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Response of Anestrus Rural Buffaloes (*Bubalus bubalis*) to Intravaginal Progesterone Implant and $PGF_{2}\alpha$ Injection in Summer

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Abstract

An investigation was conducted to study the genital condition and response of intravaginal progesterone implant with and without administration of $PGF_{2}\alpha$ in anestrus rural buffaloes during summer (April - June, 2001) in tropical climate. The ovarian status of 40 lactating (1st to 4th lactation) noncycling buffaloes and 15 noncycling buffalo heifers (3.5 to 5.0 years of age) maintained under village management condition were examined per rectum. Inactive genitalia with [12] and without [28] palpable corpus luteum (CL) were detected on per rectum examination in buffalo cows. The $PGF_{2}\alpha$-injection caused luteolysis and induction of estrus in 83% buffaloes having distinct corpus luteum. Implantation of progesterone in form of CIDR (Controlled Internal Drug Release device) intravaginally for 10 to 14 days induced resumption of estrus among 83% of anestrus buffaloes within 12 to 120 h after implant removal. Of those detected estrus about 80% of buffaloes conceived following natural service. Implantation of CIDR in combination with intramuscular injection of $PGF_{2}\alpha$ was more effective than CIDR alone in terms of exhibition of estrus and conception rate. Long term of 10 to 14 days CIDR implant was superior to short term of 8 days in terms of resumption of estrous cyclicity. The observation suggests that during summer about 30% of rural buffaloes were cycling but due to lack of estrus detection they were declared anestrus, though they were having distinct corpus luteum on ovary. The summer sterility can be overcome by raising progesterone level for 10 to 14 days in buffaloes under rural managemental condition.

Key words: anestrus, buffaloes, progesterone, $PGF_{2}\alpha$

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Introduction

Breeding of dairy animals throughout the year plays a vital role for maintaining dairy as a viable unit. Buffaloes being a predominant milk producing animals suffer from a great limitation of long prepubertal period, prolonged calving interval, seasonal nature of breeding cycle and silent estrus. The summer anestrus in buffaloes are due to lower circulating concentration of hypophyseal and gonadal hormone [14, 19] and inherently suboptimal functioning of hypothalamo-hypophyseal and gonadal axis [17]. The elevation of hypophyseal and gonadal hormone before the resumption of ovarian function at onset of rainy season [14, 19] suggests that elevated levels of these hormones are essential for resumption of ovarian cyclicity in buffaloes. The present experiment was conducted on rural buffaloes to develop a schedule of prostaglandin and progesterone treatment with a view to bring the buffaloes in estrous cyclicity even during unfavourable summer season with sole aim to breed the buffaloes throughout the year.

Materials and Methods

Selection of animals

The present experiment was conducted on rural buffaloes during summer season (April to June, 2001) when ambient temperature and relative humidity were ranging respectively from 36℃ to 42℃ and 27% to 90%. A total of 40 anestrus river buffalo cows (*Bubalus bubalis*) of 1st to 4th lactation that had not detected in estrus since 10 to 18 months after calving (producing 2 to 4 kg milk/day) and 12 buffalo heifers of 3.5 to 5 years of age (late maturing) maintained in rural areas within the radius of 15 km from capital town (Patna) of Bihar, India were included in the experiment. Their genitalia were examined per rectum. Twelve buffaloes were having palpable corpus luteum (CL) while the ovaries of 28 buffaloes were smooth and without CL. The ovaries of buffalo heifers were without CL.

Treatment schedule

The animals were covered for treatment with different treatment resimens consisting of $PGF_{2}\alpha$ and progesterone in two phases (Phase I and II).

Phase I: Six buffaloes with CL, six buffaloes without CL and six buffalo heifers (without CL) were administered $PGF_2\alpha$ (Prosolvin, 15 mg) intramuscularly (treatment group). Control group consisted of six buffaloes with CL, six buffaloes without CL and six heifers without CL. They were injected with saline solution (2 ml, im). Ten days after normal saline injection six buffaloes (with CL) of control group were again administered $PGF_2\alpha$ (Prosolvin, 15 mg). The animals of both groups were watched closely and their genitalia were examined daily for detection of estrus. Buffaloes detected in estrus were allowed for natural service.

Phase II: Thirty four buffalo cows and 12 buffalo heifers were treated with prostaglandin and progesterone in different combinations and duration allocating the animals randomly in seven different groups. $PGF_2\alpha$ (Lutalyse, 25 mg, Pharmacia and Upjohn), progesterone in the form of EASY-BREED-CIDR having 1.9 gm progesterone manufactured by M/S Inter Ag., New Zealand was used between 9 a.m. and 11 a.m. in this phase constituting following groups:

Group I (10 buffaloes)
- Day 0: CI¡DR was implanted intravaginally.
- Day 14: CIDR was removed.

Group II (6 buffaloes)
- Day 0: CIDR was implanted intravaginally.
- Day 12: $PGF_2\alpha$ was injected intramuscularly.
- Day 14: CIDR was removed.

Group III (6 buffaloes)
- Day 0: CIDR was implanted intravaginally.
- Day 10: $PGF_2\alpha$ injected intramuscularly.
- Day 12: CIDR was removed.

Group IV (6 buffaloes)
- Day 0: CIDR was implanted intravaginally.
- Day 8: $PGF_2\alpha$ was injected intramuscularly.
- Day 10: CIDR was removed.

Group V (6 buffaloes)
- Day 0: CIDR was inplanted intravaginally.
- Day 6: $PGF_2\alpha$ was injected intramuscularly.
- Day 8: CIDR was removed.

Group VI (6 heifers)
- Day 0: CIDR was implanted intravaginally.
- Day 14: CIDR was removed.

Group VII (6 heifers)
- Day 0: CIDR was implanted intraveginally.
- Day 12: $PGF_2\alpha$ was injected intramuscularly.
- Day 14: CIDR was removed.

All animals were watched closely for change in external genitalia after CIDR removal. Internal genitalia of each animal was examined per rectum at 24 h interval after removal of CIDR. The animals detected in estrus were allowed for natural service. Those which did not exhibited behavioural estrus were adviced for fixed time natural service between 50 and 55 h after CIDR removal.

The estrus behavior was classified as strong, moderate and weak. Those exibiting mounting behavior and bellowing along with other genital changes such as vaginal mucus discharge, congested vaginal mucus membrane, oedematous and enlarged vulva and vagina, uterine tone and increased frequency of micturition were classified as strong response. The moderate behavior estrus symptom consisted of vaginal mucus discharge, congested vaginal mucus membrane, oedematous and enlarged vulva and vagina, increased frequency of micturition and presence of uterine tone, while just presence of uterine tone, some congestion in vaginal mucus membrane was considered as weak estrus symptom.

Analysis of variance and *t*-test was done to see the difference and interval of response to $PGF_2\alpha$ and CIDR treatment between Phases and different groups of same phase [25].

Results and Discussion

Out of 40 anestrus buffaloes 12 (30%) were having CL while 28 (70%) were without CL. The ovaries of such buffaloes (without CL) were apparently smaller than those of buffaloes with distinct CL. About 75% (9) of CL was detected on the right while 25% (3) on the left ovaries (Table 1). Prostaglandin injection caused luteolysis in all buffaloes within 48 to 72 hours. Growing palapable follicles on ovaries were detected on day 3 (72 h) after $PGF_2\alpha$ injection in 5 (83%) buffaloes with CL, two (33%) buffaloes without CL and in one (8%) heifer. Exhibition of the strong estrus with developed follicle on both right (6) and left (2) ovaries within 96 to 144 h. After $PGF_2\alpha$ injection was observed in buffaloes having distinct CL on the day of $PGF_2\alpha$ injection. Two buffaloes and one heifer were detected respectively in moderate and week estrus with developed follicles. On natural service buffaloes exhibiting strong (8) and moderate (1) estrus were conceived (Table 1). But those detected in week estrus though ovulated but did not conceive. None of the buffaloes responded to the saline injection.

A little swelling in external genitalia with whitish thin discharge was observed between day 8 to 12 after CIDR implant in 5, 3, 2, 2, 3 and 3 animals respectively, of group I, II, III, IV, VI and VII. Slight uterine tone with growing follicles were observed at 24 to 48 h after implant removal in 7, 5, 6, 5, 2, 4 and 5 animals respectively, of group I, II, III, IV, V, VI and VII. The response of buffaloes for different regimen of CIDR implant in terms of the number of animals detected in estrus, interval from CIDR removal to onset of estrus, intensity of estrus and conception has been presented in Table 2.

The animals with distinct CL exhibited more pronounced behavioral estrus than those animals without palpable CL. The 10 to 14 days (Group II to IV Table 2) CIDR implant was superior in respect of exhibition of overt sign of estrus, to eight days CIDR implant (Group V). Two heifers of each group VI and VII exhibited overt sign of estrus 12 h.

Table 1. Response of PGF_2 a (prosolvin) injection on ovarian status and cyclicity among rural anestrus buffaloes in summer (Phase I)

Particular of Animals	Duration of anestrus (months)	Ovarian status: Before treatment		Ovarian status: No. of Follicles After treatment		Interval from PGF_2 to onset (h)	Duration of estrus (h)	Inten-sity of estrus	No. of animals conceived
		Right ovary	Left ovary	Right ovary	Left ovary				
Treatment Group (Prosolvin, 15 mg, im) Buffaloes									
With CL (6)	12 to 20	CL(4)	CL(2)	(4)	(1)	72 to 96	12 to 24	Strong	(3)
Without CL (6)	10 to 18	Smooth	Smooth	(2)	0	72 to 84	12 to 20	Moderate	(1)
Heifers (6)	36 to 55	Smooth	Smooth	(1)	0	30	12	Weak	(0)
Control Group (Normal saline, 2 ml, im) Buffaloes									
With CL (6)	10 to 18	CL(5)	CL(1)	0	0	Nil	Nil	Nil	
Without (6)	12 to 18	Smooth	Smooth	0	0	Nil	Nil	Nil	
Heifers (6)	36 to 50	Smooth	Smooth	0	0	Nil	Nil	Nil	
Control buffaloes With CL (6) treated With Prosolvin (15 mg, im)	10 to 18	CL(5)	CL(1)	(4)	(1)	24 to 96	20.00	Strong	(4)

* Figures in parenthesis indicate number of animals.

Table 2. Response of prostaglandin and progesterone (CIDR) implant in buffaloes in summer (Phase II)

Groups	Duration of anestrus (Months)	Ovarian status: Before CIDR implant		Ovarian status: No. follicles after CIDR removal		Number of buffaloes detected in estrus	Interval from CIDR removal to estrus	Intensity of estrus			No. of animals conceived
		Right ovary	Left ovary	Right ovary	Left ovary			Strong	Moderate	Weak	
I (10 buffaloes)	10 to 18	Smooth	Smooth	4	3	5 (50%)	48 to 120	3	1	1	5 (50%)
II (6 buffaloes)	10 to 14	Smooth	Smooth	3	2	5 (83%)	48 to 120	3	2	0	4 (66%)
III (6 buffaloes)	9 to 14	Smooth	Smooth	4	2	5 (83%)	72 to 120	2	2	1	4 (66%)
IV (6 buffaloes)	10 to 18	Smooth	Smooth	4	1	5 (83%)	60 to 120	1	3	1	3 (50%)
V (6 buffaloes)	10 to 16	Smooth	Smooth	2	0	2 (33%)	72 to 96	0	1	2	Nil
VI (6 heifers)	42 to 60	Smooth	Smooth	3	1	4 (67%)	12 to 96	1	1	1	2 (33%)
VII 6 heifers	42 to 60	Smooth	Smooth	3	2	5 (83%)	12 to 120	1	2	1	4 (66%)

after CIDR removal. Swelling of external genitalia with thin white tinged vaginal discharge was observed on day 8 to 14 during CIDR implant in almost all buffaloes.

Presence of distinct CL in 30% of the anestrus baffaloes suggests that these buffaloes were cycling and might have come in ovulatory estrus for shorter duration without any pronounced heat symptom. Due to lack of technique for estrus detection in rural managemental condition the owners could not detect estrus. Silent estrus and ovulation in which did not precede by overt sign of estrus and detection of 70% of buffaloes anestrus without CL during present experiment, is common phenomenon in buffaloes [8]. Besides, total ovarian inactivity is one of the major causes of reproductive failure in buffaloes under Indian village management [6, 17]. The presence of distinct and embedded CL on the ovaries of respectively 33% and 20% of noncycling buffaloes maintained in rural management system has been detected [24]. The response of these noncyling buffaloes having CL on the ovaries to the administration of 25 mg prostaglanding with intravaginal estradiol and progesterone implement in respect of exhibition of fertile estrus during summer month has also been reported [23].

High concentration of plasma prolacting [11] associated with lower peak of FSH and LH [12] and plasma progesterone [13, 18] could explain the high incidence of anestrus during summer. A highly marked seasonal trend in breeding of water buffaloes has been observed in nearly all the countries [2, 9, 10, 20].

The presence of CL in nine buffaloes on right ovaries while in three on left ovaries suggests that right ovaries in buffaloes were more active than left during summer. This is in confirmity with the similar observation reported earlier [26]. Folliculogenesis following $PGF_{2}\alpha$ injection and subsequently exhibition of estrus in buffaloes with and without CL (Table 1) reveals that smooth ovaries (without palpable CL) of buffaloes might have luteal tissue embaded in the ovarian cortex and secreting higher concentration of progesterone which degenerated in the similar fashion as degenaration of distinct CL following prostaglandin injection [7].

The small ovaries in buffaloes without CL than the ovaries with CL as observed in the present experiment is a common phenomenon in buffaloes during summer [2, 17]. The presence of CL on day 9 to 11 after $PGF_{2}\alpha$ injection on the ovary of one buffalo cow and one buffalo heifer exhibited respectively, moderate and weak estrus symptom suggest that folliculogenesis and ovulation was occurred in these buffaloes. Confirmation of pregnency in only seven buffaloes having distinct CL and one buffalo without CL at the time of $PGF_{2}\alpha$ injection (Table 1) reveals that these buffaloes were having functional CL secreting threshold level of progesterone for priming of the hypothalamo-hypophyseal and gonadal system for its sensitivity and feedback mechanism for optimum follicular granulosa cell development, secretion of large quantities of estrogen, oocyte maturation and ovulation leading to successful fertilization and maintenance of CL and pregnency. The follicular development and exhibition of estrus in buffalo cows and heifers after CIDR removal in each group of phase II suggests that the increasing level of progesterone might have been released from CIDR implanted intravaginally in these buffaloes and was absorbed subsequently in circulation through the vaginal mucus membrane. That increased circulatory concentration of progesterone might have sensitised hypothalmic-pituitary system. Elevation of progesterone before onset of ovarian cyclicity works as primer sensitizer on the level of hypothalamus and pituitary [4] for initiation of their own function [5] and for the reception of other reproductive hormones. Similar response of anesturs buffaloes to crestar ear implant and crestar (estrogen and progesterones) injection on resumption of estrus cyclicity in cows has been reported [15].

Lack of response of CIDR in five buffalo cows and one buffalo heifer of group I (Phase II) reveals that these buffaloes had either luteal tissue secreting threshold level of progesterone that inhibited hypothalamic pituitary system or these system did not respond to the progesterone released from CIDR. Higher level of progesterone in cattle and buffaloes inhibits the release of GnRH and gonadotrophin respectively, from hypothalmus and pituitary and its withdrawal reverse the situation [7, 16].

The exhibition of overt sign of estrus and conception following natural service in animals of group II indicates that these animals responded to the CIDR implant and ovulated following CIDR removal. Equal response of CIDR implant for 10, 12 and 14 days in summer indicates that elevation of progesterone for at least 10 days over basal value in circulation was sufficient to sensitize the hypothalamo-hypophyseal and gonadal system of buffalo for resumption of estrous syclicity. Similar observation of exhibition of estrus and conception has been reported after treatment with CIDR and different combinations in buffaloes [1] and elevation of progesterone towards onset of puberty in buffalo heifers [14, 22]. The previous research reported that the increase in progesterone concentration before breeding is positively associated with conception rate and also reduced interval from the start of breeding season to conception in lactating cows treated with progesterone and $PGF_{2}\alpha$ [27, 28].

The observation suggests that about 30% anestrus buffaloes of rural areas were having corpus luteum. They can be brought to estrus to achieve conception by administering leuteolytic agents. However, the true noncycling buffaloes might be suffering from incoordination among hypothalamo-hypophyseal and gonadal system. They can be bred by raising the progesterone level through intravaginal progesterone implant even during unfavourable summer season in traditional village managemental condition.

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