



EFFECT OF VITAMIN A SUPPLEMENTATION WITH OVSYNCH PROTOCOL ON FERTILITY IN REPEAT BREEDER COWS DURING DIFFERENT SEASONS

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ABSTRACT

This study was carried out to determine the effect of supplementation of vitamin A along with Ovsynch protocol on the pattern of estrus, conception rate and serum progesterone and estradiol-17 concentration in repeat breeder cows during different seasons. One hundred and twenty repeat breeder cows were equally divided into three experimental groups viz., Group I, II (Treatment groups) and Group III (Control group) during high breeding season (HBS: *i.e.*, from October to March) and low breeding season (LBS: *i.e.*, from April to September) consisting of 20 cows in each group. The group I cows were treated with Ovsynch protocol. Group II cows were administered with 12,00,000 IU of Vitamin A along with Ovsynch during first GnRH injection and group III (control) cows were inseminated during the observed estrus. Hundred percent estrus response was observed in both HBS and LBS. The mean time taken for the onset of induced estrus and the mean duration of induced estrus in groups I and II of HBS was significantly different (P 0.05) from the corresponding group of LBS. The overall percentage of intense estrus intensity following synchronization of ovulation in cows during HBS was high (26.67 percent) when compared to the cows treated during LBS (10.00 %). The overall conception rate was higher in group II in both the seasons (80.00 and 60.00 %) followed by group I (50.00 and 40.00 %) and group III (30.00 and 20.00%). All the groups of HBS and LBS had less than 1ng/ml of mean (\pm SE) serum progesterone at the time of selection. Increased serum progesterone and estradiol-17 concentration was observed in group II and it might be related with the vitamin A supplemented with Ovsynch protocol. From this study it is concluded that vitamin A supplementation along with Ovsynch protocol can be used to further improve the fertility in repeat breeder cows during different breeding seasons.

KEYWORDS: ovulation, high breeding season, low breeding season, conception rate, progesterone, estradiol-17 .

INTRODUCTION

Among the various reproductive disorders, repeat breeding syndrome alone is said to contribute to the tune of 2.29 to 42.7 percent¹. The most common causes for repeat breeding syndrome include inadequate estrus detection, nutrition, ovulatory defects, inadequate luteal function, heat stress, infection or managerial factors². Heat stress due to hot weather is a major contributing factor for the low fertility in repeat breeder cows during summer. There was a decrease in the percentage of pregnant cows per artificial insemination (AI) during hot season³. The causes of repeat breeding may originate either during early stages of follicle maturation or during preovulatory period⁴. Ovulation synchronization (ovsynch) protocol, developed by Pursley *et al.*⁵, is a biotechnological tool substantially improves fertility in normal cycling cows can also be used to improve fertility in repeat breeder cows^{6,7}. Hence, this protocol is currently used across the world, key improvements should be made by its modification for further improvement in conception rate in repeat breeder cows for applicable in different breeding seasons. It has been documented that the β -carotene and vitamin A were having a beneficial effect on reproduction and also in controlling various maladies causing reproductive

failure^{8,9}. Vitamin A deficiency resulted in extended duration of estrus, delayed ovulation, retarded development of corpus luteum and higher incidence of ovarian cyst which led to lower conception rates and abortion in early pregnancy¹⁰. Aslan *et al.*¹¹ reported that supplementation of vitamin A has beneficial effect on the fertility rate in cows whereas Jukola *et al.*¹² recorded no significant relationship between concentration of vitamin A and fertility disorders or success of AI. Vitamin A plays a crucial role in the synthesis of steroid hormones, such as progesterone⁸. Dietary β -carotene/vitamin A prevents oxidative stress and plays a crucial role in immune-stimulation¹³. Its addition may improve the reproductive outcome by altering the ovarian function and uterine environment. Hence the study was formulated to investigate the addition of vitamin A along with Ovsynch synchronization programme on the pattern of estrus, conception rate and hormonal concentration in repeat breeder cows during different seasons under Indian conditions.

MATERIALS & METHODS

Selection of repeat breeder cows

A total number of 120 pluriparous, healthy crossbred cows which failed to conceive after three or more consecutive

artificial inseminations with good quality semen were selected during estrus for the study. The selected cows were between 2nd and 5th parity. They were subjected to a thorough gynaeco-clinical examination at the time of selection and those cows with no palpable abnormalities in the genital tract and negative for white side test with uterine discharge were utilized for the experiment. All the selected cows at random were equally divided into three experimental groups *viz.*, Group I, II (Treatment groups) and Group III (Control group) during high breeding season (HBS: *i.e.*, from October to March) and low breeding season (LBS: *i.e.*, from April to September). The experiment was designed with 60 cows in each season consisting of 20 cows in each group. All the cows were kept outdoors, fed with hay and concentrate twice daily and provided with ad libitum water.

Experimental design

The group I cows were treated with Ovsynch protocol as described by Pursley *et al.*⁵. The Ovsynch protocol consisted of intramuscular injections of 10µg of GnRH (2.5 ml, Buserelin acetate, Ovulanta[®], Vet Mankind, New Delhi, India) on the day of start of synchronization of ovulation (d 0), 25 mg of PGF₂ (5 ml, Dinoprost tromethamine, Lutalyse[®], Pfizer India Ltd, Mumbai) seven days later (d 7) and another 10 µg of GnRH (2nd GnRH) 48 hours after the PGF₂ (d 9) and Timed Artificial Insemination (TAI) at 16-18 hours after the second GnRH injection (d 10). The group II cows were treated with the Ovsynch protocol as described for Group I animals. In addition, during the start of Ovsynch protocol (*i.e.*, at the time of first GnRH injection) 12,00,000 IU of Vitamin A injection (4 ml, Vitamin A[®], Virbac Animal Health India Pvt Ltd., Mumbai, India) was administered intramuscularly to all the cows. The group III (control) cows were observed for estrus from the time of selection and artificial insemination was done during the observed estrus.

Collection of blood samples

Blood collection was done in groups I and II at the (i) time of selection of repeat breeder cows (ii) initiation of Ovsynch protocol (iii) time of PGF₂ injection (iv) TAI and (v) 7 days following TAI. In group III animals, blood was collected at the (i) time of selection of animals (ii) AI and (iii) 7 days following AI. The serum was separated and stored at -20°C until progesterone and estradiol-17 estimation by radioimmunoassay (RIA) technique.

Pregnancy diagnosis

All the inseminated cows were monitored regularly following TAI at induced estrus in groups I and II and at observed estrus in group III for non-returning of estrus. The cows which have not expressed heat signs after TAI were confirmed for pregnancy by rectal palpation and ultrasound scanning at 45 days post insemination. The conception rate was expressed in percentage.

Statistical analysis

The Completely Randomized Design (CRD) method and independent 'T' - test were followed for the experiment¹⁴ and the data collected were analysed using SPSS[®] 20.0. software package. Post hoc analysis was done by Tukey's Honestly Significance Difference.

RESULTS & DISCUSSION

Estrus response

All the repeat breeder cows of experimental groups in HBS and LBS exhibited estrus (100%) following the synchronization of ovulation protocol (Table 1). The entire control group (group III) cows in HBS and LBS exhibited natural estrus (100 per cent). Similar finding was reported by Viramani *et al.*¹⁵ in cows and Ravikumar¹⁶ in buffaloes following Ovsynch treatment. However, reduced estrus response of 90¹⁷, 45¹⁸ and 18¹⁹ percent were reported in cows following Ovsynch protocols. The 100 percent estrus response following Ovsynch protocols in groups I and II might be due to the fact that these cows were in diestrus at the time of start of Ovsynch protocol as indicated by the blood progesterone concentration. Start of Ovsynch protocol during early diestrus might prevented premature estrus between first GnRH and PGF₂ injection and resulted in increased estrus response^{20,21,22}. From this study, it was clear and evident that Ovsynch protocol was effective to yield 100 percent estrus response in repeat breeder cows. In control group (group V) of HBS and LBS, there was 100 percent estrus response in this investigation which was equal to experimental groups.

Onset of induced estrus

The overall mean time taken for the onset of induced estrus in HBS was significantly lower than that of LBS (Table 1). The result indicated that the season had influence on the onset of induced estrus following synchronization of ovulation in repeat breeder cows. Statistical analysis revealed that during HBS and LBS, no significant difference in the mean onset of induced estrus among the experimental groups. The mean interval to onset of induced estrus in groups I and II of HBS was significantly different (P 0.05) from the corresponding group of LBS. The mean time taken for the onset of induced estrus in this study was in accordance with Sathiamoorthy¹⁷ and Vijayarajan *et al.*²³ who treated cyclic crossbred cows with Ovsynch protocol. Similarly, Stevenson *et al.*²⁴ reported 54.00 ±13.00 and 55.00 ±4.40 hours as mean time to onset of estrus in Ovsynch 33 and Ovsynch 48 treated dairy cows, respectively. In this experiment, mild variations were observed in the time of onset of induced estrus in both HBS and LBS. The increased time for the onset in the experimental group of HBS recorded than the LBS in the present study indicates the seasonal influence in repeat breeder cows. Incidence of different climatic factors either alone or in combination had distinct or indirect effect on the ovarian activity and expression of estrus in cows²⁵. The increased heat stress during LBS might be the reason for delayed onset of estrus in this study²⁶.

Duration of induced estrus

The overall mean (±SE) duration of induced estrus in experimental groups during HBS and LBS were 27.03 ±0.56 and 21.60 ±0.38 hours, respectively. During HBS, the mean (±SE) duration of induced estrus in groups I and II had no significant difference (P 0.05) among them. However, the group III had significant difference (P 0.05) with group II and had no significant difference (P 0.05) with group I during HBS, respectively. In LBS, the mean (±SE) duration of estrus in groups I and II showed no significant difference (P 0.05) among them. But group III

had significant difference ($P < 0.05$) with group II during LBS. The mean (\pm SE) duration of induced estrus in each group from I to III during HBS significantly differed ($P < 0.05$) from the corresponding group of LBS.

In both the seasons, the group II (GnRH + vitamin A) cows had slightly longer duration of estrus than other groups. Vitamin A was considered to have specific role in reproduction and was involved in the formation of estradiol-17 from tertiary follicles²⁷ and was evidenced by the serum estradiol-17 levels obtained in this group. In this study, comparatively lower duration of natural estrus was recorded in control group than induced estrus in

treatment groups in both the seasons. Similar to this investigation, Dhande and Kadu²⁸ recorded 18.20 \pm 0.04 hours as duration in natural estrus and 18.90 \pm 0.49 hours as induced estrus in cows. In this investigation, all the treatment and control groups of HBS had longer mean (\pm SE) duration of induced estrus than LBS. It proved the presence of seasonal effect on the duration of induced estrus in repeat breeder cows. In this study, the mean serum estradiol-17 concentrations during the LBS were lesser than the HBS in all the repeat breeder cows. It might be the reason for reduced duration of estrus in LBS than in HBS^{25,29}.

TABLE 1: Estrus response, onset, duration and intensity of induced estrus following synchronization of ovulation in repeat breeder cows during high (HBS) and low (LBS) breeding seasons

S. No.	Treatment groups	No of repeat breeder cows treated	Estrus response (%)	Onset of induced estrus (Mean \pm SE) (hours)	Duration of estrus (Mean \pm SE) (hours)	Intensity of the estrus			
						Intense	Intermediate	Weak	
1.	High breeding season (October to March)	Group I	20	100	46.70 ^{ap} \pm 1.27	26.60 ^{abp} \pm 0.61	6 (30.00)	10 (50.00)	4 (20.00)
		Group II	20	100	44.70 ^{ap} \pm 1.11	27.21 ^{bp} \pm 1.52	8 (40.00)	12 (60.00)	0 (0.00)
		Overall			45.70 \pm 0.61	27.03 \pm 0.56	-	-	-
2.	Low breeding season (April to September)	Group I	20	100	52.80 ^{aq} \pm 1.10	21.15 ^{abq} \pm 0.83	2 (10.00)	8 (40.00)	10 (50.00)
		Group II	20	100	53.20 ^{aq} \pm 0.92	22.15 ^{bq} \pm 0.71	4 (20.00)	10 (50.00)	6 (30.00)
		Overall			53.00 \pm 0.49	21.60 \pm 0.38	-	-	-
3.	Total	Group III	20	100	-	20.10 ^{aq} \pm 0.53	0 (0.00)	10 (50.00)	10 (50.00)
		Overall	60			Overall	16 (26.67)	32 (53.33)	12 (20.00)
		Overall	60			overall	6 (10.00)	28 (46.67)	26 (43.33)

Means bearing different superscripts (a, b) in high and low breeding season among groups within a same column of particular season differ significantly ($P < 0.05$).

Means bearing different superscripts between seasons of same groups (p, q) differ significantly ($P < 0.05$).

Figures in the parentheses are in percentage

Group I - Ovsynch, **Group II** - Vitamin A + Ovsynch and **Group III** – Control

Intensity of induced estrus

The intensity of estrus following synchronization of ovulation in repeat breeder cows in the present study was classified as intense, intermediate and weak³⁰. The percentage of intense and intermediate estrus intensity was high in group II (Ovsynch+Vitamin A) in both HBS and LBS. The higher estradiol-17 levels recorded in this group might be the reason for the more intense estrus in this group than other groups. Gaikwad *et al.*³¹ reported diets deficient in vitamin A led to low estrus intensity. In group I also, no much of variation in the estrus intensity was observed. It might be due to prompt follicular development following synchronization with GnRH and PGF₂³². Further, mineral mixture supplemented could have resulted in higher estrus expression rates in experimental cows. Minerals act as co-enzymes for the production of reproductive hormones especially in steroidogenesis³³.

The overall percentage of intense estrus intensity following synchronization of ovulation in repeat breeder cows during HBS was high (26.67%) when compared to the repeat breeder cows treated by synchronization of ovulation protocol during LBS (10.00 %). Also the overall percentages of intermediate estrus intensity in repeat breeder cows during HBS were higher (53.33) than the cows treated by synchronization of ovulation protocol

during LBS (46.67%). The overall percentage of weak estrus intensity in repeat breeder cows during HBS was low (20.00) when compared to LBS (43.33 %). In the current experiment, higher incidence of intense and intermediate intensities of estrus during HBS than during LBS was recorded as observed by Mustafa *et al.*²⁵ in cows. Heat stress during HBS might reduce the intensity of estrus in dairy cattle³⁴. Further, reduced concentration of serum estradiol-17 in cows during LBS might be the reason for higher rate of weak estrus intensities during LBS.

Pregnancy diagnosis

During HBS, the percentage of first service, second service and overall conception rates observed in this study were 30.00, 20.00 and 50.00 in group I; 50.00, 30.00 and 80.00 in group II and 10.00, 20.00 and 30.00 per cent in group III, respectively. The percentage of first service, second service and overall conception rates in groups I, II and III during LBS were 20.00, 20.00 and 40.00; 40.00, 20.00 and 60.00 and 10.00, 10.00 and 20.00 per cent, respectively. The overall first service, overall second service and overall conception rates recorded following synchronization of ovulation in repeat breeder cows were 30.00, 23.33 and 53.33 percent during HBS and 23.33, 16.67 and 40.00% during LBS, respectively. Synchronization of ovulation programmes in repeat

breeder cows yielded 53.33 and 40.00% overall conception rate during HBS and LBS, respectively and results confirmed the influence of season on fertility in repeat breeder cows.

The overall pregnancy rates following Ovsynch + vitamin A (group II) treatment during HBS and LBS was higher than group I and III in the respective seasons. The conception rates of 21.6³⁵ and 33.3³⁶ per cent were reported in dairy cows following Ovsynch plus β -carotene treatment. It has been documented that the β -carotene and vitamin A were having a beneficial effect on reproduction and also in controlling various maladies causing reproductive failure^{8,9,11}. Vitamin A in particular might play a potential role in follicular development because epithelial tissues were highly dependent on the sufficient supply of vitamin A for normal cellular differentiation³⁷. Schweigert *et al.*³⁸ reported that intra-follicular vitamin A

concentrations were indirectly indicating the function and size of the follicles. β -carotene and vitamin A were considered to have specific role in reproduction and was involved in the formation of estradiol-17 in tertiary follicles and progesterone from corpus luteum²⁷ (Kumar *et al.*, 2010). The estradiol-17 and progesterone concentrations obtained in this group on both the seasons indicated the advantage of vitamin A added along with Ovsynch protocol and might be the reason for the higher conception rate found in this group during both the seasons.

Serum progesterone

In the current study, the mean serum progesterone concentration ranged from 0.41 \pm 0.01 to 3.72 \pm 0.12 ng/ml during different phases of treatments in HBS and LBS both in pregnant and non-pregnant cows (Table 2).

TABLE 2: Serum progesterone (P₄) levels (mean \pm SE) before, during and after synchronization of ovulation in repeat breeder cows during high and low breeding seasons

S.No.	Treatment groups		P ₄ (ng/ml)				
			At the time of selection	Day 0 (GnRH injection)	Day 7 (PGF ₂ injection)	Day 10 (Timed AI)	7 days post AI
High Breeding Season							
1.	Group I (Ovsynch)	P	0.55 ^{pa} \pm 0.04	2.26 ^{qa} \pm 0.41	3.00 ^{ra} \pm 0.51	0.51 ^{pa} \pm 0.03	3.14 ^{ra} \pm 0.07
		NP	0.97 ^{pb} \pm 0.14	1.46 ^{qa} \pm 0.14	1.95 ^{ra} \pm 0.24	0.92 ^{pa} \pm 0.21	2.54 ^{sb} \pm 0.15
		Overall	0.77 ^p \pm 0.11	1.87 ^q \pm 0.25	2.47 ^r \pm 0.31	0.73 ^p \pm 0.11	2.83 ^r \pm 0.12
2.	Group II (Vitamin A + Ovsynch)	P	0.48 ^{pa} \pm 0.05	1.65 ^{qa} \pm 0.09	3.33 ^{ra} \pm 0.16	0.45 ^{pa} \pm 0.03	3.72 ^{sa} \pm 0.12
		NP	0.72 ^{pb} \pm 0.01	1.29 ^{qa} \pm 0.14	2.29 ^{rb} \pm 0.01	0.72 ^{pb} \pm 0.06	3.04 ^{sb} \pm 0.09
		Overall	0.53 ^p \pm 0.05	1.58 ^q \pm 0.09	3.12 ^r \pm 0.19	0.51 ^p \pm 0.05	3.58 ^s \pm 0.13
3.	Group III (Control)	P	0.53 ^{pa} \pm 0.05	-	-	0.54 ^{pa} \pm 0.02	3.00 ^{qa} \pm 0.19
		NP	0.82 ^{pa} \pm 0.07	-	-	0.90 ^{pb} \pm 0.07	2.53 ^{qa} \pm 0.14
		Overall	0.70 ^p \pm 0.07	-	-	0.72 ^p \pm 0.06	2.71 ^q \pm 0.13
Low Breeding Season							
1.	Group I (Ovsynch)	P	0.46 ^{pa} \pm 0.04	1.44 ^{qa} \pm 0.11	2.51 ^{ra} \pm 0.07	0.43 ^{pa} \pm 0.02	3.11 ^{sa} \pm 0.02
		NP	0.71 ^{pb} \pm 0.02	1.13 ^{qa} \pm 0.10	1.67 ^{rb} \pm 0.11	0.67 ^{pb} \pm 0.01	2.51 ^{sb} \pm 0.03
		Overall	0.62 ^p \pm 0.03	1.28 ^q \pm 0.07	2.01 ^r \pm 0.14	0.57 ^p \pm 0.03	2.76 ^s \pm 0.16
2.	Group II (Vitamin A + Ovsynch)	P	0.38 ^{pa} \pm 0.04	1.45 ^{qa} \pm 0.06	3.05 ^{ra} \pm 0.12	0.38 ^{pa} \pm 0.03	3.47 ^{ra} \pm 0.08
		NP	0.59 ^{pb} \pm 0.03	1.23 ^{qb} \pm 0.05	2.01 ^{ra} \pm 0.04	0.60 ^{pb} \pm 0.01	2.69 ^{sb} \pm 0.17
		Overall	0.46 ^p \pm 0.04	1.36 ^q \pm 0.05	2.63 ^r \pm 0.18	0.47 ^p \pm 0.04	3.16 ^s \pm 0.15
3.	Group III (Control)	P	0.41 ^{pa} \pm 0.01	-	-	0.46 ^{pa} \pm 0.05	2.97 ^{qa} \pm 0.14
		NP	0.56 ^{pb} \pm 0.03	-	-	0.60 ^{pa} \pm 0.04	2.21 ^{qb} \pm 0.06
		Overall	0.53 ^p \pm 0.03	-	-	0.57 ^p \pm 0.04	2.36 ^q \pm 0.12

Means bearing different superscripts (p,q,r,s) among different days of blood collection within same row differ significantly (P 0.05). Means bearing different superscripts (a,b) between rows within a column differ significantly (P 0.05).

During HBS and LBS, in both pregnant and non-pregnant cows, the mean serum progesterone levels ranged from 1.13 \pm 0.10 to 2.26 \pm 0.41 ng/ml on the d 0 (day of initiation of Ovsynch) of the experiment. It clearly showed that the first GnRH was administered during diestrus in these animals. Similarly Vasconcelos *et al.*²⁰ initiated the Ovsynch protocol during diestrus of the cycle. The luteal activity as indicated by elevated serum progesterone concentration at the start of synchronization of ovulation protocol confirmed the increase in conception rates in treatment groups over the control group in this study. In the same experimental groups the mean serum progesterone levels ranged from 1.68 \pm 0.12 to 3.33 \pm 0.16 ng/ml on d 7 (PGF₂ injection) of experiment. It clearly indicated that on day of PGF₂ injection, the cows were in

late diestrus. In all the cows, at the time of induced estrus (d 10) there was a drastic reduction (P 0.05) in the serum progesterone level (<1 ng/ml, ranged from 0.38 \pm 0.03 to 0.92 \pm 0.11 ng/ml) observed. It visibly indicated that in all the cows, complete luteolysis occurred in response to the administration of PGF₂ injection on d 7 of the trial. The reduction in mean serum progesterone concentration in this experiment during induced estrus proved that all the treatment protocols followed for synchronization of ovulation in this study effectively controlled the estrus and it might be the reason for improved conception in treated groups of HBS and LBS. Duchens *et al.*³⁹ suggested that elevated progesterone level at estrus might lead to asynchrony between onset of estrus and ovulation and consequently cause failure of conception. In the present

study, the mean serum concentration of progesterone at 7 days post AI ranged from 2.21 ± 0.06 to 3.72 ± 0.12 ng/ml. It was in concurrence with the findings of Takkar *et al.*⁴⁰, Selvaraju *et al.*⁴¹ in cows and Berber *et al.*⁴² in buffaloes. The elevated progesterone in all the groups of this study was correlated well with ovulatory response following synchronization of ovulation as described by Vasconcelos *et al.*⁴³ and Gaja *et al.*⁴⁴. In group II, the vitamin A

administered along with Ovsynch protocol might be the reason for definite elevation of progesterone and improved fertility.

Serum estradiol-17

In the current study, the mean serum estradiol-17 concentration ranged from 12.92 ± 0.35 to 41.24 ± 1.17 pg/ml during different phases of treatments in HBS and LBS both in pregnant and non-pregnant cows (Table 3).

TABLE 3: Serum estradiol-17 levels (mean \pm SE) before, during and after synchronization of ovulation in repeat breeder cows during high and low breeding seasons

S.No.	Treatment groups		Estradiol-17 (pg/ml)				7 days post AI
			At the time of selection	Day 0 (GnRH injection)	Day 7 (PGF ₂ injection)	Day 10 (Timed AI)	
High Breeding Season							
1.	Group I (Ovsynch)	P	40.16 ^{qa} \pm 1.73	16.19 ^{pa} \pm 0.25	17.70 ^{pa} \pm 0.36	41.10 ^{qa} \pm 2.26	16.88 ^{pa} \pm 0.42
		NP	34.56 ^{rb} \pm 1.54	14.65 ^{pb} \pm 0.48	16.36 ^{pa} \pm 0.54	34.78 ^{rb} \pm 0.92	18.25 ^{qb} \pm 0.40
		Overall	37.36 ^f \pm 1.44	15.42 ^p \pm 0.36	17.03 ^p \pm 0.38	38.31 ^f \pm 1.64	17.57 ^{pa} \pm 0.36
2.	Group II (Vitamin A + Ovsynch)	P	39.81 ^{qa} \pm 1.13	17.70 ^{pa} \pm 0.32	18.88 ^{pa} \pm 0.27	41.24 ^{qa} \pm 1.17	18.18 ^{pa} \pm 1.15
		NP	35.63 ^{qa} \pm 2.24	15.77 ^{pb} \pm 0.44	18.74 ^{pa} \pm 0.88	37.85 ^{qa} \pm 2.31	19.68 ^{pb} \pm 0.33
		Overall	38.97 ^f \pm 1.11	17.31 ^p \pm 0.37	18.85 ^p \pm 0.25	40.18 ^f \pm 1.14	19.38 ^{qa} \pm 0.37
3.	Group III (Control)	P	40.82 ^{qa} \pm 1.13	-	-	40.46 ^{qa} \pm 2.29	17.35 ^{pa} \pm 0.37
		NP	35.38 ^{qb} \pm 1.55	-	-	37.62 ^{qa} \pm 1.45	18.84 ^{pa} \pm 0.26
		Overall	37.56 ^f \pm 1.33	-	-	39.04 ^{qa} \pm 1.34	17.95 ^p \pm 0.34
Low Breeding Season							
1.	Group I (Ovsynch)	P	35.65 ^{qa} \pm 1.88	14.13 ^{pa} \pm 0.23	15.32 ^{pa} \pm 0.31	36.21 ^{qa} \pm 1.74	14.67 ^{pa} \pm 0.33
		NP	30.41 ^{rb} \pm 1.10	12.92 ^{pb} \pm 0.35	14.54 ^{qa} \pm 0.43	30.57 ^{rb} \pm 0.80	16.08 ^{qb} \pm 0.44
		Overall	32.51 ^f \pm 1.27	13.41 ^p \pm 0.29	14.85 ^{pa} \pm 0.30	33.96 ^f \pm 1.58	15.23 ^{qa} \pm 0.34
2.	Group II (Vitamin A + Ovsynch)	P	34.75 ^{qa} \pm 1.35	15.74 ^{pa} \pm 0.31	16.74 ^{pa} \pm 0.26	36.76 ^{qa} \pm 1.39	15.16 ^{pa} \pm 0.65
		NP	30.40 ^{qa} \pm 1.24	13.32 ^{pb} \pm 0.34	15.07 ^{pa} \pm 0.93	32.19 ^{qa} \pm 0.84	17.00 ^{pb} \pm 0.31
		Overall	33.01 ^q \pm 1.15	14.77 ^p \pm 0.45	16.07 ^p \pm 0.46	34.33 ^q \pm 1.04	16.27 ^p \pm 0.42
3.	Group III (Control)	P	33.79 ^{qa} \pm 1.32	-	-	36.50 ^{qa} \pm 0.64	15.28 ^{pa} \pm 0.29
		NP	30.53 ^{qa} \pm 0.74	-	-	32.23 ^{rb} \pm 0.64	17.26 ^{pb} \pm 0.68
		Overall	31.18 ^q \pm 0.75	-	-	33.09 ^q \pm 0.76	15.68 ^p \pm 0.36

Means bearing different superscripts (p,q,r) among different days of blood collection within same row differ significantly (P 0.05).
Means bearing different superscripts (a,b) between rows within a column differ significantly (P 0.05).

The mean (\pm SE) serum estradiol-17 concentrations during HBS was higher than LBS in repeat breeder cows treated with various synchronization of ovulation protocols. The repeat breeder cows were selected during estrus for the experiment and the mean (\pm SE) estradiol-17 concentration ranged between 30.41 ± 1.10 and 40.82 ± 1.13 pg/ml at the time of selection. A sharp rise (P 0.05) in mean (\pm SE) estradiol-17 concentration during induced estrus and followed by a drastic reduction on day 7 post AI was observed. Statistical analysis revealed a significant (P 0.05) difference in mean (\pm SE) estradiol-17 concentration between pregnant and non-pregnant cows on day 7 except in group III of HBS. Among the various groups, the estradiol-17 concentration was higher in group II during various stages of treatment.

Upadhyay *et al.*⁴⁵ and Ravikumar¹⁶ stated that low estradiol-17 level on the day of estrus during summer in buffaloes might be a factor for poor expression of estrus. It might be also a reason in this study for the less intense estrus and weaker estrus intensity percentage in cows of LBS. Plasma estradiol concentrations were reduced by heat stress in dairy cows^{46,47} and the effect was consistent with decreased concentration of luteinizing hormone and

reduced dominance of selected follicle, although this effect had not been observed always⁴⁸. Further the serum estradiol-17 concentration during induced estrus in group II was higher in both the seasons when compared to group I and III. The vitamin A administered along with 1st GnRH of Ovsynch might be the reason for the higher levels in this group. Vitamin A in particular might play a potential role in follicular development because epithelial tissues were highly dependent on the sufficient supply of vitamin A for normal cellular differentiation³⁷. Schweigert *et al.*³⁸ reported that intra-follicular vitamin A concentrations were indirectly indicating the function and size of the follicles. Hence the cows in this group have expressed more intense and intermediate estrus intensity when compared to other groups in both the seasons.

From this study it is concluded that Ovsynch protocol can be used to augment fertility in repeat breeder cows during different breeding seasons. Vitamin A supplementation along with Ovsynch protocol favorably affects the serum estradiol-17 and progesterone concentration and thereby improves the pregnancy rate in repeat breeder cows during high and low breeding seasons.

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