

## IMPACT OF DIFFERENT ESTRUS INDUCTION AND OVULATION SYNCHRONIZATION PROTOCOLS IN ADDRESSING INFERTILITY IN BUFFALOES

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### ABSTRACT

The efficacy of six estrus induction/synchronization protocols, viz., Ovsynch, Ovsynch Plus, PRID/Triu-B, PRID+PMSG, Doublesynch and Estradoublesynch was compared for their estrus induction response, and conception rates at FTAI and overall of three cycles post-treatment including monitoring of plasma progesterone ( $P_4$ ) at different time intervals in postpartum anestrus and repeat breeding buffaloes (20 to 35 animals in each protocol) under field conditions. All infertile animals with average BCS were injected initially once with Inj. Ivermectin 100 mg s/c, Inj. organic phosphorus and Vit AD<sub>3</sub>E, 10 ml each i/m and multi-minerals 1 bolus orally for 7 days. The repeat breeders also received additional i/m Inj. of single shot Enrofloxacin 40 ml. Induction and synchronization of estrus with establishment of normal cyclicity was found in 100% animals with PRID, PRID+PMSG, Doublesynch, Estradoublesynch protocols, but the response with Ovsynch and Ovsynch Plus was 92 to 94 %. The conception rates obtained at induced estrus/FTAI in anestrus buffaloes under Ovsynch, Ovsynch Plus, PRID/Triu-B, PRID +PMSG, Doublesynch and Estradoublesynch protocols were 30.0, 21.7, 30.0, 45.0, 34.2 and 23.3%, respectively, with

overall post-treatment 3 cycles' conception rates of 53.3, 39.1, 70.0, 75.0, 57.9 and 40.0%, respectively. In repeat breeder buffaloes, the conception rates obtained at induced estrus/FTAI with Ovsynch, Ovsynch Plus, Doublesynch and Estradoublesynch protocols were 31.8, 20.0, 47.6 and 40.0%, respectively, and overall 3 cycles' conception rates were 59.1, 46.7, 76.2 and 60.0%, respectively. Among untreated anestrus and repeat breeding control animals only 20% and 35% animals conceived over 60 to 90 days follow up. The basal level of plasma  $P_4$  in anestrus animals and higher one in repeat breeders on the day of initiation of treatment confirmed their reproductive status. The plasma  $P_4$  profile was significantly improved on day 7 to 9, i.e. just before last PGF<sub>2</sub> $\alpha$  injection and dropped significantly to the basal level in most of the animals in next 2 to 3 days with induced estrus. The plasma  $P_4$  was significantly higher in conceived than non-conceived animals on day 12 post-AI in all the groups suggesting anovulatory induced estrus and/or luteal insufficiency in non-conceived animals. Thus, it was concluded that the progestagen based protocols followed by Doublesynch and Ovsynch protocols were the best in anestrus animals, and doublesynch followed by Estradoublesynch and Ovsynch protocols in repeat breeders to economically improve the conception

rates at induced estrus and overall of 3 cycles.

**Keywords:** *Bubalus bubalis*, buffalo, cyclic, acyclic, synchronization protocols, estrus induction, conception rate, plasma progesterone

## INTRODUCTION

Buffalo is known for long postpartum anestrus and silent estrus contributing to its low reproductive efficiency. Prolonged postpartum anestrus and repeat breeding are considered major causes of economic loss to buffalo breeders and to the dairy industry (El-Wishy, 2007). Effective treatment of infertile buffaloes is the efficient means of improving their milk production. Various hormonal preparations and protocols are being used to modulate the circulating plasma progesterone levels to make acyclic and repeat breeding buffaloes to cycle normally and to improve their reproductive efficiency, thereby reducing the inter-calving interval economically viable (Mirmahmoudi *et al.*, 2014; Mungad *et al.*, 2017). But the results reported in the literature are inconsistent (Ghuman *et al.*, 2009; Nakrani *et al.*, 2014; Savalia *et al.*, 2014; Mungad *et al.*, 2017) largely due to nutritional status, faulty management, ovarian changes, endocrine events and even uterine infection. Hormonal therapies have good therapeutic value to enhance reproductive efficacy only in infection free infertile animals with good nutritional status (Malik *et al.*, 2010; Savalia *et al.*, 2014; Buhecha *et al.*, 2016). Very meagre information is available on use of recently developed Doublesynch, Estradoublesynch and PRID+PMSG protocols on cyclic/acyclic buffaloes. Hence, this study was planned to evaluate the comparative efficacy of different estrus induction

and ovulation synchronization protocols through monitoring plasma progesterone and conception rates in anestrus and repeat breeding buffaloes under field conditions.

## MATERIALS AND METHODS

### Selection of infertile animals

The study was carried out during 2015 to 2017 under the milk shed areas of Amul dairy, Anand and Panchamrut dairy, Godhra, Gujarat, India. The buffaloes having average BCS and postpartum true anestrus (>90 days acyclic) or repeat breeding (>140 days cyclic) were thoroughly screened gynaeco-clinically for their reproductive status. Anestrus animals were confirmed per rectum by palpating smooth small inactive ovaries twice 10 days apart. Repeat breeders were confirmed on the basis of regular cyclicality and failure to conceive after AI for more than 3 times without visible or palpable genital abnormality.

### Pre-synchronization treatment

All the infertile animals selected were injected once with 100 mg Ivermectin s/c (Inj. Ivetin, 10 ml, Indian Immunologicals Ltd), and injection organic phosphorus (Inj. Tonophosphan, MSD Animal Health) and multivitamins AD<sub>3</sub>E (Inj. Intavita-H, Intas Pharma) 10 ml each, and bolus Minotas (Intas Pharma) 1 bolus PO for 7 days. In repeat breeders, a single shot i/m injection of Enrofloxacin (Inj. Flobac SA, 40 ml, Intas Pharma) was also given to rule out invisible genital infection, if any. The buffaloes were then subjected randomly to following six estrus induction/synchronization protocols, keeping one group each of untreated acyclic and cyclic controls. Progestagen based PRID (Triu-B) and PRID + PMSG protocols were

used only in anestrus buffaloes.

### **Ovsynch and ovsynch plus protocols**

The acyclic and cyclic buffaloes under Ovsynch protocol were administered with i/m Inj. of 20 µg GnRH analogue, i.e. Buserelin acetate (Receptal, 5 ml, MSD) on day 0, Inj. of 500 µg PGF<sub>2</sub>α analogue, i.e. Cloprostenol sodium (Estrumate, 2 ml, MSD) on day 7, and second Inj. of 10 µg of GnRH on day 9, followed by FTAI twice at 0 and 24 h later. In Ovsynch Plus protocol, buffaloes received an additional i/m Inj. of PMSG 500 IU (Folligon, MSD) 2 days before the actual Ovsynch protocol.

### **Doublesynch and estradoublesynch protocols**

In Doublesynch protocol, acyclic and cyclic buffaloes were administered with i/m Inj. of 500 µg PGF<sub>2</sub>α analogue, i.e. Cloprostenol sodium on day 0, 20 µg GnRH analogue, i.e. Buserelin acetate on day 2, and second Inj. of 500 µg PGF<sub>2</sub>α on day 9 and 10 µg GnRH on day 11, followed by FTAI twice at 16 and 24 h later, while in Estradoublesynch protocol, the infertile buffaloes received an Inj. of estradiol benzoate 1 mg (Sigma, USA) on day 10, in place of second GnRH injection on day 11 in Doublesynch, with FTAI twice at 48 and 60 h post-estradiol injection.

### **PRID and PRID plus PMSG protocols**

Under PRID protocol, Triu-B (0.96 g progesterone in elastic rubber molded over a nylon spine, Virbac Animal Health Ltd) was inserted intra-vaginally for 7 days in anestrus buffaloes. On the sixth day, the buffaloes were treated with i/m injection of 500 µg Cloprostenol sodium. Estradiol benzoate 0.75 mg (Sigma) was given i/m on day 7 while removing the insert. FTAI was done 24 h post-EB treatment. In PRID plus PMSG protocol,

an Inj. of PMSG 500 IU (Folligon, MSD) replaced the estradiol benzoate in PRID protocol, and FTAI was done twice at 48 and 60 h later.

### **Control groups**

Twenty cyclic repeat breeder and 20 acyclic buffaloes served as untreated controls in which only pre-synchronization treatment was given as above without any hormonal intervention, and were followed for spontaneous estrus and insemination.

### **Blood sampling and progesterone Assay**

Blood samples were collected from 12 representative animals of each treatment protocol in heparinized vacutainers by jugular vein puncture, just before initiation of treatment (i.e. day 0), at the time of last PGF<sub>2</sub>α injection (i.e. on day 6/7/9/10), at induced estrus/FTAI (i.e. on day 8/9/10/12 as per protocol) and on day 12 post-AI. The blood samples were centrifuged at 3000 rpm for 15 minutes, and plasma separated out was stored at -20°C with a drop of merthiolate (0.1%). Plasma progesterone concentrations were determined by employing standard Radio-Immuno-Assay (RIA) technique of Kubasic *et al.* (1984). Labelled antigen (I<sup>125</sup>), antibody coated tubes and standards were procured from Immunotech-SAS, France.

### **Data recording and analysis**

For animals that failed to conceive at FTAI, the visual heat detection both in the morning and evening and frozen semen inseminations were practiced for two more cycles. Pregnancy was confirmed in non-return cases by per rectal palpation 60 days of last AI. The observations on estrus responses and conception rates were recorded and compared between different groups statistically by using Chi-square test and those of

progesterone profile by using ANOVA and NMRT (Snedecor and Cochran, 1986).

## RESULTS AND DISCUSSION

### Estrus synchronization and fertility

The estrus induction responses and conception rates observed in acyclic and cyclic buffaloes under different synchronization protocols and under control groups are presented in Tables 1 and 2, and Figures 1 and 2.

Among 20 to 38 acyclic/true anestrus buffaloes each subjected to PRID/Triu-B, PRID+PMSG, Doublesynch and Estradoublesynch protocols, 100% animals exhibited synchronized estrus with excellent behavioural signs, but the response with Ovsynch and Ovsynch plus was 92 to 94%. The conception rates obtained at induced estrus/FTAI in anestrus buffaloes under Ovsynch, Ovsynch plus, PRID, PRID+PMSG, Doublesynch and Estradoublesynch protocols were 30.0, 21.7, 30.0, 45.0, 34.2 and 23.3%, respectively. Good number of animals conceived at subsequent second and third natural cycle post-treatment also. Thus, the corresponding overall 3 cycles' conception rates post-treatment with said protocols were 53.3, 39.1, 70.0, 75.0, 57.9 and 40.0%, respectively. Among untreated anestrus control Group 4 out of 20 buffaloes exhibited estrus over a period of 90 days follow up and conceived giving overall conception rate of only 20%. The conception rates achieved were the best at induced estrus and overall of 3 cycles' with progestagen based protocols, followed by Doublesynch and Ovsynch protocol in anestrus buffaloes, but the results with Estradoublesynch and Ovsynch plus protocols were relatively poor (Table 1, Figure 1).

Among 15 to 22 repeat breeder buffaloes

each managed with Ovsynch, Ovsynch plus, Doublesynch and Estradoublesynch protocols with FTAIs, 100% showed synchronized estrus, and the conception rates obtained at induced estrus/FTAI were 31.8, 20.0, 47.6 and 40.0%, respectively, and overall 3 cycles' conception rates post-treatment were 59.1, 46.7, 76.2 and 60.0%, respectively. Among untreated control group, all animals continued to cycle and 7 out of 20 buffaloes conceived within 2 to 3 repeated cycles over 60 days follow up giving overall conception rate of only 35%. Like anestrus animals, the conception rates achieved in repeat breeder buffaloes were also the best at induced estrus and overall of 3 cycles' with Doublesynch followed by Estradoublesynch and Ovsynch protocols (Table 2, Figure 2).

The estrus induction and synchronization rates of 100% achieved with Ovsynch protocol in both anestrus and repeat breeder buffaloes were in harmony with the earlier results of Navrange *et al.* (2012); Nakrani *et al.* (2014); Savani *et al.* (2017), while many other researchers documented lower response in the range of 80.00 to 87.50% (Campanile *et al.*, 2005; Ali and Fahmy, 2007; Thorat *et al.*, 2014; Buhecha *et al.*, 2016; Mungad *et al.*, 2017) in different breeds of buffaloes. The estrus induction rate of 90% achieved with Ovsynch plus protocol in anestrus buffaloes coincided well with the result of 93.7 to 100% reported by Kumar *et al.* (2016<sup>a</sup>) in anestrus buffaloes and Virmani *et al.* (2013) in Sahiwal cows, but no report was available with Ovsynch Plus protocol to compare the present 100% estrus response in repeat breeder buffaloes.

The estrus induction rate of 90% achieved with doublesynch protocol in both acyclic and cyclic buffaloes was in harmony with the earlier results of Miramahmoudi and Prakash (2012) and Miramahmoudi *et al.* (2014), however Parida *et al.* (2015) found 70 % response in anestrus buffaloes

using same protocol. The estrus synchronization rate of 90 to 100% achieved in acyclic and cyclic buffaloes with Estradoublesynch protocol also concurred with the earlier results of 83.3 to 100% by Miramahmoudi *et al.* (2014); Parida *et al.* (2015). Further, the 100% estrus response obtained with PRID and PRID+PMSG protocols were in close agreement with the reports of Zaabel *et al.* (2009); Kajaysri *et al.* (2015); Mungad *et al.* (2017) with PRID protocol in anestrus buffaloes.

The present conception rates of 30.0 and 31.8% obtained at induced estrus in acyclic and cyclic buffaloes under Ovsynch protocol were in range of 27.42 to 40.00% reported by Ravikumar *et al.* (2007); Parmar *et al.* (2015) in repeat breeder buffaloes, and 29.41 to 37.50% reported by Campanile *et al.* (2005); Ali and Fahamy (2007); Buhecha *et al.* (2016) in anestrus buffaloes. Further, the present 53.3 and 59.1% overall three cycles' conception rates in anestrus and repeat breeders under Ovsynch protocol were in line with 43.70 to 60% CR reported by Tiwari *et al.* (2005); Ali and Fahamy (2007); Parmar *et al.* (2015) with the same protocol in cyclic buffaloes, and 45 to 60% CR reported by Savalia *et al.* (2014); Buhecha *et al.* (2016); Mungad *et al.* (2017) in anestrus buffaloes. However, higher overall conception rates of 83.33 to 89% in cyclic animals (Ravikumar *et al.*, 2007; Dahham *et al.*, 2014), and 71.43 to 85.71% in anestrus buffaloes (Thorat *et al.*, 2014; Nakrani *et al.*, 2014; Savani *et al.*, 2017) were reported by others.

The conception rates of 21.7 and 20% obtained with Ovsynch plus protocol at induced estrus and 39.1 and 46.7% for overall 3 cycles' in anestrus and repeat breeding buffaloes closely concurred with the earlier conception rates of 20 to 28% at FTAI reported by Virmani *et al.* (2013); Rathore *et al.* (2015) in anestrus animals, but were

quite lower than 53.30 and 75% reported by Kumar *et al.* (2016a). No report was available on use of Ovsynch Plus protocol in repeat breeding buffaloes to compare the present findings.

The present first service conception rates of 34.2 and 47.6% obtained with Doublesynch protocol in anestrus and repeat breeder buffaloes are little lower than earlier reports of 55 to 62% by Mirmahmoudi and Prakash (2012); Mirmahmoudi *et al.* (2014); Parida *et al.* (2015). The present 57.9 and 76.2% overall three cycles' conception rates found in anestrus and repeat breeder buffaloes with Doublesynch protocol closely corroborated with the findings of Parida *et al.* (2015); Dhindsa *et al.* (2016) in anestrus buffaloes. However, the relatively lower conception rates than present ones were reported by Kumar *et al.* (2016b) during summer and winter season (40.00 and 48%) in anestrus buffaloes.

Moreover, the conception rates of 23.3 and 40% obtained in acyclic and cyclic animals at induced estrus with Estradoublesynch protocol were lower than earlier results of 60 to 64% found by Mirmahmoudi *et al.* (2014). Further, the present 40 and 60% overall three cycles' conception rates with this protocol were closer to the earlier finding of 59.45% in repeat breeder cattle (Sharavanan *et al.*, 2016) and 62.50% in anestrus buffaloes (Parida *et al.*, 2015).

The first service and overall three cycles' conception rates of 30 and 70% obtained with Triu-B/PRID in anestrus buffaloes were in close agreement with the findings of Naseer *et al.* (2013); Nakrani *et al.* (2014); Mungad *et al.* (2017), while Vikash *et al.* (2016) obtained higher overall three cycles conception rate of 80%. Further, the first service and overall three cycles' conception rates of 45 and 75% achieved with PRID+PMSG protocol were much higher than 50.9% overall conception

rate documented by Carvalho *et al.* (2013).

The better results with PRID and PRID+PMSG probably were due to longer and better negative feedback of progesterone on hypothalamus and anterior pituitary gland, and also due to the fact that the PGF<sub>2</sub>α increases pituitary responsiveness in postpartum buffaloes (Randel *et al.*, 1996) and hence the released GnRH after CIDR/PRID removal effectively stimulates the pituitary gonadotrophins with subsequent estrus induction (Zaabel *et al.*, 2009). It seems that progesterone alone has the capability to induce fertile heat in a proportion of buffaloes, while GnRH (with prostaglandin) was partially able to do so. The combination of progesterone with PMSG causes increased estradiol production leading to behavioural estrus followed by ovulation and thus favours conception (Carvalho *et al.*, 2013).

### Progesterone profile

The mean plasma progesterone concentrations in anestrus buffaloes under all protocols were significantly ( $P<0.05$ ) higher on day 7 to 10 of protocol (i.e. just before last PGF<sub>2</sub>α injection) and day 12 post-AI compared to day 0 and day of FTAI, while in repeat breeders the concentrations on day 0 were also significantly higher compared to day of FTAI (Table 3). These results confirmed the reproductive status of two categories of animals at the start of treatment. The higher mean plasma progesterone concentrations in PRID+PMSG protocol on day 12 post-AI as compared to different days of treatment, might be due to more number of animals conceived in this group. Sarvaiya *et al.* (1991) reported progesterone levels more than 3.0 ng/ml between days 12 and 22 post-breeding in conceived buffaloes. Higher mean plasma progesterone level recorded on day 6 of treatment in buffaloes under PRID and

PRID+PMSG protocols might be due to continuous release of the exogenous progesterone from the progesterone implant inserted in the anterior vagina. In Doublesynch and Estradoublesynch protocols the rise in mean progesterone levels noted on day 9 might be due to luteinisation of some of the growing follicles and/or ovulation of dominant follicle and formation of CL under the influence of GnRH. In Estradoublesynch protocol, the estradiol benzoate injected on day 10 might also have triggered positive feedback effect of estrogen on hypothalamus and pituitary resulting into ovulatory LH surge and thereby improved conception rate in that group. The trend and levels of plasma progesterone observed under different protocols closely concurred with the previous reports of Mirmahmoudi and Prakash (2012); Mirmahmoudi *et al.* (2014); Savalia *et al.* (2014); Buhecha *et al.* (2016); Mungad *et al.* (2017) with Ovsynch, Doublesynch and CIDR protocols.

In conceived and non-conceived groups the mean plasma progesterone concentrations on day 0 and on day of FTAI were very low or basal ( $<0.5$  ng/ml) in all four treatment protocols and did not differ statistically. The mean values in samples collected just before PGF<sub>2</sub>α injection (day 6 to 10) in both conceived and non-conceived buffaloes had increased significantly in all six protocols. The conceived buffaloes of both anestrus and repeat breeder category had higher mean plasma progesterone as compared to non-conceived ones on day 12 post-AI in all the protocols with significant ( $P<0.05$ ) differences in Ovsynch, Doublesynch, Estradoublesynch, and PRID+PMSG protocols due to varying number of animals that conceived under these protocols and probable anovulatory induced estrus and/or luteal insufficiency in non-conceived animals. The present findings on plasma progesterone profile

Table 1. Efficacy of different estrus synchronization protocols in terms of estrus induction response and conception rates in acyclic/anestrus buffaloes.

Synchronization protocol	No. of cow	% Estrus induction response	Conception rate (%)			
			Induced/ 1 <sup>st</sup> estrus	2 <sup>nd</sup> cycle	3 <sup>rd</sup> cycle	Overall of 3 cycles
Ovsynch	32	93.7 (30/32)	30.0 (9/30)	21.1 (4/19)	21.4 (3/14)	53.3 (16/30)
Ovsynch plus	25	92.0 (23/25)	21.7 (5/23)	18.8 (3/16)	10.0 (1/10)	39.1 (09/23)
Doublesynch	38	100	34.2 (13/38)	29.2 (7/24)	13.3 (2/15)	57.9 (22/38)
Estra doublesynch	30	100	23.3 (7/30)	15.0 (3/20)	12.5 (2/16)	40.0 (12/30)
PRID/Triu-B	20	100	30.0 (6/20)	53.8 (7/13)	20.0 (1/5)	70.0 (14/20)
PRID+PMSG	20	100	45.0 (9/20)	50.0 (5/10)	25.0 (1/4)	75.0 (15/20)
Anestrus control	20	20.0 (4/20)	50.0 (2/4)	50.0 (1/2)	100.0 (1/1)	20.0 (04/20)

Table 2. Effect of different estrus synchronization protocols in terms of estrus induction response and conception rates in cyclic/repeat breeder (RB) buffaloes.

Synchronization protocol	No. of cow	% Estrus induction response	Conception rate (%)			
			Induced/ 1 <sup>st</sup> estrus	2 <sup>nd</sup> cycle	3 <sup>rd</sup> cycle	Overall of 3 cycles
Ovsynch	22	100	31.8 (7/22)	26.7 (4/15)	20.0 (2/10)	59.1 (13/22)
Ovsynch plus	15	100	20.0 (3/15)	25.0 (3/12)	12.5 (1/8)	46.7 (07/15)
Doublesynch	21	100	47.6 (10/21)	40.0 (4/10)	33.3 (2/6)	76.2 (16/21)
Estra doublesynch	20	100	40.0 (8/20)	18.2 (2/11)	22.2 (2/9)	60.0 (12/20)
RB control	20	100	15.0 (3/20)	11.76 (2/17)	13.32 (2/15)	35.0 (07/20)

with respect to effect of Ovsynch, PRID/Triu-B and Doublesynch protocols closely corroborated with the reports of Mirmahmoudi *et al.* (2014). Nakrani *et al.* (2014); Savalia *et al.* (2014); Buhecha *et al.* (2016); Savani *et al.* (2017) in cyclic and/or acyclic buffaloes treated with similar protocols. The plasma progesterone assay confirmed the estrus response and conception or anovulatory estrus and/or luteal insufficiency in infertile buffaloes treated with different protocols.

### CONCLUSION

The study showed excellent induction and synchronization of estrus with establishment of normal cyclicality with all the protocols in both anestrus and repeat breeder buffaloes. However, the conception rates achieved were the best at induced estrus and overall of three cycles' post-

treatment with progestagen based protocols followed by Doublesynch and Ovsynch in anestrus buffaloes, and with Doublesynch followed by Estradoublesynch and Ovsynch protocols in repeat breeding buffaloes. Ovsynch Plus protocol in fact suppressed conception rate even with expensive injection of PMSG in place of estradiol in anestrus animals. It is thus concluded that the PRID, Doublesynch or Ovsynch protocol can be used to manage the nutritionally sound infertile buffaloes on large scale to make them productive in shorter time and reduce calving interval under field conditions with economic benefit.

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Table 3. Plasma progesterone profile on different days of synchronization and on day 12 post-AI in anestrus and repeat breeder buffaloes.

Status	Synchronization protocol	No.	Plasma progesterone (ng/ml)			
			D-0	D-6/7/9/10 (PGF <sub>2</sub> α Inj)	D-8/9/10/12 (FTAI)	D-12 post-AI
Anestrus	Ovsynch	12	0.23±0.09	1.82±0.78	0.23±0.08	3.05±1.08
	Ovsynch plus	12	0.32±0.13	1.96±0.72	0.21±0.07	3.29±0.81
	Doublesynch	12	0.47±0.18	1.54±0.47	0.10±0.01	3.41±0.75
	Estra doublesynch	12	0.26±0.10	1.93±0.76	0.11±0.01	2.88±0.84
	PRID	12	0.16±0.02	2.88±0.21	0.15±0.02	2.16±0.54
	PRID+ PMSG	12	0.21±0.05	2.54±0.33	0.25±0.03	4.37±0.52
Repeaters	Ovsynch	12	0.93±0.29	1.51±0.67	0.21±0.09	2.98±0.93
	Ovsynch plus	12	1.03±0.33	2.06±0.77	0.32±0.08	1.28±0.41
	Doublesynch	12	2.11±0.58	2.03±0.47	0.12±0.02	3.04±0.59
	Estra doublesynch	12	2.13±0.89	2.63±0.49	0.38±0.19	3.39±0.91

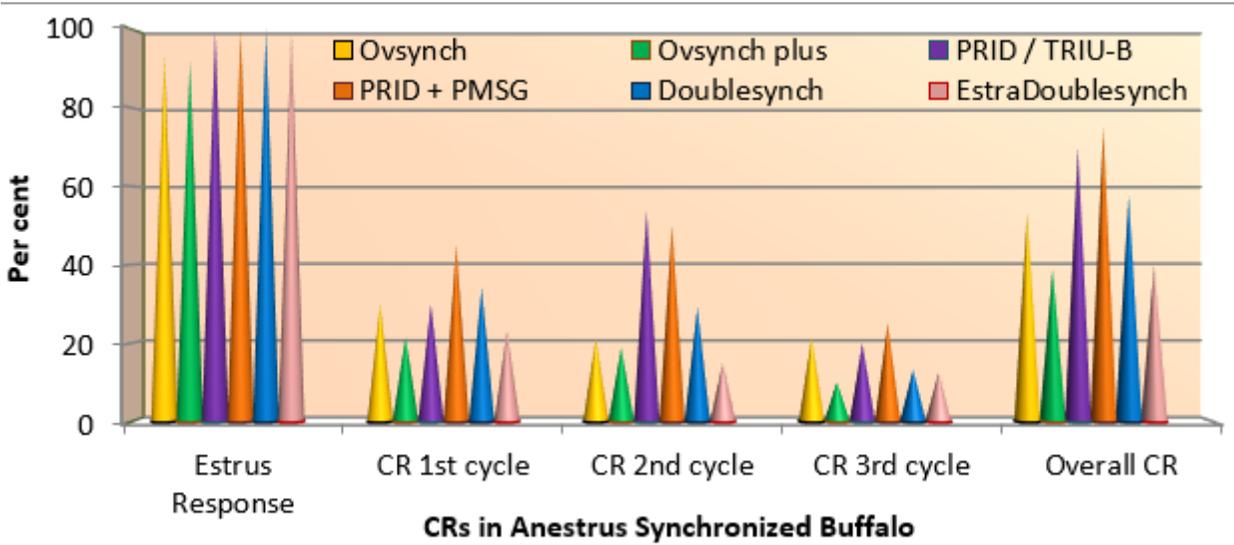


Figure 1. Estrus response and conception rates at induced and subsequent cycles with different synchronization protocols in anestrus buffaloes.

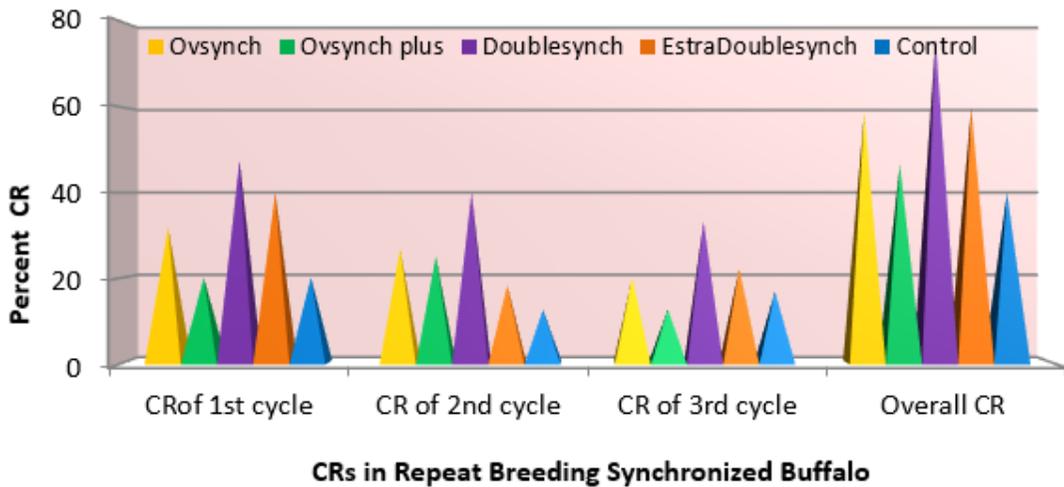


Figure 2. Conception rates at induced and subsequent cycles with different estrus synchronization protocols in repeat breeder buffaloes.

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