful in meeting the market demand of milk during the time when calving is less frequent [7]. However, further studies are required to under stand the exact mechanisms through which melatonin control the neuroendocrine axis of reproduction.

In buffaloes, hyper-prolactinemia has been found to be associated with hot summer season [62]. Prolactin concentrations has been reported to be many folds higher during summer compared to winter months also in buffalo heifers [70] and may be due to an influence of photoperiod on the pineal gland. These high concentrations of prolactin have been reported to be associated with low estrogen and progesterone concentrations and resultant summer infertility [71,72] may be via reduction of pulsatile and oestradiol-induced LH secretion by acting on the brain level as reported in other species [73] which needs to be confirmed with further experiments in buffaloes. However, a direct adverse effect of high prolactin level on ovaries via altering LH receptors and making the ovaries refractory to LH and FSH culminating in summer infertility has also been suggested [74]. In addition, use of antiprolactin drug, norprolac in ovosynch protocol during summer months increased the efficacy of this treatment to a great extent in terms of oestrus induction and conception rate and increased oestrus behavior via increased estradiol levels [72].

Factors affecting seasonal behavior

Till today, it is unclear to the buffalo reproductive physiologists whether poor reproductive efficiency during summer or seasonal breeding of buffaloes is attributed to genetic characteristics of buffalo or environmental factors or nutrition. However, evidence suggests a strong influence of biometeorological factors on the endocrine system of buffaloes (i.e., day length, ambient temperature, relative humidity and rain fall [13]. The seasonal pattern particular poor reproductive efficiency during summer season may also be attributed to meager availability of green fodder during these months [7]. However, this claim has been refuted by one study in which buffalo displayed a different breeding pattern than cattle kept under similar environmental conditions and fed similar diet [13]. In addition, Italian buffalo exhibit seasonal pattern of reproduction in spite of providing constant balanced diet throughout the year [75]. Certain reports suggest that reduced sexual activity in the buffalo coincides with both an increase in ambient temperature [31] and increasing day length [12], but effects of photoperiod and other environmental factors individually has not been reported to suggest their relative role. The incidence of true anestrus was significantly correlated with mean maximum and minimum air temperature (r=0.8 and 0.9, respectively) and with mean relative humidity (r=−0.7). The proportion of buffaloes exhibiting estrus during the period of short day length was significantly greater than during the period of long day length (74% versus 26%, respectively) [12].

Decreasing day length may be a stronger determinant of the onset of postpartum ovarian activity, whereas ambient temperature and relative humidity may have relatively lesser influence [76]. The adverse effect of different biometeorological factors on poor reproductive efficiency in buffaloes during summer months needs to be investigated with further experiments via looking at the effect of nutrition, high ambient temperature, photoperiod, humidity etc. individually or in combination during different seasons [19].

Effect of seasonality on induction/control of estrus cycle:
Reproductive similarities between bubaline (*Bubalus bubalis*) and bovine (*Bos taurus* and *Bos indicus*) reproduction allow reproductive management technologies developed in cattle to be applied to buffalo [77]. Based upon the information gathered through the investigations on endocrinological aspects of female buffaloes and results obtained in cattle, different hormonal preparations e.g. PGF2 alpha, progestagens, GnRH etc. alone or in combination with eCG, FSH or PMSG have been used by different workers for induction and synchronization oestrus and controlling ovulation in buffaloes. The efficacy of different hormonal regimen treatments has been reviewed indicating a dramatic seasonal variation [43].
Oestrus induction rate with single or double injection of PGF2 alpha was found to between 60-80% which resulted a conception rate of 45-50% during breeding season [78,43] whereas induction rate of 44-63% and conception rate of 13-25% was observed during non-breeding season [79,80]. However, when FSH or GnRH or PMSG was given alongwith PGF2 alpha it considerably improved the conception during anestrous condition during summer [81].

Progestagens in the form of CIDR, PRID, CRESTAR with or without estradiol valerate/benzoate (at the start of treatment) and PG or eCG (at the end of treatment) have been tried to induce oestrus and to synchronize the oestrus cycle in buffaloes with variable results irrespective of the season. An oestrus induction of 80-90% with 51% conception rate following treatment with PRID in post-partum lactating buffaloes was achieved [82]. However, during summer season, treatment of cyclic and acyclic buffaloes with PRID induced oestrus in 70-100% buffaloes but a poor conception rate (0-20%) was achieved [83] which was significantly improved (28-60%) by PMSG treatment at the time of PRID withdrawal [7,84]. However, Singh *et al.* [85] using PRID only achieved 35.8% oestrus induction in summer anoestrus buffaloes and only 54% of buffaloes detected in oestrus conceived following treatment. However, a very good pregnancy rate of 70.5% was achieved in acyclic buffaloes during increasing day length with CIDR plus PMSG regimen [86]. Similarly, Singh [80] induced cyclicity in anoestrus buffaloes with CIDR during summer season with 83% induction of cyclicity and 80% of the detected animals in oestrus conceived following treatment. However, during summer, treatment with progesterone/norgestomet induced oestrus only in 62-66% anoestrus buffaloes with 40-50% conception of the detected animals [87]. These studies indicate better results with CIDR as compare to progestagens and PRID. Further, in anestrous buffaloes during progesterone-based estrus synchronization protocol, administration of eCG equine chorionic gonadotropin (eCG) at the time of progesterone withdrawal, improved the ovulation rate in buffaloes [88,89].

The Ovsynch protocol was found to be effective in buffaloes for induction of ovulation, particularly in cyclic buffaloes where during breeding and non-breeding season efficacy of treatment was found to be similar with 81-93 % ovulation and 33-50% conception rate [90,91,84 92]. However, acyclic buffaloes respond poorly to the Ovsynch protocol yielding poor conception/pregnancy rates, compared to cyclic buffalo cows [93,94]. During increasing day though the efficacy of treatment was similar in cyclic buffaloes with regards to ovulation (80-85%) but conception rate was poor in acyclic compared to cyclic buffaloes (17 % Vs 40-45%) [93,86]. During summer, high degree of ovulation was achieved with ovsynch protocol in cyclic buffalo (80-100%) [94,70] as compare to acyclic but with poor conception rate in acyclic buffaloes owing to asynchronous ovulation [94]. This poor pregnancy rates in non-cyclic/anestrous buffaloes following ovosynch protocol during summmer was improved by adding exogenous progesterone with an intra-vaginal progesterone device [93], injection of eCG at the time of PG [95] and addition of norprolac (anti-prolactin drug) to the ovsynch protocol [70]. Administration of PG adopting same protocol at the time of AI in cyclic buffaloes during breeding season by enhancing progesterone profiles also increased conception rate [86]. However, the efficacy of presynch ovsynch and ovsynch protocol was found to be similar with regard to ovulation (85-90%) and conception rate 35-45% [96].

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INTERNATIONAL JOURNAL OF
PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

ISSN 2231-4490

International Journal of Plant, Animal and Environmental Sciences