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**Vishwanath Kurella**  
MVSc. Scholar, College of  
Veterinary Science,  
Rajendranagar, Hyderabad  
Telangana, India

**Sunil Anand Kumar E**  
Associate Professor, Veterinary  
Clinical Complex, Mamnoon,  
Warangal, Telangana, India

**Ramsingh L**  
Associate Professor, Veterinary  
Clinical Complex, Korutla,  
Jagityal, Telangana, India

**Padmaja K**  
Professor and Principal, A.H.  
Polytechnic, Mahbubnagar,  
Telangana, India

**Corresponding Author:**  
**Vishwanath Kurella**  
MVSc. Scholar, College of  
Veterinary Science,  
Rajendranagar, Hyderabad  
Telangana, India

## Efficacy of estrus synchronization protocols in graded Murrah Buffaloes

**Vishwanath Kurella, Sunil Anand Kumar E, Ramsingh L and Padmaja K**

### Abstract

To assess the efficacy of Doublesynch, Estradoublesynch and Modified doublesynch protocols on estrus induction and conception rate in postpartum anestrous Graded Murrah buffaloes. A study was conducted with forty (40) Pluriparous Graded Murrah Buffaloes between 2-4 calvings exhibiting postpartum anestrous between 90-180 days were selected and were randomly divided into four groups (n=10). Group-I (Control group), Group-II (Doublesynch protocol), Group-III (Estradoublesynch protocol) and Group-IV (Modified doublesynch protocol). Results revealed, that the estrus response rate was significantly ( $p < 0.05$ ) higher in Group II (90%) and Group III (90%) followed. The mean time taken for the onset of induced estrus from PGF $2\alpha$  was significantly ( $p < 0.01$ ) shortest in Group-IV ( $59.63 \pm 1.21$  hr). However, the mean score for estrus intensity was significantly ( $p < 0.05$ ) higher in Group-III ( $19.33 \pm 1.87$ ) followed by Group-II ( $17.44 \pm 1.71$ ). The mean duration of estrus was significantly ( $p < 0.05$ ) higher in Group-III ( $20.67 \pm 1.13$  hrs). The overall conception rate was 20.00, 80.00, 70.00 and 60.00 per cent in Group-I, Group-II, Group-III and Group-IV respectively. The present investigation concluded that Doublesynch protocol could be used as best hormonal protocol to induce the estrus as well as improve the conception rate in postpartum anestrous Graded Murrah buffaloes.

**Keywords:** Anestrous, doublesynch protocol, estra-doublesynch protocol, estrus synchronization and modified doublesynch protocol

### Introduction

India is known as the 'Oyster' of the global dairy industry in which Buffalo (*Bubalus bubalis*) contributes more than two-third of the total milk production. Incidence of anestrous in buffaloes is more common in India (Thakor and Patel, 2013) [46] due to poor gonadotropic support from hypothalamo-pituitary axis (Madan *et al.*, 1983) [21] which is associated with low secretion of FSH and LH and variable plasma concentration of progesterone during the luteal phase (Murugavel *et al.*, 2009) [25] which leads to anestrous.

Estrus synchronization is one such valuable reproductive and manage mental technique for farmers to breed the animals at a definite time (Sunil Anand Kumar E *et al.* 2018) [45]. Various synchronization protocols are being used to modulate the circulating plasma progesterone levels to make acyclic buffaloes to cycle normally and to improve their reproductive efficiency, thereby reducing the inter-calving interval making buffalo farming economically viable (Mirmahmoudi *et al.*, 2014a) [24].

Cirit *et al.* (2007) [6] developed a new synchronization protocol "Doublesynch" and the pregnancy rates increased by 30.8% in buffaloes (Mirmahmoudi and Prakash, 2012) [23]. While, Mirmahmoudi and Prakash (2014a) [24] further developed a "Estradoublesynch protocol" that resulted in 62% pregnancy rates in cyclic Murrah buffaloes. However, Modified doublesynch (or hCG doublesynch) is a new novel synchronization protocol in which the exogenous administration of LH hormone (human chorionic gonadotropin hormone) in place of second GnRH used in Doublesynch protocol. Alternative use of hCG to GnRH, enhances pituitary release of luteinizing hormone (LH) to cause the ovulation and showing longer period of LH like stimulation of ovulatory follicle due to extended half-life of hCG in blood (Schmitt *et al.*, 1996) and have better satisfactory pregnancy rates (De Rensis *et al.*, 2008a) [9].

### Material and Methods

#### Experimental location

The present study was conducted in an organized private dairy farm located in Thurkayamjal area, K.V. Ranga Reddy district, Telangana State (latitude: 17° 16'58.2"N; longitude: 78° 35' 30.9"E; altitude: 510.m above mean sea level) during the period between June 2020 to November 2020.

### Experimental animals

A forty (40) Pluriparous Graded Murrah Buffaloes between 2-4 calvings which were free from all venereal diseases and exhibiting postpartum anestrus between 90-180days, having history of normal calving and without any palpable abnormalities in genital tract were selected based on history and detailed gynaecological examination and kept under intensive housing system and fed as per nutritional requirement.

### Pre-synchronization treatment

All the experimental postpartum anestrus Graded Murrah buffaloes were dewormed with Oxfendazole and Ivermectin single Bolus (VOXMEC™ BOLUS, Vvaan Lifesciences Pvt. Ltd) per orally and supplemented with mineral mixture (Area specific Mineral mixture prepared for Livestock nutrition by dept of Animal Nutrition, C. V. Sc, Rajendranagar, Hyd.) 50gm/animal/day orally for one month prior to the synchronization.

These buffaloes were randomly divided into four groups (with 10 buffaloes in each group) and different protocols were employed i.e., Control group (untreated), Doublesynch protocol group, Estradoublesynch protocol group and Modified doublesynch protocol group in postpartum anestrus buffaloes.

### Doublesynch, Estradoublesynch and Modified doublesynch protocols

Under Doublesynch protocol, ten postpartum anestrus Graded Murrah buffaloes were injected with 500 µg PGF2α analogue injection i.e., Cloprostenol sodium (PRAGMA, Intas Pharmaceuticals Ltd., 2ml i/m) on day 0 and day 9 and 20µg of GnRH analogue injection, i.e. Buserelin acetate (GYNARICH, Intas Pharmaceuticals Ltd., 5ml) on day 2 and second inj. 10 µg GnRH on day 11 and Fixed time AI was done after 16 and 24 hours of the 2<sup>nd</sup> dose GnRH of injection. In Estradoublesynch protocol, administration of 1mg Estradiol benzoate i/m (PREGHEAT, Virbac Animal Health Pvt Ltd.) on day 10, in place of second GnRH injection on day 11 of Doublesynch and Fixed time AI was done after 48 and 60 hours of the Estradiol benzoate injection. Whereas in Modified doublesynch protocol buffaloes received an Inj. of 1500 IU of hCG i.e., human Chorionic Gonadotropin (CHORULON, MSD Animal Health) on day 11, in place of

second GnRH injection on day 11 of Doublesynch and Fixed time AI was done along and 12 hours of the hCG injection.

### Control group

Bbuffaloes were untreated in which only pre-synchronization treatment was advocated as above without any hormonal treatment. Whenever the animal exhibit estrus was inseminated.

### Data recording and analysis

For inseminated buffaloes that failed to conceive at FTAI, the visual heat detection were observed for estrus signs up to 45 days both in the morning and evening and were re-inseminated for two more cycles and the buffaloes not returned to estrus were examined per rectally for confirmation of pregnancy after 60 days of AI and conception was calculated.

The observations on estrus response rate, intensity of estrus and conception rates were recorded and compared between different groups statistically by using Chi-square test and the data on time taken for induction of estrus and duration of estrus were analyzed using one way analysis of variance (ANOVA) to compare variation between groups (Snedecor and Cochran, 1994) [44].

### Results and Discussion

#### Estrus synchronization and Conception rates

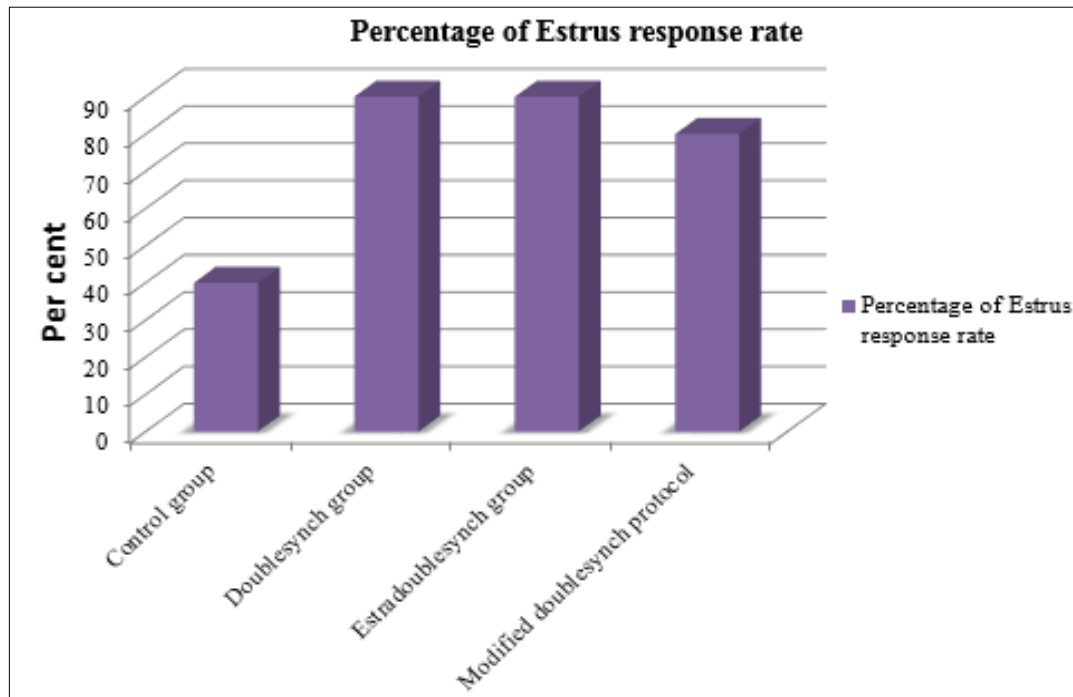
In the present investigation, the estrus response rate, time taken for induction of estrus, duration of estrus, intensity of estrus and conception rates observed in postpartum anestrus buffaloes under different synchronization protocols and control groups are presented in Tables and Figures.

The estrus response rate was significantly higher (90.00%) in Doublesynch and Estradoublesynch protocol with excellent behavioral signs, greater uterotonic, and vaginal mucus congestion and followed by 80.00 per cent in Modified doublesynch protocol and Control group (40%). Statistically there was a significant ( $p < 0.05$ ) difference among the synchronized and Control group of Graded Murrah buffaloes (Table No.1 and figure no. 1). The higher estrus response rate in synchronized group than Control group may due to fact that double dose of PGF2α increases pituitary responsiveness in postpartum anestrus buffaloes subsequent estrus induction.

**Table 1:** Estrus response rate in postpartum anestrus Graded Murrah buffaloes

S. No	Groups	Number of treated buffaloes	Number of buffaloes exhibited estrus	Per cent of Estrus response	Chi-Square value
1	Control group	10	4	40.00	9.06*
2	Doublesynch	10	9	90.00	
3	Estradouble synch	10	9	90.00	
4	Modified doublesynch protocol	10	8	80.00	

\*Estrus response rate between groups differed significantly ( $p < 0.05$ )



**Fig 1:** Estrus response rate in postpartum anestrus Graded Murrah buffaloes

In present findings, the estrus response rates in Double synch protocol were in accordance with the earlier studies of Miramahmoudi and Prakash, 2012 [23]; Chaudhary *et al.* (2018) [5]; however 100 per cent estrus response rates are proposed by many authors (Dhami *et al.*, 2020) [10]. But, lower estrus response rates i.e., 70 per cent was documented by Dash *et al.* (2020) [8] in anestrus buffaloes. The estrus response rates using Estradoublesynch protocol was in same alignment with investigations of several researchers studies (Behera, 2018; and Raju *et al.*, 2018) [3, 35]. While the 100 per cent estrus response rate was reported by Prajapati (2018) [32]. But, Parida *et al.* (2015) [28] reported 80 per cent estrus response rate which is close to present findings. The estrus response rate with Modified doublesynch protocol was in

concurrency with Purohit *et al.* (2019). However, the 100 per cent estrus response rate was reported by Lingaswamy (2017) [20]. In Contrast, Kuru *et al.* (2017) [19] recorded lower estrus response rate which is ranges from 32.8- 43.5 per cent.

The mean time taken for induction of estrus from PGF2 $\alpha$  injection was significantly ( $p < 0.01$ ) lower with Modified doublesynch protocol ( $59.63 \pm 1.21$ hr) which is comparably quicker in onset of estrus than Control group ( $92.50 \pm 1.26$  hr), Double synch protocol ( $64.56 \pm 1.19$  hr) and Estradoublesynch protocol ( $62.67 \pm 1.55$  hr) in Graded Murrah buffaloes (Table No.2). Alteration in the onset of estrus between treatment and control groups might be due to possible changes in their inherent endocrine status as well as degree of luteinization of small follicle.

**Table 2:** Mean time taken for induction of estrus among various groups of postpartum anestrus Graded Murrah buffaloes.

S. No	Group	Number of treated buffaloes	Induction of estrus Mean $\pm$ S.E (hrs)
1	Control group	10	$92.50 \pm 1.26^a$
2	Doublesynch	10	$64.56 \pm 1.19^b$
3	Estradoublesynch	10	$62.67 \pm 1.55^{bc}$
4	Modified doublesynch protocol	10	$59.63 \pm 1.21^c$

\*Means bearing different superscripts differed significantly ( $p < 0.01$ ).

The mean time taken for induction of estrus from PGF2 $\alpha$  injection in Doublesynch protocol was in harmony with previous reports of several researchers (Chaudhary *et al.*, 2018; Patel *et al.*, 2018) [5, 30]. However, the shorter and longer onset of induced estrus with Doublesynch protocol recorded by Raju *et al.* (2018) [35] and Prajapati *et al.* (2019) [31], respectively. The mean time taken for induction of estrus in Estradoublesynch was in agreement with Prajapati *et al.* (2018a) [33]. In contrast, Raju *et al.* (2018) [35] and Prajapati *et al.* (2019) [31] recorded shorter and longer time taken for onset of induced estrus with Estradoublesynch protocol, respectively. The mean time taken for induction of estrus in Modified doublesynch protocol was in close contact with investigations of Selvaraju *et al.* (2008) [39]. In contradiction, the shorter and longer onset of estrus was estimated by Coyan *et al.* (2003) [7] and Senthilkumar and Chandrahasan (2015)

[41], respectively.

The mean duration of estrus from onset to cessation of estrus in Estradoublesynch protocol ( $20.67 \pm 1.13$  hrs) group was significantly ( $p < 0.01$ ) longer than Doublesynch ( $18.56 \pm 1.06$  hrs) protocol, Modified doublesynch ( $16.38 \pm 1.18$  hr) protocol and Control ( $13.75 \pm 1.25$ ) group (Table No.5). The variation in duration of estrus in Estradoublesynch group might be owing to the fact to after Estradiol benzoate injection, duration of estrogen surge will be longer and subsequently increases the duration of estrus and also due to inherent endocrine – hormonal status of the individual buffaloes.

The mean duration of estrus in Double synch protocol were in harmony with studies of Harichandan (2018) [15]. But, In Contradict to present results, the longer and shorter duration of estrus was recorded by Mirmahmoudi and Prakash (2012)

[23] and Raju *et al.* (2018) [35] using Doublesynch protocol. Under Estradoublesynch protocol, the mean duration of estrus was in same alignment with studies of Shrivanan and Kulasekar (2016) [42]. But, the longer and shorter duration of estrus was recorded by Dudi *et al.* (2017) [12] and Dash *et al.* (2020) [8], respectively. In Modified doublesynch, the mean duration of estrus was in corroboration with Yendraliza *et al.* (2011) [47]. But, In Contradiction, Ahmed *et al.* (2016) [2] documented the longer duration estrus.

The mean score for estrus intensity in Estradoublesynch ( $19.33 \pm 1.87$ ) was significantly ( $p < 0.05$ ) higher than Doublesynch protocol ( $17.44 \pm 1.71$ ), Modified doublesynch ( $15.75 \pm 1.81$ ) protocol and Control group ( $10.00 \pm 2.45$ ) with six out of nine buffaloes expressed intense heat in

Estradoublesynch whereas, five out of nine buffaloes in Doublesynch and four out of eight buffaloes in Modified doublesynch protocol exhibited intense estrus with vulval mucosal congestion, clear vaginal discharges and with excellent behavioural estrus signs (Table No.3 and 4 and figure no. 2). Variation of estrus intensity between treatment and Control groups may be due to administration of double dose of PGF2 $\alpha$  causing rapid regression of corpus luteum which may be the reason for more intense estrus in synchronized buffaloes whereas Estradiol benzoate inj. on the 10<sup>th</sup> day of protocol which decreases the progesterone level in synchronized animals during pro-estrus and subsequently increases estrus intensity.

**Table 3:** Intensity of estrus expressed in postpartum anestrus Graded Murrah buffaloes

Sl. No	Name of the group	Number of treated buffaloes (n)	Number of buffaloes exhibited estrus	Estrus Intensity		
				Intense (Percent)	Normal (Percent)	Weak (Percent)
1	Control group	10	4	25.00	50.00	25.00
2	Doublesynch	10	9	55.56	33.33	11.11
3	Estradoublesynch	10	9	66.67	22.22	11.11
4	Modified doublesynch protocol	10	8	50.00	25.00	25.00
Overall per cent				53.33	30.00	16.67
Chi-square value				2.57 <sup>NS</sup>		

NS: Non Significant

**Table 4:** Mean Score for estrus intensity in postpartum anestrus Graded Murrah buffaloes

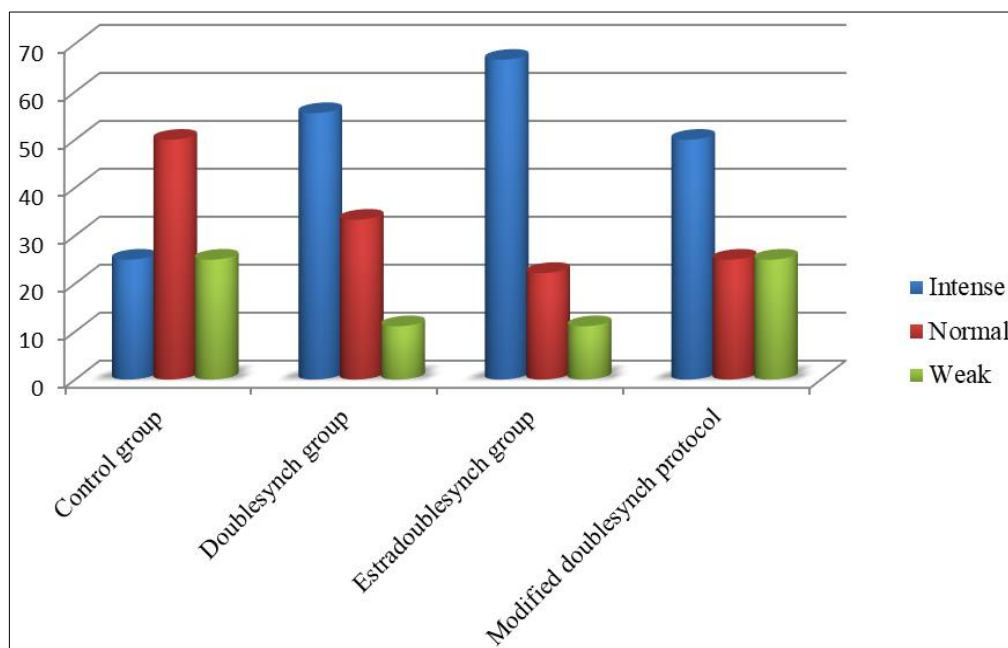
Sl. No	Group	Number of treated buffaloes (n)	Number of buffaloes exhibited estrus	Estrus score (Mean $\pm$ S.E)
1	Control group	10	4	$10.00 \pm 2.45^b$
2	Doublesynch	10	8	$17.44 \pm 1.71^a$
3	Estradouble synch	10	9	$19.33 \pm 1.87^a$
4	Modified doublesynch protocol	10	8	$15.75 \pm 1.81^{ab}$

Means bearing different subscripts differed significantly ( $p < 0.05$ )

**Table 5:** Mean duration of estrus in postpartum anestrus Graded Murrah buffaloes.

Sl. No	Name of the group	Number of Buffaloes treated (n)	Mean duration of estrus (hours)
1	Control	10	$13.75 \pm 1.25^c$
2	Doublesynch	10	$18.56 \pm 1.06^{ab}$
3	Estradoublesynch	10	$20.67 \pm 1.13^a$
4	Modified doublesynch protocol	10	$16.38 \pm 1.18^{bc}$

Means bearing different subscripts differed significantly ( $p < 0.01$ )



**Fig 2:** Intensity of estrus in postpartum anestrus buffaloes.



Under Doublesynch protocol, Intensity of estrus was in accordance with Chaudhary *et al.* (2018) [5]. However, contrary to Agarwal and Purbey (1983) where they recorded the lesser percentage of intense estrus (15.38%). The results obtained with Estradoublesynch protocol was in agreement with Chaudhary *et al.* (2018) [5] and Raju *et al.* (2018) [35] and in contrary to Harichandan (2018) [16]. Whereas in Modified doublesynch protocol, intensity of estrus was in parallel with Rao and Kodagali (1983) [36]. However, Gunasekaran *et al.* (2007) [13] recorded intense and moderate estrus in murrah buffaloes which is almost similar to present intensity rates. In contrast, Lingaswamy (2017) [20] recorded lesser estrus intensity rate.

The conception rates achieved with Double synch group (80%) was significantly ( $p < 0.05$ ) higher than Estradoublesynch (70%) group, Modified doublesynch (60%) group and Control group (20%). Variation in conception rates is due to higher release of LH and the higher rate of ovulation detected after the first and second GnRH injection of Doublesynch protocol

The conception rates obtained in postpartum anestrus buffaloes under Doublesynch, Estradoublesynch and Modified doublesynch protocols were after first service were 40.00, 30.00, 30.00 per cent, respectively and conception rate achieved after second service was recorded as 33.33, 28.57 and 28.57 per cent. But, the conception rate achieved after third service was recorded as 50.00, 40.00 and 20.00 per cent, respectively. Thus, the corresponding overall 3 cycles' conception rates post-treatment with protocols were 80.00, 70.00 and 60.00 per cent, respectively. The conception rates obtained were the best when treated under overall of

3cycles'with Doublesynch followed with Estradoublesynch protocol but the results with Modified doublesynch protocol were relatively lower in postpartum anestrus buffaloes, (Table 6, Figure 3 and 4).

The overall conception rate achieved in Doublesynch group was in concurrence with the results of Prajapati *et al.* (2019) [31]. The results achieved in first service (FTAI) of Doublesynch protocol was in accordance with Panchal *et al.* (2017) [27]. Whereas, the second service (FTAI) was in corroboration with Prajapati *et al.* (2018) [32] and Singh *et al.* (2019) [43]. But, Cirit *et al.* (2007) [6] and Dhami *et al.* (2020) [10] recorded the pregnancy rates which were nearly equal to present overall conception. However, comparatively lower conception rates were reported by Mirmahmoudi and Prakash (2012) [23] and Sahoo *et al.* (2017) [37].

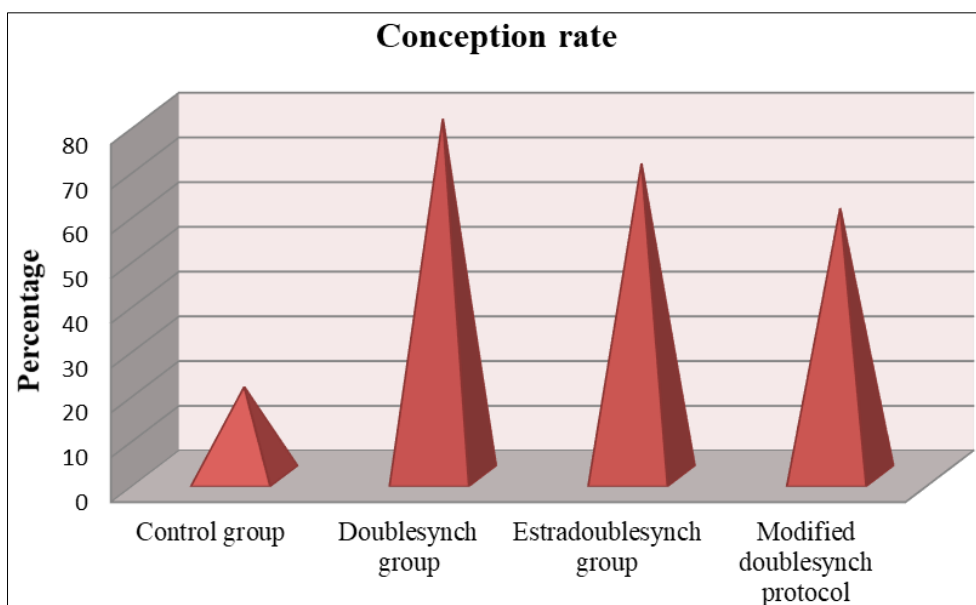
Under Estradoublesynch, the overall conception rate is in corroboration with results reported by Behera (2018) [3] and Harichandan *et al.* (2018) [16]. However, Prajapati *et al.* (2019) [31] and Dash *et al.* (2020) [8] are nearly accordance with present overall conception rate. In contrast, Raju *et al.* (2018) [35] recorded the lower pregnancy rates.

The overall conception rate in Modified doublesynch protocol was in corroboration with results reported by Coyan *et al.* (2003) [7] and Lingaswamy (2017) [20]. While the conception rate achieved after first service (FTAI) was in alignment with Binversie *et al.* (2012) [4] and Kashyap *et al.* (2018) [17]. Whereas the conception rate achieved after second service (FTAI) is in accordance with Metin Pancarci *et al.* (2013) [22]. In contrast, Senthilkumar *et al.* (2017) [40] and Keskin *et al.* (2010) [18] recorded higher conception rates and lower conception rates.

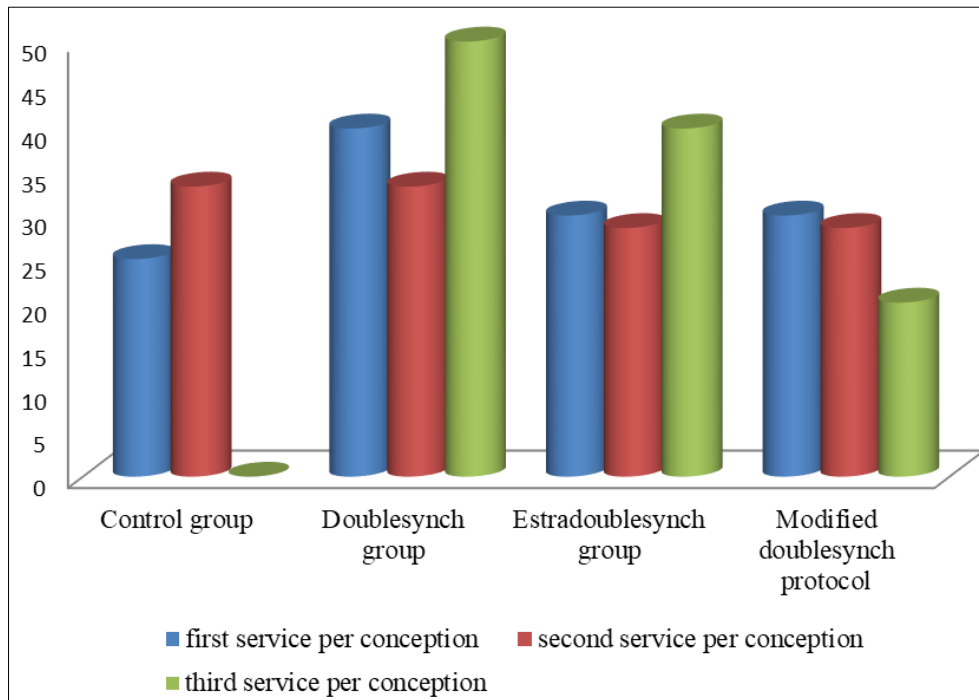
**Table 6:** Conception rate in postpartum anestrus Graded murrah Graded Murrah buffaloes.

Sl. No	Name of the group	Number of Buffaloes treated	Number of Buffaloes inseminated	Number of Buffaloes conceived	Conception rate	Chi- square test
1	Control group	10	4	2	20	8.49*
2	Doublesynch	10	10	8	80	
3	Estradoublesynch	10	10	7	70	
4	Modified doublesynch protocol	10	10	6	60	
	TOTAL	40	34	23	67.64	

\*Conception rates between groups differed significantly ( $p < 0.05$ ).



**Fig 3:** Conception rate in postpartum anestrus Graded Murrah buffaloes.



**Fig 4:** service wise conception rate in postpartum anestrous buffaloes

### Conclusions

The current study carried out Doublesynch and Estradoublesynch protocols which are efficient synchronization protocols for acyclic and cyclic buffaloes/cattle. It is therefore, suggested that administration of hCG in the place second GnRH of Doublesynch protocol also favour LH surge and improve the conception rates. But, Further reports warranted to support the effect of hCG in the place second GnRH of Doublesynch protocol on large number of clinical trials under farm as well as field level conditions. In nut shell, it is recommended that Double synch protocol could be used as best hormonal protocol to induce the estrus as well as improve the conception rate in postpartum anestrous Graded Murrah buffaloes.

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