Review Article

Buffalo Bulletin (April-June 2023) Vol.42 No.2

https://doi.org/10.56825/bufbu.2023.4222415

ESTRUS AND OVULATION SYNCHRONY OF BUFFALOES (*Bubalus bubalis*): A REVIEW

Gh Rasool Bhat[1,*] and Gurucharan Singh Dhaliwal[2]

Received: 02 February 2021

Accepted: 27 June 2023

ABSTRACT

Synchronizing estrus and ovulation in buffalo using timed inseminations (TAI) protocols circumvents the limitations associated with estrus detection. Spontaneous estrus in buffalo may achieve more conception rate on insemination than those subjected to synchronization of estrus and timed insemination (TAI). The advantages of insemination following synchronization programs include higher detection rates combined with decreased embryonic losses and reduction in days open. A synchonization programme should ensure control of estrus cycle which entirely depends on ovarian status of animals before starting any protocol. Ovarian follicular status at the beginning of treatment protocols plays vital role for effective response shown by animals. Studies show that more precise alterations in follicular waves may be needed to achieve better synchrony of ovulation and more fertility. The protocols which use Gonadotropic Releasing Hormone (GnRH), follicle stimulating hormone (FSH), luteinizing hormone (LH), equine chorionic gonadotropin (eCG), human chorionic gonadotropin (hCG), prostaglandins, progesterone and estradiol have been helpful in achieving the estrus and ovulation synchrony. Use of estradiol esters and progesterone per-vaginum implants have also been incorporated in synchronization programs. Synchronous emergence of follicular waves using trans-vaginal ultrasound guided follicular ablation and estradiol administration is a recent concept for synchronization in buffalo. Further, a recent approach has been to allow the follicle of second follicular wave to ovulate as the fertility has been lesser when first wave follicle ovulates. Moreover, controlling environmental influences, nutritional and managemental strategies remain pre-requisite to achieve desired goals though hormonal administration. This is more particular in buffaloes suffering from anestrum more so in low or non-breeding season and pre-pubetal anestrum. This review will cover the available documented literature regarding estrous cycle physiology with emphasis on follicular wave dynamics and the future area of work to be focused to achieve more reproductive efficiency in buffalo.

Keywords: *Bubalus bubalis*, buffaloes, estrus,

[1]Division of Veterinary Clinical Complex, Shere Kashmir University of Agricultural Sciences and Technology of Kashmir, Kashmir, India, *E-mail: rasool_roshan0127@rediffmail.com

[2]Department of Teaching Veterinary Clinical Complex, Guru Angad Dev Veterinary and Animal Sciences, Ludhiana, India

ovulation, synchronisation, anestrus

INTRODUCTION

Estrus or ovulation synchronization refers to procedures for manipulating estrus cycle of a group of female animals so that they can be brought into estrus at a predetermined time (Odde, 1990). Buffaloes can adapt harsh environmental conditions, thrive poor quality forage production and compromise reproductive efficiencies. The species often encounters sexual maturity later, postpartum anestrus longer, expression of estrus poorer, conception rate lesser and calving intervals longer (Barile, 2005). Compared to cattle, their ovaries are smaller bearing fewer primordial follicles (Danell, 1987). Their puberty and sexual maturity depend on genotypes, nutritional aspects, managemental strategies and climatic conditions. Puberty at favorable conditions is seen at 15 to 18 months and 21 to 24 months in river and swamp buffalo respectively (Perera, 2011). Buffaloes have the capability to exhibit breeding throughout the year although they have seasonally governed ovarian activity. This is attributed to rainfall patterns and temperature stresses in the tropics. Rainfall affects the feed availability while temperature stresses raise prolactin levels and disturb gonadotropins. Besides photoperiodic changes and melatonin secretion pattern in temperate regions also alters the ovarian cyclicity (Roy and Prakash, 2007). Dairy type buffaloes exposed to restricted or no suckling exhibit estrus cyclicity 30 to 60 days post calving. Swamp buffalo breeds with free suckling exposure show cyclicity 60 to 90 days after parturition. (Perera *et al*., 1987).

The potential of production in buffalo is largely affected by the efficiency of reproduction or ovarian activity during different seasons (Singh *et al*., 2000). Also in buffaloes, due to lesser efficiency of heat detection, variable estrus duration (4 to 64 h, Baruselli *et al*., 2001) and the more unpredictable timing of ovulation, artificial insemination (AI) remains a limited technique. This leads to further lowering of reproduction efficiency in buffaloes. Though this lower efficiency of reproduction is a major concern, but recently developed protocols have been able to control the estrus cycle. This fixes the ovulation window, confines estrous period and lowers need of estrus detection in buffalo (De Rensis and Lopez-Gatius, 2007). For synchronization protocols to be applicable, understanding hormone regulation of the estrus cycle, follicular dynamics, and efficient heat detection (estrus signs being less overt or a problem of sub estrus in the species) is required. The concept of premature luteolysis by prostaglandins, luteal phase prolongation by progestogens or ovulation induction using gonadotropins and estrogens have been practiced manipulating estrus cycle physiology so far in buffaloes (De Rensis, 2007). More recent approaches are being targeted to manipulate follicular development to achieve greater estrus synchrony and improved fertility. Synchronous emergence of follicular wave prior to estrus and synchronization protocols providing ovulation synchrony allowing fixed-time AI without detecting estrus (Honparkhe, 2010) are the latest advances in this regard. In our one recent study (Bhat *et al*., 2015) we observed a good synchrony of follicular waves following estradiol-based synchronization regimen in buffalo and also in our other findings (Bhat *et al*., 2014) we achieved an optimal size of dominant follicle and subsequent conception rate in anestrous buffalo following estradiol-17β+ Controlled Internal Drug Release Device (CIDR) protocol for synchronization.

Further buffaloes tend to be seasonal breeders exhibiting summer anestrum particularly in tropics. Hence protocols need to modify for different reproductive behaviors. Based on review of literature progestogen regimens adjunct by prostaglandins, gonadotropins (GnRH, hCG and eCG) and estradiol esters help in improving reproductive efficiency, but more comprehensive studies have to be designed to improve conception rate in buffalo.

Estrus cycle physiology in buffalo

Buffaloe's mean cycle length is 21 days, showing greater variation than cows. The estrus duration being 5 to 27 h and ovulation happens for a period of 24 to 48 h after the onset of estrus (Perera *et al*., 1987; Perera, 1999). Circulatory hormonal changes occurring being similar to cattle, but the peak concentrations of progesterone and oestradiol-17β are always lesser (Roy and Prakash, 2009; Singh C, 2001). The luteal phase duration and inter-ovulatory intervals are governed by the number of follicular waves in one cycle (1 to 3 waves in buffalo). The overall estrus cycle length in buffalo is similar to cattle, having 17 to 26 days range and 21 days mean (Jainudeen and Hafez, 2000). Variations have been recorded in cycle length in buffalo, with incidences of shorter and longer cycles, associated mainly with adverse environmental and nutritional conditions (Nanda *et al*., 2003). Estrus duration in river and swamp buffalo has been documented to be almost similar (5 to 27 h) and ovulation happens 34 h after onset of estrus, or 14 h after the end of estrus (Kanai *et al*., 1990). Further this duration is shorter, and signs are exhibited towards night hours or early morning in hot seasons. More specifically, Italian breeds, as reported by Zicarelli (Kumar), estrus cycle duration varies from less than to greater than 48 h. In the short and medium cycles ovulation has been reported to occur variably after the end of estrus or 24 to 60 h after the onset of estrus. And longer cycles exhibited ovulation before the end of estrus. Also, in the case of buffalo estrus signs is not overt and homosexual behavior is rare compared to cows (Sharma, 2011).

Buffaloes's ovarian follicular dynamics is defined by a wave-like pattern showing all stages of emergence, growth, dominance and atresia or ovulation of follicles (Garcia *et al*., 1998). Normally 1 or 2 anovulatory follicular waves followed by an ovulatory wave has been reported (Adams *et al*., 1995). Moreover buffalo's ovaries are smaller and have lower number of follicles per follicular wave (due to smaller pool of primordial follicles) compared to cattle (Gimenes *et al*., 2009; Danell, 1987).

Follicular dynamics in buffaloes

Ovarian follicular dynamics in buffaloes has been described during estrus cycle (Taneja *et al*., 1996), early pregnancy (Kumar *et al*., 2001) and of ovaries young female calves (Presicce *et al*., 2004). Being reported similar to those in cattle (Baruselli *et al*., 1996), follicular development has a predominance of two waves in buffalo. The first wave has been observed around Day 0 (day of ovulation) and the second wave around Day 9 or 10 (Ali *et al*., 2003). One, two and three waves have been reported respectively in 3%, 63% and 33% Brazilian Murrah buffaloes. The first wave usually starts on day of ovulation in buffaloes exhibiting 1, 2 or 3 waves, while the 2^{nd} wave emerged on days 10.8 and 9.3 for the buffaloes with 2 and 3 waves, respectively, and the 3^{rd} wave emerged on day 16.8 in 3 wave cycles (Yindee, 2010). 2 and 3 follicular wave cycles differ in the mean luteal phase duration (10.4 vs. 12.7 days) and the inter-ovulation intervals (22.3 vs. 24.5 days; Baruselli *et al*., 1997). On contrary, the majority of Indian suckled buffalo possess single follicular wave cycles and less number exhibit two wave pattern (Taneja *et al*., 1995). The mean inter-ovulatory interval was 21.0 days in one wave cycles and 22.7 days in two wave cycles. The growth profile of the dominant follicle in one wave cycle is atypical following growth, regression and re-growth and ending at ovulation (Awasthi *et al*., 2006). Interestingly, another study on Egyptian buffalo revealed three follicular waves in the majority of cycles (54%, Barkawi *et al*., 2009). Italian buffaloes exhibited differences in follicular dynamics between heifers and buffaloes (Presicce, 2004). The results of above studies warrant further investigations on follicular dynamics in buffaloes to delineate whether the number of follicular waves influences the oocyte quality that is destined for ovulation. Each follicular wave, when started, is preceded by a transient increase FSH levels and this often peaks at or near the time of emergence of future dominant follicle. Raised FSH levels serve as a stimulus for the recruitment of the cohort of ovarian follicles and hence for a new wave emergence (appearance of ≥4 mm sized follicles on ovaries (Wiltbank *et al*., 2002; Binelli *et al*., 2006).

In general, in bovines, ovaries show emergence of 2 and/or 3 successive and wave emergence is observed on ovulation day (day 0) and day 10 in case of 2-wave cycle and on days 0, 9 and 16 in 3-waves cycles (Garcia *et al*., 1998). Gonadotropins basal levels remain sufficient to allow emergence of waves about every 7 to 9 days. Further it is the time of luteolytic in relation to appearance of the last follicular wave which governs two or three wave cycles of follicular development.

Going to other aspect of ovarian dynamics in buffalo, first postpartum ovulation in suckled Thai swamp buffalo is followed by a short estrus cycle (10.2±0.38 days) in majority of the animals and improves thereafter (Yindee *et al*., 2010). According to Yindee *et al.* (2007), the mean diameters of ovulatory follicles increased between the first and second ovulation (13.50±0.52 in first and 14.31±0.38 mm in second ovulation) whereas mean size of ovulatory follicle and corpus luteum was larger in pregnant animals at that ovulation. The reason for the different results have been the influence on the quality of the oocyte in the dominant follicle destined for ovulation. Shorter estrus cycles or anovulatory cycles in buffalo often lead to postpartum anestrus and infertility resulting in economic loss to buffalo breeders in many countries. In Egypt, India, and Pakistan only 34 to 49% of buffaloes exhibit estrus during the first 90 days postpartum while about 31 to 40% remain in anestrum up to 150 days of calving (EL-Wishy 2007).

Estrus synchronization in buffaloes

Satisfactory results have been achieved when estrus cycles were synchronised on the basis of manipulation of ovarian activity and prediction of time of ovulation in cattle. Such manipulation and prediction could be possible by controlling the estrus cycle in buffaloes which has already been reviewed by previous studies (Perera, 2011). The estrus cycle may be controlled by either controlling the luteal phase or follicular phase such as follicular development and ovulation. So far, luteal phase has been controlled by prostaglandin or progesterone analogues while follicle development and ovulation has been manipulated successfully using combination protocols of prostaglandins, progesterone, gonadotropins (GnRH, hCG, eCG) and estradiol esters (Lopez Gartius, 2009).

Latest synchronization programs in

buffalo are based on cattle studies but different results have been reported because that buffaloes are characterized by more intense nonbreeding season especially in hot summer months owing to reduced ovarian activity. Some other inherent problems such as delayed sexual maturity, seasonal pattern of reproduction, anestrus and long inter-calving interval hamper reproductive efficiency in buffalo (Nanda *et al*., 2003; Das and Khan, 2010). Besides, sparse knowledge documented regarding buffalo reproduction physiology, our progress towards countering the reproductive problems and improving production efficiency is lesser than expected. Follicular fluid microenvironment provides insight into the normal follicular development processes and pathogenesis of infertility problems in buffalo (Das and Khan, 2010). The GnRH and $PGF_{2\alpha}$ based estrus synchronization is reported to be very helpful for synchronizing estrus in cattle (Amaya-Montoya *et al*., 2007) and buffaloes (Ghuman *et al*., 2010, Bhat *et al*., 2015).

Previous studies regarding synchronization estrus in buffalo have been based on premature luteolysis induction using prostaglandins or luteal phase extention using progestagens (Perera, 1987). However, recent trends focus on ovarian follicular wave dynamics (Zicarelli *et al*., 1997; Brito *et al*., 2002) and its manipulation during follicular development to achieve greater estrus synchrony and improved fertility (De Rensis and Lopez-Gatius, 2007, Bhat *et al*., 2015). So far, various combinations of prostaglandins, progesterone devices, GnRH and eCG have been applied but poor results in buffalo are reported especially in during the periods of marginal breeding activity or seasonal anestrus (Parera, 2011). Targeting follicular development stages of dynamics may be needed to achieve more precise synchrony of ovulation and acceptable fertility. Researchers thus are turning their attention to evaluate new hormonal programs using combination protocols of GnRH, FSH, LH, eCG, hCG, prostaglandins, progesterone, and estradiol esters in buffalo. Administering prostaglandins in buffalo alone or in combination with GnRH has been evaluated with conception in the range of 7 to 56%, while using progesterone insert devices alone or in combination with GnRH, eCG or hCG give conception rates in the ranged of 8 to 64%. However, success rate in many studies was lower in buffalo particularly when regimens were used during the periods of low breeding activity or seasonal anestrus. Other studies have tried various modified protocols to overcome the limitations. The two main and efficient protocols appear to be "Ovsynch" and its supplementation with progesterone for 7 days and progesterone 10-14 days supplementation along with GnRH or estradiol administration at progesterone insertion and prostaglandin plus eCG at withdrawal (De Rensis and López-Gatius, 2007,). Ovsynch protocol (GnRH, PG and GnRH on days 0, 7 and 9 respectively) has good results in buffalo, achieving ovulation synchronization and conception to the extent of 70 to 90% and 33 to 60% respectively (Baruselli, 1999; Paul and Prakash, 2005). Ovsynch supplemented with CIDR has even more results in buffalo as reported in literature. Moreover, Jabeen *et al*., 2013 reports positive response by 87.5% Nili Ravi buffaloes in peek breeding season using ovsynch protocol in term of oestrus and ovulation synchronization while the response was only 36.36% during low breeding season. Work is on the progress for establishing regimens in buffalo which can produce higher conception by reducing ovulation window and make timed insemination an effective technique and circumvent estrus detection need which otherwise has serious consequences in buffalo reproduction.

Synchronisation of ovarian follicular growth and induction of ovulation of a dominant follicle at predetermined time to allow insemination known day and hour, without the need of estrus detection (Baruselli *et al*., 2004) has been made now a practical in cattle as well as buffalo. Many ovulation synchronization protocols now synchronize wave emergence by simultaneous administration of progesterone and estradiol (Cavalho *et al*., 2008; Bhat *et al*., 2015) and reduce exogenous and endogenous circulating progesterone levels by progesterone removal plus prostaglandin for CL regression and timed ovulation. At the end, final growth and/or maturation of the follicle for synchronized ovulation allows more effective insemination at a pre-determined moment (Bo *et al*., 2003).

Little information in literature about buffalo reproduction continues to hamper more progress towards better understanding of reproduction physiology and efficient tools to improve fertility in this species. Follicular status of animals is necessary to be evaluated before start of any treatment protocols due to variable responses shown by buffalo to the established regimens. In a study on ewes by Gonzalez *et al.* (2000) ovarian response was better following super ovulatory treatment at the beginning or early luteal phase of the estrous cycle. This allows corpus luteum appearance along with the progestogen treatment which controls follicular development for better synchrony. Similarly, in buffalo a comprehensive study of ovarian dynamics needs to do before start of synchronisation protocols. This will allow to determine ideal time of treatment to achieve better synchrony of estrus and ovulation.

Estrus cycle manipulations

The procedures to interfere with normal estrus cycle physiology are based on manipulating the corpus luteum by its premature luteolytic using prostaglandins or to extend the luteal phase using progestogens. In buffaloes hormonal changes occurring during synchronization are poorly understood. Plasma progesterone reflected by concentration of faecal progesterone metabolite determines functional stage of the corpus luteum. Although plasma progesterone profiles give good evidence of the reproductive status of the animal, but its analysis may not provide accurate evaluation of the ovarian status.

For effective estrus cycle manipulations in buffalo, good body condition score, minimal stress during the treatment and at insemination, less seasonal differences and peak stages of the breeding are some of the factors to determine success rate of protocols. Moreover, the problem of silent estrus in buffalo results in poor reproductive efficiency (Baruselli *et al*., 2001). Various estrus synchronization regimens such as progesterone, progestogens and prostaglandin (Palta *et al*., 1995) have been utilized to to successfully modify estrus cycle physiology but their efficacy is lesser due to the need of visual estrus detection aids (Erven and Albaugh, 1987). Future research efforts in this area should try to identify both factors determining seasonal low fertility and factors affecting patterns of estrus behavior, understanding hormone physiology of cycle in buffalo. Hence, more understanding of reproductive physiology or patterns in buffalo will help improve future therapies.

Protocols used to control the luteal phase of cycle

The luteal phase duration can be disturbed through administration of prostaglandins or progesterone.

$PGF_{2\alpha}$ administration in buffalo

In cattle and buffaloes, the effect of $PGF_{2\alpha}$ on luteal phase is very similar. $PGF_{2\alpha}$ injection right from day 5 of the estrus cycle has been reported to cause regression of the corpus luteum. This follows progesterone decline rapidly to basal concentrations within 24 h and ultimately growth of dominant follicle for induction of estrus and ovulation (Chohan, 1998). The submucosal intra-vaginal low doses of $PGF_{2\alpha}$, ipsilateral to corpus luteum, also effectively induces luteolysis (Subramaniam *et al*., 1989). In buffalo, single or double shots of $PGF_{2\alpha}$ induced estrus and ovulation in about 60 to 80% of animals during the breeding season (Brito *et al*., 2002). $PGF_{2\alpha}$ treatment to estrus interval reported in few studies was 88 h (78% cases from 72 to 96 h), while $PGF_{2\alpha}$ to ovulation was 100 h (81% cases from 84 to 108 h). Further the intervals are shorterfollwing $PGF_{2\alpha}$ administration in the early luteal phase (Berardinelli and Adair, 1989).

The double PGF2 alpha plus GnRH protocol

45 to 50% pregnancy rates have been achieved following double prostaglandin treatment (Neglia, 2003) which in some studies appears to be similar to those of natural estrus. Reduced efficacy does exist during the non-breeding season, even when a majority of buffaloes display standing estrus following $PGF_{2\alpha}$ treatment (Jabeen *et al*., 2013). In breeding season buffaloes treated with two doses of $PGF_{2\alpha}$ at 13 days interval showed estrus on examining ovarian activity two days after the second $PGF_{2\alpha}$. Animals with a Dominant Follicle (10 mm) inseminated 16 to 22 h later had acceptable conception rate. Pregnancy rates in some studies were similar to the animals managed under the classic ovsynch protocol (48% versus 50%, respectively). However, interval between $PGF_{2\alpha}$ treatment and the onset of estrus in buffalo varies according to the stage of follicular development at the beginning of protocol (De Rensis, 2007). Animals having pre-dominance stage of development when treated exhibited estrus 1 to 2 days later compared to those treated in the presence of a dominant follicle. This puts a limitation on timed insemination in such protocols. In other findings, double $PGF_{2\alpha}$ 12 days apart in cycling buffaloes is more effective in synchronizing ovulation and allows fixed-time AI at 72 h after second-$PGF_{2\alpha}$. Also, here the interval to ovulation was not observed to be affected by the presence of corpus luteum or ovulatory follicle at the beginning of treatment. Moreover, there appeared to be no effect of administering GnRH injection 48 h after second-PGF2α (Dadarwal *et al*., 2009). Warriach *et al.* (2008) reported larger ovulatory follicle size and more estrus and to ovulation duration in $PGF_{2\alpha}$-induced luteolysis. Determining ovarian activity by ultrasound may help to establish the ideal time of $PGF_{2\alpha}$ treatment (De Rensis and Lopez-Gatius, 2007).

Modified synchronization protocols involving ovulation of second follicular wave

Till date partial success has been achieved with the used estrus synchronization protocols in buffaloes (Ghuman *et al*., 2010). Gain in information regarding the effect of extending the growth of dominant follicle and its effect on subsequent fertility might be useful. Therefore, there is need to establish a hormonal regimen in buffaloes that will allow the dominant follicle to grow under a high progesterone environment for a prolonged period so that acceptable pregnancy rate can be achieved consistently. Modified synchronization protocols involving ovulation of dominant follicle of second follicular wave have been applied in buffaloes (Bilal *et al*., 2016).

Fonseca *et al.* (1983) reported for the first time that Holstein and Jersey cow breeds becoming pregnant had greater concentrations of P_4 in a 12 days period prior to insemination. Other studies have documented P_4 concentration during the development of pre-ovulatory follicle influence fertility, since dominant follicle of first and second wave use to grow under different P_4 environment (Denicol *et al*., 2012). Sartori *et al.* (2004); Denicol *et al.* (2012), have observed that first wave dominant follicle grows under sub luteal phase P_4 level (P<1.5 ng/ml) followed by luteal phase level of P_4, whereas dominant follicle of ovulatory wave grows under luteal level (>2 ng/ml) of P_4, prior to luteolysis. Various studies have demonstrated favourable effect of P_4 environment during DF development on subsequent corpus luteum (CL) development, CL functionality and fertility in cattle (Lonergan 2011; Wiltbank *et al*., 2012; Dadarwal *et al*., 2013). Moreover, Low circulating concentrations of P_4 immediately before insemination have been linked with low pregnancy rate and early pregnancy losses in bovines (Diskin and Morris, 2008; Lonergan, 2011). Bisinotto *et al.* (2010) has reported that increasing progesterone supports growth of dominant (ovulatory) follicle and improve subsequent pregnancy rate following FTAI. Effective control on follicle and CL development following $PGF_{2\alpha}$ and GnRH pre-synchronization and Ovsynch protocol 7 days later in cyclic buffaloes has been reported in buffalo (Bilal *et al*., 2016). Also, a higher pregnancy rate has been reported on delaying $PGF_{2\alpha}$ by 1 day in synchronisation. The pregnancy rates obtained were in confirmation with earlier studies by Cunha *et al.* (2008); Hoque *et al.* (2014) comparing conception rate following ovulation dominant follicle of second wave. Hence it is ascertained that delay in luteolysis causes dominant follicle of second follicular wave to grow under high progesterone milieu, thereby improving the oocyte competence and subsequent pregnancy rate in buffaloes (Ganaie *et al*., 2018).

Progesterone based synchronization regimens in buffalo

Compared to prostaglandins, progesterone-based regimens using Controlled Internal Drug Release (CIDR) and Progesterone Releasing Intravaginal Device, PRID or CRESTAR in most of the protocols have limited outcomes in cattle and buffalo. As per information available, ovulation and estrus following progesterone withdrawal occurs in 40 to 96 h and 43 to 117 h respectively. Also, pregnancy rates following such treatments varies from 20 to 50%. Bodhipaksha *et al.* (1994) after comparing pregnancy rates in following artificial insemination and natural breeding in 40 buffaloes, treated with progestagen for 12 days, obtained similar pregnancy rates (40%) in both groups. Studies indicate that progestagen treatments during the breeding season have acceptable conception rates in buffalo.

Progesterone supplementation prior to first postpartum ovulation reduces the occurrence of short estrus cycles (Perry *et al*., 2004; Sa Filho *et al*., 2006). Also, the majority of high-producing dairy cows showing anestrum when treated with CIDR show estrus cycles (Galvao *et al*., 2004; Chebel *et al*., 2006). However, CIDR in Ovsynch based fixed time AI protocols in buffalo induces ovulation in majority of repeat breeding cattle with acceptable conception rates (61%, P<0.05, Ghuman *et al*., 2010). PRID insertion for 15 days in anestrus buffalo induces fertile estrus in heifers showing

60% first service conception rate after insemination at 48 h and 72 h of PRID removal. Fertility results achieved also depend upon the life span of ovulatory follicle during and post-PRID period (Singh *et al*., 2009). Furthermore progesterone-based hormone regimen (Gonadotropin-Prostaglandin-Gonadotropin + CIDR) followed by insemination at 72 (day 10) and 96 (day 11) h after CIDR withdrawal is most favorable for optimization of fertility status in the lactating anestrus buffaloes (Ghuman *et al*., 2011). The buffaloes that failed to conceive at induced estrus in different synchronization regimens exhibited spontaneous onset of estrus in the subsequent 16 to 35 days. Further high proportion of buffaloes of CIDR-based regimen exhibited estrus after failure of conception at induced-estrus compared to other regimens (Ghuman *et al*., 2011). In this regard more comprehensive studies needs to be framed to evaluate efficiency of CIDR application during timed AI protocols in high producing animals

Controlling follicular development and ovulation with different hormone combinationsTreatments involving GnRH-prostaglandin

GnRH leads to LH surge during any stage of the estrus cycle and promotes the ovulation of a dominant follicle (Ghuman *et al*., 2010). It has also been reported that GnRH administration in buffaloes induces ovulation in 60 to 86% of treated animals and the interval between GnRH administration and ovulation is 3.3 to 8.3 h (Aboul-Ela MB, 1988). So far most of the estrus and ovulation GnRH synchronization protocols allowing timed artificial insemination programmes without estrus detection in cattle and buffalo have been developed. All these regimens promote ovulation by GnRH, CL regression by prostaglandin 7 days later, and thereafter the control of ovulation of the new ovulatory follicle by a second injection of GnRH. Presence of dominant follicle assured by ultrasound examination of the ovary (De Rensis *et al*., 2005) or in some cases by double prostaglandin pre-synchronization. Although conception rates following these protocols are different during transition from the breeding to non-breeding season and dramatically decreases during non-breeding season (Baruselli, 2001).

Combination protocols of Progesterone, GnRH, estradiol, hCG and eCG

Prostaglandins, estradiol esters and hCG have been successfully used for decades to improve the synchrony of estrus and improving conception rates in progestagen based protocols (Saini, 1988). Many protocols are used to intensify estrus behavior using different hormonal combination in buffalo to prevent problem of wrong time insemination. We have recently compared estradiol-17β in place of first GnRH in a conventional ovsynch protocol. The observations showed us more intensified estrus using estradiol which simplified estrus detection and improved conception rate. (Bhat *et al*., 2018). CIDR protocol Barile *et al.* (2005) used eCG at the time of progesterone device withdrawal and double insemination at 72 and 96 h later and reported higher conception rate.

Using GnRH in such protocols helps to improve conception. Compared to non-pregnant buffaloes, the pregnant buffaloes had a larger CL only on day 5 post ovulation, whereas plasma progesterone concentration was high throughout the post-ovulation luteal phase (Pandey *et al*., 2010). One of the important reproductive wastages in buffalo is the early embryonic mortality. Thus, protocols using GnRH and hCG may help to combat luteal inadequacy by promoting luteinisation of follicles and formation of accessary corpora lutea, this luteal insufficiency otherwise can be an established cause of earlier embryonic mortality (Lynch *et al*., 2010). GnRH injection increases the large luteal cell proportion on day 10 of estrus cycle (Mee *et al*., 1993), conceptus development (Mann and Lamming, 1999), ability of embryo to secrete IFN-τ, embryo viability (Stronge *et al*., 2005) and ultimately conception rates (Campanile *et al*., 2008).

Non-breeding season and estrus synchronization in buffalo

Both reproduction and production status of buffalo is impeded in non-breeding season. Longer day length and elevated environmental temperature with high humidity pre-dispose to poor nutrition status of buffaloes. Besides environmental and nutritional factors, management system also impedes reproduction, better exemplified by problem of summer anestrus in buffalo. More day length and high environmental temperature are associated with hyper-prolactinaemia, gonadotrophin suppression leading to alterations in ovarian steroidogenesis in buffalo (Das and Khan, 2010). Because of ovarian inactivity in summer as a result of endocrine profile aberrations, buffaloes fail to exhibit estrus (El-Wishy, 2007). Irregular progesterone profiles during summer indicate disturbances in the normal development of the corpus luteum (Tegegne, 1993). Heat stress produced during summer also affects folliculogenesis, follicular fluid microenvironment and oocyte quality. Heat stress results in alteration of follicular development and depression of follicular dominance and early emergence of pre-ovulatory follicle. Heat stress during the hot summer months in India is a major cause of anestrus in buffalo and is associated with elevated blood concentrations of prolactin influencing ovarian activity and causing sub-fertility, silent estrus and repeat breeding by decreasing progesterone secretion (Roy and Prakash, 2007).

During seasonal anestrus, resumption ovarian activity is delayed after each parturition (38 to 64 days in breeding season to 116 to 148 days during non-breeding season) and parturition to first estrus interval is prolonged compared to the rest of the year (Madan *et al*., 1988). Moreover, there is impact on the duration of estrus (8 to 10 h versus 18 h) in hot months. Also, unobserved heats (silent or subestrus) are common during summer months. However, ovarian activity not decreased in all animals during the non-breeding season, as a good number of females exhibited variable degrees of ovarian activity and only 30% of cyclic animals show no estrus behavior during summer (Tailor *et al*., 1990). Accordingly, some investigations have focused on protocols that are effective at inducing ovarian activity, stimulating estrus behavior and controlling ovulation during seasonal anestrus. Many hormonal regimens have been tried with varying degree of efficacy in terms of estrus induction and conception rate in summer. Treatment with progestagens and eCG can induce the resumption of estrus in anestrus buffalo cows, yielding good pregnancy rates. Similarly, progesterone-based protocols during the non-breeding season leads to pregnancy in non-productive animals. Implantation of intra-vaginal progesterone devices like CIDR for 10 to 14 days induced resumption of estrus among 83% of anestrus buffaloes within 12 to 120 h after implant removal. Implantation of CIDR and $PGF_{2\alpha}$ injection is reported more effective than CIDR alone in terms of exhibition of estrus and conception rate. Long exposure of CIDR (10 to 14 days) was superior to short exposure of 8 days in terms of resumption of estrus cyclicity in cattle and buffalo. Long exposure of progesterone may overcome summer sterility in buffaloes under rural managemental condition (Singh, 2003). Further, anestrus buffaloes reared under village or farm conditions during summer show more responses to norgestomet because of higher rates of ovulation and conception than CIDR or PRID treated animals. Numerous researchers have evaluated the potential of using a norgestomet and other hormonal combinations as a tool to synchronize estrus in cyclic and anestrus animals. Buffaloes showing absence of estrus after last calving show intense heat and satisfactory conception following $PGF_{2\alpha}$ 10 days apart (Day -12 and Day-2), pregnant mare serum gonadotropin (PMSG) Day-5, and hCG on the day of AI (Day 0). In buffaloes, pregnancy rates achieved were acceptable following Ovsynch during the non-breeding season (Warriach *et al*., 2008). While other earlier studies report poor pregnancy rates (26% in detected estrus and 7% in Ovsynch, Chohan, 1998). Ovulation in Nili-Ravi buffalo occured 30 h after the onset of standing estrus and buffaloes successfully synchronized with optimum fertility using either PGF2α alone (detected estrus) or using (Ovsynch protocol) during non-breeding season in order (Warriach *et al*., 2008). Conception rated in other studies were variable during non-breeding than breeding season in buffalo (Murugavel, 2009). Moreover many hormonal treatments aimed at controlling luteal and follicular functions provide exciting results after timed artificial insemination during the non-breeding season (Baruselli, 2005). Few studies reflect ovsynch protocol to be less effective during the non-breeding season, but CIDR application increased fertility during this period (Jabeen *et al*., 2012). This indicates ovsynch + CIDR a more effective regimen in non-breeding seasons in buffalo. Acyclic buffaloes treated with Ovsynch+CIDR, PRID+PMSG display follicular growth, dominance, ovulation, and good conception rates (Presicce *et al*., 2005). Future investigations need to be targeted at identifying factors determining seasonal impact on reproduction, patterns of estrus behaviour, physiology of estrus cycle and induction of follicular growth during non-breeding season in buffalo. Also, ovulation and its regulation by hormones needs emphasis to overcome the problem of silent or unobserved estrus behavior in buffaloes which otherwise often lead to wrong time insemination, conception failure and repeat breeding problems in this species.

Effect of equine chorionic gonadotropins (eCG) in synchronization

eCG when added to any progesterone-based estrus synchronization protocol substantially improves ovulation rate in non-cyclic buffaloes. But for unpredictability in timing of ovulation in buffalo, double inseminations and (Barile, 2005) was carried out to obtain acceptable pregnancy rates. Studies on anestrus buffaloes have shown that eCG incorporation at progesterone withdrawal in progesterone-based estrus synchronization protocols improved the ovulation rate. However, evaluations of such estrus synchronization protocols under field conditions in tropical climates have not been widely designed. Most of the protocols are applied to well managed animals reared under farm conditions (De Rensis, 2007). The CIDR + eCG treatment in cows and buffaloes improves ovulation rate in non-cyclic buffaloes and pregnancy rates in cyclic as well as non-cyclic animals (Murugavel *et al*., 2009). eCG improves the efficacy of a timed artificial insemination protocols by improving follicular growth reponse, luteal dynamics and fertility in buffalo during the non-breeding season although in heifers interval from progesterone withdrawal to ovulation and its synchrony has not been influenced (Baruselli *et al*., 2004).

Pre-pubertal anestrus and synchronization protocols

Anestrus condition in pre-pubertal buffaloes is characterized by the development of follicles to ovulatory size without ovulations. Anestrus condition in pre-pubertal buffaloes needs stimulation push to existing endogenous gonadotropins to start the ovulatory activity compared to strict seasonal breeders like ewe (Malpaux *et al*., 1998). Following second GnRH injection on day 9 in prepubertal anestrum, majority of the animals ovulate between 24 to 48 h (Baruselli *et al*., 2003). So far, only a few trials to induce and synchronize estrus in pre-pubertal anestrus buffaloes have been carried out, although these trails lack the understanding of ovarian activity (Zicarelli *et al*., 1997). In recent past, treatments that stimulate gonadotrophin secretion, follicular development, and ovulation such as Melatonin (Malpaux *et al*., 1998), ovsynch protocol (Ghuman *et al*., 2010), and progesterone implants (Ghuman *et al*., 2011) have been thoroughly investigated in pre-pubertal buffaloes displaying anestrus. Pre-pubertal anestrus buffaloes exhibited ovulatory response between 48 to 72 h subsequent to PRID removal. Possibly, prolonged progesterone exposure increases hypothalamic responsiveness to endogenous estradiol produced by dominant non-ovulatory follicles and hence occurrence of a proper LH surge leads to ovulation (Singh *et al*., 2009). PRID has been used in pre-pubertal conditions with 60% CR in PRID-treated pre-pubertal buffaloes inseminated 48 and 72 after PRID removal. There is emergence of an ovulatory follicle after PRID removal (12.4±1.0 mm; life span, 17.0±1.0 D), but heifers, emergence of 1 or 2 dominant non-ovulatory follicles (13.6±0.69 mm and ovulatory follicle (14.7±0.77 mm; life span, 11.7±0.9D) which ovulate 48 h after PRID removal (Singh *et al*., 2009). Ovsynch protocol has been found 100% successful for inducing ovulatory estrus which was synchronized in 82% anestrus buffalo heifers, thus requiring insemination only at 24 h subsequent to second GnRH but investigations are required to have better first service conception rate after ovsynch program in acyclic buffalo heifers during summer season (Ghuman *et al*., 2009). Underlying mechanisms for the induction of estrus could be priming of hypothalamus with progesterone and estrogen.

CONCLUSION

Recent developments in synchronization protocols ensure control on ovarian follicular growth and luteal dynamics in buffalo. This is aimed for better synchrony of estrus and fixed time ovulation of resulting dominant follicle and allowing inseminations at predetermined time. Many of the ovulation synchronization protocols control wave emergence and growth of follicles successfully in cattle but still need evaluation in buffalo. Further most of the combination protocols are designed for plasma progesterone decline at ovulation by removing exogenous progesterone source and administration of prostaglandin to promote luteal regression/endogenous source to improve timed ovulation rate. This way such protocols may serve as good tools for control of reproduction in buffalo. But extensive investigations are lacking in literature. $PGF_{2\alpha}$ based synchronization is more economical and effective in buffalo but success entirely depends on the presence of mature corpus luteum hence it may not work in anestrus or acyclic animals. Further success rate is poor when synchronization treatment is done during the periods of marginal breeding activity or in pre-pubertal anestrum condition in buffalo. Thus, effective synchronization protocols controlling/ synchronizing follicular and luteal processes need to be comprehensively designed for buffalo, particularly in different breeding seasons and near puberty. This could have an economic impact on buffalo production by breeding heifers exhibiting pre-pubertal anestrus at an optimal age and to enhance lifetime reproductive performance and productivity. As an overall conclusion, hormonal interventions along with improved management practices can better enhance reproductive performance in buffalo. Future investigations with this regard should be aimed at identification factors determining seasonal low fertility as well as patterns of estrus behaviour in buffalo. Insight into or better clarification of factors affecting estrus cycle physiology will help to achieve precise control of estrus cycle in buffalo. This would ultimately ensure more synchrony and acceptable ovulation and conception rates in fixed time insemination protocols in infertile buffaloes.

REFERENCES

Aboul-Ela, M.B., R.M. Khattab, F.E. El-Keraby, M.M. El-Shafie and L.H. Bedeir. 1988. Patterns of ovarian and estrous activity and induction of cyclic activity during the postpartum period in Egyptian buffaloes, p. 239-253. *In Optimizing Grazing Animal Productivity in the Mediterranean and North African Regions with the use of Nuclear Techniques*, IAEA, Vienna, Austria.

Adams, G.P. and R.A. Pierson. 1995. Bovine model for study of ovarian follicular dynamics in humans. *Theriogenology*, **43**(1): 113-120. DOI: 10.1016/0093-691X(94)00015-M

Ali, A., S. Abdel-Razek, S. Abdel-Ghaffar and P.S. Glatzel. 2003. Ovarian follicular dynamics in buffalo cows (*Bubalus bubalis*). *Reprod. Domest. Anim*., **38**(3): 214-572. DOI: 10.1046/j.1439-0531.2003.00428.x

Amaya-Montoya, C., M. Matsui, K.G. Kawashima, G. Hayashi, E. Matsuda, K. Kaneko, A. Kida, Y. Miyamoto and Miyake. 2007. Induction of ovulation with GnRH and PGF(2 alpha) at two different stages during the early postpartum period in dairy cows: ovarian response and changes in hormone concentrations. *J. Reprod. Develop*., **53**(4): 867. DOI: DOI: 10.1262/jrd.18163

Awasthi, M.K., A.F. Khare, F.S. Kavani, G.M. Siddiquee, M.T. Panchal and R.R. Shah. 2006. Is one-wave follicular growth during the estrous cycle a usual phenomenon in water buffaloes (*Bubalus bubalis*)? *Anim. Reprod. Sci*., **92**(3-4): 241-253. DOI: 10.1016/j.anireprosci.2005.05.024

Barile, V.L. 2005. Improving reproductive efficiency in female buffaloes. *Livest. Prod. Sci*., **92**(3): 183-194. 10.1016/j.livprodsci.2004.06.014

Barkawi, A.H., Y.M. Hafez, S.A. Ibrahim, G. Ashour, A.K. El-Asheeri and N. Ghanem. 2009. Characteristics of ovarian follicular dynamics throughout the estrous cycle of Egyptian buffaloes. *Anim. Rep. Sci*., **110**(3-4): 326-333. DOI: 10.1016/j.anireprosci.2008.02.016

Baruselli, P.S. 2001. Control of follicular development applied to reproduction biotechnologies in buffalo. *In Proceedings of the 1st Congresso Nazionalesull'allevamento del Bufalo*, *Book of the Congress*, p. 128-146.

Baruselli, P.S. and N.A.T. Carvalho. 2005. Biotechnology of reproduction in buffalo (*Bubalus bubalis*). *Revista Brasileira de Reprodução Animal*, **29**: 4-17.

Baruselli, P.S., N.A.T. Carvalho, L.U. Gimenes and G.A. Crepaldi. 2007. Fixed-time artificial insemination in buffalo. *Ital. J. Anim. Sci*., **6**(2S): 107-118. aDOI:10.4081/ijas.2007.s2.107

Baruselli, P.S., E.H. Madureira, V.H. Barnabe, R.C. Barnabe and R.C. de Araújo Berber. 2003. Evaluation of synchronization of ovulation for fixed timed insemination in buffalo (*Bubalus bubalis*). *Braz. J. Vet. Res. Anim. Sci*., **40**(6). DOI: 10.1590/S1413-95962003000600007

Baruselli, P.S., M.O. Marques, N.A.T. Carvalho, E.H. Madureira and E.P. Filho. 2002. Efeito de diferentes protocolos de inseminação artificial em tempo fixo na eficiência reprodutiva de vacas de corte lactantes. *Revista Brasileira de Reprodução Animal*, **26**(3): 218-221.

Baruselli, P.S., M.O. Marques, L.F.T. Nasser, E.L. Reis and G.A. Bo. 2003. Effect of eCG on pregnancy rates of lactating zebu beef cows treated with CIDR-B devices for timed artificial insemination. *Theriogenology*, **59**(1): 214. DOI: 10.1016/S0093-691X(02)01253-0

Baruselli, P.S., G.A. Mucciolo, V.C. Visintin, R.P.V. Arruda and E.H. Madureira. 1997. Ovarian follicular dynamics during the estrous cycle in buffalo (*Bubalus bubalis*). *Theriogenology*, **47**: 1531-1547. DOI: 10.1016/s0093-691x(97)00159-3

Baruselli, P.S., R.G. Mucciolo, R.P. Arruda, E.H. Madureira, R. Amaral and M.E.O.A. Assumpcao. 1999. Embryo recovery rate in superovulated buffalo. *Theriogenology*, **51**: 401.

Baruselli, P.S., R.G. Mucciolo, J.A. Visintin, W.G. Viana, R.P. Arruda, E.H. Madureira, C.A. Oliviera and J.R. Molero-Filho. 1996. Ovarian follicular dynamics during the estrus cycle in buffalo (*Bubalus bubalis*): Preliminary research. *Theriogenology*, **47**: 1531-1547. DOI: 10.1111/j.1749-6632.1996.tb53547.x

Baruselli, P.S., E.L. Reis, N.A.T. Carvalho and J.B.P. Carvello. 2004. eCG increase ovulation rate and plasmatic progesterone concentration in Nelore (*Bos indicus*) heifers treated with progesterone releasing device [abstract]. *In 16th International Congress on Animal Reproduction*, **1**: 117.

Baruselli, P.S., E.L. Reis, M.O. Marques, L.F. Nasser and G.A. Bo. 2004. The use of hormonal treatments to improve reproductive performance of anestrous beef cattle in tropical climates. *Anim. Reprod. Sci*., 82-83; 479-486. DOI: 10.1016/j.anireprosci.2004.04.025

Baruselli, P.S., O. Bernandes, F.B. Barufi, D. Braga, D Araujo and H. Tonathi. 2001. Calving distribution throughout the year in buffalo raised all over Brazil. *In Proceedings of the 6th World Buffalo Congress*, p. 234-340.

Baruselli, P.S., E.H. Madureira, V.H. Barnabe, R.C. Barnabe and R.C.A. Berber. 2003. Evaluation of synchronization of ovulation for fixed timed insemination in buffalo (*Bubalus bubalis*). *Brazilian Journal of Veterinary Research and Animal*

Science, **40**: 431-442. DOI: 10.1590/S1413-95962003000600007

Baruselli, P.S., E.H. Madureira, J.A. Visintin, V.H. Barnabe, R.C. Barnabe and R. Amaral. 1999. Timed insemination using synchronization of ovulation in buffaloes. *Revista Brasileira de Reprodução Animal*, **23**: 360-362.

Berardinelli, J.G. and R. Adair. 1989. Effect of prostaglandin F2 alpha dosage and stage of estrous cycle on the estrous response and corpus luteum function in beef heifers. *Theriogenology*, **32**(2): 301-314. DOI: 10.1016/0093-691x(89)90320-8

Bhat, G.R. and G.S. Dhaliwal. 2018. Characteristics and quantification of induced-estrus following estradiol-based and ovsynch-based synchronization protocols in Murrah buffalo (*Bubalus bubalis*). *SKUAST Journal of Research*, **20**(1): 111-113.

Bhat, G.R., G.S. Dhaliwal, S.P.S. Ghuman and M. Honparkhe. 2015. Comparative efficacy of E-17β and GnRH administration on day 0 of a controlled interval drug release (CIDR) based protocol on synchrony of wave emergence, ovulation and conception rates in Murrah buffalos (*Bubalus bubalis*). *Iran. J. Vet. Res*., **16**(1): 53-58.

Bhat, G.R., G.S. Dhaliwal, S.P.S. Ghuman and M. Honparkhe. 2014. Size of dominant follicle, plasma progesterone and estradiol levels on the day of ovulation and subsequent conception rate in buffalo (*Bubalus bubalis*) following modified ovsynch + CIDR protocol. *J. Appl. Anim. Res*., **43**(3): 314-317. DOI: 10.1080/09712119.2014.964249

Bilal, A.G., S.S. Dhindsa, M. Honparkhe, S.P.S. Ghuman, R. Jindal and P.S. Brar. 2016. Effect of delaying Luteolysis during second follicular wave on subsequent fertility following timed artificial insemination in the buffalo. *Indian Vet. J*., **93**(7): 84-85. Available on: https://www.researchgate.net/profile/Sarvpreet-Ghuman/publication/309060078_Effect_of_delaying_luteolysis_during_second_follicular_wave_on_subsequent_fertility_following_timed_artificial_insemination_in_the_buffalo/links/5a190737a6fdcc50ade7ee9f/Effect-of-delaying-luteolysis-during-second-follicular-wave-on-subsequent-fertility-following-timed-artificial-insemination-in-the-buffalo.pdf

Binelli, M., B.T. Ibiapina and R.S. Bisinotto. 2006. Bases fisiológicas, farmacológicas e endócrinas dos tratamentos de sincronização do crescimento folicular e da ovulação. *Acta Sci. Vet*., **34**(1): 1-7.

Binelli, M., W.W. Thatcher, R. Mattos and P.S. Baruselli. 2001. Antiluteolytic strategies to improve fertility in cattle. *Theriogenology*, **56**(9): 1451-1463. DOI: 10.1016/s0093-691x(01)00646-x

Bisinotto, R.S., R.C. Chebel and J.E.P. Santos. 2010. Follicular wave of the ovulatory follicle and not cyclic status influences fertility of dairy cows. *J. Dairy Sci*., **93**(8): 3578-3587. DOI: 10.3168/jds.2010-3047

Bo, G.A., G.P. Adams and R.A. Pierson. 1995. Exogenous control of follicular wave emergence in cattle. *Theriogenology*, **43**(1): 31-40. DOI: 10.1016/0093-691X(94)00010-R

Bo, G.A., P.S. Baruselli and M.F. Martínez. 2003. Pattern and manipulation of follicular development in *Bos indicus* cattle. *Anim. Reprod. Sci*., **78**: 307-326.

Bo, G.A., P.S. Baruselli and M.F. Martinez. 2003. Pattern of manipulation of follicular development in *Bos indicus* cattle. *Anim. Reprod. Sci*., **78**(3-4): 307-326. DOI: 10.1016/s0378-4320(03)00097-6

Bo, G.A., P.S. Baruselli, D. Moreno, L. Cutaia, M. Caccia, R. Tribulo, H. Tribulo and R.J. Mapletoft. 2002. The control of follicular wave development for self-appointed embryo transfer programs in cattle. *Theriogenology*, **57**(1): 53-72. DOI: 10.1016/s0093-691x(01)00657-4

Brito, L.F.C., R. Satrapa, E.P. Marson and J.P. Kastelic. 2002. Efficacy of PGF2 alpha to synchronize estrus in water buffalo cows (*Bubalus bubalis*) is dependent upon plasma progesterone concentration, corpus luteum size and ovarian follicular status before treatment. *Anim. Reprod. Sci*., **73**(1-2): 23-35. DOI: 10.1016/s0378-4320(02)00124-0

Campanile, G., D. Vecchio, G. Neglia, R. Di Palo, A. Prandi and M.J. Occhio. 2008. Progesterone and pregnancy status in buffaloes treated with a GnRH agonist. *Livest. Sci*., **115**(2-3): 242-248. DOI: 10.1016/j.livsci.2007.08.001

Carvalho, J.B., N.A. Carvalho, E.L. Reis, M. Nichi, A.H. Souza and P.S. Baruselli. 2008. Effect of early luteolysis in progesterone-based timed AI protocols in *Bos indicus*, *Bos indicus* x *Bos taurus*, and *Bos taurus* heifers. *Theriogenology*, **69**(2): 167-175. DOI: 10.1016/j.theriogenology.2007.08.035

Chebel, R.C., J.E.P. Santos, R.L.A. Cerri, H.M. Rutigliano and R.G.S. Bruno. 2006. Reproduction in dairy cows following progesterone insert presynchronization and resynchronization protocols. *J. Dairy Sci*., **89**(11): 4205-4219. DOI: 10.3168/jds.S0022-0302(06)72466-3

Chohan, K.R. 1998. Estrus synchronization with lower dose of PGF2α and subsequent fertility in subestrous buffalo. *Theriogenology*, **50**(7): 1101-1108. DOI: 10.1016/S0093-691X(98)00211-8

Craig, J., M. Orisaka, H. Wang, S. Orisaka, W. Thompson, C. Zhu, F. Kotsuji and B.K. Tsang. 2007. Gonadotropin and intra-ovarian signals regulating follicle development and atresia: The delicate balance between life and death. *Front. Biosci*., **12**: 3628-3639. DOI: 10.2741/2339

Cunha, A.P., J.N. Guenther, M.J. Maroney, J.O. Giordano, A.B. Nascimento, S. Bas, H. Ayres and M.C. Wiltbank. 2008. Recent advances in ovulation synchronization and superovulation in dairy cattle. *J. Dairy Sci*., **6**(1): 194. Available on: https://www.animal-reproduction.org/article/5b5a606ef7783717068b4767/pdf/animreprod-6-1-194.pdf

Dadarwal, D., S.P.S. Ghuman, M. Honparkhe and J. Singh. 2009. Synchronization of ovulation and subsequent fertility in buffaloes following PGF2α-PGF2α protocol, with or without GnRH. *Indian J. Anim. Sci*., **79**(9): 861-865.

Dadarwal, D., R.J. Mapletoft, G.P. Adams, C. Creelman and J. Singh. 2013. Effect of progesterone concentration and duration of proestrus on fertility in beef cattle after fixed-time artificial insemination. *Theriogenology*, **79**(5): 859-866. DOI: 10.1016/j.theriogenology.2013.01.003

Danell, B. 1987. *Oestrousbehaviour, ovarian morphology and cyclical variation in folicular system and endocrine pattern in water buffalo heifers*. Ph.D. Thesis, Faculty of Veterinary Medicine, University of Agricultural Sciences, Sveriges Lantbruksuniversitet, Uppsala, Sweden. p.

54-94.

Das, G.K. and F.A. Khan. 2010. Summer anestrus in buffalo - A review. *Reprod. Domest. Anim.*, **45**(6): 483-494. DOI: 10.1111/j.1439-0531.2010.01598.x

De Rensis, F. and L. Gatius. 2007. Protocols for synchronizing estrus and ovulation in buffalo (*Bubalus bubalis*): A review. *Theriogenology*, **67**(2): 209-216. DOI: 10.1016/j.theriogenology.2006.09.039De

Rensis, F., G. Ronci, P. Guarneri, B.X. Nguyen, G.A. Presicce, G. Huszenicza and R.J. Scaramuzzi. 2005. Conception rate after fixed time insemination following ovsynch protocol with and without progesterone supplementation in cyclic and non-cyclic Mediterranean Italian buffaloes (*Bubalus Bubalis*). *Theriogenology*, **63**(7): 1824-1831. DOI: 10.1016/j.theriogenology.2004.07.024

Denicol, A.C., G.Jr. Lopes, L.G. Mendonca, F.A. Rivera, F. Guagnini, R.V. Perez, J.R. Lima, R.G. Bruno, J.E. Santos and R.C. Chebel. 2012. Low progesterone concentration during the development of the first follicular wave reduces pregnancy per insemination of lactating dairy cows. *J. Dairy Sci.*, **95**(4): 1794-1806. DOI: 10.3168/jds.2011-4650

Diskin, M.G. and D.G. Morris. 2008. Embryonic and early foetal losses in cattle and other ruminants. *Reprod. Domest. Anim.*, **43**(2): 260-267. DOI: 10.1111/j.1439-0531.2008.01171.x

El-Wishy, A.B. 2007. The postpartum buffalo. II. Acyclicity and anestrus. *Anim. Reprod. Sci.*, **97**(3-4): 216-236. DOI: 10.1016/j.anireprosci.2006.03.003

Erven, B.L. and D. Arbaugh. 1987. Artificial Insemination on US dairy farms. *Report of a Study Conducted in Cooperation with the National Association of Animal Breeders*, National Association of Animal Breeders, Columbia, USA.

Fonseca, F.A., J.H. Britt, B.T. Mcdaniel, J.C. Wilk and A.H. Rakes. 1983. Reproductive traits of Holsteins and Jerseys - Effects of age, milk yield, and clinical abnormalities on involution of cervix and uterus, ovulation, estrous cycles, detection of estrus, conception rate, and days open. *J. Dairy Sci.*, **66**(5): 1128-1147. DOI: 10.3168/jds.S0022-0302(83)81910-9

Galina, C.S. and A. Orihuela. 2007. The detection of estrus in cattle raised under tropical conditions: What we know and what we need to know. *Horm. Behav.*, **52**(1): 32-38. DOI: 10.1016/j.yhbeh.2007.03.025

Galvao, K.N., J.E.P. Santos, S.O. Juchem, R.L.A. Cerri, A.C. Coscioni and M. Villasenor. 2004. Effect of addition of a progesterone intravaginal insert to a timed insemination protocol using estradiol cypionate on ovulation rate, pregnancy rate, and late embryonic loss in lactating dairy cows. *J. Anim. Sci.*, **82**(12): 3508-3517. DOI: 10.2527/2004.82123508x

Ganaie, B.A., S.S. Shahbaz, D. Dadarwal and M. Honparkhe. 2018. Timed artificial insemination methods involving ovulation of second follicular wave *vis-a-vis* extension in growth of dominant follicle-new alternatives to enhance fertility in buffalo (*Bubalis bubalis*). *J. Anim. Res.*, **8**(3): 521-524. DOI: 10.30954/2277-940X.06.2018.30

Garcia, A. and M. Salah-ed-dine. 1998. Effects of repeated ultrasound-guided transvaginal follicular aspiration on bovine oocyte recovery and subsequent follicular development. *Theriogenology*, **50**(4): 575-

585. DOI: 10.1016/s0093-691x(98)00162-9

Ghuman, S.P.S., J. Singh, M. Honparkhe, D. Dadarwal, G.S. Dhaliwal and A.K. Jain. 2010. Induction of ovulation of ovulatory size non-ovulatory follicles and initiation of ovarian cyclicity in summer anoestrous buffalo heifers (*Bubalus bubalis*) using melatonin implants. *Reprod. Domest. Anim*., **45**(4): 600-607. DOI: 10.1111/j.1439-0531.2008.01310.x

Ghuman, S.P.S, M. Honparkhe, J. Singh and V.K. Gandotra. 2010. Ovsynch plus CIDR-based fixed-time AI protocol as a therapeutic strategy in repeat-breeder crossbred dairy cattle. *Indian J. Anim. Sci*., **81**(3): 257-259. Available on: https://www.researchgate.net/profile/Sarvpreet-Ghuman/publication/286531361_Ovsynch_plus_CIDR-based_fixed-time_AI_protocol_as_a_therapeutic_strategy_in_repeat-breeder_crossbred_dairy_cattle/links/567a332608ae361c2f681ca8/Ovsynch-plus-CIDR-based-fixed-time-AI-protocol-as-a-therapeutic-strategy-in-repeat-breeder-crossbred-dairy-cattle.pdf

Ghuman, S.P.S., M. Honparkhe and J. Singh. 2010. Optimizing ovarian and reproductive events of anestrus buffaloes using intravaginal progesterone, GnRH, and PGF 2α, p. 82-83. *In Proceedings of International Buffalo Conference held at National Agricultural Science Centre Complex*, New Delhi, India.

Ghuman, S.P.S., M. Honparkhe, J. Singh, D.S. Dhami, A. Kumar, G. Nazir and C. Ahuja. 2011. Fertility response using three estrus synchronization regimens in lactating anestrous buffaloes. *Indian J. Anim. Sci*., **82**(2): 162-166.

Ghuman, S.P.S., J. Singh, M. Honparkhe and D. Dadarwal. 2009. Induction of ovulatory estrus using Ovsynch protocol and subsequent fertility in true anestrus buffalo heifers. *Indian Journal of Animal Reproduction*, **30**(2): 1-5.

Gimenes, P., N. Fantinato, J.S.P. Arango, H. Ayres and P.S. Baruselli. 2009. Follicular dynamics of *Bos indicus*, *Bos taurus* and *Bubalus bubalis* heifers treated with norgestomet ear implant associated or not to injectable progesterone. *Anim. Reprod*., **6**(1): 256.

Gonzalez, B.A., G.R.M. Garcia, J. Santiago, S.A. Lopez and M.J. Cocero. 2002. Effect of follicular status on superovulatory response in ewes is influenced by presence of corpus luteum at first FSH dose. *Theriogenology*, **58**(8): 1607-1614. DOI: 10.1016/s0093-691x(02)01078-6

Gumen, A. and M.C. Wiltbank. 2005. Length of progesterone exposure needed to resolve large follicle anovular condition in dairy cows. *Theriogenology*, **63**(1): 202-218. DOI: 10.1016/j.theriogenology.2004.04.009

Hahn, N.V., Q.X. Hou and J. Sulon. 2007. Estrus synchronization, artificial insemination, and pregnancy diagnosis in water buffaloes (*Bubalus bubalis*). *Reprod. Fert. Develop.*, **19**(1): 198. DOI: 10.1071/RDv19n1Ab162

Honparkhe, M. 2011. *Ovulation synchrony and superovulatory response after synchronization of follicular wave emergence in buffaloes*. Ph.D. Dissertation, Submitted to Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India.

Hoque, M.N., A.K. Talukder, M. Akter and M. Shamsuddin. 2014. Evaluation of ovsynch protocols for timed artificial insemination in water buffaloes in Bangladesh. *Turk. J. Vet. Anim. Sci*., **38**(4): 418-424. DOI: 10.3906/vet-1302-35

Jabeen, S., M. Anwar, S.M.H. Andrabi, A. Mehmood, S. Murtaza and M. Shahab. 2013. Determination of Ovsynch efficiency for estrus synchronization by plasma LH and P4 levels in Nili Ravi buffalo during peak and low breeding seasons. *Pak. Vet. J*., **33**(2): 221.

Jainudeen, M.R. and E.S.E. Hafez. 2000. *Reproduction in Farm Animals*, 7th ed. Lea and Febiger, Philadelphia, USA. p. 315-329.

Kanai, Y., T. Abdul-Latief, N. Ishikawa and H. Shimizu. 1990. Behavioural and hormonal aspects of the estrus cycle in Swamp buffaloes reared under temperate conditions, p. 113-120. *In Domestic Buffalo Production in Asia, International Atomic Energy Agency*, Vienna, Austria.

Kaur, H. and S.P. Arora. 1994. Annual pattern of plasma progesterone in normal cycling buffaloes (*Bubalus bubalis*) fed two levels of nutrition. *Anim. Reprod. Sci*., **7**(4): 323-741. DOI: 10.1016/0378-4320(84)90017-4

Khan, F.A., G.K. Das, M. Pandey, M.K. Pathak and M. Sarkar. 2011. Biochemical and hormonal composition of follicular cysts in water buffalo (*Bubalus bubalis*). *Anim. Reprod. Sci*., **124** (1-2): 61-64. DOI: 10.1016/j.anireprosci.2011.02.020

Kulick, L.J., K. Kot, M.C. Wiltbank and O.J. Ginther. 1999. Follicular and hormonal dynamics during the first follicular waves in heifers. *Therigenology*, **52**(5): 913-921. DOI: 10.1016/S0093-691X(99)00182-X

Lonergan, P. 2011. Influence of progesterone on oocyte quality and embryo development in cows. *Theriogenology*, **76**(9): 1594-1601. DOI: 10.1016/j.theriogenology.2011.06.012

Lynch, C.O., D.A. Kenny, S. Childs and M.G. Diskin. 2010. The relationship between periovulatory endocrine and follicular activity on corpus luteum size, function, and subsequent embryo survival. *Theriogenology*, **73**(2): 190-198. DOI: 10.1016/j.theriogenology.2009.08.012

Malpaux, B,, C. Viguie, D.C. Skinner, J.C. Thiéry, J. Pelletier and P. Chemineau. 1996. Seasonal breeding in sheep: mechanism of action of melatonin. *Anim. Reprod. Sci*., **42**(1-4): 109-117. DOI: 10.1016/0378-4320(96)01505-9

Malpaux, B., A. Daveau, F. Maurice-Mandon, G. Duarte and P. Chemineau. 1998. Evidence that melatonin acts in the premammillary hypothalamic area to control reproduction in the ewe: Presence of binding sites and stimulation of luteinizing hormone secretion by *in situ* microimplant delivery. *Endocrinology,* **139**(4): 1508-1515. DOI: 10.1210/endo.139.4.5879

Manik, R.S., P. Palta, S.K. Singla and V. Sharma. 2002. Folliculogenesis in buffalo (*Bubalus bubalis*): A review. *Reprod. Fert. Develop*., **14**(5-6): 315-325. DOI: 10.1071/rd01126

Mann, G.E. and G.E. Lamming. 1999. The influence of progesterone during early pregnancy in cattle. *Reprod. Domest. Anim*., **34**(3-4): 269-274. DOI: 10.1111/j.1439-0531.1999.tb01250.x

Mee, M.O., J.S. Stevenson, B.M. Alexander. and R.G. Sareer. 1993. Administration of GnRH at estrus influences pregnancy rates, serum concentrations of LH, FSH, estradiol-17 beta, pregnancy-specific protein B, and progesterone, proportion of luteal cell types, and *in vitro* production of progesterone in dairy cows. *J. Anim. Sci*., **71**(1): 185-198.

DOI: 10.2527/1993.711185x

Murugavel, K., D. Antoine, M.S. Raju and F.L Gatius. 2009. The effect of addition of equine chorionic gonadotropin to a progesterone-based estrous synchronization protocol in buffaloes (*Bubalus bubalis*) under tropical conditions. *Theriogenology*, **71**(7): 1120-1126. DOI: 10.1016/j.theriogenology.2008.12.012

Nanda, A.S., P.S. Brar and S. Prabhakar. 2003. Enhancing reproductive performance in dairy buffalo: Major constraints and achievements. *Reproduction*, **61**: 27-36.

Neglia, G., B. Gasparrini, R. Di Palo, C. De Rosa, L. Zicarelli and G. Campanile. 2003. Comparison of pregnancy rates with two estrus synchronization protocols in Italian Mediterranean buffalo cows. *Theriogenology*, **60**(1): 125-133. DOI: 10.1016/S0093-691X(02)01328-6

Noseir, W.M.B. 2003. Ovarian follicular activity and hormonal profile during estrous cycle in cows: The development of 2 versus 3 waves. *Reprod. Biol. Endocrin.*, **1**: 50. DOI: 10.1186/1477-7827-1-50

Odde, G.K. 1990. A review of synchronization of estrusin postpartum cattle. *J. Anim. Sci.*, **68**(3): 817-830. DOI: 10.2527/1990.683817x

Pandey, A.K., G.S. Dhaliwal, S.P.S. Ghuman and S.K. Agarwal. 2010. Impact of pre-ovulatory follicle diameter on plasma estradiol, subsequent luteal profiles and conception rate in buffalo (*Bubalus bubalis*). *Anim. Reprod. Sci.*, **123**(3-4): 169-174. DOI: 10.1016/j.anireprosci.2010.12.003

Parmeggiani, A., E. Seren, L. Esposito, A. Borghese, R. Di Palo and G.M. Terzano. 1992. Plasma levels of melatonin in buffalo cows. *In Proceedings of the International Symposium 'Prospects of buffalo production in the Mediterranean and the Middle East'*, Cairo, Egypt. **62**: 401-403.

Paul, V. and B.S. Prakash. 2005. Efficacy of the Ovsynch protocol for synchronization of ovulation and fixed-time artificial insemination in Murrah buffaloes (*Bubalus bubalis*). *Theriogenology*, **64**(5):1049-1060. DOI: 10.1016/j.theriogenology.2005.02.004

Perera, B.M.A.O. 1999. Reproduction in water buffalo: Comparative aspects and implications for management. *J. Reprod. Fertil.*, **54**: 157-168.

Perera, B.M.A.O. 2008. Reproduction in domestic buffalo. *Reprod. Domest. Anim.*, **43**(Suppl. 2): 200-206. DOI: 0.1111/j.1439-0531.2008.01162.x

Perera, B.M.A.O. 2011. Reproductive cycles of buffalo. *Anim. Reprod. Sci.*, **124**(3-4): 194-199. DOI: 10.1016/j.anireprosci.2010.08.022

Perera, B.M.A.O., L.N.A. de Silva, V.Y. Kuruwita and A.M. Kurunaratne. 1987. Postpartum ovarian activity, uterine involution and fertility in indigenous buffaloes at a selected village location in Sri Lanka. *Anim. Reprod. Sci.*, **14**(2): 115-127. DOI: 10.1016/0378-4320(87)90091-1

Perry, G.A., M.F. Smith and T.W. Geary. 2004. Ability of intravaginal progesterone inserts and melengestrol acetate to induce estrous cycles in postpartum beef cows. *J. Anim. Sci.*, **82**(3): 695-704. DOI: 10.2527/2004.823695x

Perry, G.A., M.F. Smith, M.C. Lucy, J.A. Green, T.E. Parks, M.D. MacNeil, A.J. Roberts and T.W. Geary. 2005. Relationship between follicle size at insemination and pregnancy success. *P. Natl. Acad. Sci. USA*, **102**(14): 5268-5273. DOI: 10.1073/pnas.0501700102

Presicce, G.A., D. Rath, P. Klinc, E.M. Senatore and M. Pascale. 2005. Buffalo calves born following AI with sexed semen. *Reprod. Domest. Anim*., **40**: 349.

Presicce, G.A., E.M. Senatore, A. Bella, G. De Santis, V.L. Barile, G.J. De Mauro, G.M. Terzano, R. Stecco and A. Parmeggiani. 2004. Ovarian follicular dynamics and hormonal profiles in heifer and mixed-parity Mediterranean Italian buffaloes (*Bubalus bubalis*) following an estrus synchronization protocol. *Theriogenology*, **61**(7-8): 1343-1355. DOI: 10.1016/j.theriogenology.2003.08.013

Roy, K.S. and B.S. Prakash. 2007. Seasonal variation and circadian rhythmicity of the prolactin profile during the summer months in repeat-breeding Murrah buffalo heifers. *Reprod. Fert. Develop*., **19**(4): 569-575. DOI: 10.1071/rd06093

Roy, K.S. and B.S. Prakash. 2009. Plasma progesterone, oestradiol-17β and total oestrogen profiles in relation to oestrous behaviour during induced ovulation in Murrah buffalo heifers. *J. Anim. Physiol. An. N*., **93**(4): 486-495. DOI: 10.1111/j.1439-0396.2008.00830.x

SaFilho, O.G., C.C. Dias and J.L.M. Vasconcelos. 2006. Effect of progesterone or 17β-estradiol on luteal lifespan in anestrus Nelore cows, *J. Anim. Sci*., **84**: 207.

Saini, M.S., M.M. Galhotra, M.L. Sagwan and M.M. Razdan. 1988. Use of PRID in inducing estrus and its effect on the sexual behavior of Murrah buffalo heifers. *Indian J. Dairy Sci*., **41**: 40-42.

Sartori, R., J.M. Haughain, R.D. Shaver, G.J. Rosa and M.C. Wiltbank. 2004. Comparison of ovarian function and circulating steroids in estrous cycles of Holstein heifers and lactating cows. *J. Dairy Sci*., **87**(4): 905-920. DOI: 10.3168/jds.S0022-0302(04)73235-X

Savio, J.D., W.W. Thatcher, G.R. Morris, K. Entwistle, M. Drost and M.R. Mattiacci. 1993. Effects of induction of low plasma progesterone concentrations with a progesterone-releasing intravaginal device on follicular turnover and fertility in cattle. *J. Reprod. Fert*., **98**(1): 77-84. DOI: 10.1530/jrf.0.0980077

Shah, S.N.H. 1988. Comparative studies of seasonal influence on breeding behaviour and conception rate of dairy buffalo and zebu cattle. *In Proceedings of the 11th International CD Congress on Animal Reproduction and Artificial Insemination*, Universiy College Dublin, Dublin, Ireland, **3**: 538-542.

Singh, C. 2003. Response of anestrus rural buffaloes (*Bubalus bubalis*) to intravaginal progesterone implant and PGF2 alpha injection in summer. *J. Vet. Sci*., **4**(2): 137-141. DOI: 10.4142/jvs.2003.4.2.137

Singh, J., S.P.S. Ghuman, M. Honparkhe and N. Singh. 2009. Investigations on dominant follicle development, estrus response, ovulation time and fertility in PRID-treated anestrous buffalo heifers. *Indian J. Anim. Sci*., **79**(8): 773-777.

Singh, J., M. Honparkhe, D. Dadarwal, S.P.S. Ghuman and G.S. Dhaliwal. 2009. Optimization of the estrus period characteristics in crossbred cattle exhibiting prolonged estrus using prostaglandin analogue, p. 166-167. *In Proceedings of Silver Jubilee Annual Convention of ISSAR and International Symposium*, Namakkal, Chennai, India.

Singh, J., A.S. Nanda and G.P. Adams. 2000. The reproductive pattern and efficiency of female buffaloes. *Anim. Reprod. Sci*., **60-61**: 593-604. DOI: 10.1016/s0378-4320(00)00109-3

Stronge, A.G., J.M. Sreenan, M.G. Diskin, J.F. Mee, D.A. Kenny and D.G. Morris. 2005. Post-insemination milk progesterone concentration and embryo survival in dairy cows. *Theriogenology*, **64**(5): 1212-1214. DOI: 10.1016/j.theriogenology.2005.02.007

Subramaniam, P.S., J.D.S. Sundarsingh and K.P. Devarajan. 1989. Estrus synchronization with 844 PGF2 alpha in buffaloes. *Indian Vet. J*., **66**: 538-540.

Tailor, S.P., L.S. Jain, H.K. Gupta and J.S. Bhatia. 1990. Oestrus and conception rates in buffaloes 846 under village conditions. *Indian J. Anim. Sci*., **60**(8): 1020-1021.

Taneja, M., A. Ali and G. Singh. 1996. Ovarian follicular dynamics in water buffalo. *Theriogenology*, **46**(1): 121-130. DOI: 10.1016/0093-691X(96)00147-1

Taneja, M., S.M. Totey and A. Ali. 1995. Seasonal variation in follicular dynamics of superovulated Indian water buffalo. *Theriogenology*, **43**(2): 451-464. DOI: 10.1016/0093-691x(94)00038-v

Tegegne, A., K.W. Entwistle and E. Mukasa-Mugerwa. 1993. Plasma progesterone and blood metabolite profiles in post-partum small east African zebu cows. *Trop. Anim. Health Pro*., **25**(2): 101-110. DOI: 10.1007/BF02236516

Viguie, C., A. Caraty, A. Locatelli and B. Malpaux. 1995. Regulation of luteinizing hormone-releasing hormone (LHRH) secretion by melatonin in the ewe. I. Simultaneous delayed increase in LHRH and luteinizing hormone pulsatile secretion. *Biol. Reprod*., **52**(5): 1114-1120. DOI: 10.1095/biolreprod52.5.1114

Warriach, H.M., A.A. Channa and N. Ahmad. 2008. Effect of oestrus synchronization methods on oestrus behaviour, timing of ovulation and pregnancy rate during the breeding and low breeding seasons in *Nili-Ravi* buffaloes. *Anim. Reprod. Sci*., **107**(1-2): 62-67. DOI: 10.1016/j.anireprosci.2007.06.007

Wiltbank, M.C., A. Gumen and R. Sartori. 2002. Physiological classification of anovulatory conditions in cattle. *Theriogenology*, **57**(1): 21-52. DOI: 10.1016/s0093-691x(01)00656-2

Wiltbank, M.C., A.H. Souza, J.O. Giordano, A.B. Nascimento, J.M. Vasconcelos, M.H.C. Pereira, P.M. Fricke, R.S. Sarjus, F.C.S. Zinsly, P.D. Carvalho, R.W. Bender and R. Sartori. 2012. Positive and negative effects of progesterone during timed AI protocols in lactating dairy cattle. *Anim. Reprod*., **9**(3): 231-241. Available on: http://www.cbra.org.br/pages/publicacoes/animalreproduction/issues/download/v9n3/pag231-241%20(AR530).pdf

Xu, Z., H.A. Garverick, G.W. Smith, M.F. Smith, S.A. Hamilton and R.S. Youngquist. 1995. Expression of follicle-stimulating hormone and luteinizing hormone receptor messenger ribonucleic acids in bovine follicles during the first follicular wave. *Biol. Reprod*., **53**(4): 951-957. DOI: 10.1095/biolreprod53.4.951

Yindee, M., M. Techakumphu, C. Lohachit, S. Sirivaidyapong, A. Na-Chiangmai, M.H. Rodriguez, G.C. Vander Weyden and B. Colenbrander. 2010. Follicular dynamics and oestrous detection in Thai postpartum swamp buffaloes (*Bubalus bubalis*). *Reprod. Domest. Anim*., **46**(1): 91-96. DOI: 10.1111/j.1439-0531.2010.01647.x

Yindee, M., M. Techakumphu, C. Lohachit, S.

Sirivaidyapong, A. Na-Chiangmai and B. Colenbrander. 2007. Ovarian activity and sexual behavior in the postpartum Swamp buffalo (*Bubalus bubalis*). *Ital. J. Anim. Sci*., **6**(2): 632-635. DOI: 10.4081/ijas.2007.s2.632