RESEARCH ARTICLE

Impact of Various Ovulation Synchronization Protocols on Serum Triglycerides and Cholesterol Profile in Repeat Breeder Cows during Different Breeding Seasons

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Abstract

The present study evaluated whether various ovulation synchronization protocols modulate cholesterol and triglycerides profile and enhance fertility in repeat breeding crossbred cows under field conditions. The study was conducted in 100 repeat breeder cows which were divided into five experimental groups, *viz.,* Group I, II, III, IV (Treatment groups) and Group V (Control group) during the high breeding season (HBS: *i.e.,* from October to March) and low breeding season (LBS: *i.e.,* from April to September). Fifty cows were used during HBS and 50 cows during LBS. The Ovsynch, Presynch+Ovsynch, Ovsynch+Post-AI GnRH, and Ovsynch+Vitamin A protocols were followed to treat the Group I, II, III, and IV cows, respectively, and artificial insemination was performed during induced estrus. The group V cows were inseminated during observed estrus. There was an increasing trend in cholesterol concentration from selection to 7 days post-AI in all the groups in both HBS and LBS. Further, the cholesterol concentration was higher during HBS than LBS. The pregnant cows had significantly ($p<0.05$) higher mean (±SE) serum cholesterol levels than non-pregnant cows in both seasons. There was no much variation in serum triglycerides concentration between pregnant and non-pregnant cows. The study showed that the cholesterol concentration was affected by the season of the year, whereas the serum triglyceride concentration was not affected. The synchronization protocols used to affect the serum cholesterol concentration rather than serum triglycerides in repeat breeder cows during different seasons.

Keywords: Cholesterol, Repeat breeder cows, Season, Synchronization of ovulation, Triglycerides.

Ind J Vet Sci and Biotech (2021): 10.21887/ijvsbt.17.2.5

Introduction

Repeat breeding is one of the major reproductive problems causing great economic loss to the farmers by affecting the fertility in dairy cattle (Das *et al*., 2009). The reasons for repeat breeding are multifactorial, involving many extrinsic and intrinsic factors coupled to the individual animal (Abhijit *et al*., 2015). The deficiency of biochemical constituents can impair reproductive efficiency, which may lead to reproductive failures. Among the various components the glucose, protein, cholesterol, and triglycerides appear to be critical nutrients affecting fertility and cyclicity in farm animals (Park *et al.,* 2010). Cholesterol, a constituent of plasma lipoproteins, is involved in the lipid transport system of the body and is an essential precursor for steroidogenesis in gonads (Rowlands *et al.,* 1980). Triglycerides concentration in the maternal circulation was positively correlated with the physiology of fertilization and implantation (Patel, 1988).

The synchronization of ovulation is a recent biotechnological tool used to augment fertility in repeat breeder cows (Manokaran *et al.,* 2016). To synchronize the ovulation in a short period and enable the time bound insemination in the GnRH-prostaglandin regimen, an additional GnRH dose was included at 48 hr after $PGF_2\alpha$ treatment (Pursley *et al.,* 1995), which improved the precision of ovulation over

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How to cite this article: Manokaran, S., Napolean, R.E., Selvaraju, M., Balasubramaniam, G.A., Palanisamy, M., & Geetha, T. (2021). Impact of Various Ovulation Synchronization Protocols on Serum Triglycerides and Cholesterol Profile in Repeat Breeder Cows during Different Breeding Seasons. Ind J Vet Sci and Biotech, 17(2): 25-29.

Source of support: Nil

Conflict of interest: None.

Submitted: 20/03/2021 **Accepted:** 26/04/2021 **Published:** 25/05/2021

an 8 hrs period from 24 to 32 hrs. This standard Ovsynch or timed artificial insemination (TAI) protocol allowed successful fixed-time AI without the need for estrus detection (Pursley *et al.,* 1995). The studies on the effect of various ovulation synchronization protocols on the alteration of cholesterol and triglycerides concentration during different breeding seasons are limited. Hence, the present study was aimed to evaluate whether different ovulation synchronization protocols modulate cholesterol and triglycerides profile and enhance fertility in repeat breeding crossbred cows during high (October-March) and low (April-September) breeding seasons under field conditions.

Materials and Methods

The research work was carried out in the Department of Veterinary Gynaecology and Obstetrics, Veterinary College and Research Institute, Namakkal, Tamil Nadu. It included 100 healthy, pluriparous Jersey crossbred repeat breeder cows, which failed to conceive even after three or more consecutive artificial inseminations (AI). The selected cows were between 2^{nd} and 5^{th} parity. All the selected cows at random were equally divided into five experimental groups, *viz.,* Group I, II, III, IV (Treatment groups) and Group V (Control group) during the high breeding season (HBS, October to March) and low breeding season (LBS, April to September). The experiment was designed with 50 cows in each season, consisting of 10 cows in each group. All the cows were kept outdoors, fed with hay, and concentrate twice daily and provided *ad libitum* water. The animals of different groups were treated after one week of selection.

The schedule of synchronization protocols used in different groups was: Gr-I administered ovsynch protocol only, Gr-II was administered 25 mg of $PGF_2\alpha$ and 10 µg of GnRH 8 days and 6 days before ovsynch protocol, respectively. Gr-III cows were given ovsynch protocol and 10 µg of GnRH i/m 7 days after TAI. Gr-IV was administered 12 lakhs IU of Vitamin A on day zero of ovsynch protocol. Cows of treatment Group I to IV were bred by timed artificial insemination (TAI) at 16-18 hours after the last GnRH injection. In group V (control) AI was done during the observed estrus without any treatment. The animals which returned to estrus following TAI or observed estrus and AI were subjected to repeat AIs in the subsequent estrus. The cows that did not express heat signs after TAI were confirmed for pregnancy by rectal palpation and ultrasound scanning 45 days post-insemination. The conception rate was expressed in percentage.

Table 1: Serum triglycerides levels (mean ± SE, mg/dl) before, during and after synchronization of ovulation in repeat breeder cows during high (October to March) and low (April to September) breeding seasons

				Ovsynch and related protocols			
Season	*Treatment groups*	*Pregnancy Status*	*At the time of selection*	*Day 0 (GnRH inj.)*	*Day 7 ($PGF_2\alpha$ inj.)*	*Day 10 (Timed AI)*	*7 days Post-AI*
High breeding seasons (October to March)	Group I (Ovsynch)	P = 5	$30.19^{pq} \pm 0.82$	$30.49^{pq} \pm 0.69$	$30.38^{p} \pm 0.24$	$30.66^{pq} \pm 0.26$	$31.29^{qa} \pm 0.07$
		NP = 5	29.86 ± 0.40	29.97 ± 0.37	30.19 ± 0.44	29.78 ± 0.46	$29.51^{b} \pm 0.14$
	Group II (Presynch + Ovsynch)	P = 7	$29.91^{p} \pm 0.28$	$30.51^{pq} \pm 0.23$	$30.21^{p} \pm 0.07$	$30.57^{pq} \pm 0.42$	$30.57^{q} \pm 0.42$
		NP = 3	29.13 ± 0.68	29.97 ± 0.25	29.74 ± 0.36	28.70 ± 0.86	30.70 ± 0.86
	Group III (Ovsynch + post-AI GnRH)	P = 8	$30.32^{p} \pm 0.25$	$30.21^{pa} \pm 0.10$	$30.36^{pa} \pm 0.07$	$30.31^{p} \pm 0.20$	$31.75^{qa} \pm 0.28$
		NP = 2	29.24 ± 1.32	$28.90^{b} \pm 0.61$	$29.47^{b} \pm 0.88$	29.44 ± 0.99	$29.82^{b} \pm 0.83$
	Group IV (Vitamin A + Ovsynch)	P = 8	$30.18^{pa} \pm 0.08$	$30.46^{p} \pm 0.21$	$30.25^{p} \pm 0.17$	$30.60^{p} \pm 0.25$	$31.42^{qa} \pm 0.36$
		NP = 2	$29.51^{b} \pm 0.14$	29.63 ± 0.21	29.72 ± 0.31	29.86 ± 0.10	$30.60^{b} \pm 0.13$
	Group V (Control)	P = 3	$30.33^{p} \pm 0.11$			$30.39^{pa} \pm 0.05$	$31.42^{qa} \pm 0.36$
		NP = 7	29.62 ± 0.24			$29.59^{b} \pm 0.18$	$29.62^{b} \pm 0.13$
Low breeding seasons (April to Septe-mber)	Group I (Ovsynch)	P = 4	$29.92^{p} \pm 0.19$	$30.01^{p} \pm 0.16$	$29.93^{p} \pm 0.22$	$29.99^{p} \pm 0.20$	$31.83^{qa} \pm 0.51$
		NP = 6	29.27 ± 0.22	29.47 ± 0.18	29.54 ± 0.19	29.67 ± 0.20	$29.44^{b} \pm 0.31$
	Group II (Presynch + Ovsynch)	P = 5	$29.92^{p} \pm 0.22$	$30.05^{p} \pm 0.20$	$30.18^{p} \pm 0.19$	$30.31^{p} \pm 0.16$	$31.77^{q} \pm 0.89$
		NP = 5	29.05 ± 0.43	29.51 ± 0.26	29.64 ± 0.32	29.74 ± 0.26	30.08 ± 0.48
	Group III (Ovsynch + post-AI GnRH)	P = 7	$29.53^{p} \pm 0.43$	$29.78^{pq} \pm 0.28$	$30.16^{pqa} \pm 0.17$	$30.34^{qr} \pm 0.19$	$31.97^{ra} \pm 0.25$
		NP = 3	$28.84^{p} \pm 0.20$	$29.04^{p} \pm 0.27$	$29.13^{pqb} \pm 0.30$	$29.55^{pq} \pm 0.33$	$29.93^{qb} \pm 0.37$
	Group IV (Vitamin A + Ovsynch)	P = 6	$29.67^{p} \pm 0.22$	$29.91^{p} \pm 0.20$	$29.86^{p} \pm 0.19$	$29.98^{p} \pm 0.23$	$31.08^{qa} \pm 0.20$
		NP = 4	28.63 ± 0.28	29.20 ± 0.20	29.35 ± 0.37	29.46 ± 0.34	$29.66^{b} \pm 0.27$
	Group V (Control)	P = 2	$30.19^{p} \pm 0.04$			$30.85^{pa} \pm 0.41$	$31.89^{qa} \pm 0.56$
		NP = 8	29.15 ± 0.24			$29.32^{b} \pm 0.22$	$29.66^{b} \pm 0.20$

Means bearing different superscripts (p,q,r) among different days of blood collection within same row differ significantly ($p \leq 0.05$).
Means bearing different superscripts (a,b) between rows within a column for pregnancy status differ significantly ($p \leq 0.05$).

The blood samples were collected from animals of all groups at the time of selection and then on days 0, 7, 10 (TAI) of ovysnch and day 7 post-AI. The serum was separated and stored at -20°C until analyzed for triglycerides and cholesterol by using commercial kits (Span Diagnostic Ltd., Surat, Gujarat, India). The data were analyzed using SPSS® 20.0. software package. Tukey's Honestly Significance Difference did post hoc analysis.

Results and Discussion

The percentage of conception rate obtained in Group I, II, III, IV, and V was 50, 70, 80, 80, and 30 % during HBS, and 40, 50, 70, 60, and 20 % during LBS, respectively. The results indicated that synchronization of ovulation protocols had increased the conception rates in repeat breeder cows. The results were in concurrence with the observations of Selvaraju *et al.* (2008 and 2009) in repeat breeder cows treated with $PGF_2\alpha$ and synchromate-B, respectively.

The mean (±SE) serum triglycerides and cholesterol levels (mg/dl) before, during, and after synchronization of ovulation protocols in repeat breeder cows during high and low breeding seasons are presented in Tables 1 and 2.

In this study, the mean serum triglycerides were found to be a little higher in pregnant/ conceived cows than non-pregnant cows in all the groups in both HBS and LBS on all the sampling days, except on day 7 post-AI in all groups, on day 7 of treatment in Gr-III and on day of TAI in Gr-V., which differed significantly ($p<0.05$) (Table 1). Similar to this finding, increased serum triglycerides in pregnant cows were observed by Patel *et al.* (2014) in repeat breeder cows. Triglycerides concentration in the maternal circulation positively correlated with the physiology of fertilization and implantation (Patel, 1988). In the present investigation, the mean serum triglycerides from the day of selection to 7 days post-AI showed an increasing trend, particularly in the conceived group in both HBS and LBS, which might be due to the altered lipid metabolism towards the conception, as stated by Ravikumar (2014) in buffaloes and Velladurai *et al.* (2018) in cows.

In all the experimental and control groups, the mean serum cholesterol levels were significantly lower in LBS than the HBS. Hence, it indicated that season influenced the mean serum cholesterol concentrations in repeat breeder cows. In both HBS and LBS, the pregnant or conceived cows on all days of sampling in all groups had significantly ($p<0.05$) higher mean serum cholesterol levels than non-pregnant cows. Further, there was an increasing trend from the time of selection to 7 days post-AI in all the groups of HBS and LBS.

Table 2: Serum cholesterol levels (mean ± SE, mg/dl) before, during and after synchronization of ovulation in repeat breeder cows during high (October to March) and low (April to September) breeding seasons

				Ovsynch and related protocols			
Season	*Treatment groups*	*Pregnancy Status*	*At the time of selection*	*Day 0 (GnRH inj.)*	*Day 7 ($PGF_2\alpha$ inj.)*	*Day 10 (Timed AI)*	*7 days Post-AI*
High breeding seasons (October to March)	Group I (Ovsynch)	P = 5	$155.32^{pa} \pm 2.16$	$162.98^{qa} \pm 1.65$	$166.60^{qra} \pm 0.88$	$166.88^{qra} \pm 0.69$	$170.30^{ra} \pm 0.58$
		NP = 5	$152.82^{pa} \pm 1.82$	$156.20^{pb} \pm 1.51$	$162.23^{qa} \pm 2.22$	$163.83^{qa} \pm 1.55$	$166.18^{qb} \pm 1.17$
	Group II (Presynch + Ovsynch)	P = 7	$158.97^{pa} \pm 2.31$	$163.23^{pqra} \pm 2.28$	$165.15^{pqra} \pm 1.79$	$167.43^{qra} \pm 1.43$	$168.94^{ra} \pm 1.08$
		NP = 3	$151.16^{pb} \pm 0.89$	$157.21^{qra} \pm 1.42$	$159.36^{rsb} \pm 1.52$	$162.04^{stb} \pm 1.75$	$164.12^{tb} \pm 2.10$
	Group III (Ovsynch + post-AI GnRH)	P = 8	$161.06^{pa} \pm 1.63$	$165.27^{qa} \pm 1.04$	$166.31^{qra} \pm 1.37$	$168.65^{rsa} \pm 0.84$	$170.86^{sa} \pm 0.65$
		NP = 2	$153.26^{pb} \pm 4.87$	$156.51^{pqb} \pm 4.04$	$161.58^{pqa} \pm 2.26$	$162.44^{pqb} \pm 1.88$	$163.77^{qb} \pm 0.05$
	Group IV (Vitamin A + Ovsynch)	P = 8	$161.15^{pa} \pm 0.87$	$164.34^{qa} \pm 1.19$	$166.33^{qra} \pm 0.78$	$168.24^{rsa} \pm 0.70$	$170.35^{sa} \pm 0.50$
		NP = 2	$155.10^{pb} \pm 3.27$	$157.08^{pb} \pm 3.36$	$159.07^{pb} \pm 4.39$	$159.45^{pb} \pm 5.10$	$163.53^{pb} \pm 4.19$
	Group V (Control)	P = 3	$161.00^{pa} \pm 3.34$			$165.57^{qa} \pm 2.25$	$165.70^{qa} \pm 1.53$
		NP = 7	$152.01^{pa} \pm 1.85$			$155.78^{qb} \pm 1.74$	$158.22^{qb} \pm 1.34$
Low breeding seasons (April to Septe-mber)	Group I (Ovsynch)	P = 4	$150.71^{pa} \pm 2.09$	$151.70^{pa} \pm 2.16$	$154.39^{pqa} \pm 1.35$	$155.35^{pqa} \pm 1.25$	$157.45^{qa} \pm 1.94$
		NP = 6	$146.12^{pa} \pm 2.01$	$149.00^{pqa} \pm 1.90$	$150.57^{pqa} \pm 2.09$	$151.23^{qa} \pm 1.30$	$152.75^{qa} \pm 1.60$
	Group II (Presynch + Ovsynch)	P = 5	$148.47^{pa} \pm 1.33$	$154.62^{ra} \pm 0.50$	$156.90^{rsa} \pm 0.41$	$158.09^{sta} \pm 0.51$	$159.78^{ta} \pm 0.61$
		NP = 5	$145.70^{pa} \pm 1.44$	$149.76^{pqrb} \pm 1.91$	$151.06^{pqrb} \pm 2.25$	$152.88^{qrb} \pm 1.93$	$153.83^{rb} \pm 1.90$
	Group III (Ovsynch + post-AI GnRH)	P = 7	$151.86^{pa} \pm 0.80$	$154.02^{qa} \pm 0.91$	$155.60^{qra} \pm 0.82$	$157.34^{rsa} \pm 0.78$	$160.82^{sa} \pm 0.76$
		NP = 3	$146.63^{pb} \pm 1.49$	$148.04^{pqb} \pm 1.68$	$149.70^{pqb} \pm 2.08$	$151.39^{pqb} \pm 1.91$	$153.07^{qb} \pm 2.18$
	Group IV (Vitamin A + Ovsynch)	P = 6	$153.81^{pa} \pm 1.21$	$156.13^{qa} \pm 1.02$	$157.71^{qra} \pm 0.80$	$158.31^{rsa} \pm 0.37$	$159.30^{sa} \pm 0.36$
		NP = 4	$146.97^{pb} \pm 1.90$	$149.39^{pqb} \pm 1.53$	$150.87^{qrb} \pm 1.29$	$153.33^{rsb} \pm 1.03$	$154.94^{sb} \pm 0.32$
	Group V (Control)	P = 2	$152.40^{pa} \pm 1.06$			$155.83^{qa} \pm 0.39$	$157.29^{ra} \pm 0.43$
		NP = 8	$149.81^{pa} \pm 0.95$			$152.79^{qa} \pm 0.84$	$153.99^{qb} \pm 0.74$

Means bearing different superscripts (p,q,r) among different days of blood collection within same row differ significantly ($p \leq 0.05$).
Means bearing different superscripts (a,b) between rows within a column for pregnancy status differ significantly ($p \leq 0.05$).

However, on day 7 post-AI, there was a significant ($p \leq 0.05$) elevation of mean serum cholesterol levels in pregnant cows than non-pregnant cows in all the groups of HBS and LBS, except group I of LBS (Table 2). These results were in concurrence with the studies made by Viramani *et al.* (2011) in cows and Ravikumar (2014) in buffaloes.

The increased mean serum cholesterol levels during HBS could be one of the reasons for enhanced fertility during HBS than LBS. In this present study, on day 10 (first AI) and 7 days post-AI, high serum cholesterol levels were noticed compared to other days of estimation. A similar finding during estrus was reported in buffaloes (Sarvaiya and Pathak, 1992). Blood cholesterol was found to be lower during summer than winter in cows, probably due to increased environmental temperature (Marai and Habeeb, 2010). The marked increase in glucocorticoid hormone levels in heat-stressed animals might be another factor causing the decline in blood cholesterol (Marai and Haeeb, 2010). The lower plasma concentration of cholesterol in repeat breeder cows when compared to fertile cows is indicative of subnormal energy status that affects the function of the pituitary gland, thereby reducing the secretion of gonadotropins which might lead to the failure of follicular development, increased follicular atresia, and reduced conception rate (Pandey *et al.,* 2009). Savalia *et al.* (2014) and Prajapati *et al.* (2018) reported a significantly higher plasma cholesterol concentration in non-conceived than in conceived animals following different synchronization protocols.

Cholesterol, a constituent of plasma lipoprotein, is involved in the lipid transport system of the body and is an essential precursor for steroidogenesis in gonads. The increased levels of serum cholesterol in pregnant cows might have caused the secretion of steroid hormones and resulted in conception, as stated by Rowlands *et al.* (1980). Rajagopal *et al.* (2011) reported a significant increase in serum cholesterol concentration in repeat breeder crossbred cows treated with Ovsynch. The elevated cholesterol after treatment might be due to GnRH administration which could have influenced lipoprotein metabolism in a positive manner (Grummer and Carroll, 1998).

Conclusion

The study concluded that the summer season has an adverse influence on the serum cholesterol concentration and that the cholesterol concentration increases during ovulation synchronization protocol till 7 days post-AI in both the seasons, particularly in pregnant/conceived cows. Serum triglycerides levels did not show such a trend but had elevated levels on day 7 post-AI in all the groups in both the seasons. The hormonal protocols of synchronization of ovulation affect serum cholesterol concentration coupled with enhanced conception rates in repeat breeding crossbred cows.

Acknowledgement

The authors are thankful to Tamil Nadu Veterinary and Animal Sciences University, Chennai, for the facilities provided to conduct the research work.

References

Abhijit, B., Batabyal, S., Ghosh, S., Saha, D., & Chattopadhyay, S. (2015). Plasma mineral profiles and hormonal activities of normal cycling and repeat breeding crossbred cows: A comparative study. *Veterinary Word, 8,*42-45.

Das, J.M., Dutta, P., Deka, K.C., Biswas, R.K., Sarmah, B.C., & Dhali, A. (2009). Comparative study on serum macro and micro mineral profiles during oestrus in repeat breeding crossbred cattle with impaired and normal ovulation. *Livestock Research and Rural Development*, 21. (http://www.lrrd.org/lrrd21/5/das21072.htm).

Grummer, R.R., & Carroll, D.J. (1998). A review of lipoprotein cholesterol metabolism: importance to ovarian function. *Journal of Animal Science*, *66,* 3160-3173.

Manokaran, S., Napolean, R.E., Selvaraju, M., Doraisamy, K.A., & Balasubramaniam, G.A. (2016). Effect of Ovsynch protocol on conception rate in repeat breeder cows during high and low breeding seasons. *Indian Veterinary Journal, 93*, 22-24.

Marai, I.F.M., & Habeeb, A.A.M. (2010). Buffalo's biological functions as affected by heat stress: A review. *Livestock Science, 127*, 89-109.

Pandey, V., Singh, A.K., & Sharma, N. (2009). Blood biochemical profile in fertile and repeat breeding crossbred cows under field conditions. *Veterinary Practitioner, 10*, 44-46.

Park, M.S., Yang, Y.X., Shinde, P.L., Choi, J.Y., Jo, J.K., Kim, J.S., Lohakare, J.D., Yang, B.K., Lee, J.K., Kwon, I.K., & Chae, B.J. (2010). Effects of dietary glucose inclusion on reproductive performance, milk compositions and blood profiles in lactating sows. *Journal of Animal Physiology and Animal Nutrition, 94*, 677-684.

Patel, A.V. (1988). Lipid composition in reproductive tract tissues and blood serum in Surti buffalo heifers during different phases of reproduction. Ph.D., Thesis submitted to Gujarat Agricultural University, Anand, India.

Patel, J.A., Dhami, A.J., Kavani, F.S., & Rani, R.G. (2014). Modulation of conception rate and plasma biochemical profile in repeat breeding Holstein Friesian cows by hormonal therapy. *International Journal of Cow Science, 1,* 47-52.

Prajapati, A.R., Dhami, A.J., Hadiya, K.K., & Patel, J.A. (2018). Influence of estrus synchronization protocols on plasma profile of progesterone, protein and cholesterol in acyclic Holstein Friesian crossbred cows. *Indian Journal of Veterinary Science and Biotechnology, 13*, 5-11.

Pursley, J.R., Mee, M.O., & Wiltbank, M.C. (1995). Synchronization of ovulation in dairy cows using $PGF_2\alpha$ and GnRH. *Theriogenology, 44*, 915-923.

Rajagopal, K., Veerabramhaiah, K., Naidu, S.,Rao, V.V., & Kumar, R.V.S. (2011). Effect of synchronization protocols on serum cholesterol concentration and pregnancy rate in repeat breeder crossbred cows. *Theriogenology Insight, 1*, 79-82.

Ravikumar, K. (2014). Synchronization of ovulation in postpartum buffaloes during peak and low breeding seasons using Ovsynch with CIDR protocols. *Ph.D. Thesis*. Tamil Nadu Veterinary and Animal Sciences University, Chennai.

Rowlands, G.J., Manstona, R., Starka, A.J., Russella, A.M., Collisa, K.A., & Collisa, S.C. (1980). Changes in albumin, globulin, glucose and cholesterol concentrations in the blood of dairy cows in late pregnancy and early lactation and relationships with subsequent fertility. *Journal of Agricultural Science, 94*, 517-527.

Savalia, K.K., Dhami, A.J., Hadiya, K.K., Patel, K.R., & Sarvaiya, N.P. (2014). Influence of controlled breeding techniques on fertility and plasma progesterone, protein and cholesterol profile in true anestrus and repeat breeding buffaloes. *Veterinary World, 7*, 727-732.

Sarvaiya, N.P., & Pathak, M.M. (1992). Profile of progesterone, estradiol-17β, triiodothyronine and blood biochemical parameters in Surti buffalo heifers. *Buffalo Journal, 8*, 23-30.

Selvaraju, M., Veerapandian, C., Katiresan, D, Kulasekar, K., & Chandrahasan, C. (2008). Pattern of induced oestrus and fertility rate following hCG injection at early luteal phase in $PGF_2α$ treated repeat breeder cows. *Journal of Veterinary and Animal Sciences, 39*, 1-4.

Selvaraju, M., Veerapandian, C., Katiresan, D, Kulasekar, K., & Chandrahasan, C. (2009). Pattern of oestrus, oestrous cycle length and fertility rate following Synchromate-B treatment in repeat breeder cows. *Indian Journal of Animal Reproduction, 30*, 22-25.

Velladurai, C., Napolean, R.E., & Selvaraju, M. (2018). Ovsynch treatment protocol improved the conception rate in retained fetal membranes treated cows. *Indian Veterinary Journal, 95*, 24-26.

Viramani, M., Malik, R.K., Singh, P., & Dalal, S.S. (2011). Studies on blood biochemical and mineral profiles with the treatment of acyclicity in postpartum anoestrus Sahiwal cows. *Haryana Veterinary Journal, 50,* 77-79.