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Fertility response and ovulatory response following 'Ovsynch-plus' and 'Modified Ovsynch-plus' protocol in peri-pubertal acyclic Murrah buffalo heifers (*Bubalus bubalis*) in different seasons

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Abstract
Background: Fertility is one of the key determinants in the life time performance of animal. The success of the dairy cattle and buffalo husbandry lie in ensuring proper and optimal reproductive rhythm in herd. The application of Real Time Ultrasonographic monitoring in bovine reproduction has made it possible to monitor follicular population during estrus cycle and hormonal treatment for estrus synchronization and induction protocol. To rescue acyclic animals several hormonal treatment protocols have been employed with variable results.
Methods: The peripubertal anestrous 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. Subsequently, the animals during each season, were randomly divided into two groups and received either 'Ovsynch-Plus' (Group I; summer, n = 7; winter, n = 6) or 'Modified Ovsynch-Plus' (Group II; summer, n = 7; winter, n = 6) treatment. The follicular dynamics was monitored by ultrasonography daily during the period of treatment till day 12 and then after insemination at weekly interval i.e., at day 7, 14, 21 and 28 post insemination. All the treated females were inseminated at fixed time on day 12 and 13. Pregnancy was diagnosed by ultrasonography on day 28 after insemination. Administration of eCG increased the number as well as size of dominant follicles (DF) on day 2nd and 3rd after injection.
Results: A combination of eCG and GnRH at 3 days apart resulted in formation of CL in 100% animals during summer season and in 96.15% of heifers during winter season. Administration of hCG (on day 3) in 'Modified Ovsynch-Plus' treated group resulted in availability of large persistent follicles that were not responsive to GnRH injection given on day 12 of treatment. Only those females conceived who responded by ovulation to eCG/1st GnRH/hCG and 2nd GnRH administrations. Conception rate was found to be better in 'Ovsynch-Plus' treated heifers compared to that of 'Modified Ovsynch-Plus' treated heifers. In conclusion, start of the 'Ovsynch' protocol with hCG did not increase ovulation and conception rates in heifers. However, increasing ovulation rate in response to pre-hormonal administration of 'Ovsynch' with eCG have a significant effect on conception rate in anoestrous buffalo heifers.

Keywords: acyclicity, dominant follicle, fertility, FTAI, ovulation response, pregnancy rate

1. Introduction
In India and particularly in Haryana, buffalo is considered as the primary milch animal contributing about 55.5% of total milk production to the country (Dairy India, 2007) [7]. It has been observed that about 50% of rural people in India are dependent on livestock for their live hood (Thorton *et al*., 2002) [37]. On the basis of utility and production potential, the buffalo is so-called - *"The Black Gold"* (Acharya and Bhat, 1988) [1]. However, 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) [25] and this low reproductive efficiency has been attributed to some inherent problems like late maturity, silent estrus, poor expression of estrus and inactive ovaries (Madan 1988) [27].
The application of ultrasonography in bovine reproduction has made it possible to monitor the follicular population during estrous cycle (Pierson and Ginther, 1987a, b, 1988) [29, 30], anestrus period (Henao *et al*., 2000) [19], postpartum period (Ruiz-Cortes and Olivera-Angel, 1999) [34] and during hormonal treatment (Pierson and Adam, 1999) [28]. Similarly, Karen and Darwish (2010) [22] studied the follicular dynamics and efficiency of 'Ovsynch' protocol in cyclic and acyclic Egyptian buffaloes during summer season.

Earlier trials with GnRH in cattle and buffaloes recorded excellent results in induction of LH surge (Sheth *et al*., 1978) [36]. Several research workers tried to induce estrus in postpartum buffaloes with single as well as multiple GnRH dose schedules but with variable results (Balasundram *et al*., 1981; Krishnaswamy, 1985) [3, 24]. Now a day, a novel protocol, 'Ovsynch' which was developed by Pursley *et al*., (1995) is being extensively used to synchronize ovulation in cattle. The precise synchrony achieved following the treatment allows for successful AI without the need for detection of estrus and has been applied widely in cattle breeding. Berber *et al*. (2002) [7] compared the efficiency of replacing GnRH with LH in ovulation synchronization protocol in buffaloes and monitored the response by ultrasonography, which revealed a similar ovulation rates if second dose of GnRH was replaced with LH. Khanna (2003) [23] studied estrus induction and fertility response of 'Ovsynch' protocol in early postpartum suckled buffaloes (days 35-45) during breeding season and observed better fertility following insemination according to estrus rather than fixed time inseminations. Recently a modified 'Ovsynch' protocol is being used to induce cyclicity in non-cycling cattle. Geary *et al*., (2001) [18] replaced GnRH with hCG and evaluated the conception rate in primiparous and multiparous cows and concluded that hCG was not a suitable replacement for GnRH to synchronize ovulation in multiparous cows. Similarly, Carvalho *et al.,* (2007) [11] observed that when using 'Ovsynch' protocol for fixed time AI in buffalo, the replacement of the last GnRH administration with hCG increases the plasma P_4 concentration, however, no positive effect on the conception rate was observed.

However, scanty reports are available on the study of follicular activity and fertility in anestrous buffalo heifers following treatment with 'Ovsynch-Plus' (eCG + Ovsynch) and a 'Modified Ovsynch-Plus' (replacing 1st GnRH with hCG), therefore, the present study was designed to evaluate the fertility response to 'Ovsynch-Plus' (eCG + Ovsynch) and a 'Modified Ovsynch-Plus' (1st GnRH replaced with hCG) in anestrous Murrah buffalo heifers.

2. Material and Methods

2.1 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 anestrous 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 anestrous heifers for each season were selected at random after a proper ultrasound examination of the ovaries for this study for two seasons i.e. Summer (n = 14) and winter (n = 12).

2.2 Housing, feeding and management

Experimental buffalo heifers were housed in half-walled pucca sheds with asbestos sheet roof. Heifers were let loose daily for pasture grazing for 3-4 hr. In addition, the animals were stall-fed with adequate quantity of green fodder and wheat-straw. The concentrate feed, supplemented with mineral mixture and common salt prepared in the institute, was also provided to the animals. Drinking water was available *ad libitum*. Animals were protected from the inclement weather and given water shower bath daily during summer season.

2.3 Schedule for ultrasonographic monitoring

2.3.1 Summer season

The ovaries of the experimental animals were scanned for a period of 20 days on every alternate day and follicular dynamics was monitored during these days and subsequently, after then these animals were given treatment as per technical program of work. 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 follicles, growth and regression rate of Dominant follicles, growth rate and regression rate of Corpus luteum were monitored. 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.-2.1) or "Modified Ovsynch-Plus" (Group II, Fig.-2.2) protocol as follow:

Group I

In this group the experimental heifers received 'Ovsynch-Plus' treatment as follow

- Day 0: eCG (500 IU, I.M. Folligon, Intervet)
- Day 3: GnRH (Buserelin Acetate 16µg, I.M. Receptal, Intervet)
- Day 10: $PGF_2\alpha$ (Tiaprost 750µg, I.M. Iliren, Intervet)
- Day 12: GnRH (Buserelin Acetate 16µg, I.M. Receptal, Intervet)

Group II

The heifers of this group received 'Modified Ovsynch-Plus' treatment as follow

- Day 0: eCG (500 IU, I.M. Folligon, Intervet)
- Day 3: hCG (3000 IU, I.M. Chorulon, Intervet)
- Day 10: PGF2α (Tiaprost 750µg, I.M., Iliren, Intervet)
- Day 12: GnRH (Buserelin Acetate 16µg, I.M. Receptal, Intervet)

2.3.2 Winter season

During winter season similar to that of summer season 12 acyclic heifers from the herd were selected at random and all these experimental animals were subjected to ultrasonography monitoring for a period of 20 days and the follicular dynamic parameters were monitored as summer season.

2.4. Ultrasonographic monitoring during 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. After properly restraining the animal in a chute, without use of any tranquilizing agent, well lubricated transducer was inserted together with hand in the rectum after evacuating the faecal material. For orientation, the transducer was moved along the dorsal surface of the reproductive tract and then moved laterally to examine the ovaries. During examination, individual ovary and the transducer were held in the same hand. Ovaries were scanned by turn, visualizing the structures on each ovary by sliding the probe from medial to the lateral aspect of the ovary and recording all structures for their location and size. Follicular luteinization was appreciated by appearance of hyperechoic

appearance of the follicular cavity. Corpora lutea were appreciated as well defined, granular, round to oval structures with lower echogenicity than the adjoining ovarian tissue, but greater than that of the follicular fluid. Ovulation - Sudden disappearance of dominant follicle and subsequent development of corpus luteum at the same site of ovary. 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 after insemination (Day 13) at weekly intervals (Day 20, 27, 34, 41) or at day 7, 14, 21, 28 post insemination.

2.5. Artificial insemination, pregnancy diagnosis and fertility response

All the experimental buffalo heifers were inseminated at a fixed time with a good quality frozen-thawed semen on day 12 (day of 2nd GnRH injection) and again on day 13. However, in both the groups AI was not performed in those animals which had already ovulated. Pregnancy diagnosis was done by transrectal ultrasonography on Day 28 post insemination and was considered positive when non-echogenic fluid in the uterus or anechogenic embryo surrounded by foetal fluid was observed. Estrus response was defined as percentage of female exhibiting estrus of those treated. Pregnancy rate was defined as the percentage of females conceiving of the total treated. To measure the reproductive performance following the treatment protocol, the following parameters were observed

a. Animals ovulated in response to eCG injection treatment alone.
b. Animals ovulated to eCG/1st GnRH/hCG injections.
c. Time to ovulation in response to 1st GnRH/hCG injections (-24 hr, 0-24 hr, > 24 hr.)
d. CL formation after 1st GnRH/hCG injections.
e. Animals ovulated to 2nd GnRH injection (-24 hr, 0-24 hr, > 24 hr.)
f. Animals having CL formation on Day 7, 14, 21 and 28 post AI.
g. Animals conceived to fix time artificial insemination (FTAI).
h. Animals returned to acyclic status/acyclicity.

2.6. 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).

3. Results and Discussion

In the present study, two groups of acyclic peri-pubertal buffalo heifers were subjected to hormonal treatment ('Ovsynch-Plus' and 'Modified Ovsynch-Plus') and out of 26 treated animals, 7/14 animals (50%) during the summer season and 3/12 animals (25%) during winter season ovulated in response to eCG injection alone (Table-1; Fig.-1) Subsequently after injection of 1st GnRH/hCG, all the treated females (100%) during summer season and 11 out of 12 during winter season ovulated (Table-1, Fig.-1). It was further observed that almost equal number of females treated during the summer season ovulated 24 hours before and after the 1st injection of GnRH/hCG whereas during winter season majority of the female 8/12 (66%) ovulated after the GnRH/hCG injection (Table 1).Moreover, to the eCG/ 1st GnRH injection, 100% females (13/13) ovulated in 'Ovsynch-Plus' treated and 12 of 13 heifers ovulated to eCG/hCG injection in 'Modified Ovsynch-Plus' group. The results of the present study showed that 100% females ovulated in 'Ovsynch-Plus' group and 96.15% of heifers in 'Modified Ovsynch-Plus' group had ovulations after the 1st GnRH/hCG injection. In previous studies, an ovulation rate of approximately 90% in cyclic and 50% in non-cyclic buffalo (Ali and Fahmy, 2007) [2] was also observed. Administration of eCG on day 0 resulted in better ovulation rate in present study compared to the reports of Neglia *et al.,* (2003) and Ali and Fahmy, (2007) [2]. In the present study 38.5% experimental animal's ovulated following eCG treatment alone. Derar *et al.,* (2012) [15] observed ovulation rate of 87.5% and 100% in cyclic buffalo heifers and buffaloes following the 1st GnRH administration following 'Ovsynch' protocol. Karen and Darwish, (2010) [22] after the administration of 1st GnRH, observed that 50% (5 of 10) of acyclic buffalo cows and 33% (1 of 3) of acyclic heifers responded by ovulation or luteinization of the follicles. The better ovulation rate in present study may be due to the administration of eCG injection at day 0 (day of start of treatment). eCG stimulated the follicular development and increased the size and number of dominant follicles that responded by ovulation.

In the present study a wider range of ovulation time (hrs) was observed. The ovulation time observed after 1st GnRH/hCG treatment was -24 hrs, 0-24 hrs and 24-48 hrs in 38.5%, 19.23% and 38.5% treated heifers, respectively. Ovulation before GnRH/hCG injection indicates ovulation in response to eCG and this was because of LH like activity in the hormone. A slightly more number of treated females ovulated to 2nd GnRH injection during the summer season (6/14) as compared to that of winter season (4/12). However, majority of the heifers of the both treated groups during summer season ovulated after the 2nd GnRH injection whereas almost equal number of treated female during the winter season ovulated before and after the 2nd GnRH injection (Table-1). Multiple ovulations following treatment were observed more commonly during winter season and the time of ovulation ranged from -24 hr to 48 hr following 1st GnRH/hCG and 2nd GnRH treatment during both seasons. Follicle diameter is not the only parameter that can affect ovulation rates. In a recent study, it was demonstrated that follicle size in buffalo that ovulated compared to those that did not ovulated is quite similar (Campanile *et al.,* 2008) [8]. Moreover, the stage of follicular development (growth or regression phase) greatly affects its response to GnRH treatment (Dharani *et al.,* 2010) [16]. It has been noted that the first GnRH injection was successful in synchronizing a new follicular wave 1-3 days after treatment (Ali and Fahmy, 2007) [2]. In cattle, the new wave started 1-2 days after GnRH treatment, regardless of the incidence of ovulation (Hussein, 2003; Hussein *et al.,* 2004) [20, 21]. A similar trend was also observed in the present study.

In all the ovulated females during both summer and winter season, ovulation was subsequently confirmed by the presence of CL on day 7 and 14 post AI. Further presence of CL was confirmed till day 28 post AI unless it regressed in some treated animals (Table-2). After the 2nd GnRH injection the follicles responded with the development of three types of structures *viz.* NR, LF and CL with a frequency of 2 of 13

(15.4%), 4 of 13 (30.8%) and 7 of 13 (53.8%), respectively in 'Ovsynch-Plus' Group heifers. The animals of 'Modified Ovsynch-Plus' group also responded with the development of three types of structures *viz*. NR, LF and CL with the frequency of 4 of 13 (30.8%), 4 of 13 (30.8%) and 5 of 13 (38.5%) after the 2nd injection, respectively. Sharma *et al*., (2012) also observed the similar pattern of response with variable frequencies after treatment with different GnRH doses in prepubertal Murrah buffalo heifers. This finding of present study indicates that administration of GnRH/hCG always does not induce either ovulation or atresia of DFs.

Karen and Darwish, (2010) [22] observed ovulation rate in 60% (6/10) of acyclic cows and in 33% (1/3) of acyclic heifers, respectively after the administration of 2nd GnRH during summer. In the present study slightly higher ovulation rate (42.85%, 3 of 7) in heifers of both the treatment groups during summer season was observed and overall 38.46% (5 of 13) heifers ovulated in both the 'Ovsynch-Plus' and 'Modified Ovsynch-Plus' treated groups. The ovulation response to 2nd GnRH injection in both the 'Ovsynch-Plus' and 'Modified Ovsynch-Plus' protocol was better than observed by Karen and Darwish in 'Ovsynch' protocol 33% (1/3). Karen and Darwish (2010) [22] observed that ovulation in response to 2nd GnRH injection occurred over a wider range of time in the non-cyclic buffalo cows (26±4.8 hrs; range: 12-36 hrs) and in heifer (12 hrs). In the present study the ovulation in response to 2nd GnRH injection also occurred with a wider range of time and was -24 hrs, 0-24 hrs and 24-48 hr in 11.5%, 15.38% and 15.38% treated heifers, respectively. In the present study, an ovulation rate of 38.46% was observed in the both the treatment groups. This difference may be due to the acyclic status of experimental heifers or low breeding seasons. The lower ovulation response to 2nd GnRH injection may be probably due to 1) premature regression of induced CLs resulting insufficient progesterone priming to maintain blood progesterone level in terms of duration and level of hormone resulting asynchronous hormonal events and availability of large persistent follicles that were unresponsive to 2nd GnRH injection. These large persistent follicles may not have sufficient LH receptors to respond to the GnRH injection. Even ovulation of such large follicles may not result in a successful pregnancy.

Fertility is associated with progesterone levels at induction of luteolysis, size, and function of preovulatory follicle. In addition, another critical factor affecting the overall outcome of 'Ovsynch' is follicular size at the time of the second GnRH/AI, which affects ovulatory response. In our study, despite the fact that the 'Modified Ovsynch-Plus' and 'Ovsynch-Plus' groups had almost similar ovulatory response to the first GnRH/hCG treatment of 'Ovsynch', animals in the 'Ovsynch-Plus' group had tendency to produce to smaller preovulatory follicle's size at the time of AI than animals in 'Modified Ovsynch-Plus' group. In contrast to the findings of the present study, Lopes *et al.,* (2007) [26] reported that presence of a larger preovulatory follicle size in the treated animals increased pregnancy rate. On the other hand, Vasconcellos *et al.,* (1999) indicated that lower ovulatory follicle size produces more pregnancy in lactating dairy cows. In the present study, although preovulatory follicle`s size was lower in the 'Ovsynch-Plus' (9.30±0.33 mm to 11.60±2.52 mm) than the 'Modified Ovsynch-Plus' group (14.57±1.44 mm to 20.82±3.39 mm), conception rate was lower in the 'Modified Ovsynch-Plus' (15.38%) than 'Ovsynch-Plus' (30.8%) groups. This result support Vasconcellos's hypothesis and shows that follicle size at the time of AI could not affect subsequent fertility of animals. Contrary to our results, some studies have indicated that fertility was greater after the ovulation of a follicle of approximately 16 mm in diameter (Perry *et al.*, 2005) [6].

A lesser number of treated females of both the groups (2/14) conceived during summer season. Whereas a higher number of treated females (4/12) conceived during winter season (Table-3). The pregnancy was confirmed on day 28 and later post AI by ultrasonography (Sonograph-1and 2). It was further observed that during summer season more number of treated heifers (5/7) became acyclic following the 'Modified Ovsynch-Plus' treatment, whereas (3/7) treated heifers became acyclic following the 'Ovsynch-Plus treatment. Similarly, during winter season all of treated females remained cyclic following 'Ovsynch-Plus' treatment whereas 33% of treated females following 'Modified Ovsynch-Plus' treatment became acyclic after treatment (Table 2). After injection of 1st GnRH/hCG, 100% females (13/13) ovulated out of which 7 animals (53.85%) ovulated to 2nd GnRHand subsequently 4 animals conceived (30.77%) at the induced estrus and 3 animals (23.1%) became acyclic after treatment 'Ovsynch-Plus' treatment. In the heifers treated with 'Modified Ovsynch-Plus', after the injection of 1st GnRH/hCG, 91.7% females (11 of 12) ovulated out of which 5 animals (38.5%) ovulated to 2nd GnRH and subsequently 2 animals conceived (15.38%) at the induced estrus and 7 animals (53.8%) became acyclic after treatment. In contrast to the present study, Ali and Fahmy (2007) [2] using 'Ovsynch' protocol in anestrous buffaloes recorded that 62.5% (5/8) buffaloes responded to first GnRH and 100% (8/8) responded to 2nd GnRH ultimately leading to 37.5% (3/8) conception rate. Derar *et al.,* (2012) [15] observed 100% and 88.9% ovulatory response with 62.5% and 22.7% conception rate in buffalo heifers and buffalo cows, respectively following 'Ovsynch' treatment. Similarly, a 15.0 vs. 51.4% pregnancy rate was observed in Swamp buffalo heifers v/s cows (Chaikhun *et al.,* 2010) [12] after using the 'Ovsynch' protocol with timed insemination, respectively. Parity, season and cyclic status might result in higher pregnancy rate. Low breeding season is also considered an important factor for low conception rates in buffaloes.

Baruselli *et al.,* (2001) [5] reported a lower pregnancy rate of 7% when 'Ovsynch' was used during seasonal anestrous period in buffaloes. Previous studies in cattle indicated the conception rate following 'Ovsynch' varied from 27 to 39% (Burke *et al.,* 1996; Pursley *et al.,* 1998) [33]. A very low conception rate of 4.7% (1/21) has been reported by De Rensis *et al.,* (2005) [14] after synchronized ovulation with FTAI in non-cyclic buffaloes but conception rates were significantly increased to 30% (6 of 20) when 'Ovsynch' protocol was supplemented with progesterone. In the current study the conception rate in the 'Ovsynch-Plus' and 'Modified Ovsynch-Plus' treated heifers were 30.77% and 15.38%, respectively. The overall conception rate observed in the present study was 23.1% which was superior to 9.5% reported by Karen and Darwish (2010) [22] and 6.9% Baruselli *et al.,* (2003) [4] when applied timed-AI after using 'Ovsynch' protocol during non-breeding season in buffaloes. Lower fertility response may be probably due to fixed time AI, as in the present study ovulation time was variable (ranges from -24 hrs to 48 hrs). Therefore, heifers should be monitored closely immediately after PG injection and females detected in estrus should be inseminated. Animals not detected in

estrus may be inseminated at fixed time at 12 and 24 hrs after 2nd GnRH injection.

4. Conclusion and Acknowledgement

The author is highly acknowledged for kind co-operation of ICAR-CIRB, Hisar institute staff members, for providing necessary facilities like experimental heifers and well equipped lab facilities for present investigation. From this investigation it is concluded that only those females conceived who responded by ovulation to eCG/1st GnRH/hCG and 2nd GnRH injections. Conception rate was found to be better in 'Ovsynch-Plus' treated heifers compared to that of 'Modified Ovsynch-Plus' treated heifers and beginning of the 'Ovsynch' protocol with hCG did not increase ovulation and conception rates in heifers. Thus, hCG is not a suitable replacement for the first GnRH of 'Ovsynch'. However, increasing ovulation rate in response to pre-hormonal administration of 'Ovsynch' with eCG have a significant effect on conception rate. The author is highly acknowledged for kind co-operation of ICAR-CIRB, Hisar institute staff members, for providing necessary facilities like experimental heifers and well equipped lab facilities for present investigation.

Table 1: Ovulation response of treated heifers

Sr. No.	Parameter		n	Summer n (%)	n	Winter n (%)
1.	Animals ovulated in response to eCG treatment alone		14	7 (50)	3/12	3 (25)
2.	Animals ovulated to eCG/1st GnRH/hCG injection in both treated group		14/14	14 (100)	12	11 (91.7)
3.	Animals ovulated to 2nd GnRH injection in 'Ovsynch-Plus' group		3/7	42.85	2/6	33.33
4.	Animals ovulated to 2nd GnRH injection in 'Modified Ovsynch-Plus' group		3/7	42.85	2/6	33.33
5.	Time of ovulation in response to 1st GnRH/hCG					
	(i)	- 24 hr.	7/14	50	3/12	25
	(ii)	0-24 hr.	1/14	7.14	4/12	33
	(iii)	24 hr and later	6/14	42.86	4/12	33
6.	Total CL* formed after (n)					
	(i)	'Ovsynch-Plus' treated group	7	9 (128.6)	6	11 (183.3)
	(ii)	'Modified Ovsynch-Plus' treated group	7	8 (114.3)	5	13 (260)
7.	% of animal ovulated to 2nd GnRH with in					
	(i)	-24 hr.	1/14	7.14	2/12	16.67
	(ii)	0-24 hr.	2/14	14.28	2/12	16.67
	(iii)	>24 hr and later	3/14	21.43	0	0

[8 animals did not respond during summer due to the presence either small (n = 5) or luteinised follicles (n = 3); 8 animals did not respond during winter due to the presence either persistent CL (n = 1) or small (n = 3), luteinised follicles (n = 4)]; n-number of animals.

Table 2: Ovulatory response and conception rate in treated heifers

Sr. No.	Parameter	N	Summer (%)	N	Winter (%)
1.	Animals having CL on day 7 post A.I.	6/14	42.85	4/12	33.33
2.	Animals having CL on day 14 post A.I	6/14	42.85	6/12	50
3.	Animals having CL on day 21 post A.I.	5/14	35.7	9/12	75
4.	Animals having CL on day 28 post A.I.	6/14	42.85	8/12	66.67
5.	Animals found pregnant in 'Ovsynch-Plus' treated group	1/7	14.28	3/6	50
6	Animals found pregnant in 'Modified Ovsynch-Plus' treated group	1/7	14.28	1/6	16.67
7.	Animals became acyclic in 'Ovsynch-Plus' treated group	3/7	42.85	0/6	0
8.	Animals became acyclic in 'Modified Ovsynch-Plus' treated group	5/7	71.42	2/6	33.33

*In 3 Heifers (2 in GnRH group and 1 in hCG group during the winter season, CL was observed before treatment). In winter season in 'Ovsynch-Plus' treated group, n = 3 became pregnant and n = 3 became cyclic while in 'Modified Ovsynch-Plus' treated group n = 3 became cyclic.

Table 3: Table showing response of heifers to 'Ovsynch-plus' treatment, pregnancy and cyclicity status of heifers

S. No.	Animal No.	Response to 1st GnRH/hCG	Response to 2nd GnRH	Cyclicity status of animal	Pregnancy status
1	S-2593	CL	CL	Pregnant	P
2	S-2630	CL	NR	Cyclic	NP
3	S-2677	CL	LF	Cyclic	NP
4	S-2681	CL	CL	Cyclic	NP
5	S-2696	CL	LF	Acyclic	NP
6	S-2702	CL	LF	Acyclic	NP
7	S-2713	CL	CL	Acyclic	NP
8	W-2453-O	CL	CL	Pregnant	P
9	W-2664	CL	NR	Cyclic*	NP
10	W-2820	CL	LF	Cyclic	NP
11	W-2800	CL	CL	Cyclic	NP
12	W-2867	CL	CL	Pregnant	P
13	W-2887	CL	CL	Pregnant	P

*Indicates delayed response, 1st GnRH response; CL = 13, 2nd GnRH response; CL = 7, NR = 2, LF = 4.

Table 4: Table showing response of heifers to 'Modified ovsynch-plus' treatment, pregnancy and cyclicity status of heifers

S. No.	Animal No.	Response to 1st GnRH/hCG	Response to 2nd GnRH	Cyclicity status of animal	Pregnancy status
1	S-2453	CL	LF	Acyclic	NP
2	S-2491	CL	CL	Acyclic	NP
3	S-2623	CL*	CL	Pregnant	P
4	S-2629	CL	NR	Acyclic	NP
5	S-2659	CL	NR	Acyclic	NP
6	S-2697	CL*	NR	Acyclic	NP
7	S-2744	CL	CL	Acyclic	NP
8	W-2574	CL	CL	Pregnant	P
9	W-2702	NR	LF	Acyclic	NP
10	W-2796	CL	LF*	Cyclic	NP
11	W-2800	CL	NR*	Cyclic	NP
12	W-2841	CL	LF	Acyclic	NP
13	W-2889	CL	CL	Cyclic	NP

*Indicates delayed response
1st GnRH response; CL = 12, 2nd GnRH response; CL = 5, NR = 4, LF = 4
S = Summer season; W = Winter season
P = Pregnant, NP = Non Pregnant
CL = Corpus Luteum,
NR = No Response, LF = Luteinized Follicle

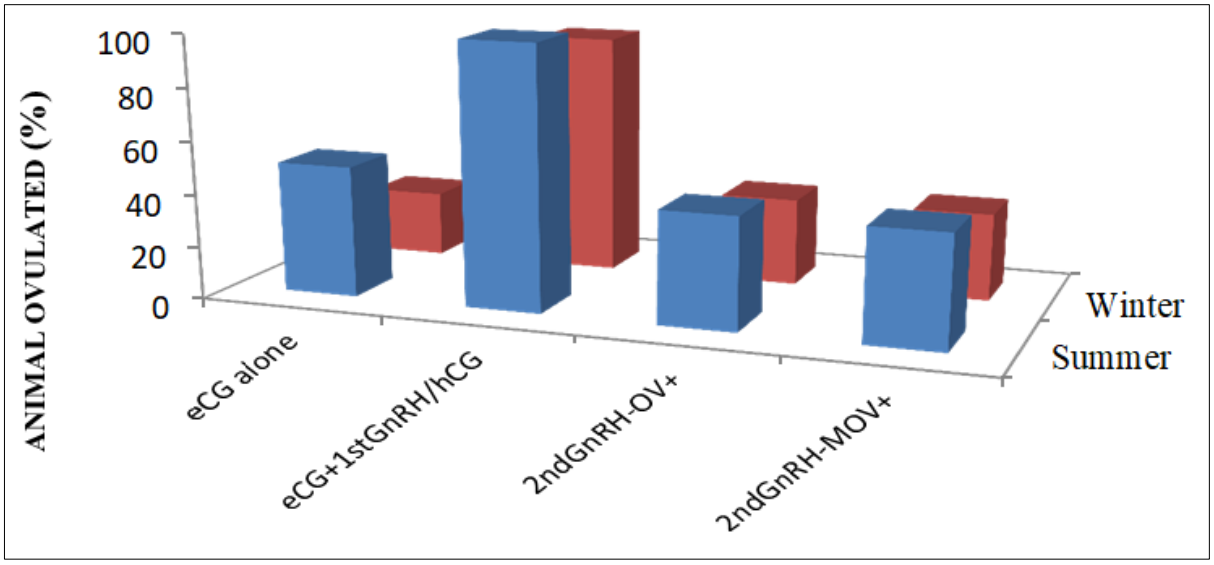
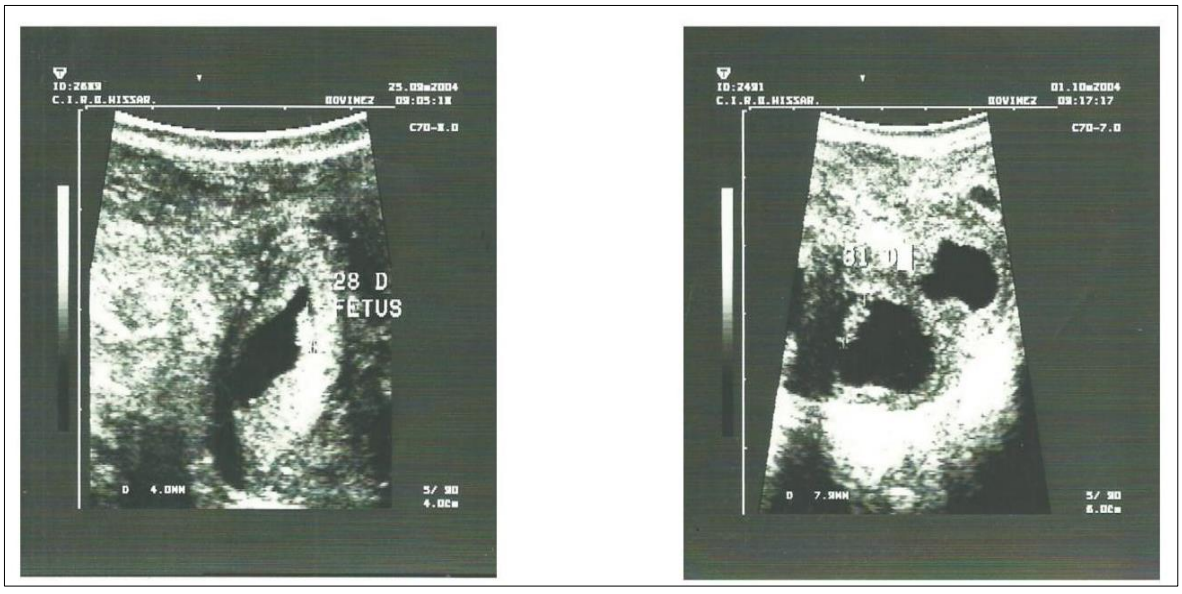


Fig 1: Percentage of animals ovulated to treatment

Ultrasonograph-1 Ultrasonograph-2



28 days foetus in pregnant Heifer 31 days foetus in pregnant Heifer

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