Haryana Vet. (March, 2022) **61(SI)**, 14-20 **Research Article**

FOLLICULAR PARAMETERS IN PERI-PUBERTAL ACYCLIC MURRAH BUFFALO HEIFERS FOLLOWING "OVSYNCH-PLUS" AND "MODIFIED OVSYNCH-PLUS" PROTOCOLS

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ABSTRACT

The peri-pubertal acyclic Murrah buffalo heifers (*Bubalus bubalis*) during summer (Jun-Aug; n=14) and winter season (Dec-Feb; n=12) were selected at random from herd and the ovaries of the experimental animals were scanned daily during the study period (Day 0: day of start of treatment). The animals during each season, were randomly divided into two groups and received either Ovsynch-Plus (Group I; summer season, n=7; winter season, n=6) or Modified Ovsynch-Plus (Group II; summer season, n=7; winter season, n=6) treatment. The follicular dynamics was monitored by ultrasonography daily during the period of treatment till day 12. Multiple ovulations following treatment were more common during winter season. The induced CLs formed after treatment was of shorter duration and started regressing before prostaglandin injection was administered. It can be concluded that Modified Ovsynch-Plus resulted in better response in treated buffalo heifers compared to Ovsynch-Plus, particularly in winter. Therefore, from the present study it is concluded that administration of eCG increased the number as well as size of dominant follicles (DF) and a combination of eCG and GnRH resulted in formation of CL in100% animals during summer season and in 96.15% of heifers during winter season.

Keywords: Acyclicity, Buffalo heifers, Dominant follicle, Follicular wave, Modified Ovsynch-Plus, Ovsynch-Plus, Ultrasonography

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India's largest livestock resources, among the world, play an important role in uplifting the socio-economic status of country's rural population. Low reproductive efficiency in buffalo remains a major economic problem, throughout the world, but with still a higher incidence in India (Kumar *et al.*, 2009) attributed to some inherent problems like late maturity, silent estrus, poor expression of estrus and inactive ovaries. The application of ultrasonography in bovine reproduction has made it possible to monitor the follicular population during estrous cycle (Pierson and Ginther, 1988) and hormonal treatment (Pierson and Adam, 1999). Two wave follicular growth is more common in buffaloes during breeding season (Baruselli *et al.*, 1997) and predominance of it has been observed in buffaloes during winter season (Manik *et al.*, 1998). Karen and Darwish (2010) studied the follicular dynamics and efficiency of "Ovsynch" protocol in cyclic and acyclic Egyptian buffaloes during summer season. Likewise, the efficacy of synchronization protocols for improving fertility in postpartum crossbred dairy cows and ewes (Ratnaparkhi *et al.*, 2020; Yadav *et al.*, 2021) has been observed. The present study was designed to monitor the ovarian follicular activities and study follicular turnover in peri-pubertal acyclic Murrah buffalo heifers following 'Ovsynch–Plus' protocol (eCG+Ovsynch) and 'Modified Ovsynch–Plus' protocol (replacing 1st GnRH with hCG) during summer and winter seasons.

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MATERIALS AND METHODS

Study Location and Animals: The present study was conducted at ICAR-Central Institute for Research on Buffaloes, Hisar during summer (Jun-Aug) and winter season (Dec.-Feb.) on 26 peri-pubertal acyclic Murrah buffalo heifers (*Bubalus bubalis*). All these heifers were between age groups of 30-36 months weighing 300-340 kg body weight having good body condition of 2.75-3.0. From the herd, 26 acyclic heifers for both seasons were selected after a proper ultrasound examination of the ovaries for this study for two seasons i.e. summer (n=14) and winter (n=12).

Schedule for ultrasonography and treatment: During each season, during the period of treatment with 'Ovsynch-Plus' and 'Modified Ovsynch-Plus' in all the animals, ovarian response was monitored by ultrasonography daily {(i.e. Day 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and on 13th day i.e. on Day of Insemination) (Day 0-day of start of treatment)}. In these experimental animals, the numbers and size of Dominant follicles, growth and regression rate of Dominant follicles, response to eCG/1st GnRH/ hCG and 2nd GnRH injection, growth rate and regression rate of Corpus luteum were monitored.

In summer season: After ultrasonographic monitoring during summer season, all the experimental heifers were randomly divided into two groups (7 in each group) to receive either "Ovsynch-Plus" (Group I, Fig. 1) or "Modified Ovsynch-Plus" (Group II, Fig. 2).

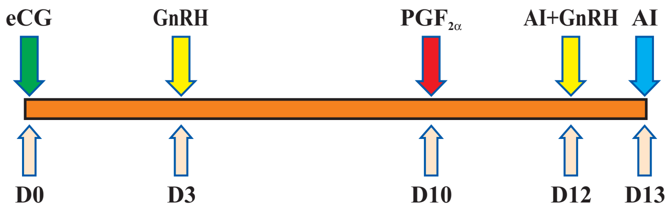


Fig. 1. 'Ovsynch-Plus' Protocol

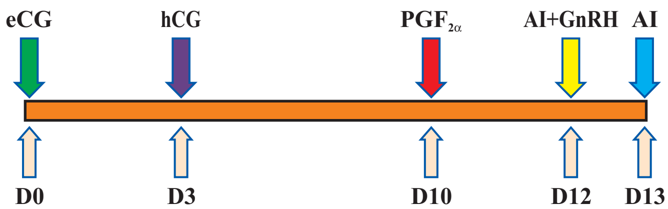


Fig. 2. 'Modified Ovsynch-Plus' Protocol

In winter season: During winter season similar to that of summer season, 12 acyclic heifers from the herd were selected and all these experimental animals were subjected to ultrasonographic monitoring. Subsequently these 12 heifers were divided in two groups (n = 6 each) to receive either 'Ovsynch-Plus' (Group I) or 'Modified Ovsynch-Plus' (Group II) treatment as described earlier i.e. summer season.

Ultrasonographic monitoring for follicular dynamic following treatment protocol: Ovarian follicular changes were monitored in all experimental buffalo heifers with a real time B-mode ultrasound scanner (Just Vision 200–Model SSA-320A, Toshiba, Japan) equipped with a convex array multi frequency transducer using frequency of 7.0 MHz. Total follicular population on an ovary was recorded by noting down each follicle observed during scanning of individual ovary through its entire surface, from medial to lateral aspect on any given day. Smallest appreciable follicles over 2 mm in diameter were recorded. The positions and sizes of follicles (≥ 3 mm) were traced at each scanning and the exact location of follicles was recorded. The follicles were categorized as small, medium and large having size of ≥ 2 mm to 4.9 mm, ≥ 5 - 8.9 mm and ≥ 9 mm, respectively. During this period of study, the following parameters were recorded in all experimental animals in each season in both groups -

a. Size of DF at the time of eCG injection (mm) i.e at day 0.

b. Growth rate of DF on day of eCG injection and after eCG injection (mm/day).

c. Size of dominant follicle at day 3 of protocol (mm).

d. Size of dominant follicle at day of $PGF_{2\alpha}$ treatment of protocol (mm) i.e. at day10.

e. Size of dominant follicle 24 hours after administration of $PGF_{2\alpha}$ injection (mm) i.e. at day 11.

f. Size of dominant follicle at day of fixed time AI/ administration of 2^{nd} dose of GnRH of treatment protocol (mm) i.e. at day 12.

g. Size of DF at 24 hours post 2^{nd} GnRH injection (mm) i.e. at day 13.

h. Growth and regression of Corpus Luteum (CL) at day 6, 8, 10 and 12 of treatment protocol (mm^2).

Statistical analysis: The data obtained in the present study were subjected to Analysis of Variance and students T-test analysis (Snedecor and Cochran, 1994) to draw scientific inferences. Characteristics associated with ovarian follicular development were examined with General Linear Model Procedures in the statistical software package for Windows Version 9.0.1 (SPSS Inc. Chicago, IL).

RESULTS AND DISCUSSION

In Ovsynch-Plus protocol, an injection of eCG was given on day 0 of treatment to enhance the follicular growth in heifers. The first GnRH/hCG injection was designed to enhance the ovulation of the largest functional follicle and induce a new follicular wave. It is well accepted that administration of GnRH agonist at any stage of the estrous cycle in buffalo increases the number of medium-sized follicles within 3 days of treatment, eliminating the large follicles by ovulation or atresia and induces the emergence of a new follicular wave within 2 to 3 days of treatment (Jazayeri *et al.*, 2010). The subsequent use of $PGF_{2\alpha}$ (on day 10) increases the percentage of synchronized animals by lysis of the CL resulting from ovulation of the dominant follicle (Pursley *et al.*, 1995). The second GnRH administration after two days post $PGF_{2\alpha}$ injection (on day 12 of the protocol) causes an induced LH surge responsible for ovulation of the dominant follicle and formation of a new CL (Senger, 2003) (Fig. 3).

Mean diameter of DF during summer was slightly more than winter season (8.89± 0.24 mm and 7.99 ± 0.6 mm, Table 1). The number of DF in summer and winter were 14 and 12, respectively in the study groups. However, mean diameter of maximal largest follicle did not differ significantly between the study groups. Karen and Darwish (2010) observed similar size of DF (on day 0) 8.4

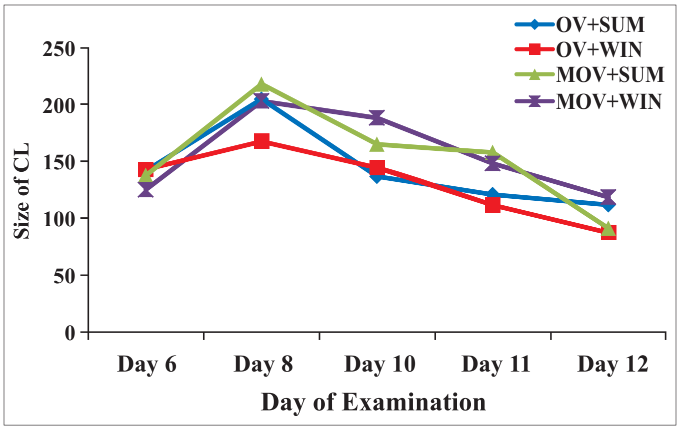


Fig. 3. Growth and regression of CL during treatment period $(mm)^2$

± 1.4 and 9.2 ± 1.5 mm in cyclic and non-cyclic Egyptian buffalo heifers treated with 'Ovsynch' treatment in summer season. On day 3, the size of the DF during both summer and winter were comparable in both groups. No significant difference was observed in the size of DF in GnRH and hCG treated heifers during winter (12.32 ± 0.87 and 11.66 ± 0.76) and summer (11.27 ± 0.47 and 10.02 ± 0.98, Table 1). The size of DF increased significantly on day 3 of treatment in both groups and seasons compared to day 0 of the treatment. The increase in size and number of follicular populations on day 2 or 3 indicates favourable effect of exogenous eCG on recruitment and selection of follicles as it enhances ovarian stimulation. Following treatment, small and medium follicles of day 0 were converted into medium and large follicles, respectively, ultimately resulting in maximum diameter (large follicle) on day 2 and 3 of treatment (Fig. 4 & 5).

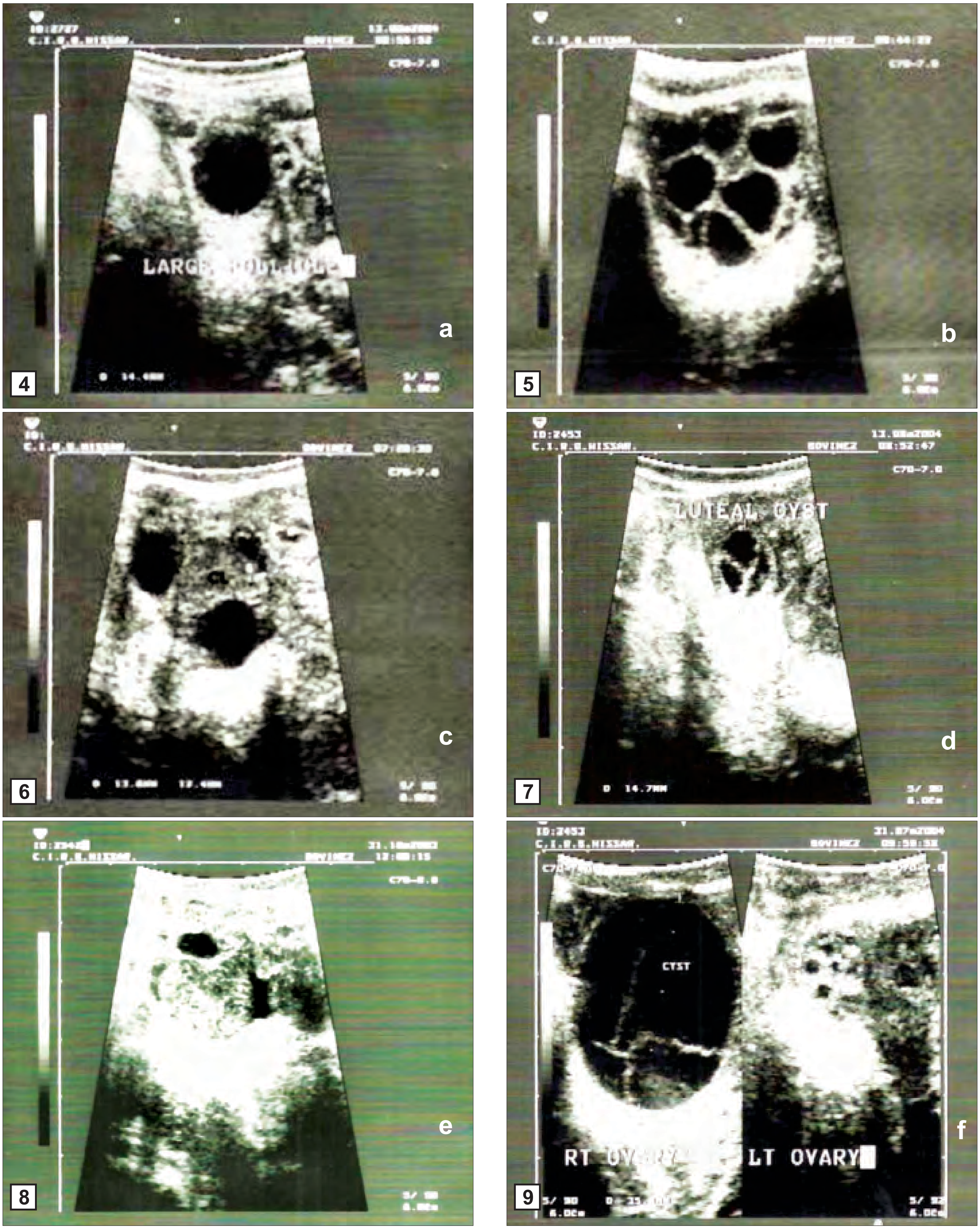
The growth rate of DF increased faster after eCG administration and observed almost double than before treatment during both the seasons. The growth rate of DF observed before eCG injection was slightly higher during winter season. However, the growth rate was significantly higher during both the season (1.1 ± 0.16 and 1.82 ±0.16 mm) following eCG injection and it was observed that the growth rate of DF following eCG injection was significantly higher (1.82 ± 0.16 mm) in the heifers treated during winter season compared to summer (1.1± 0.16 mm) (Table 1). Overall growth of DF increased at faster rate after administrating of eCG during summer (1.1 ± 0.1 mm/day) as well as winter season (1.82 ± 0.16 mm/day). Moreover, the growth was significantly higher during winter season (2.01±0.24 mm/day) than summer (1.14 ± 0.13 mm/day) in eCG treated heifers (Table 2). Anestrous buffaloes respond better to Ovsynch treatment if a follicle of > 9 mm was present in the ovaries at the start of the treatment (Rohilla *et al.*, 2005). The stage of the follicle at the time of the initial GnRH injection thus represents a major limitation in synchronization programs (Navanukraw *et al.*, 2004). The mean diameters of preovulatory dominant follicles at the time of 1st GnRH injection during summer and winter season varied from 10.02±0.98 to 12.32±0.87 mm in both groups. Karen and Darwish (2010) after the administration of 1st GnRH, observed an 8.1±0.6 mm vs 9.2±1.5 mm mean diameters of the dominant follicles in the cyclic and acyclic buffalo heifers, respectively. The larger size of the follicle observed in the present study might be due to administration of eCG injection on day 0 of the treatment protocol. Kumar (2010) observed a smaller diameter of DF at 1st GnRH treatment of 'Ovsynch' in anestrous postpartum buffaloes. On day 10 of the treatment, it was observed that DF continued to increase in size progressively up to day 13 (Table 3). It was observed that the size of DF was significantly higher (14.57±1.44 mm vs 9.30±0.33 mm) in those heifers which were given hCG injection as compared to those receiving GnRH injection during summer season. The same pattern was observed during winter season also (17.82±2.93 mm vs 10.70±2.44 mm). The size of DF was higher during winter season compared to summer on each day of examination in both treated groups. However, in one of the animal (Fig. 7) treated with Modified Ovsynch-Plus protocol during summer, a large cyst of 21.3 mm size was observed on the right ovary on day 10 and 11 of treatment

Table 1

Mean diameter and Growth of DF at Day 0 and Day 3 following treatment

Sr. No.	Parameter	N/n	Summer (Mean ±SEM)	N/n	Winter (Mean ±SEM)
1	Mean Diameter (mm) of DF at the time of eCG injection (Day 0) in both protocol	14/14	$8.89±0.24^a$	12/12	$7.99±0.69^a$
2.	Size (mm) of DF at day 3 of protocol in 'Ovsynch-Plus' treated group	3/7	$11.27±0.47^b$	4/6	$12.32±0.87^b$
3.	Size (mm) of DF at day 3 of protocol in 'Modified Ovsynch-Plus' treated group	4/7	$10.02±0.98^b$	5/6	$11.66±0.76^b$

N- Number of follicles, n- Numbers of animals
Means bearing different superscripts (a, b) differ significantly in a column (P<0.05)



Figs. 4-9. **(4)** Ovary with dominant follicle; **(5)** Ovary with multiple follicles after eCG treatment; **(6)** Ovary with large follicles and CL during treatment; **(7)** Ovary with luteal cyst; **(8)** Ovary with recently formed multiple CLs at Day 4 in hCG treated group; **(9)** Cyst on right ovary and multiple small follicles on left ovary

Table 2

Growth rate of dominant follicle (DF) on day of eCG and after eCG treatment

Growth rate (mm) of DF		N/n Mean+SEM	Summer	N/n Mean+SEM	Winter
	On day of eCGtreatment	18*/14	0.63±.09[a]	28A/12	0.71±0.06[a]
	After eCGtreatment	18*/14	1.1±0.16[b]	23B/12	1.82±0.16[b]
Growth rate (mm) of DF	'Ovsynch-Plus' group	(9/7)**	1.05±0.15[b]	11C/ 6	1.62±0.2[b]
after treatment in	'Modified Ovsynch-Plus' group	(9/7)**	1.14±0.13[b]	12D/6	2.01±0.24[c]

N- Number of follicles, n- Numbers of animals; *in 4 animals 2 DF, in 10 animals 1 DF were observed during this study.
**in both treated groups in 2 animals 2 DF and in 5 animals 1 DF was observed during this study.
A - in 7 animals 2 DF, in 3 animals 3 DF, in 1 animals 1 DF and in 1 animals 4 DF were observed.
B - During study DF observed with the frequency of 1 (n=3), 2 (n=4), 3 (n=1), 4 (n=1) and 5 (n=1) during winter season.
C - During study DF observed with the frequency of 1 (n=3), 2 (n=2), and 4 (n=1) during winter season in 'Ovsynch -Plus' treated group.
D - During study DF observed with the frequency of 2 (n=2), 3 (n=1), and 5 (n=1) during winter season in 'Modified Ovsynch -Plus' treated group
Means bearing different superscripts (a, b, c) differ significantly in a column (P<0.05)

Table 3

Size (mm) of the largest DF on and after $PGF_{2\alpha}$ injection

S. No.	Day	'Ovsynch-Plus' Protocol				'Modified Ovsynch-Plus' Protocol			
		N/n	Summer (mean± SEM)	N/n	Winter (mean± SEM)	N/n	Summer (mean± SEM)	N/n	Winter (mean± SEM)
1.	10	7/7	9.30±0.33[a]	6/6	10.70±2.44[a]	7/7	14.57±1.44	5*/6	17.82±2.93
2.	11	7/7	9.51±0.34[a]	6/6	10.93±2.16[a]	6*/7	15.47±2.30	5*/6	18.18±2.97
3.	12	7/7	9.59±0.36[a]	6/6	11.25±1.85[b]	6*/7	15.95±1.84	4C/6	20.82±3.39
4.	13	*6/7	10.05±0.47[b]	3A/6	11.60±2.52[c]	4B/7	15.62±2.96	4C/6	19.23±3.48

N- Number of follicles, n= numbers of animals.; *indicate ovulation of 1 D F in animals
A- Indicates ovulation of 3 DF; B- Indicates ovulation of 2 DF and 1 LF formation.; C- Indicates ovulation of 2 DF
Means bearing different superscripts (a, b, c) differ significantly in a column (P<0.05)

Table 4

Growth and regression $(mm)^2$ of CL during treatment

S. No.	Day	'Ovsynch-Plus' Protocol				'Modified Ovsynch-Plus' Protocol			
		N/n	Summer (mean± SEM)	N/n	Winter (mean± SEM)	N/n	Summer (mean± SEM)	N/n	Winter (mean± SEM)
1.	6	8/7	141.52±13.08[a]	10*/6	142.68±12.99[c]	7/7	137.54±27.32[b]	10C/6	124.97±9.11[a]
2.	8	8/7	204.41±15.60[b]	10*/6	167.29±14.93[c]	7/7	217.49±28.43[c]	10C/6	202.67±21.16[c]
3.	10	8/7	136.44±14.70[a]	10*/6	144.22±19.19[c]	7/7	164.79±29.67[b]	10C/6	187.88±18.0[b]
4.	11	9/7	120.71±14.09[a]	12A/6	111.35±15.94[b]	7/7	157.64±23.69[b]	13D/6	147.94±13.49[a]
5.	12	7/7	111.58±15.02[a]	12A/6	87.20±16.23[a]	6B/7	91.17±9.70[a]	13D/6	118.37±12.18[a]

N- Number of follicles, n- Numbers of animals; In 'Ovsynch-Plus' protocol on day 6, 8, and day10 in one animal two CL was observed while on day 11 in 2 animals 2 CL were observed.
*on Day 6,8,10 during winter season in 'Ovsynch-Plus' treated group 10 CL observed with the frequency of 1CL (n=4), 2 CL (n=1) and 4CL (n=1).
A- During winter season in 'Ovsynch-Plus' treated group of heifers 12 CL were observed on day 11 and 12 with a frequency of 1 CL (n=3), 2 CL (n=2) and 5 CL (n=1).
B- 1 animal became cyclic in 'Modified Ovsynch-Plus' treated group in summer season.
C- on Day 6,8,10 during winter season in 'Modified Ovsynch-Plus' treated group 10 CL observed with the frequency of 1 CL (n=3), 2 CL (n=1) and 5 CL (n=1).
D- on Day 11 and 12 during winter season in 'Modified Ovsynch-Plus' treated group 13 CL observed with the frequency of 1 CL (n=3), 2 CL (n=1), 3 CL (n=1) and 5 CL (n=1). Means bearing different superscripts (a, b, c) differ significantly in a column (P<0.05)

period while the left ovary was having small multiple follicles (Fig. 9). If a CL resulted from the initial injection of GnRH, the 7-day interval should have provided sufficient time for the CL to mature in order to respond to $PGF_{2\alpha}$ (Ali and Fahmy, 2007). In the present study, almost all treated heifers (13/13) in Ovsynch-Plus and 12/13 heifers in Modified Ovsynch-Plus group showed at least one or more CL on the day of $PGF_{2\alpha}$ treatment (Fig. 8). There was no significant difference in size of corpus luteum formed during summer or winter season at different days of treatment after administering either GnRH or hCG injection. The size of CL increased up to day 8 of treatment

(Fig. 3). However, CL started regressing before the day of $PGF_{2\alpha}$ administration. The size of CL decreased gradually after day 10 of treatment and this CL was not visible beyond day 13 of treatment (Table 4; Fig. 3).The finding indicates that CL formed in response to GnRH/hCG injections were of shorter duration. This hypothesis supports the findings of Sharma *et al.* (2012) in prepubertal heifers. Premature regression of CL probably indicates that these CL were either simply luteinized follicle or not having sufficient luteal cells to sustain their viability for full cycle length.

Present finding supports the hypothesis that ovulation induced by GnRH administration results in a high proportion of pre-mature luteal regression (De Rensis *et al.*, 2005), which was detected in the heifers in present investigation. A pre-mature luteal regression was observed by Kumar (2010) in postpartum anestrous buffaloes after treatment with Ovsynch+Hydroxy progesterone. Pursley *et al.* (1995) observed smaller diameter of the induced corpus luteum than the natural one in cattle. On day 10, the size of DF in Modified Ovsynch-Plus group heifers was significantly higher (14.57±1.44, summer; 17.82±2.93 mm, winter) as compared to those of 'Ovsynch-Plus' treated group (9.30±0.33, summer; 10.70±2.44 mm, winter). After PG injection, the size of these follicles in both group increased progressively from day 10 to day 12 (when an injection of GnRH was given in both groups). Karen and Darwish (2010) observed 8.1±0.6 mm and 8±1.9 mm size of follicle at the $PGF_{2\alpha}$ treatment of Ovsynch in non-cyclic buffaloes and heifers, respectively. The increased size of DF in present study may be due to follicular/ovarian hyperstimulation caused by eCG administration on day 0. The size of dominant follicles on the day of 2nd GnRH injection (day 12) was smaller in Ovsynch-Plus group (9.59±0.36mm, summer; 11.25±1.85 mm, winter) as compared to Modified Ovsynch-Plus group (15.95±1.84 mm, summer; 20.82 ± 3.39 mm, winter) confirming the faster growth of DF during winter as compared to summer season.

However, it was interesting to observe a much larger size of DF in Modified Ovsynch-Plus group. This group was administered hCG on day 3 of treatment in place of GnRH. The findings suggest that following hCG treatment, large follicles ovulated (Fig. 8) whereas medium sized follicles continue to increase in size without atresia or ovulation and formation of luteinized follicle or luteal cyst (Fig. 7). On the other hand, GnRH injection on day 3 of treatment in Ovsynch-Plus treated group resulted in ovulation of large follicles and subsequent atresia of medium sized follicles resulting in availability of relatively smaller sized follicles at 2nd GnRH treatment. The size of ovulatory DF was observed almost similar in Ovsynch-Plus treated heifers during the both seasons. However, the size of ovulatory DF observed in Modified Ovsynch-Plus treated heifers was significantly higher during winter season as compared to summer. Karen and Darwish (2010) observed 11.9 mm and 11.9±0.6 mm size of ovulatory follicles in acyclic buffaloes and buffalo heifers in Ovsynch treatment, respectively. Bartolomeu *et al.* (2007) recorded the diameter of ovulatory follicle at 2nd GnRH injection as 10.5±1.1 mm in Ovsynch+CIDR treated nuliparous buffaloes and these results are not in agreement with the findings of present study in Modified Ovsynch-Plus group where the diameter of DF was much higher 15.95±1.84 mm and 20.82±3.39 mm during summer and winter, respectively at day of 2nd GnRH injection. Kumar (2010) observed the diameter of DF 11.90±0.36 mm at 2nd GnRH treatment in buffaloes. Rastegarnia *et al.* (2004) recorded the average diameter of dominant follicle as 12.7±0.54 mm in norgestomet +GnRH treated buffaloes.

Therefore, from the present study it is concluded that administration of eCG increased the number as well as size of dominant follicles (DF) and a combination of eCG and GnRH resulted in formation of CL in100% animals during summer season and in 96.15% of heifers during winter season. Likewise, administration of hCG in 'Modified Ovsynch-Plus' group resulted in availability of large persistent follicles (PF) that were not responsive to GnRH. As many heifers responded with development of multiple follicles following eCG injection, the dose of eCG may further be reduced in order to have a lesser number of large follicles at the time of GnRH injection particularly during winter season.

REFERENCES

Ali, A. and Fahmy, S. (2007). Ovarian dynamics and milk progesterone concentrations in cycling and non-cycling buffalo-cows (*Bubalus bubalis*) during Ovsynch program. *Theriogenology.* **68**: 23–28.

Baruselli, P.S., Mucciolo, R.G., Vistin, J.A., Viana, W.G., Arruda, R.P., Maduriera, E.H., Oliveira, C.A. and Molero-Filho, J.R. (1997). Ovarian follicular dynamics during the estrous cycle in buffalo. *Theriogenology.* **47**: 1531-1547.

Bortolomeu, C.C., Del Rei, A.J., Alvares, C.T.G. and Vilar, G.D. (2007). Follicular dynamics during synchronization of ovulation of nulliparous buffaloes cows during unfavourable reproductive station. *Italian J. Anim. Sci.* **6(Suppl.)**: 589–592.

De Rensis, F., Ranci, G., Guarneri, P., Nguyen, B.X., Presicces G.A., Huszenicza, G. and Scaramuzzi, R.J. (2005). Conception rate after fixed time insemination following Ovsynch protocol with and without progesterone supplementation in cyclic and non-cyclic Mediterranean Italian buffaloes (*Bubalus bubalis*). *Theriogenology.* **63**: 1824-1831.

Jazayeri, S.P., Kohram, H. and Salehi, R. (2010). Hormonal responses to GnRH injection given at different stages of the estrous cycle in water buffaloes. *Afr. J. Biotechnol.* **9**: 2169–2172.

Karen, A.M. and Darwish, S.A. (2010). Efficacy of Ovsynch protocol in cyclic and acyclic Egyptian buffaloes in summer. *Anim. Reprod. Sci.* **119(1-2)**: 17–23.

Kumar, R., Saxena, A. and Niranjan, P.S. (2009). Estrus detection by serum progesterone concentration in buffaloes. *Indian Vet. J.* **86**: 326-327.

Kumar, S. (2010). Follicular dynamics in anestrous Murrah buffaloes during cyclicity induction using Ovsynch in different combinations. M.V.Sc. Thesis submitted to CCS HAU, Hisar, India.

Manik, R.S., Singla, S.K., Palta, P. and Madan, M.L. (1998). Real time ultrasound evaluation of changes in follicular population during estrus cycle in buffaloes. *Indian J. Anim. Sci.* **68(11)**: 1157-1159.

Navanukraw, C. Redmer, D.A., Reynolds, L.P., Kirsch, J.D., Grazul-Bilska, A.T. and Fricke, P.M. (2004). A modified presynchronization protocol improves fertility to timed artificial insemination in lactating dairy cows. *J. Dairy Sci.* **87(5)**: 1551–1557.

Pierosn, R.A. and Adams, G.P. (1999). Remote assessment of ovarian response and follicular status using visual analysis of ultrasound images. *Therigenology.* **51**: 47–57.

Pierson, R.A. and Ginther, O.J. (1988). Ultrasonic imaging of the ovaries and uterus of cattle. *Theriogenology.* **29(1)**: 21–37.

Pursely, J.R., Mee, M.O. and Wiltbank, M.C. (1995). Synchronization of ovulation in dairy cows using PGF_{2alpha} and GnRH. *Theriogenology.* **44**: 915–923.

Rastegarnia, A., Niasari-Naslaji, A., Hovareshti, P., Sarhaddi, F., Safaei, M. (2004). The effect of different doses of Gonadorelin on ovarian follicle dynamics in river buffalo (*Bubalus bubalis*). *Theriogenology.* **62**: 1283–1291.

Ratnaparkhi, A.R., Deshmukh, S.G., Birade, H.S., Kale, V.B., Harkal, S.B. and Jadhao A.D. (2020). Comparative efficacy of synchronization protocols for improving fertility in postpartum crossbred dairy cows. *Haryana Vet.* **59(SI)**: 23–26.

Rohilla, N., Singh, U., Sharma, R.K. and Singh, I. (2005). Ultrasonographic study of ovarian status in summer anestrus postpartum Murrah buffaloes. *Indian J. Anim. Reprod.* **26(2)**: 95–98.

Senger P.L. (2003). Reproductive cyclicity-luteal phase. In: Pathways to Pregnancy and Parturition. Senger, P.L. (2nd Edn.), Pulman, WA, Current Conceptions, Inc.

Sharma, R.K., Singh J.K., Khanna, S. and Singh, I. (2012). Ovarian response of pre-pubertal Murrah heifers to exogenous GnRH. *Anim. Reprod. Sci.* **133(3-4)**: 153–158.

Yadav, V., Chandolia, R.K., Ranga, L.C., Bisla, A., Saini, G., Dutt, R., Singh, G., Patil, S. and Kumar, A. (2021). Estrus synchronization to combat reproductive seasonality in crossbred ewes. *Haryana Vet.* **60(SI)**: 47–50.