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The Effect of hCG or Receptal During Diestrous and Proestrous in the Synchronization, Super Ovulation Corpus Luteum (CL) Development in Buffaloes Cows

Azizi-Moghadam.Ahmad
Department of Clinical Sciences,
Faculty of Veterinary College, University of Zabol, Zabol, Iran
Email: azizi@uoz.ac.ir

Abstract **– This study was managed to evaluate synchronization, super-ovulatory responses, corpus luteum (CL) development in buffaloes. Hundred -three cyclic, non lactating buffaloes (Murrah) were hCG versus 2.5 ml of receptal (Hoechst) containing 0.01mg/2.5ml Buserelin injected intra muscularly (Gn-RH) during diestrous and proestrous. In Experiment 1, 55 buffaloes received a vaginal sponge of a 3 mg synthetic progestogene, norgestomet for 14 d to achieved estrus synchronization (day 0), with PG $F_{2\alpha}$ after vaginal sponge removal. 5 days after vaginal sponge insertion, all follicles ≥10 mm were ruptured, 14 days after vaginal insertion, buffaloes injected either with 3, 4,5 cc hCG or 2.5 ml of receptal. On day 14, The ovulatory response to5 cc hCG (82%) or 100 µgGnRH (70%) compared to 3cc hCG (31%) was showing significantly greater response, but not significantly different from that of 4cc hCG (56%). In Experiment 2, days 7after after estrus signs, those buffaloes injected with PGF, and, 36 hr later with hCG or receptal (as in Experiment 1). Buffaloes presented in both experiments I and II were received 75 NIH units of Super-Ov in a descending dose schedule spread over the first 3 days (twice daily) after ovulation (day 0).**

The ovulation time was most variable among buffaloes given3cc hCG compared to 4, 5 cc hCG or 2.5 ml receptal; only 50% of 5 cc hCG ($P < 0.05$) injected buffaloes ovulated during the initial 24 hr of estrous sign. Buffaloes injected with 5 cc hCG or 2.5 ml of receptal had larger CL compared to those injected with 3cc hCG . This study concluded that, the diestrous buffalo cows injected with 5 cc hCG their ovulatory response did not differ from that of those injected with 2.5 ml of receptal. Proestrous buffaloes given 5 cc hCG or 2.5 ml of receptal, their ovaries showing larger CL compared to those injected with 3cc hCG. Immediately after first mating each buffalo cow was administered either with 2.5 mL of receptal or 3, 4, 5 cc of hCG to ensure that the buffaloes ovulated. Non surgically embryos collection was done on Days 6 and 7 after buffaloes showing estrus signs (Day0) and then collected embryos were subjected to comparative studies. A majority of buffaloes cows (19/24) exhibited estrus within 8 hr after vaginal sponge removal . The adverse affect of heat stress on the number of CL, un-ovulated follicles and overall ovarian activity, estrus behavior, super ovulation and embryos quality for summer and winter seasons were recorded for buffaloes during the summer and winter seasons.

Keywords **– Corpus Luteum, hCG, Ovulation, Receptal.**

I. INTRODUCTION

The buffalo reproductive system showing low performance. The methods used inthe recent days for estrous synchronization in buffaloes introduced by[1] . [2] inferred that,efficacy of synchronize estrus in water buffalo cows (bubalus bubails) is dependent upon corpus luteum size. The two-dose regime of prostaglandin can overcomes some of the limitations of manipulation of multiple ovulation to achieve better synchrony and super ovulatory rate. Therefore, in recent days a new methods introduced for synchronization of ovulation and super ovulation in Murrah buffaloes (*Bubalusbubalis*), including GnRH or gonadotrophins,hCG; which can be successful in achieving higher Conception rate. The initial success of Super ovulation and non-surgical embryo collection and embryo transfer in riverine buffalo reported 1st time in the USA [3] was followed by the birth of buffalo calves in Bulgaria [4] and India [5]. In 1991, a riverine buffalo calf (2n = 50) was born out of transfer in a swamp buffalo recipient (2n = 48) [6]. These initial successes were transfers of embryos derived in vivo.

The low ovulatory response among treated animals remains the greatest impediment to the use of super-ovulation in an embryo transfer system for water buffaloes. Attempts to apply in vitro maturation and in vitro fertilization (IVM/IVF as part of the buffalo embryo transfer is a recent development. Indeed, the first in vitro-derived embryo calf was born only in 1991 [7].

In real sense, the status of super ovulation and embryo transfer and other advanced reproductive technologies in buffalo in many countries have confirmed that buffaloes have lower super ovulatory response a further limitation appears to be the relatively low rate of transfer of oocytes / embryos in the reproductive tract [8]. The most current protocols include FSH (Super-Ov) with prostaglandins, for successful synchronizing and super ovulation in buffaloes[9] ,but earlier synchronization of estrus have been reviewed in buffaloes reported earlier by [10]. Similarly, super ovulation using either PMSG or FSH , much more earlier have been established in buffaloes and reported by [11]. These works has shown that, the superovulatory response in the buffalo was quite variable and differed with ovarian status, season, age and the stage of the cycle at which administration of exogenous hormones was initiated [12],[13], [14]. More recently, synchronization protocols with fixed time inseminations in buffaloes have been shown to yield good results [10].

Therefore for commercial application in buffalo getting help from biotechnologies is widely feel. Recently a considerable scientific efforts to improve fertility rate and increased reproductive efficiency in buffaloes suggested that the rate of transferable embryo yield remains poor ([15],[16], [17], [18],[19].

Various authors have reported the use of PGF_2ð or one of its potent synthetic analogues in estrus control in buffaloes, often using an 11-day interval between two consecutives doses [20]; or in combination with GnRH for pre-determined [21]. [22] can improve synchronization efficiency.

The luteolytic effects of a small dose of cloprostenol (100 ug) administered by the intra vulva route in river buffaloes was also reported by [23]. They found this dose to be as effective as the larger one (500 ug) given by intramuscular injection. The decline in progesterone concentration and the onset of estrus after prostaglandin treatment was found to be slower in buffaloes treated by the intra vulva sub-mucosal route (8 mg dose) compared with those injected intramuscularly (25 mg dose) [24].

In estrus control, and more particularly, in the treatment of anestrus in buffaloes, intra vaginal devices impregnated with progesterone (PRIDs and CIDRs) and ear implants impregnated with a potent progestagen (norgestomet) have been widely used by [25], [26], [27].

Therefore, the current study of synchronization and super ovulation in buffalo confirmed, that buffaloes have lower synchrony and super ovulatory response attributed mainly to the smaller population of recruited follicles in the ovary. Therefore, the aim of the present study was to use synchronization , super ovulation , non invasive method of embryo collection in buffalo to overcome these bottlenecks.

II. MATERIALS

This study was conducted at the Hissar buffaloes centre , Haryana and Punjab agricultural University ,India with cooperation with the Islamic Republic of Iran as a joint venture with the University of Zabol, College of veterinary sciences. These buffaloes were non pregnant, non lactating, healthy buffaloes (Murrah buffaloes) ,achieved synchronized ovulation (day 0 = date of ovulation) then received a total dose of 75 NIH units of FSH (Super-Ov, AUSA International, U.S.A.) beginning on day 1 twice daily at 12 hr interval for three consecutive days according to the following dosage regimen: 16,16, 12, 12, 9.5, 9.5 NIH FSH (Super-Ov, AUSA International, U.S.A.) After 36 hs, all follicles larger than 5 mm were aspirated (day 0) by trans-vaginal ultrasound-guided procedure (Tokyo, Japan), with an attached 18-gauge stainless steel needle was used for the aspiration of the follicles. Ultrasound scanning of the ovaries was done with ultrasound scanner (Tokyo, Japan) 72 hr after synchronization and super-ovulation.

Experiment 1

Fifty-five cyclic, non lactating buffaloes (Murrah) were given vaginal sponge insertion(17α-aceto-methyl19-nor preg-4-en-3,20 dione) containing3 mg synthetic progestogene, norgestomet for 14 d, and 2.5 ml PGF2α was administered , at time of sponge removal. Estrus detection was done for 60 min 3 times daily with the aid of Murrah Bull heat detector. On Day 5 (estrus = day 0), follicles larger than 8 mm in diameter were ablated by a trans vaginal ultrasound-guided procedure in all buffaloes to synchronize follicular wave emergence. On day 14 (diestrous phase), when 5- 6-days-old growing dominant follicle was expected, buffaloes were allocated randomly to one of four groups to receive 3, 4 ,5 cc hCG or 0.01mg/2.5ml Buserelin (Receptal),Hoechst company. Trans rectal ultra sonography was performed at 72 hr post to 1st mating , to seen pre ovulatory (diameter ≥ 10 mm) follicle. Fifty five non lactating buffaloes were given vaginal sponge of a 3 mg synthetic progestogene, norgestomet. for 14 d. Estrus was detected and on day 5 (estrous = day 0), ultrasound-guided follicular ablation was performed to synchronize follicular wave emergence.

Day 14, buffaloes were randomly selected to one of four treatment) groups to receive 3, 4, 5 cc or 100 µg;2.5 ml receptal. Trans rectal sonography (U/S) was performed at 72 hr after 1st mating to determine ovulation.

- Perior to removal of the vaginal sponge , 75 IU of FSH-P(Super Ov ;Ausa International ,USA) was administered to each buffaloes according to the following schedule.
  Day -2: 2×16 IU at 12 hr interval.
  Day -1:2×12 IU at 12 hr interval.
  Day 0:2×9. 5 IU at 12 hr interval.
- The vaginal sponge was removed on the morning of day 0, before the last injection of FSH-P.
- Eight hours after vaginal sponge removal , buffaloes were observed for estrus signs by teaser buffaloes bulls.
- Upon detection of estrous , cows were allowed two mating with buffaloes bulls.
- After first mating ,each buffaloes was administered 2.5 mL receptal containing 0.01 mg of Buserelin to ensure that the buffaloes ovulated.

Non invading flushing method of embryos

All buffaloes s were flushed non surgically with a silicone specific Catheter to allow the oviducts and uterine horns to be flushed on Day6 and Day 7 post mating,for winter and summer season) using Dulbecco's Phosphate Buffer Salines as the flushing medium . In general , on Day 6 and Day 7 post mating both oviducts as well as the uterine horns were flushed. All synchronization and super ovulatory responses in buffaloes were recorded as described previously by[28].

Sonography was performed on alternate days, from day 12 to 24 (2 to 14 d after ovulation) to determine CL diameter and on days 14, 16 and 22 for pixel intensity.

Measurement of CL diameter, were done on days 14, 16, and 22 (4, 6, and 12 d after ovulation).

Experiment 2

Forty eight cyclic non lactating buffaloes (Murrah) were given vaginal sponge insertion(17α-aceto-methyl19-nor preg-4-en-3,20 dione) containing3 mg synthetic progestogene, norgestomet for 14 d ,followed by twice injection with 2.5 ml PGF2α at the termination of Experiment 1(day=0) and 10 d later, a second PGF2α, estrus detection (estrous = day 0) was performed as described in Experiment 1. PGF2α treated buffaloes (proestrous phase), were allocated to one of four groups to receive3, 4, or 5 cc hCG or 100 µg ,2.5 ml receptal.

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Cyclic, non lactating buffaloes were treated with PGF2α twice (10 d apart) were given a third PGF2α injection 7 d after estrus. Thirty-six hours later, buffaloes were allocated to one of four treatment groups to receive either 8, 4, or 5 cc hCG or 2.5 ml receptal. Sonography (U/S) was d at 72 hr after 1st mating to determine ovulation.

Perior to removal of the vaginal sponge, 75 IU of FSH-P(Super Ov ;Ausa International, USA) was administered to each buffaloes according to the following

Day -2: 2×16 IU at 12 hr interval.
Day -1:2×12 IU at 12 hr interval.
Dy 0:2×9. 5 IU at 12 hr interval.

- The vaginal sponge was removed on the morning of day 0, before the last injection of FSH-P.
- Eight hours after vaginal sponge removal , buffaloes were observed for estrus signs by teaser buffaloes bulls
- Upon detection of estrous, cows were allowed two mating with buffaloes bulls.
- After first mating each buffaloes was administered 2.5 mL receptal containing 0.01 mg of Buserelin to ensure that the buffaloes ovulated.

All buffaloes s were flushed non surgically with a silicone specific Catheter to allow the oviducts and uterine horns to be flushed on Day6 and Day 7 post mating,for winter and summer season) using Dulbecco's Phosphate Buffer Salines as the flushing medium . In general, on Day 6 and Day 7 post mating both oviducts as well as the uterine horns were flushed. All synchronization and super ovulatory responses in buffaloes were recorded as described previously by [28].

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Measurement of CL diameter, were done on days 14, 16, and 22 (4, 6, and 12 d after ovulation).

The statistical analysis of data were made by using the methods described by [29].

III. Results

Experiment 1

The proportion of buffaloes ovulated after injection with either 5 cc hCG (82%) or 2.5 ml receptal (70%) were showing greater significant compared to those after injection with 3 cc hCG (31%). Ovulatory response to injection with 4cc hCG (56%) tended to be lower ($P < 0.07$) than those injected with 5 cc hCG .

In the present study, irrespective of seasons, majority of the buffaloes cows in Experiment 1,exhibited estrus 24 hr after vaginal sponge removal and in the summer season only 1 buffaloes cows exhibited estrus at 12 hr and 9 buffaloes at 48 hr after vaginal sponge removal).

Although CL diameter (mm) was larger among buffaloes injected with 5 cc hCG , 2.5 ml receptal (20.20 ± 2.60 mm) compared to those injected with 3cc hCG (12.10 ± 2.70 mm), it did not differ from those injected with 4cc hCG (16.8 ± 2.30 mm).

There was no significant correlations between pre ovulatory follicle diameter and CL diameter and there was no significant effect of type of injection, time, or time by treatment interaction for heterogeneity of the CL diameter. Irrespective of the seasons, majority of buffaloes exhibited estrus 24 hr after vaginal sponge removal, in summer season only 1 buffaloes exhibited estrus at 12 hr and 11 at 48 hr after implant removal).No significant differences were observed within experiments 1 and 2 except in summer season which exhibited heat 48 hr after vaginal sponge removal compare with winter season.

The total number of ovulation, C.L development following super ovulation and total number of embryos recovery for the right ovary and uterine horn compared with the left ovary and uterine horn were much higher non significantly and it was just similar to the observation inferred by other scientists in other species of domestic animals .All results found to be more pronounce in winter season compared to summer season. All results found to be more pronounce in winter season compared to summer season, When different lines of injection with hCG and receptal were follows after 1st mating to enhanced super ovulatory responses, no significant differences were observed in either experiments 1.

Table I: Occurrence of estrus in buffaloes cows following super ovulation in Experiment 1

S. No	Seasons	No. of Donors	Hours to estrus following implant withdrawal					Interval to estrus(h)	Duration of estrus (h)
			12	24	36	48	60		
1	Winter Season	30	0	28	2	0	0	24.00±24.00	20.75±1.16
2	Summer Season	25	1	14	0	9	0	31.00±5.00	16.00±4.05

Table II: Ovarian response of buffaloes cows following super ovulation during the winter season in Experiment 1

Number of animals			Corpora Lutea			Unovulated follicles			Overall Ovarian activity
FSH-P Treated	Right Ovary	Left Ovary	Total	Right Ovary	Left Ovary	Total	Right Ovary	Left Ovary	Total
30	95	64	159	54	27	81	149	91	240
Mean±se	3. 60 ±0.70	2.1 0±0.33	5.30±0.15	0.98±0.19	0.49±0.36	1.47±0.56	4.o9±0.66	3.03±0.33	8.00±0.01

Table III: Ovarian response of buffaloes cows following super ovulation during the summer season in Experiment 1.

Number of animals			Corpora Lutea			Unovulated follicles			Overall Ovarian activity
FSH-P Treated	Right Ovary	Left Ovary	Total	Right Ovary	Left Ovary	Total	Right Ovary	Left Ovary	Total
25	82	54	136	34	10	44	116	64	180
Mean±se	3.02±0.08	2. 1±0.06	6.02±1.53	0.72±0.27	0.18±0.10	0.90±0.34	4.64±0.04	2. 56±0.02	7.20±0.10

Table IV: Embryo recovery, quality and developmental stages in super ovulated buffaloes cows during the winter season in Experiment 1

Number of animals	Day of flushing	Ovulations	Embryos Recovered	Developmental stages R.24.8.16.M.B	Transferable embryos	Degenerated embryos
15	6	82	33	-.--.-.21.12.-.-	33	
Total Mean±SE		82 5.46±0.66	33 2.20±0.10	-.-.-.21.12.-.-	33 2.20±0.10	-
15	7	34	16	.-.-.-.9.6.1	16	

Table V: Embryo recovery, quality and developmental stages in super ovulated buffaloes cows during the summer season in Experiment 1

Number of animals	Day of flushing	Ovulations	Embryos Recovered	Developmental stages R.24.8.16.M.B	Transferable embryos	Degenerated embryos
12	6	65	26	-.--.-.18.8.-.-	26	
Total Mean±SE		65 5.41±0.66	26 1.03±0.33	-.-.-.18.8.-.-	26 1.03±0.33	-
13	7	43	22	1.-.-.-.15.4.2	21	1

Experiment 2

No significant differences were observed among treatment groups for either plasma P4 concentration during proestrous or pre ovulatory follicle diameter (14.3 ± 0.6 mm,). All buffaloes ovulated after treatment. The mean interval from synchronization to ovulation followed by injection either with hCG or receptal after 1st mating did not differ among injected groups ,but was most significant variable in buffaloes injected with 3 3cc hCG compared with for 5, 4cc hCG and or 2.5 ml receptal, respectively. Buffaloes ovulating within 72 hr after synchronization followed with injection either with 3, 4, or 5 cc hCG or 2.5 ml receptal (12 buffaloes / group) after 1st mating ,mean interval after synchronization followed with injection either with hCG or 2.5 ml receptal up to ovulation did not differ among injected groups but was most significantly variable among buffaloes injected with 3 cc hCG . During the initial 27-hr period, ovulation percentage was lower among buffaloes injected with 3cc hCG compare to those injected with 5 cc hCG.

Although CL diameter (mm) was larger among buffaloes injected with 5 cc hCG , 2.5 ml receptal (20.20 ± 2.60 mm) compared to those injected with 3cc hCG (12.10 ± 2.70 mm), it did not differ from those injected with 4cc hCG (16.8 ± 2.30 mm).

There was no significant correlations between pre ovulatory follicle diameter and CL diameter and there was no significant effect of type of injection, time, or time by treatment interaction for heterogeneity of the CL diameter.

Irrespective of the seasons, majority of buffaloes exhibited estrus 24 hr after vaginal sponge removal, in summer season only 1 buffaloes exhibited estrus at 12 hr and 11 at 48 hr after implant removal).No significant differences were observed within experiments 1 and 2 except in summer season which exhibited heat 48 hr after vaginal sponge removal compare with winter season. As it was seen in other species of domestic animals in this study also right ovary shows more activity compared to the left ovary. The total number of ovulation, C.L development following super ovulation and total number of embryos recovery for the right ovary and uterine horn compared with the left ovary and uterine horn were much higher non significantly and it was just similar to the observation inferred by other scientists in other species of domestic animals. All results found to be more pronounce in winter season compared to summer season, When different lines of injection with hCG and receptal were follows after 1st mating to enhanced super ovulatory responses, no significant differences were observed in either experiments 1 and2. At the last ,if any degenerated or retarded embryos were harvested , it was seen during summer season, so it is showing suppressing effect of summer season on reproductive performance of buffalo cows .

Table VI: Occurrence of estrus in buffaloes cows following super ovulation in Experiment 2.

S. No	Seasons	No. of Donors	Hours to estrus following implant withdrawal					Interval to estrus(h)	Duration of estrus (h)
			12	24	36	48	60		
1	Winter Season	24	0	20	3	1	0	26.00±4.00	20.00±2.18
2	Summer Season	24	1	13	0	11	0	33.00±8.00	17.75±4.05

Table VII: Ovarian response of buffaloes cows following super ovulation during the winter season in Experiment 2

Number of animals			Corpora Lutea			Unovulated follicles			Overall Ovarian activity
FSH-P Treated	Right Ovary	Left Ovary	Total	Right Ovary	Left Ovary	Total	Right Ovary	Left Ovary	Total
48	99	68	167	55	26	81	154	94	248
Mean±SE	2.06 ±0.25	1.41±0.66	3.47±0.91	1.14±0.44	0.47±0.16	1.62±0.61	3.28±0.33	0.64±0.58	5.18±0.75

Table VIII: Ovarian response of buffaloes cows following super ovulation during the summer season in Experiment 2

Number of animals			Corpora Lutea			Unovulated follicles			Overall Ovarian activity
FSH-P Treated	Right Ovary	Left Ovary	Total	Right Ovary	Left Ovary	Total	Right Ovary	Left Ovary	Total
48	82	54	136	30	9	39	112	63	175
Mean±se	1.70±0.33	1.12±0.50	2.08±0.33	0.64±0.28	0.18±0.36	0.82±0.34	20±0.33	1.31±0.83	3.64±0.58

Table IX: Embryo recovery, quality and developmental stages in super ovulated buffaloes cows during the winter season in Experiment 2

Number of animals	Day of flushing	Ovulations	Embryos Recovered	Developmental stages R.24.8.16.M.B	Transferable embryos	Degenerated embryos
12	6	85	28	-.--.-.19.9.-.-	28	
Total		85	28	-.-.-.19.9.-.-	28	-
Mean±SE		7.08±3.33	2.33±3.33		2.33±3.33	
12	7	46	22	-.-.-.-.16.4.2	22	
Total	46	22	22	-.-.-.-.16.4.2		
Mean±SE	3.83±3.33	1.83±3.33		1.83±3.33		

Table X: Embryo recovery, quality and developmental stages in super ovulated buffaloes cows during the summer season in Experiment 2

Number of animals	Day of flushing	Ovulations	Embryos Recovered	Developmental stages R.24.8.16.M.B	Transferable embryos	Degenerated embryos
12	4	64	34	-.--.-.21.12.-.-	34	
Total		64	34	-.-.-.21.12.-.-	34	-
Mean±SE		5.25±0.83	2.75±0.83		2.75±0.83	
12	6	34	16	-.-.-.-.8.7.1	16	
Total	34	16	-.-.-.-.8.7.1	16		
Mean±SE	2.83±0.83	1.33±0.33		1.33±0.33		

IV. Discussion

The hypothesis revealed that buffaloes with hCG, even at lower doses, increase ovulatory response and CL function compared to those treated with receptal was not supported by this research results. In this study, the ovulatory response in buffaloes to 4or 5 cc hCG injection after 1st mating did not differ from that to receptal injection. It could be due to decreased responsiveness affect of the anterior pituitary to receptal, suppressing CL

function and decrease ovulation rate, so in this research ,buffaloes injected with receptal were expected to have suppressed CL function and reduced ovulatory response, but did not occurred so . Conversely, we expected that the ovulatory response of buffaloes treated with hCG would not be affected by CL function, because hCG acts directly at the ovarian level. Earlier studies in buffaloes inferred by [14] reported decreased superovulatory response when FSH treatment was initiated in the presence of dominant follicle which is suggested to have inhibitory effect on the other follicles of the wave. The differences in the superovulatory response may be due to the individual variability. This difference can be related to the size of corpus luteum and follicular status before the administration of PGF2α [2]. In our study, lower number of corpora lutea were comparable with those reported earlier in buffaloes [12], [30]. Estrus synchronization programs improve reproduction efficiency by reducing the length of breeding and calving seasons and increasing calf weaning weights.

The major limiting factor for optimum reproductive performance in buffalo is failure to detect estrus in a timely and accurate manner. A number of controlled or breeding programs have been developed for synchronization can be directed to buffaloes that pass a corpus luteum test as determined by rectal palpation of the ovaries and for further administering PGF2α to these animals. An important requirement in this study was the presence of functional CL as directed by an expert veterinary officer. It was observed that all the buffaloes were in heat 72hr post PGF2α injection.

For this protocol, rectal palpation was done to assess ovarian status (presence of CL). The, hCG, GnRH injection stimulates the release of luteinizing hormone which causes the follicle to ovulate and synchronize ovulation. For testing protocols and adopting a breeding program one must think of drug cost and anticipated success rate. This study demonstrates accuracy of palpation of functional CL resulting majority of buffaloes showing heat signs 72h post PGF2α injection. Benefits of any synchronization program will be obtained only through a systematic, timely and efficient management of events related to reproduction.

In the current study, the ovulatory response of diestrous buffaloes injected with 5 cc hCG did not differ from those injected with 2.5 ml receptal and had greater ovulation rates compared to those injected with 3cc hCG , whereas buffaloes injected with 4cc gave intermediate results. Thus, we concluded that 3cc hCG did not reliably induce ovulation in diestrous buffaloes.

The main reason for synchronizing estrus was to facilitate use of AI. Till date in a place like India effectiveness of current estrus synchronization strategies is limited, because they rely on visual estrus detection, which is inefficient under field conditions, hence accurate timing for AI is not possible, which leads to decrease conception rate. Since, the veterinary officer was an expert and availability of an able stock man, at the time period for hormone administration and fixed time insemination and drug administration was strictly followed, a pregnancy rate increased for buffaloes species is expected for this study. In this study, buffaloes heat detection was done by bull detector and instead AI, the current study used natural mating, yet acceptable results for pregnancy rates to first service in buffaloes were obtained (50-56%). In addition, the CL function after the 5 cc hCG injection was higher compared to other concentration treatments either injected 2.5 - 9 hr post 1st mating or up to 10 hr post 1st mating, and not returning to basal levels by the end of the collection period. Even with lower doses of hCG (4or3cc), CL function remained active for a considerably longer interval compared to those after receptal administration.

[10] Reported 60% ovulation rate, when receptal was used in postpartum buffaloes. Interval period after receptal administration to ovulation was36 hr in another study in buffaloes, which reported by [10]. Therefore, it appears that receptal-PGF2α protocol can synchronize anoestrous buffaloes; however, regular heat detection is necessary.

In another protocol [10] reported ovulation synchronization effect of GnRH-PGF2α-Gn-RH in buffaloes, where difficulty of heat detection has been overcome when fixed time insemination was preferred. Brito *et al*, (2002) inferred that, the CL in buffaloes may be more developed by nine days after 1st mating followed by Gn-RH administration, as its sensitivity to luteolytic effects of PGF2α increases with time.

Administration of GnRH causes larger follicles to ovulate and a new follicular wave emergence take place within 3 to 4 days after treatment with Gn-RH at any stage of the estrous cycle . The large follicle of this wave becomes ovulatory follicle after PGF2α induced luteolysis.

Buffaloes in Experiment 2 ovulated, regardless of treatments with hCG or receptal , the authors suggested that buffalo heifers treated with 5 cc hCG may have shown an earlier emergence of CL formation. Furthermore, pregnancy rate after first time mating was numerically lower with hCG injection in buffaloes heifers compared to those subjected to 2.5 ml receptal injection. In this protocol with 5 cc hCG injection after 1st mating pregnancy rates improved significantly ,although an extended hCG exposure may have enh hCG anced oocyte maturation, fertilization, and embryo survival effects remain to be investigated in a protocol proposed in near future .but showing a strong correlation between the diameter of CL and hCG administration during luteal development in buffaloes heifers. However, the size of CL were differ among synchronization followed by treatment groups either by injection with hCG and or receptal , study suggested it was probably due to larger size of CL.

This study concluded , buffaloes injected with 5 cc hCG or 2.5 ml receptal showing more pronounce ovulations compared with 3cc hCG injection , whereas 4cc hCG gave intermediate responses. Buffaloes injected with 5 cc hCG or 2.5 ml receptal during proestrous had more synchronous ovulatory response and increased CL diameter compared to those injected with 3cc hCG . A synchronization treatment followed by injection with 4 cc hCG after 1st mating resulted in pronounce synchronous ovulation rate. This study concluded a low dose (3cc) of hCG was clearly inadequate for the induction of ovulation during diestrous

in non lactating dairy buffalo. In other words , reduced doses of hCG (3or4cc) was not as effective as either with 4, 5 cc hCG or 2.5 ml receptal to synchronize ovulations, particularly during diestrous in mature buffaloes. Therefore, using reduced doses (<3cc) of hCG for this protocol is not recommended. As known , the ovarian follicular growth during the estrous cycle in buffalo is similar to that observed in cattle, is characterized by waves of follicular recruitment, growth and regression.

This study clearly showed that follicular recruitment occurred when synchronized ovulation take placed. In this study, however the total number of growing follicles at days 6 and 7 were higher, the number of small follicles decreased in both groups from day 6 to day 7, and the number of large follicles increased at day 7. These results indicated that the follicular growth from small to middle and from middle to larger size were up at an early period of the follicular wave. Improved follicular growth may be due to the stimulation under Super-Ov administration. Follicle rupture enhances endogenous release of FSH and increased endogenous release of FSH may overlap with the exogenous FSH, together make the concentration of the plasma FSH higher .According to the present data, the number of recruited follicles and C.L formation was expected to increase as a result of endogenous increase of FSH after follicle rupture, which acted concomitantly with the exogenous FSH to produce such an effect. Studies by [31], [32], [33] have confirmed that buffaloes have lower super ovulatory response, attributed mainly due to smaller population of recruited follicles in the buffaloes ovary and same was occurred in this study, which inferred a lower embryo recovery rate.

Although improved protocols of estrous synchronization and super ovulation can overcome these problems, there are many other factors such as nutritional status, seasonality and reproductive management that need to be addressed to achieve success. Embryo technologies that include synchronization and super ovulation have been vigorously studied over the past two decades, but the success rates remain below that achieved in cattle, because of many inherent biological features that are unique to the buffalo. Once the technological problems are overcome, the successful practical application of these methods will need to be preceded by measure to overcome the managerial and nutritional causes of infertility that are common in the majority of current buffalo farming systems as well as for this research farming system

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