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Estrus synchronization in buffaloes: Prospects, approaches and limitations

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Abstract
Estrus synchronization and timed inseminations can overcome two distinct problems of buffalo breeding: poor overt estrus expression and seasonality of breeding. Evidence is accumulating that, similar to cattle, the synchronization of estrus in buffalo is dependent on the presence of dominant follicle (DF) and corpus luteum (CL) on the ovary. Approaches for estrus synchronization govern either the progression of DF to ovulation following luteolysis (prostaglandins PG) or regression of a DF, followed by growth of a new DF that progresses to ovulation (progestins and GnRH). The use of prostaglandins for estrus synchronization has led to estrus rates (ER) of 60-80% and conception rates (CR) of 12.5%-60% in buffalo heifers and ER of 70-100% and CR of 40-80% in adult buffaloes. Such an approach has the disadvantage of poor response in postpartum anestrus buffaloes and buffalo heifers with absence of CL and follicular growth. Moreover, the effects are suboptimal during the non-breeding season and during nutritional inadequacies. Progestagen treatments are more useful in buffalo heifers and adult buffaloes during the non-breeding season when combined with estradiol, eCG, prostaglandins and GnRH/hCG. A slightly longer duration (12-14 d) of progesterone therapy is suggested during the non-breeding season along with estradiol at the time of progesterone implant insertion, as this effectively regresses large follicles and/or initiates a new follicular growth. Such therapies effectively produce additional pregnancies during the unfavourable season, which is clearly advantageous. The ovsynch is the most commonly used GnRH based estrus synchronization protocol used in buffaloes with conception rates of up to 60% during the breeding season however, during the non-breeding season and in buffalo heifers the conception rates are suboptimal (11-20%). The nutritional status of buffaloes appears important before initiating estrus synchronization. In conclusion, estrus and ovulation can be effectively synchronized in buffaloes using ovsynch protocols during the breeding season; however during the non-breeding season progestagen based protocols in combination with estradiol, eCG, PG and GnRH are a better option for timing insemination and planning calvings.

Keywords: Buffalo, estrus synchronization, PG, progestagens, ovsynch

Introduction

The success of estrus synchronization and timed inseminations in dairy and beef cattle (Bridges and Lake, 2011; Colazo and Mapletoft, 2014) [15, 30] has led to increased adoption of these approaches in the buffalo, a species with inherent problems of poor estrus expression and seasonality of reproduction. It is almost around 45 years since the first report on estrus synchronization appeared in buffalo (Baruselli *et al*., 2013) [10] yet there are still considerable limitations. The wide scale adoption of this technique can become popular in a very few buffalo rearing countries on account of smaller number of buffaloes per herd, poor awareness of farmers and poor responses of most estrus synchronization regimens especially during the hot summer months. Although buffaloes have been shown to have 2-3 follicular growth waves during estrous cycle, with 2 waves being common (Baruselli *et al*., 2013) [10], yet the number of follicles recruited per follicular wave is lower in buffalo compared to cattle (Gimenes *et al*., 2009; Campanile *et al*., 2010) [46, 21]. Thus, the results of estrus synchronization in buffaloes are expected to be low. Similar to cattle, approaches for estrus synchronization in the buffalo have utilized prostaglandins, progestins and GnRH alone or in combination (De Renis and Lopez-Gatius, 2007) [34]. These pharmaceutical agents regulate corpus luteum (CL) regression, follicle growth/regression and ovulation. Studies have shown that the dominant follicle (DF) and CL on the ovary are pivotal to estrus synchronization protocols. The mechanism of estrus synchronization involves either the progression of DF to ovulation following luteolysis (prostaglanidns), or the regression of DF, followed by growth of a new DF that progresses to ovulation (progestins and GnRH based protocols).

Estrus synchronization protocols in swamp buffaloes have been recently reviewed (Yendraliza *et al*., 2015) [115]. In the present review, the prospects, approaches and limitations of estrus synchronization in river buffaloes are discussed.

Prospects and prerequisites

The benefits of estrus synchronization in dairy cows have been analysed previously. It was reported that the use of ovsynch reduced intervals to first AI, days open and/or culling of cows for infertility (Tenhagen *et al*., 2004) [105]. The pregnancy rates can be increased further by accurate estrus detection methods to re-inseminate cows that spontaneously return to estrus, concurrent with routine pregnancy diagnosis and re-synchronization of non-pregnant cows (Galvao *et al*., 2013) [42].

Estrus synchronization in buffaloes can address two distinct problems of breeding. First, estrus signs are less marked in buffaloes (Roy and Prakash, 2009) [90] which can be efficiently managed by estrus synchronization, followed by timed insemination without estrus detection (Baruselli *et al*., 2013) [10] and second, anestrus postpartum buffaloes can be effectively impregnated during the hot summer, which would have otherwise continued to be non-pregnant during the non-breeding season, thus reducing days open and achieving yearly calving (Kumar *et al*., 2012) [63]. This has been shown in many studies (Malik *et al*., 2011b; Ghuman *et al*., 2014) [112, 44]. Using estrus synchronization, 50-75% pregnancy rate can be achieved during the first month of the breeding season without estrus detection (Crudeli and de La Sota, 2011; Baruselli *et al*., 2013) [10, 31]. Additional pregnancies during non-breeding season would result in calvings during a time of milk deficit improving overall profitability of buffalo farming. Pregnancy rates are, however, dependent upon many factors such as estrus detection, nutritional status, body condition score and postpartum interval. Low pregnancy rates during low breeding season result from high embryonic mortalities (Campanile *et al*., 2005; Russo *et al*., 2010) [19, 46]. Animals should be in good body condition before initiating estrus synchronization. It is beneficial to examine animals by transrectal palpation and transrectal ultrasonography to evaluate uterine and ovarian status (De Rensis and Lopez Gatius, 2007) [34] before initiating estrus synchronization. Buffaloes with a DF and a CL are good candidates for inclusion in the synchronization programs. Buffalo heifers should have attained around 60% of their adult body weight and a minimum of 50% of heifers should have normal estrous cycles. Use of high fertility buffalo bulls and high fertility semen are also essential for attaining success with estrus synchronization.

Approaches and limitations

Similar to cattle, estrus synchronization approaches in the buffalo have used prostaglandins, progestins and GnRH based protocols in combination with other hormones such as estradiol and hCG. The use of a particular approach would depend upon many factors which should be considered prior to use of a particular product.

Prostaglandins

Use of prostaglandins is one of the oldest approaches for synchronization of estrus in buffaloes (De Rensis and Lopez-Gatius, 2007) [34]. The admsinistration of natural or synthetic prostaglandins to the buffalo results in a marked decrease in progesterone due to luetolysis (Chohan *et al*., 1992; Markandeya and Bharkad, 2002) [7, 69] and animals return to estrus within 50-96 hr of administration. Thus, for prostaglandins to be effective animals should be cyclic. The administration of PG causes luteolysis and this permits the dominant follicle (DF) of the follicular growth wave to grow to a stage that allows estrus and ovulation (Day and Geary, 2005) [33]. An obvious increase in the size of the DF (from 0.97 ± 0.07 cm to 1.4 ± 0.09 cm) was recorded in Egyptian buffaloes on Day 2 of PG injection, with concomitant decrease in plasma progesterone and estrus in 3-4 days post injection (Noseir *et al*., 2014) [79]. The return to estrus would depend upon the status (Size and dominance) of dominant follicle and CL (at the time of PG administration). Most buffaloes with a CL and a DF below 1.0 cm would respond to PG however, buffaloes with a CL and a DF above 1.0 cm respond poorly (Brito *et al*., 2002) [16].

Since buffaloes are known to have a preponderance of two wave cycle (63.3%) (Baruseli *et al* 2013) and a lower number of follicles are recruited per follicular wave (Campanile *et al* 2010; Baruseli *et al*., 2013) [10, 21, 19], the probability of buffalo returning to estrus following PG administration is lower compared to cows. Palpation of a CL in buffaloes appears difficult due to poor projection on the ovarian surface (Sharifuddin and Jainudeen, 1983) [96]. However, estrus rates after a single PG administration following palpation of a CL or without palpation of CL in different studies (Table 1) appear to be high. A smaller number of animals (>25) were utilized in most of these studies, conducted during the breeding season. Besides many variables like, day's postpartum (Usmani, 2001) [109], nutritional status (Hussein and Abdel-Raheem, 2013) [52] and others that regulate effects of prostaglandins, an important determinant appears to be the season of administration. Administration of a single PG to buffalo heifers resulted in estrus rates of 60% during the low breeding season with poor subsequent conception rates (22.8 - 25.6%) (Chohan *et al*., 1993) [27], whereas, during the breeding season estrus rates were 86.6% and conception rates varied from 47.8 to 53% (Table 1).

Results of estrus synchronization using PG in pre-pubertal buffalo heifers and postpartum anestrus buffaloes may be sub-optimal (Honparkhe *et al*., 2008) [49]. None of the 12 postpartum acyclic buffaloes treated with PG responded to the treatment. Eight of the 12 Murrah buffalo heifers (32 months age) managed at an organized farm responded well to the PG injections with estrus and ovulation within 48 h of PG injection (Singh and Madan, 2000) [49] however, this might not be applicable to farmers buffaloes at similar age (due to diverse nutritional and managemental conditions) (Brito *et al*., 2002; Misra *et al*., 2003) [16, 71]. Thus, the nutritional status and ongoing follicular and luteal activity appears important when selecting a diverse group of buffaloes comprising of buffalo heifers and postpartum anestrus buffaloes. The intra-vulvo-submucosal route of administration of PG has been suggested for estrus induction/synchronization in buffaloes (Totewad *et al*., 2009; Lucy *et al*., 2010) [107, 64] to reduce the cost but estrus synchronization with such a treatment was suboptimal and delayed compared to the intramuscular use. Administration of 2 PG 11-12 days apart is suggested for cyclic animals in well managed buffalo herds during the breeding season with satisfactory conception rates (Table 1) employing natural service or timed artificial insemination.

Progestins

Progestin treatments inhibit the maturation of DF due to suppression of both FSH and LH which promote the atresia of all follicles present on the ovary in cattle and buffaloes (Baruselli *et al*., 2007; Suadsong, 2011) [8]. Once the DF undergoes atresia, its suppressive effects on other small follicles and FSH are removed which allows a new follicular wave to begin with formation of a DF which attains ovulatory capacity following withdrawal of progesterone implant. The use of estradiol or GnRH on the day of implant insertion significantly suppresses DF growth and enhances new wave emergence (Suadsong, 2011; Bhat *et al*., 2015) [104, 13]. Follicular waves emerged in Murrah buffaloes on days 4.22±0.12, 3.12±0.33 and 5.14±0.42 of treatment with estradiol + CIDR, GnRH + CIDR or CIDR alone (Bhat *et al*., 2015) [13]. Similarly, in another study on Italian buffaloes treated with estradiol and progestagens, a new follicular wave occurred around the 4th day of treatment (Niasari-Naslagi *et al*., 2007). The diameter of the largest follicle was reduced from about 8mm (Day 0) to 5 mm around the day of wave emergence. Also, the mean number of 4-5 mm follicles increased from 1.58 (Day 0) to 5 around the day of wave emergence (Bhat *et al*., 2015) [13].

Progesterone based treatments in combination with estradiol, PG, GnRH or hCG and timed inseminations are considered more useful during the non-breeding season in buffaloes due to poor responses of other estrus synchronization treatments such as PG and ovsynch during this time (Carvalho *et al*., 2013b; Vecchio *et al*., 2013) [22, 111]. Pre-pubertal buffalo heifers have low blood progesterone concentrations (Ahmed *et al*., 2010) [10], which peak around puberty (Haldar and Prakash, 2006) [47], thus progesterone treatments should be more suited to anestrus buffalo heifers for estrus synchronization. This was shown in a small trial (n=6) on PRID treated anestrus buffalo heifers, all of which exhibited estrus after PRID removal (Singh *et al*., 2009) [49] and similar results were shown for anestrus buffaloes treated with progesterone implants in combination with other hormones (Carvalho *et al*., 2013a; Vikash *et al*., 2014) [23, 112]. A short period of elevated progesterone concentration during anestrus period is important for the expression of estrus as well as for subsequent normal luteal function (Baruselli *et al*., 2007) [8]. A longer period of progesterone treatment (12-14 d) has been suggested to mediate these effects (Bhat *et al*., 2015) [13]. However, a recent report depicted reduction in hypothalamo-pituitary responsiveness to synchronization treatments including PRID and ovsynch during increasing daylight (Barile *et al*., 2015) [7].

Progestins have been used as oral supplements, daily injections, auricular implants (norgestomet) and vaginal implants however, use of commercially available vaginal implants (CIDR and PRID) has been most common. Use of indigenously prepared methyl-acetoxy progesterone vaginal sponges was reported in one study with estrus rates of 50-80% and conception rates of 33% in acyclic and 50% in cyclic Nili-Ravi buffaloes (Kausar *et al*., 2013) [58] sponges.

Oral supplements

Oral administration of progestin melengestrol acetate (MGA; 1-2 mg daily for 9-14 days) alone or combined with 500 µg IM estradiol 24-48 hrs before last MGA dose to buffalo heifers has been reported in a few studies with estrus rates of 62.7-100% however, conception rates were only 35% (Shanker *et al* 1996) [95]. Administration of low levels of oral progestin (MGA) in the absence of a corpus luteum can result in the formation of persistent follicles in cattle (Smith *et al*., 2011) [102] however; similar occurrence could not be recorded in trials involving small number of buffaloes.

Progesterone injections

Progesterone injections can be used as an alternative to oral feeding of progestagens, however these injections have to be administered on a daily basis for 5-8 days which limits their practical applicability. The daily administration of progesterone injections (250 mg IM) for 5 days followed by 500 IU eCG or 5 mg estradiol on day 6 to postpartum buffaloes resulted in estrus rates of 40-59% and conception rates of 45.5% (Thakur, 1989; Kumar *et al*., 2010) [62, 106].

Table 1: Effect of prostaglandins on estrus response and conception rates in buffaloes

Type	Shot	Estrus rate (%)	Conception rate (%)	Reference
Heifers	PG 1 shot	60-68	12.5-62.5	Honnapagol and Patil, (1991); Chohan *et al*. (1993) [27]; Chohan, (1998)
Heifers	PG 1 shot	60(LBS) 86.6(PBS)	22.8-5.6(LBS) 47.8-53(PBS)	Chohan *et al*. (1993) [27]; Chohan, (1998)
Heifers	PG 2 shot	71.4% 88.6(LBS)	45	Singh and Dabas, (1998) [44]
Adult	PG 2 shot	-	32.48	Phadnis *et al*., 1994 [14]
Adults	Single injection after CL palpation	76.6-80	70-80	Ribeiro *et al*. (1998) [89]
	Single injection	77.5-100	41.5-72.9	Pant and Singh, (1991) [45]; Honappagol and Patil, (1991); Khattab *et al*. (1996) [20]; Brito *et al*. (2002) [16]
	2 injection 11 d apart	25-95	22.8-83	Chohan *et al*. (1993) [27]; Diaz *et al*. (1994) [39]; Singh and Dabas, (1998) [49]; Misra *et al*. (2003) [71]; Srivastava, (2005) [105]; Hussain and Honappa, (2008) [51]
	2 injection 11 days apart TAI 72-96 h after 2nd injection	47.8-63.6	47.8-63.6	Chohan, (1998); Yuan *et al*. (2008) [117] Yuan *et al*. (2010) [118]; Dadarwal *et al*. (2009) [45]

Ear implants

Norgestomet is a synthetic progestagen ear implant that is administered subcutaneously and kept in place for 8-10 days, followed by its removal. An estradiol valerate capsule and/ or estradiol valerate (5 mg IM) is administered simultaneously to synchronize estrus. Buffaloes show estrus within 24-48 hr of withdrawal of the auricular implant. Estrus rates in buffalo heifers were high (70-100%) in trials utilizing a smaller number of animals (n=10) (Patel *et al*., 2003; Kumar and Mandape, 2004) [61, 81]. Similarly, in experiments on adult buffaloes (>15), estrus rates were high and conception rates were similar across regimens utilizing norgestomet in combination with eCG and PG or norgestom*et al*one (Table 2). A larger trial on 100 anestrus buffaloes however, yielded 75% estrus rates (Yadav *et al*., 1994) [114] only, and in another trial on 96 buffaloes the conception rates were41.4% (Phadnis *et al*., 1994) [14]. However, another study on Murrah buffaloes evidenced norgestomet to be ineffective in inducing cyclicity in postpartum anestrus buffaloes (Garg *et al*., 1999) [43].

Table 2: Estrus and conception rates in adult buffaloes administered norgestomet ear implants alone or with combination with eCG and PG

	Estrus rates (%)	Conception rates (%)	References
Norgestomet (8-10 days) + 5 mg E_2	60-100	30-70	Phadnis *et al*. (1994) [14]; Chattry *et al*. (1999); Patel *et al*. (2003) [81]
Norgestomet + 500 IU eCG	86-97.6	44.6-66.7	Luthra *et al*. (1994) [65]; Malik *et al*. (2011a) [113]
Norgestomet + PG (9 days)	100	45-66.7	Utage *et al*. (2010) [110]; Chaudhary *et al*. (2015) [25]

Timed insemination in 119 buffaloes 64-68 hr after withdrawal of norgestomet ear implants and administration of PG+ eCG before withdrawal during summer resulted in conception rates of 49.6% (Carvalho *et al*., 2013a) [24] and similar conception rates were recorded in other trials on timed insemination in adult buffaloes following estrus synchronization with norgestomet in combination with eCG, PG and GnRH (Baruselli *et al*., 2007; Diaz *et al*., 1994) [8, 39]

Vaginal progesterone implants

Two commercially available progesterone vaginal implants (CIDR and PRID) developed for cattle have been widely used in buffaloes. The implants are applied for 8-10 days (Ghuman *et al*., 2009; Murugavel *et al*., 2009) [45, 73]. Estrus and ovulation occur within 48-96 hrs after withdrawal of progestagen implant in treated buffaloes (Bhosrekar *et al*., 1994) [82].

In experiments utilizing lesser number (n=20) of buffaloes the estrus response to progesterone vaginal implants was depicted to be high (Table 3) however, the estrus response was lower in trials using a higher number of buffaloes (<50). The estrus response and subsequent conception rates are dependent upon the reproductive status, days postpartum, ovarian follicular status, season of the year and the simultaneous administration of eCG, PG and GnRH (De Rensis and Lopez-Gatius, 2007) [34]. The mean diameter of the largest follicle was significantly smaller in nulliparous Italian buffalo heifers compared to mixed parity buffaloes during the first 4 days after PRID removal (Presicce *et al*., 2004) [83], suggesting lower response of progestagen treatments in buffalo heifers. A trial in Venezuela buffalo heifers treated with PRID + E2 + PG showed no conception in treated buffalo heifers (Noguera *et al*., 2013) [78]. Anestrus buffaloes evidence a poor response, especially during the summer season when the implants are used alone (Sekerden *et al* 2005; Murugavel *et al*., 2009) [73]. It has thus been suggested to administer 1-2 mg estradiol and PG along with the CIDR/PRID (Caesar *et al*., 2011; Carvalho *et al* 2013a) [22]. The combined use of estradiol and progestagen vaginal implant at the beginning of the regime induces a new follicular wave due to atresia of all follicles present (Baruseli *et al*., 2007).

Alternatively, PG plus eCG or GnRH or both (eCG and GnRH) at withdrawal of implant have been suggested during off season use (Caeser *et al* 2011; Frares *et al* 2013) [41]. The administration of eCG at the time of withdrawal of progestagen implants significantly increased the size of the DF rapidly before ovulation (12 mm) from Day 9 to Day 12 (Malik *et al*., 2011a) [113] and the estrus and ovulation rates (Caesar *et al*., 2011). The ovulation rate can further be increased by the administration of GnRH two days after the administration of eCG and PG (Carvalho *et al* 2013c) [23].

Timed insemination at 60-72 h after withdrawal of CIDR/PRID, and repeated at 24 h, has been suggested (Carvalho *et al* 2013a) [24]. Experiments using GnRH (administered 2 days after implant withdrawal and eCG administration at withdrawal) suggest fixed time insemination 24 hr after the GnRH administration (Carvalho *et al* 2013b) [22] during the non-breeding season.

Table 3: Estrus rates and conception rates in buffaloes administered CIDR/PRID intravaginal progesterone implants in different studies

No of animals	Physiological state of buffalo	Estrus rates (%)	Conception rates (%)	References
>20	Acyclic/sub estrus	80-100	56-80	De Santis *et al*. (2003) [36]; Naseer *et al*. (2011) [74]; Yotov *et al*. (2012) [116]; Kajaysri *et al*. (2015) [55]
21-50	Anestrus	83-100	50-80	Singh *et al*. (1988) [44]; Singh, (2003) [45]
21-50	Postpartum	71.4-100	46- 85.7	Sathiamoorthy *et al*. (2007) [93]; Caesar *et al*. (2011)
>50	Anestrus	NBS-32.5-82 BS-54-93	25.3 40.7	Rao and Rao, (1983); Bhoserkar *et al*. (1994); Ghuman *et al*. (2009) [45]
>50	Postpartum/ Acyclic	-	36.0	Neglia *et al*., 2003 [76]
>50	Lactating/ Acyclic	-	36.5-46.4	Carvalho *et al*. (2013a) [23]; Noguera *et al*. (2013) [78]; Urdaneta *et al*. (2013) [108]

GnRH based Protocols

Approaches utilizing administration of GnRH alone, followed by PG to postpartum buffaloes have not become popular, although they can stimulate estrus and ovulation (Khasatiya *et al*., 2005) [59]. Pursley *et al*. (1995) [85] were the first to demonstrate that the administration of GnRH, followed by administration of PG 7 days later results in more precise control of ovarian follicle growth and estrus and the ovulation is assured by a second injection of GnRH 2 days after the PG. This has led to a wider use of GnRH in estrus synchronization protocols in cattle and such a protocol is popularly known as ovsynch protocol. By far the ovsynch protocol is the most widely used regime in buffaloes. This ovulation synchronization protocol employs the IM administration of GnRH, PG and GnRH on days 0, 7 and 9.

The administration of GnRH promotes ovulation of a DF and this initiates a new wave of follicular growth. The largest follicle present at the time of administration of first GnRH may regress or persist throughout to ovulate subsequently (Day and Geary, 2005) [33]. The success of this protocol depends on the presence of a DF at the time of first GnRH (De Rensis and Lopez-Gatius, 2007) [34] and this can be determined by ultrasound examination of the ovaries (De Rensis *et al*., 2005) [35]. In a study on Mediterranean buffaloes the largest follicle present at the time of first GnRH administration regressed in 46.6% of nulliparous and 78.5% of pluriparous animals (Presicce *et al*., 2005) [85]. In another study on postpartum anestrus Murrah buffaloes, all animals (n=15) ovulated one largest follicle in response to first GnRH treatment (Malik *et al*., 2011b) [112]. The ovulation of the largest follicle at the time of first GnRH administration is probably dependent on the size of the dominant follicle (De Rensis *et al*., 2005) [35]. When the size of the largest follicle was >8mm at the time of first GnRH injection, 87.5% of heifers and 100% of adult buffaloes ovulated (Derar *et al*., 2012) [112]. The ovulation of the DF is also dependent on the stage of the DF.

In buffaloes, Day 6 and Day 10 of the estrous cycle are considered the growth phase and regression phase of the first wave DF and the proportion of buffaloes that ovulate in response to GnRH during the growth and regression phase was 75.0 and 16.67%, respectively (Dharani *et al*., 2010) [38]. Similarly, Campanile *et al*. (2008) [20] recorded ovulation in 62% of the buffaloes when GnRH was administered on Day 5 of the estrous cycle. Moreover, the DF of the first anovulatory wave reached the maximum size earlier and remained in static phase for a greater number of days, indicating an early loss of LH receptors in buffaloes compared to cows (Sateshkumar *et al*., 2011) [87]. The ovulation of a DF initiates a new follicular wave and formation of a new DF.A new follicular wave started one day after the first GnRH dose in Egyptian buffaloes and this wave resulted in the development of a new DF with a diameter of 1.03+0.07 cm on day 7 after treatment (Day of PG administration) (Noseir *et al*., 2014) [79]. In another study, the interval from first GnRH administration to the onset of new follicular wave was 48 to 54 h and a new DF developed from this wave (Ali *et al*., 2008). The administration of PG 7 days later causes luteolysis and the new DF or the continually growing unovulated DF reaches the ovulatory size and the animal evidences estrus. The second GnRH treatment ensures ovulation of the ovulatory follicle.

The ovulation rate to first GnRH appears important and can be used as a preliminary screening of buffaloes to be inseminated, reducing timed insemination costs (Neglia *et al*., 2013) [76], as pregnancy rates were significantly higher (81%) in buffaloes that ovulated after first GnRH injection compared to 22.3% in those that did not ovulate with first GnRH treatment (Neglia *et al*., 2013) [76]. The ovsynch protocol has been used in buffaloes in a large number of countries with estrus rates varying from 41.6 to 91.9% and conception rates from 11.11 to 68.8% (Table 4). Similar pregnancy rates were recorded in studies using a combination of CIDR and ovsynch or norgestomet and ovsynch (Table 4). Although the DF at first GnRH did not ovulate or regress in 50% of buffaloes treated with CIDR in combination with ovsynch treatment, yet the addition of a progestagen implant at the time of first GnRH injection improved conception rates in acyclic postpartum buffaloes (Ali *et al*., 2008; Alyas *et al*., 2013) [6].

Estrus synchronization in buffalo heifers using ovsynch has yielded lower conception rates (18.8-40%) compared to those in adult buffaloes (Irikura *et al*., 2003; Presicce *et al*., 2005) [85, 53]. Many previous reports (Baruselli *et al*., 2002; Warriach *et al*., 2008; Jabeen *et al*., 2013) [9, 113, 54] have shown that estrus rates and conception rates in buffaloes with the ovsynch protocols are higher during the breeding season (Estrus rates 87.5% versus 36.3% and conception rates 40% versus 11.1%) (Warriach *et al*., 2008; Jabeen *et al*., 2013) [113, 54].

Table 4: Estrus rates and conception rates in buffaloes with ovsynch treatments in different reports

	Estrus Rates (%)	Conception Rates (%)	Reference
Ovsynch	46.3-91.9%	28.0 - 66.6	Berber *et al*. (2001) [11]; Francillo *et al*. (2005) [40]; Ali and Fahmy, (2007) [37]; Sathiamoorthy *et al*. (2007) [93]; Ravikumar *et al*. (2009) [88]; Derar *et al*. (2012) [112]; Hoque *et al*. (2014) [50]
Ovsynch + CIDR	58.3-86	18.18 – 63.6	Campanile *et al*. (2005) [19]; Ravikumar *et al*. (2008) [87]; Kalwar *et al*. (2015) [56]
Norgestomet + Ovsynch	-	71.4	Malik *et al*. (2010) [111]
PG + Ovsynch	-	36.4	Hoque *et al*. (2014) [50]
Ovsynch + TAI	-	18 - 59	Camelo *et al*. (2002) [18]; Karen and Darwish, (2010) [57]; Akhtar *et al*. (2013) [2]; Neglia *et al*. (2013) [76]
CIDR + Ovsynch + TAI	100	66.67	Alyas *et al*. (2013) [6]

A variant to the ovsynch protocol is the Heat synch protocol in which the second GnRH is replaced with estradiol. Heat synch protocols resulted in estrus induction rates of 100% and conception rates of 50% (Ali *et al*., 2012). Such a treatment is considered to result in better estrus expression in buffaloes, however, the conception rates were low (Akhtar *et al*., 2013) [2], especially in heavily lactating buffaloes (Berber *et al*., 2005) [12]. Although the protocol has been suggested for subestrus buffaloes for timed inseminations (Mohan *et al*., 2009; Carvalho *et al*., 2012b) [24, 72], yet due to potential dangers of milk suppression with estradiol the same should be discouraged or at least used cautiously.

Timed inseminations following ovsynch treatments are suggested 16 to 24 h after the second GnRH with variable conception rates (18 to 60%) (Malik *et al*., 2011b; Derar *et al*., 2012) [112]. Pre synchronization with PG 12 days before ovsynch treatment resulted in slight improvement in pregnancy rates with timed inseminations (Hoque *et al*., 2014) [50]. Recently, another protocol estra-doublesynch with PG on Day 0, GnRH on Day 2, PG on Day 9 and estradiol benzoate on Day 10 and timed insemination 48 and 60 h after estradiol benzoate was suggested for cyclic and anestrus Murrah buffaloes (Mirmahmoudi *et al*., 2014) [70], with pregnancy rates of 62-64%.

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