INFLUENCE OF TRIU-B, OVSYNCH AND HEATSYNCH PROTOCOLS ON ESTRUS INDUCTION RESPONSE, CONCEPTION AND BIOCHEMICAL AND MINERALS PROFILE IN ANOESTRUS CROSSBRED COWS

K.V. Buhecha, A.J. Dhami, K.K. Hadiya, C.P. Parmar, S.C. Parmar and J.A. Patel

Department of Animal Reproduction, Gynaecology and Obstetrics
College of Veterinary Science and Animal Husbandry
Anand Agricultural University, Anand, India

Received 25-7-2015 ***Accepted 29-8-2015***

Corresponding Author : ajdhami@aau.in

ABSTRACT

This study was planned to evaluate the fertility response and plasma profile of biochemical and mineral constituents in 46 postpartum anoestrus crossbred cows (>90 days) treated with different standard hormonal protocols (TriU-B/PRID, Ovsynch and Heatsynch, n=12 each), keeping untreated anoestrus control (n=10) and normal cyclic control (n=10) groups. All the cows (100 %) under TriU-B, Ovsynch and Heatsynch protocols exhibited induced estrus within mean intervals of 67.00±1.74, 69.83±0.87 and 68.17±1.24 h, respectively, from $PGF_2\alpha$ injection, with prominent to moderate estrus signs. The conception rates obtained at induced estrus in cows under TriU-B, Ovsynch and Heatsynch protocols were 33.33, 41.66 and 33.33 per cent, respectively. The corresponding overall conception rates of three cycles were 50.00, 58.33 and 41.66 per cent, respectively. In anoestrus animals under TriU-B, Ovsynch and Heatsynch protocols, and in normal cyclic control group, the overall pooled mean plasma total cholesterol concentrations found were 218.32±5.81, 218.65±6.04, 202.44 ±5.73 and 225.87±9.09 mg/dl (P<0.05), respectively. The corresponding values for total protein were 7.95±0.06, 7.57 ± 0.05, 7.94 ± 0.14 and 8.69 ± 0.16 g/dl (P<0.05), plasma calcium 9.15 ± 0.06, 9.18 ± 0.09, 10.01 ± 0.08 and 10.15 ± 0.09 mg/dl (P<0.05) and inorganic phosphorus 4.33±0.10, 4.02 ± 016, 5.00 ± 0.18 and 4.90 ± 0.10 mg/dl (P<0.05). The influence of treatment days (0, 7, 9/AI or day 21 post-AI) was not significant for any of the traits in any of the groups, except for protein in Heatsynch protocol. The levels of all four constituents were significantly higher in normal cyclic control group than the values found in most treatment groups, which were at par. The conceived cows had insignificantly higher values of total cholesterol and protein as compared to non-conceived ones in most treatment protocols and even in cyclic control groups. In general, Ovsynch protocol followed by TriU-B appeared the best for induction of estrus and improvement of fertility without influencing the blood biochemical and mineral profiles in anoestrus crossbred cows.

KEY WORDS : Anoestrus, Blood profile, Crossbred cows, Estrus synchronization, Ovsynch, Heatsynch, PRID

INTRODUCTION

Various hormonal preparations play key role in augmenting fertility in dairy animals. The variable results obtained following hormonal treatments by different workers may be largely due to varying nutritional and ovarian status at the start of treatment, endocrine events, faulty management and uterine infection apart from product potency, closeness to its deposition in vascular structures, and the quality of breeding services and its follow up provided in treated animals. Use of hormonal protocol like Ovsynch, Heatsynch, CIDR, TriU-B, Crester etc induces and synchronizes the estrus/ovulation and thus improves the conception rates and establishes cyclicity in acyclic dairy animals, thereby achieving ideal calving interval (Bhoraniya *et al.,* 2012; Nakrani *et al.,* 2014). Cholesterol is the most important sterol and is an essential component of the cell. It is a constituent of plasma

lipoproteins, which are involved in the lipid transport system of the body (Tayler *et al.,* 1966). Minerals play an important role in the regulation of reproduction and production in animals. Lower concentration of circulatory minerals results in impaired reproductive function leading to cessation of cyclic activity (Martson *et al.,* 1972). Calcium deficiency can upset the normal reproduction possibly due to lack of tone of uterine muscle. Infertility due to nutritional deficiency is usually characterized by a failure of estrus or a cessation of estrous cycle, where mineral deficiency mainly includes phosphorus than any other trace minerals. Hence, this study was planned to evaluate the comparative efficacy of TriU-B, Ovsynch and Heatsynch protocols for fertility enhancement and to see their influence on plasma biochemical and mineral profile in anoestrus rural crossbred cows.

MATERIALS AND METHODS

The present investigation was carried out under field conditions of middle Gujarat during the period of November 2014 to April 2015. In all 46 true anoestrus crossbred cows and 10 normal cyclic cows were selected through organizing special sexual health camps and thorough gynaeco-clinical examinations. All infertile animals identified were dewormed using Inj. Ivermectin. They were also treated once initially with i/m injection of inorganic phosphorus and multivitamins AD_3E, and oral supplementation of multi-minerals bolus (Minotas, Intas Pharma) @ 1 bolus daily for 7 days. The anoestrus cows were then randomly subjected to different standard estrus induction/ synchronization protocols (TriU-B/PRID, Ovsynch and Heatsynch, n=12 each, Bhoraniya *et al.,* 2012). Ten anoestrus animals were kept as anoestrus control and 10 normal cyclic cows evincing spontaneous estrus within 90 days postpartum served as normal cyclic control group. Cows in spontaneous or induced estrus were inseminated using good quality frozen-thawed semen. Cows detected in estrus subsequent to FTAI were re-inseminated up to 2 cycles and in non-return cases pregnancy was confirmed per rectum 60 days of last AI.

All the hormonally treated/untreated true anoestrus and normal cyclic cows were studied for their reproductive status and plasma profile total protein, cholesterol, calcium and inorganic phosphorus on different days of treatment. For this, jugular blood samples were collected four times in heparinized vaccutainers from treated true anoestrus animals, i.e. on day 0 - just before treatment (on diagnosis), on day 7 - at the time of $PGF_2\alpha$ administration, on day 9/10 - induced estrus/FTAI (FTAI done twice 24 hrs apart, i.e. on day 9 and 10 after initiation of treatment) and on day 21 post-AI. Blood sampling for two control groups of animals was done on the day of spontaneous estrus, and on day 21 post-AI. The samples were centrifuged at 3000 rpm for 15 min. and plasma separated out was stored at -20°C with a drop of merthiolate (0.1%) until analyzed.

Plasma profiles of total protein, cholesterol, calcium and inorganic phosphorus were estimated by using standard procedures and assay kits procured from Analytical Technologies Pvt. Limited, Baroda, on chemistry analyzer. The data on conception rate (by Chi square test) and plasma profile of biochemical and mineral constituents (ANOVA) were analyzed statistically (Snedecor and Cochran, 1994) using online SAS software.

RESULTS AND DISCUSSION

Estrus and Fertility Response to Synchronization Protocols

The estrus induction response was 100 per cent in each group of anoestrus cows (n=12) subjected to TriU-B, Ovsynch and Heatsynch treatment protocols with prominent to moderate estrus signs within mean intervals of 67.00±1.74, 69.83±0.87 and 68.17±1.24 h from $PGF_2\alpha$ injection, respectively. The conception rates obtained at induced estrus in crossbred cows under TriU-B, Ovsynch and Heatsynch protocols were 33.33, 41.66 and 33.33 per cent, respectively. The corresponding overall pregnancy rates of all three cycles post-treatment were observed to be 50.00, 58.33 and 41.66 per cent. The results of Ovsynch protocol were at par with normal cyclic control

group (n=10) having conception rates at first cycle and overall of 3 cycles as 40.00 and 60.00 per cent, In untreated anoestrus control group (n=10), only 2 cows exhibited spontaneous estrus and conceived within 90 days of follow up period giving overall pregnancy rate of only 20.00 (2/10) per cent. Moreover, by 60 days of post-induction/AI in TriU-B, Ovsynch and Heatsynch protocols 33.33, 16.66 and 33.33 per cent cows again turned out to be anoestrus.

The comparable estrus induction response and induction interval in anoestrus crossbred cows under CIDR treatment protocol (1.38 g hydroxy-progesterone caproate) has been documented by Patel *et al*. (2013) and Dhami *et al.* (2014). The mean estrus induction interval found with Ovsynch protocol in the present study was far lower than earlier reports by Patel *et al*. (2013) and Dhami *et al.* (2014) as 86.67±3.33 h and 87.23±3.53 h. There is no published report available on the use and efficacy of TriU-B, an intravaginal device simulating CIDR, recently launched by Virbac Animal Health containing 0.96 g of hydroxy-progesterone caproate. Ammu *et al*. (2012[a]), however obtained higher conception rates of 50.00, 50.00 and 33.33 per cent at induced estrus and overall of 2 cycles as 66.66, 83.33 and 83.33 with Ovsynch, CIDR and Cosynch protocol, respectively, in Gir cows and Bhoraniya *et al*. (2012) obtained 33.33, 66.66 and 16.67 per cent conception at induced estrus and overall of 3 cycles as 50.00, 83.33, 50.00 per cent with Ovsynch, CIDR and Heatsynch protocol, respectively, in anoestrus Kankrej cows. Lee *et al*. (2013) also obtained comparable overall conception rates of 60.00, 50.00, 60.00 and 40.00 per cent with CIDR, PRID, Ovsynch and $PGF_2\alpha$ protocols. However, NarenjiSani *et al*. (2011) reported lower conception rates of 32.4 and 16.6 per cent at induced estrus with Ovsynch and Heatsynch protocols.

Plasma Total Cholesterol and Total Protein

The mean levels of plasma total cholesterol and total protein recorded on day 0, 7, 9 (AI) of treatment and on day 21 post-AI in crossbred cows under TriU-B, Ovsynch and Heatsynch protocols, and on day of AI and day 21 post-AI in normal cyclic crossbred cows are presented in Table 1 and 2.

Table 1: Plasma total cholesterol concentrations (mg/dl) in anoestrus crossbred cows on different days of various estrus synchronization treatments

Estrus induction protocol	Status	No.	Days from treatment/AI				Overall
			D-0	D-7	D-9/10 (AI)	D-21 post-AI	
Triu-B	Conceived	4	212.50±20.08	230.29±10.63	237.10±10.45	210.04±20.86	223.73±7.75
	Non-conc	4	235.49±19.11	201.17±21.67	204.30±19.30	215.71±7.61	212.92±8.68
	Overall	**8**	**223.99±13.55**	**215.73±12.45**	**220.70±11.90**	**212.87±10.31**	**218.32±5.81^{xy}**
Ovsynch	Conceived	5	185.55±22.03	203.91±10.93	211.05±7.06	185.99±6.36^{p}	196.63±6.55^{p}
	Non-conc	3	219.33±8.13	250.45±19.41	225.19±20.64	232.50±7.22^{q}	231.87±7.46^{q}
	Overall	**8**	**206.66±10.67**	**233.00±14.83**	**219.89±12.81**	**215.06±9.77**	**218.65±6.04^{xy}**
Heatsynch	Conceived	4	218.93±1.51	213.13±18.39	231.78±11.75	193.56±11.90	214.35±6.74
	Non-conc	4	194.18±21.76	192.02±16.17	190.81±18.04	204.21±10.33	195.30±7.93
	Overall	**8**	**203.46±13.78**	**199.93±12.02**	**206.17±13.68**	**200.21±7.56**	**202.44±5.73^{x}**
Normal Cyclic Control	Conceived	4	-	-	242.61±14.88	210.41±14.05	226.51±9.91
	Non-conc	4	-	-	223.28±22.47	227.27±23.49	225.28±15.24
	Overall	**8**	-	-	**232.94±13**	**218.84±13.06**	**225.89±9.09^{y}**

Means bearing uncommon superscripts within column (x, y) and subgroup (p, q) differ significantly (P < 0.05). D-0 = Day of starting the treatment, D-7 = Administration of PG, D-9/10 = Fixed time AI, D-21= Day 21 post-AI.

Table 2: Plasma total protein concentrations (g/dl) in anoestrus crossbred cows on different days of various estrus synchronization treatments

Estrus induction protocol	Status	No.	Days from treatment/AI				Overall
			D-0	D-7	D-9/10 (AI)	D-21 post-AI	
TriU-B	Conceived	4	7.65±0.13	7.90±0.29	7.80±0.21	7.76±0.19	7.78±0.10
	Non-conc	4	7.99±0.07	8.19±0.08	8.20±0.08	8.09±0.09	8.12±0.04
	Overall	**8**	**7.82±0.09**	**8.04±0.15**	**8.00±0.13**	**7.92±0.12**	**7.95±0.06^{y}**
Ovsynch	Conceived	5	7.67±0.16	7.26±0.13	7.83±0.13	7.59±0.25	7.59±0.10
	Non-conc	3	7.47±0.15	7.53±0.11	7.73±0.10	7.48±0.15	7.55±0.07
	Overall	**8**	**7.55±0.11^{ab}**	**7.43±0.09^{a}**	**7.77±0.08^{b}**	**7.53±0.13^{ab}**	**7.57±0.05^{x}**
Heatsynch	Conceived	4	7.95±0.46	8.22±0.59	8.30±0.80	8.18±0.68	8.16±0.28
	Non-conc	4	7.65±0.26	7.66±0.36	7.77±0.30	8.14±0.32	7.81±0.15
	Overall	**8**	**7.76±0.22**	**7.87±0.31**	**7.97±0.33**	**8.17±0.30**	**7.94±0.14^{y}**
Normal Cyclic Control	Conceived	4	-	-	8.60±0.47	9.03±0.39	8.81±0.29
	Non-conc	4	-	-	8.61±0.17	8.54±0.24	8.57±0.14
	Overall	**8**	-	-	**8.60±0.23**	**8.78±0.23**	**8.69±0.16^{z}**

Means bearing uncommon superscripts within column (x, y, z) and subgroup (p, q) differ significantly (P < 0.05). D-0 = Day of starting the treatment, D-7 = Administration of PG, D-9/10 = Fixed time AI, D-21 = Day 21 post-AI.

The data in Table 1 do not reveal significant differences in plasma total cholesterol profile between days/periods in any of the treatment protocols or even in normal cyclic group, but did vary significantly between the groups. The level was significantly higher in normal cyclic group as compared to anoestrus treated groups, particularly Heatsynch protocol. The study of Bhoraniya *et al.* (2012) and Patel *et al.* (2013) also revealed non-significant variations in the cholesterol levels between day 0, 7, 9(AI) and on day 20/21 post-AI in Ovsynch and CIDR treated anoestrus cows. In the present study, at day 21 post-AI, there was a non-significantly lower mean plasma total cholesterol concentration (mg/dl) in conceived cows as compared to non-conceived cows in TriU-B, Heatsynch and normal cyclic control group, and significantly lower in Ovsynch group (185.99 ± 6.36 vs. 232.50 ± 7.22) of crossbred cows. The plasma total cholesterol profile in normal cyclic cows was significantly higher as compared to anoestrus treated animals. Similar results were reported by Kavani *et al*. (1987) and Ammu *et al.* (2012[b]) in Kankrej cattle and by Ahlawat and Derashri (2009) and Patel *et al.* (2013) in crossbred cows. The reduced levels of circulatory plasma cholesterol concentration recorded on day 21 post-AI in conceived cows suggests its utilization for progesterone synthesis as cholesterol is the immediate precursor of steroid hormones, and a continuous synthesis of progesterone is needed in maintaining pregnancy, thereby utilizing greater amount of plasma cholesterol.

The mean plasma levels of total proteins recorded on day 0, 7, 9/10 (AI) of treatment and on day 21 post-AI in crossbred cows under TriU-B, Ovsynch, Heatsynch protocol (Table 2) also revealed non-significant variation between sampling days in all the protocols/groups, except in Ovsynch group, in which on the day of estrus the mean total protein value was significantly higher than other days. More or less comparable findings were reported by Bhoraniya *et al.* (2012) in Ovsynch and CIDR treated anoestrus Kankrej cows, Ammu *et al*. (2012[b]) in Gir cows, Patel *et al.* (2013) in crossbred cows and Nakrani *et al.* (2014) in buffaloes. In the present study, the mean plasma total proteins concentration observed in normal cyclic cows was significantly higher than the values noted

Table 3: Plasma calcium concentrations (mg/dl) in anoestrus crossbred cows on different days of various estrus synchronization treatments

Estrus induction protocol	Status	No.	Days from treatment/AI				Overall
			D-0	D-7	D-9/10 (AI)	D-21 post-AI	
TriU-B	Conceived	4	9.14±0.04	9.06±0.16	9.24±0.11	8.93±0.29	9.09±0.08
	Non-conc	4	8.95±0.14	9.42±0.06	9.13±0.15	9.33±0.15	9.21±0.07
	Overall	**8**	**9.05±0.08**	**9.24±0.10**	**9.19±0.09**	**9.13±0.17**	**9.15±0.06^{x}**
Ovsynch	Conceived	5	9.16±0.34	9.49±0.42	9.50±0.56	9.46±0.59	9.40±0.21
	Non-conc	3	8.88±0.08	9.02±0.18	9.15±0.14	9.15±0.11	9.05±0.07
	Overall	**8**	**8.99±0.13**	**9.20±0.19**	**9.28±0.21**	**9.27±0.21**	**9.18±0.09^{x}**
Heatsynch	Conceived	4	9.34±0.36	9.79±0.32	9.92±0.21	9.79±0.19	9.71±0.14
	Non-conc	4	10.37±0.21	10.04±0.11	10.28±0.13	10.09±0.23	10.19±0.09
	Overall	**8**	**9.98±0.25**	**9.95±0.13**	**10.14±0.12**	**9.98±0.16**	**10.01±0.08^{y}**
Normal Cyclic Control	Conceived	4	-	-	9.81±0.21	10.16± 0.18	9.98±0.14
	Non-conc	4	-	-	10.20±0.14	10.43±0.08	10.32±0.09
	Overall	**8**	-	-	**10.01±0.14**	**10.30±0.11**	**10.15±0.09^{y}**

Means bearing uncommon superscripts within column (x, y) differ significantly (P < 0.05). D-0 = Day of starting the treatment, D-7 = Administration of PG, D-9/10 = Fixed time AI, D-21 = Day 21 post-AI.

Table 4: Plasma inorganic phosphorus concentrations (mg/dl) in anoestrus crossbred cows on different days of various estrus synchronization treatments

Estrus induction protocol	Status	No.	Days from treatment/AI				Overall
			D-0	D-7	D-9/10 (AI)	D-21 post-AI	
TriU-B	Conceived	4	4.02±0.18	4.08±0.29	4.22±0.27	4.11±0.45	4.11±0.14
	Non-conc	4	4.18±0.18	4.36±0.18	4.58±0.34	4.75±0.28	4.47±0.13
	Overall	**8**	**4.12±0.12**	**4.26±0.15**	**4.45±0.23**	**4.51±0.25**	**4.33±0.10^{x}**
Ovsynch	Conceived	5	4.23±0.38	4.53±0.84	3.96±0.64	4.35±0.82	4.27±0.30
	Non-conc	3	4.31±0.56	3.90±0.24	3.56±0.34	3.74±0.30	3.88±0.19
	Overall	**8**	**4.28±0.36**	**4.14±0.33**	**3.71±0.30**	**3.97±0.34**	**4.02±0.16^{x}**
Heatssynch	Conceived	4	5.22±0.42	5.07±0.41	4.93±0.55	4.93±0.48	5.04±0.21
	Non-conc	4	4.83±0.59	5.06±0.66	5.25±0.62	4.71±0.77	4.96±0.30
	Overall	**8**	**5.02±0.35**	**5.06±0.36**	**5.09±0.39**	**4.82±0.42**	**5.00±0.18^{y}**
Normal Cyclic Control	Conceived	4	-	-	4.96±0.21	5.19±0.22	5.08±0.15
	Non-conc	4	-	-	4.73±0.14	4.85±0.20	4.79±0.12
	Overall	**8**	-	-	**4.81±0.12**	**4.98±0.15**	**4.90±0.10^{y}**

Means bearing uncommon superscripts within column (x, y) differ significantly (P < 0.05).

D-0 = Day of starting the treatment, D-7 = Administration of PG, D-9/10 = Fixed time AI, D-21 = Day 21 post-AI.

in TriU-B and Heatsynch protocols and all these values were significantly higher than that in Ovsynch protocol. However, the concentrations were insignificantly higher in conceived than non-conceived cows in all the treatment protocols and even in normal cyclic control group (Table 2.) Significantly higher serum/plasma protein in cycling as compared to acyclic/anoestrus cows has also been documented earlier by many researchers (Lodhi *et al.,* 1998; Kumar *et al.,* 2007). Lack of protein or insufficient intake of protein was considered to be a cause of failure or delay in estrous cycle (Roberts, 1971).

Plasma Calcium and Phosphorus

The results on levels of plasma calcium (Table 3) obtained in anoestrus cows under TriU-B and Ovsynch protocols were statistically the same. Similarly, the mean plasma calcium concentrations noted in Heatsynch protocol and normal cyclic cows were statistically identical, but were significantly higher ($P<0.05$) as compared to other two treatment protocols. The normal cyclic cows in general had significantly ($P<0.05$) higher plasma calcium concentration than the anoestrus treated cows. Very similar observations have been recently made by some of the workers using CIDR, Ovsynch, Heatsynch and other such protocols in anoestrus cows, including normal cyclic control groups (Bhoraniya *et al.,* 2012; Ammu *et al.,* 2012[b]; Naikoo *et al.,* 2014). Calcium deficiency can upset the normal reproduction possibly due to lack of tone of uterine muscle.

The overall mean values of inorganic phosphorus (Table 4) obtained in anoestrus cows under TriU-B, Ovsynch, Heatsynch and normal cyclic cows differed significantly ($P<0.05$), the levels in cows of normal cyclic control group and Heatsynch protocol were at par and significantly higher than other two groups of cows treated with TriU-B and Ovsynch, which also however were at par. Similar trend has also been reported with various estrus synchronization protocols and in control group of Kankrej cows by Ammu *et al.* (2012[b]) and Naikoo *et al.* (2014). Infertility due to nutritional deficiency is usually characterized by a failure of estrus or a cessation of estrous cycle, where mineral deficiency mainly includes phosphorus than any other trace minerals.

Thus in general, the Ovsynch protocol appeared the best followed by TriU-B for induction of estrus and improvement of fertility without influencing the blood biochemical and mineral profiles in anoestrus crossbred cows under field conditions and hence can be suitably used by the practicing veterinarians.

ACKNOWLEDGEMENTS

We gratefully thank Dr. A.M. Thaker, Dean of the Veterinary Faculty, AAU, Anand for the funds and facilities provided for this work.

REFERENCES :

Ahlawat, A. and Derashri, H.J. (2009). *Indian J. Field Vets.*,**4**(4): 13-16.

Ammu, R., Dhami, A.J., Naikoo, M., Parmar, B.C. and Divekar, B.S. (2012[a]). *Indian J. Anim. Reprod.,* 33(1): 37-42.

Ammu, R., Dhami, A.J., Naikoo, M., Patel, S.B. and Savaliya, F.P. (2012[b]). *Indian J. Field Vets*.,**7**(4): 11-17.

Bhoraniya, H.L., Dhami, A.J. and Killedar, A. (2012). *Indian J. Anim. Reprod.,* 33(2): 14-18.

Dhami, A.J., Panchal, M.T., Hadiya, K.K., Patel, J.A. and Shah, R.G. (2014). Use of controlled breeding techniques under field conditions for estrus synchronization and conception in anoestrus crossbred cows and buffaloes. *Proc.2nd Annual Meeting of SVSBT and National Seminar on Biotechnological approaches to challenges in animal health & production,* Vet. College, DUVASU, Mathura (UP), India, March 6-7, p. 86.

Kavani, F.S., Sharma, V.K., Siddiquee, G.M. and Vadodaria, V.P. (1987). *Indian J. Anim. Reprod.,* **8**(2): 148 150.

Kumar Pravesh, Singh, M. and Vasishta, N.K. (2007). Studies on the effect of progesterone supplementation on conception rate following artificial insemination in normal cows. *Proc. XXIII Annual Convention of ISSAR and National Symposium,* 7-9 December, QUAT, Bhubaneswar, Orissa, India.

Lee, M.S., Rahman, M.S., Kwon, W.S., Chung, H.J., Yang, B.S. and Pang, M.G. (2013). *Theriogenology,* **80**: 855–861.

Lodhi, L.A., Qureshi, Z.I., Khan, A. and Hayat, S. (1998). *Pakistan J. Biol. Sci.,***1**(2): 66-68.

Martson, H.R., Allen, J.H. and Smith, R.M. (1972). *British J. Nutr.,* **27**: 127 (c.f. Sikka, P. 1992.)*Indian J. Dairy Sci.,* **45**: 159-167).

Naikoo, Mehrajuddin, Dhami, A.J., Ramakrishnan, Ammu, Parmar, B.C. and Divekar, B.S. (2014).. *Indian J. Field Vets*., **9**(4): 4-11.

Nakrani, B.B., Panchal, M.T., Dhami, A.J., Hadiya, K.K., Patel, J.A. and Gosai, R.K. (2014). *Scholars J. Agri. and Vet. Sci.,* 1(4B): 299-304.

NarenjiSani, R., Farzaneh, N., Moezifar, M., Seifi, H. A. and Alavi Tabatabei, A. (2011). *Anim. Reprod. Sci.,* **124**: 1–6.

Patel, K.R., Dhami, A.J., Hadiya, K.K., Savalia and Sarvaiya, N.P. (2013). *Indian J. Anim. Prod. Mgmt.,* **29**(3-4): 50-58.

Roberts, S.J. (1971). Veterinary Obstetrics and Genital Diseases. 2nd edn, Scientific Book Agency, Calcutta, India.

Snedecor, G.W. and Cochran, W.G. (1994). *Statistical Methods*. 8thedn. Iowa State University Press, Ames, Iowa, USA.

Tayler, R.L., Pahnish. O.F., Roubicek, C.B. and Hale, W.H. (1966). *J. Anim. Sci.,* **25**: 1035-1039.

❑