



Influence of bull biostimulation on age at puberty and reproductive performance of Sahiwal heifers

SANJAY CHOUDHARY¹, M L KAMBOJ², NITIN RAHEJA³, NISHANT KUMAR⁴, MAYAMITTA SAINI⁵ and S S LATHWAL⁶

ICAR-National Dairy Research Institute, Karnal, Haryana 132 001 India

ABSTRACT

Present study was undertaken to compare the effect of biostimulation by exposing the heifers to fenceline bull contact and fenceline + direct contact on age at puberty and reproductive performance of Sahiwal heifers. Pre-pubertal heifers (24) were allotted to 3 groups (8 animals each), i.e. non-bull exposed (NBE), fenceline bull exposed (FBE) and FBE + direct bull exposed (DBE) on the basis of age and body weight. In NBE group, heifers were not exposed to bull; in FBE group, heifers were exposed to the bull through a fenceline contact (24 h) and in FBE+DBE group, heifers were housed in a fenceline contact (24 h) with bull along with direct contact for 6 h through another bull in the heifer shed. Heifers were considered to have attained puberty if progesterone concentration was >1 ng/ml. The mean age and body weight at puberty in FBE (19.33 ± 0.36 months and 226.20 ± 6.35 kg) and FBE+DBE (19.11 ± 0.58 months and 224.19 ± 4.54 kg) heifers were almost similar but significantly lower than NBE heifers (24.13 ± 0.16 months and 262.50 ± 8.50 kg). The average age at first service and first calving was similar in FBE (20.41 ± 0.45 and 30.20 ± 0.73 months) and FBE+DBE (20.78 ± 0.36 and 29.90 ± 0.44 months) heifers but significantly lower than NBE heifers (25.15 ± 0.14 and 34.29 ± 0.53 months). We conclude that biostimulation of heifers by fenceline contact with the bull is as effective as FBE + 6 h of daily direct bull contact in hastening the age at puberty and improving reproductive performance.

Key words: Biostimulation, Direct, Fenceline, Pheromones, Ovarian cyclicity, Sahiwal, Social cues

The age at puberty of heifers, the potential replacement stock at dairy farms, is one of the major factors affecting their performance. Delay in onset of puberty causes a delay in age at first breeding and consequent delay in age at first calving increases the expenses on raising heifers (Rehman 2008). The age at puberty in the zebu breeds of cattle ranges from 27 to 32 months (Bashir 2006) and the age at first calving ranged from 37 to 42 months (Rehman *et al.* 2008, Bansal *et al.* (2017). Majority of research conducted in past for hastening age at puberty have been focused on nutritional supplementation (Abeni *et al.* 2000, Narvariya 2014) in heifers, by using of hormones (Madgwick *et al.* 2005), Narvariya (2014) and other managerial practices (Kinde *et al.* 1994). These efforts, although effective to some extent, warrant additional expenditure on feeding and hormonal treatment of animal.

Provision of social cues through biostimulation by exposing the heifers to bull during their growing period may offer a clean, green and ethical (Martin *et al.* 2004) strategy for early onset of puberty. The biostimulation has

been reported to act through bull pheromones using visual, olfactory, auditory and tactile pathways and stimulate neuro-endocrine cascade in female hypothalamus which leads to onset of puberty (Fiol and Ungerfeld 2012). Previous studies have demonstrated that direct bull exposure to pre-pubertal heifers advanced the onset of puberty in Bunaji heifers (Rekwot *et al.* 2001), Nelore heifers (Oliveira *et al.* 2009) and Hereford and Angus crossbred heifers (Fiol and Ungerfeld 2016). It is yet to be elucidated whether the direct bull exposure along with fenceline exposure (i.e. stimulus-releaser intensity) will have any additional effect on the onset of puberty. Therefore the present study was undertaken to compare the effect of biostimulation by exposing the heifers to fenceline bull contact and fenceline + direct contact on the age at puberty and reproductive performance in case of heifers of Sahiwal breed.

MATERIALS AND METHODS

Location: This research work was carried out on Sahiwal breed of cattle maintained at Livestock Research Center (LRC) of the Institute. The maximum ambient temperature in summer goes up to 43°C and minimum temperature in winter comes down to 2°C with a diurnal variation in the order of 15–20°C. The annual rainfall is 700 mm, most of which is received from July to mid-September.

Ethical approval: Experiment was approved and

Present address: ^{1,5}PhD Scholar (rajsaya07@gmail.com, saini32064@gmail.com), ^{2,6}Principal Scientist (kamboj66@rediffmail.com, lathwal1314@gmail.com), Livestock Production Management Division; ³PhD Scholar (nitinraheja23@gmail.com); ⁴Senior Scientist (drnishantvet@yahoo.com) Animal Reproduction Gynecology and Obstetrics Division.

conducted under the established standard of the Institutional Animal Ethics Committee (IAEC), constituted as per the article number 13 of the committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) rules laid down by the Government of India. IAEC Reg. No. for the present experiment was 41-IAEC-18-20.

Experimental animals: Apparently healthy Sahiwal heifers (24) were selected from the institute herd and were allotted to 3 groups (NBE, FBE and FBE+DBE) of 8 animals each on the basis of age and body weight. In NBE_group (non-bull exposed group) (age: 14.53 ± 0.97 months, body weight: 151.91 ± 10.85 kg), the heifers were not exposed to the bull; in FBE group (fenceline bull exposed) (age: 14.45 ± 0.86 months, body weight 152.41 ± 11.06 kg), the heifers were exposed to the intact bull (aged 28 and 31 months) round the clock by housing them in a fenceline contact throughout the length of the covered area as well as open paddock of the heifer shed and in FBE+DBE group (fenceline bull exposed + direct bull exposure) (age: 14.36 ± 0.88 months, body weight: 152.49 ± 11.40 kg), the heifers were exposed to the intact bull round the clock by housing them in a fenceline contact throughout the length of the covered area as well as open paddock of the heifer shed (as in case of FBE group) along with direct introduction of an another apronized intact bull for a total duration of 6 h daily (3 h in morning from 07:00 to 10:00 AM and again for 3 h in evening from 4:00 to 7:00 PM) in the heifer shed. The bull used for direct contact in these groups of heifers was weekly exchanged with the bull used for fenceline contact.

Housing, feeding and management: The experimental animals were housed under loose housing system with a covered shed and an adjoining open paddock with a total floor space of 8 m^2 per animal and common feeding through a fenceline feed barrier and common watering through plastic water tub was provided. The allocation of floor space and feeding space conformed to the Bureau of Indian Standards for housing of cattle under loose system (BIS: 1223-1987). The bulls were housed in between the houses of two treatment groups of heifers separated through 5 ft high fence made of galvanized iron pipe railings of 1.5 inches diameter. The distance between the pipe railings was 24 inches which allowed sufficient tactile interaction between bull and heifers through the fence. The floors of animal houses were made of cement concrete in the covered area and brick-on-edge paved in adjacent open paddocks. The NBE group of heifers were housed in a similar shed at a distance of about 0.5 km from the houses of other two groups of heifers in order to ward off the effect of airborne bull pheromones. The 3 groups of heifers were fed the similar ration based on the feeding standards by Indian Council of Agricultural Research (2013) for feeding of dairy cattle and buffaloes for a targeted growth rate of 500 g/day. The animals were offered seasonal farm grown green fodders (Egyptian clover, maize, black eye pea, alfalfa) and wheat straw as dry roughage. The concentrate mixture with

16-18% DCP and 70% TDN was offered at the rate of 1 kg/100 kg body weight/day (around 1.5 kg to 2.0 kg). Before the beginning of the experiment, the heifers were screened for blood and helminth parasites and appropriate treatments and vaccinations against endemic diseases were carried out. Regular treatments with anthelmintic, acaricides and antibiotics to control helminthiasis, ticks and tick-borne diseases respectively were executed throughout the experimental period.

Estrus detection and blood sampling: Experimental heifers were observed for the signs of estrus, two weeks before the commencement of the experiment, through 24 h CCTV outdoor cameras (CP Plus) recording. The ovaries of all the heifers were examined ultrasonographically using a real-time, B-mode, diagnostic scanner equipped with a transrectal 7.5 MHz, linear array transducer (Aloka, Prosound 2, Japan) for the presence of functional ovarian structures to ensure that they were non-pubertal. Jugular blood sample (5 ml) was collected by venipuncture from each heifer at the start of the experiment, serum progesterone concentration below 1 ng/ml in heifers receiving both treatments within this period were indicative of pre-puberty. In bull exposed groups, the estrus detection was done by two intact mature bulls which were used for biostimulation and monitored through 24 h CCTV cameras recording, whereas in non-exposed group estrus detection was carried out by observing estrus signs through 24 h CCTV recording and the estrus was confirmed through per rectal palpation and observing fern pattern of cervical mucus by inseminators in all the animals. Heifers were numbered on the flank and back region with black or white water paints twice a week to facilitate individual identification through CCTV cameras. Estrus was demarcated and recorded when a heifer allowing herd-mates or intact bulls to mount her. The ovaries of such a heifer were examined per rectum using an ultrasound probe for the detection of apparently functional corpus luteum after 10 days of standing estrus. For further confirmation, 5 ml blood samples were collected after 10 days from heifers showing estrus and analyzed for progesterone concentration. The collected blood samples were placed immediately on ice and serum was separated immediately by centrifugation at 3,000 rpm for 15 min and stored at -20°C until concentrations of progesterone (P4) were estimated using the Bovine Progesterone ELISA Kit (Wuhan Fine Biological Technology Co. Ltd.) with detection range and sensitivity of the kit being 0.313–20 ng/ml and <0.188 ng/ml and the intra-assay CV was $<8\%$. This ELISA kit uses Competitive-ELISA as the method. The experimental heifers which had attained about 60–65% of their mature body weight were inseminated during their second estrus.

Ovarian cyclicity using ultrasonography: Ovarian structure of all the heifers in all three groups (NBE, FBE, FBE+DBE) were monitored ultrasonographically using a real-time, B-mode, diagnostic scanner equipped with a transrectal 7.5 MHz, linear array transducer (Aloka, Prosound 2, Japan). Ultrasound examination were

performed once every fortnight. On each examination the number and diameter of the follicle in each ovary were measured.

Cervical mucus characteristics: Onset of estrus, different stages of estrus and ovulation time can be predicated by observing different properties of cervical mucus of cattle, which may increase conception rate. Cervical mucus samples were collected on the day of estrus prior to the artificial insemination by aspiration from the vagino-vestibular space using blue sterile sheath and universal artificial insemination gun through recto-vaginal method. Immediately after collection, samples were taken to the laboratory for the assessment of appearance, consistency and arborization of mucus discharge.

For appearance of mucus discharge, the mucus collected was observed visually to check the appearance, and was classified as clean (like white of an egg), cloudy (cloudy in appearance, not homogenous in look) and dirty (color like yellowish, grey, red etc.) (Deo and Roy 1971).

Consistency of the mucus was noticed by placing few drops of collected sample into a grease free glass slide and the slide was inclined to 45°. The movement of the mucus was observed and its consistency was categorized as thin (freely flowing mucus on the 45 inclined glass slides), medium (mucus moves slowly), thick (mucus is sticky and moves in lumps) (Deo and Roy 1971).

For arborization pattern of mucus discharge, few drops of cervical mucus sample were placed on a glass slide, spread uniformly over the slide and air dried. The air-dried slide was observed under microscope using low power objective (10×) for crystallization pattern of the mucus, known as fern pattern. The fern pattern of the observed mucus was classified as (Luktuke and Roy 1967) as typical (fern pattern with primary, secondary and tertiary branches), atypical (fern pattern with primary and secondary branches) and nil (fern pattern with no primary, secondary and tertiary branches) (Luktuke and Roy 1967).

Statistical analysis: Statistical analyses were performed using the statistical package for the social science (SPSS), computer software version 21. The data on mean age and body weight at puberty, reproductive performance, ovarian cyclicity using ultrasonography and progesterone concentrations among the 3 groups of heifers were analyzed using Duncan's Multiple Range Test and one-way ANOVA (Snedecor and Cochran 1994). Data on heifers reaching puberty at various ages and cervical mucus characteristic in all 3 groups of heifers were expressed as proportion (%) and no statistical test was applied.

RESULTS AND DISCUSSION

Age at puberty: The average number of heifers reaching puberty in NBE, FBE and FBE+DBE groups by the end of the experimental period was 5 (62.5%), 7 (87.5%) and 7 (87.5%), respectively (Table 1). The average age of puberty was significantly ($P \leq 0.05$) lower in FBE and FBE+DBE groups as compared to NBE group. The FBE and FBE+DBE groups of heifers were, however, similar in this respect.

Table 1. Average age and weight (Mean±SE) at puberty

Parameter	Group of experimental heifers		
	NBE (8)	FBE (8)	FBE+DBE (8)
Age at start of experiment (months)	14.53±0.97	14.45±0.86	14.36±0.88
Body weight at start of experiment (kg)	151.91±10.85	152.41±11.06	152.49±11.40
No. of heifers reaching puberty	5 (62.5%)	7 (87.5%)	7 (87.5%)
Age at puberty (months)	24.13 ^b ±0.16	19.33 ^a ±0.36	19.11 ^a ±0.58
Body weight at puberty (kg)	262.50 ^b ±8.50	226.20 ^a ±6.35	224.19 ^a ±4.54

Figures in parenthesis indicate percentage of total animals in the group. Values with different superscript in same row differ significantly ($P \geq 0.05$).

Male presence, i.e. biostimulation significantly reduced the age at puberty in both FBE and FBE+DBE groups of heifers, with a reduction of 4.7 to 4.9 months (in that order) from NBE group. Our results are in agreement with Rekwot *et al.* (2000) who reported early attainment of puberty by 3.5 months in vasectomized bulls exposed group of Bunaji heifers than non-exposed heifers. The significant ($P \leq 0.05$) reduction in age of puberty in FBE and FBE+DBE groups of heifers may have attributed due to priming of these heifers by the presence of bull through visual, tactile, olfactory and auditory cues which may have resulted in the activation of the neuro-endocrine response. Earlier reports of Izard and Vandenberg (1982) in Holstein heifers; Oliveira *et al.* (2009) in Nelore heifers; Fiol and Ungerfeld (2016) in Hereford and Angus crossbred heifers also gave a similar corollary for the reduction of age at puberty in bull exposed animals. Mean age at puberty in FBE was almost similar to FBE+DBE which may be attributed to the fenceline exposure of heifers to a bull throughout the length of animal paddock was also as effective as direct presence of bull inside the paddock as they were in continuous and close proximity to the pheromonal source (through tactile, auditory, olfactory and visual cues pheromones) for a sufficient period during which the stimuli could influence the hypothalamic-pituitary-ovarian axis to initiate resumption of ovarian activity. Fenceline bull exposure enhanced the ovarian activity in our study which is in agreement with Fike *et al.* (1996); Fernandez *et al.* (1996); Roelofs *et al.* (2007); Berardinelli and Tauck (2007) who reported early onset of ovarian activity in case of postpartum anestrus in primiparous and multiparous beef cows by fenceline bull contact and further, support the hypothesis that fenceline bull exposure hasten early onset of ovarian activity.

The average body weight at puberty in FBE and FBE+DBE groups was significantly ($P \leq 0.05$) lower than those in NBE, being similar in the both bull exposed groups. The mean live weight of 225 kg recorded at puberty in FBE

and FBE+DBE groups was ideal for the onset of puberty in Sahiwal heifers. This live weight was almost similar to that reported by Bhatti *et al.* (2007) who concluded after a detailed review of existing reports that Sahiwal heifers can attain puberty at a weight of 225 kg (about 60% of mature body weight of 375 kg). Similar finding has been observed by Abeygunawardena and Dematawewa (2004) local breed of cows in Sri Lanka and Rekwot *et al.* (2000) in local Bunaji breed in Nigeria. Roberson *et al.* (1991) reported that age at puberty is influenced by body weight and responsiveness of pubertal heifers to potential pheromonal cues from the bull, which seems to be dependent on body weight of heifers. The lower body weight of animals during bull exposure on the other hand, did not hasten the age at puberty (Macmillian *et al.* 1979).

Proportion of heifers reaching puberty at varying ages: More than two-thirds proportion of heifers in FBE and FBE+DBE group (71.4% each) attained puberty between the age of 18–20 months, whereas, all the 5 heifers which came into estrus during the experimental period in NBE reached puberty later as compared to bull exposed heifers, i.e. between the age of 23–25 months (Table 2). It is worth noting that 1 heifer out of 7 in FBE+DBE group attained puberty at a very young age (between 15–17 months). Present study results are in agreement with Rekwot *et al.* (2000) who observed higher proportion of heifers reaching puberty in bull exposed Bunaji heifers than non-exposed.

Table 2. Proportion of heifers reaching puberty at varying ages

Group of experimental Animals	No. of heifers attaining puberty	Proportion of heifers attaining puberty at different ages (month)			
		15–17	18–20	21–23	23–25
NBE	5	–	–	–	100(5)
FBE	7	–	71.4 (5)	28.6 (2)	–
FBE+DBE	7	14.3(1)	71.4(5)	14.3 (1)	–

Figures in parenthesis indicate the number of heifers.

Reproductive performance: The experimental heifers which had attained about 60% of their mature body weight were artificially inseminated during their second estrus. The average age at first service in FBE (20.41±0.45) and FBE+DBE (20.78±0.36) groups was significantly ($P \leq 0.05$) lower than that in NBE (25.15±0.14) group. There was no significant difference between the FBE and FBE+DBE groups of heifers as far as their average age at first service was concerned. These results are in agreement with Oliveira *et al.* (2009) in Nelore heifers, which may be attributed to the early onset of the puberty in bull exposed groups and were inseminated after attainment of around 65% of mature body weight. Our results showed that bull biostimulation has a positive effect on the number of services per conception and conception rate in Sahiwal heifers. The number of services per conception in NBE, FBE and FBE+DBE were 1.60±0.24, 1.17±0.17 and 1.20±0.20,

respectively which did not differ significantly among the 3 group of heifers. The conception rate was as high as 85% in both bull exposed group (FBE and FBE+DBE group), whereas it was 60% in non-bull exposed heifers (NBE group). Higher conception rate in bull exposed heifers observed in our study also corroborated with the findings of Khanh *et al.* (2012) and Ungerfeld (2009). It may be attributed to proper detection of estrus and presentation of animals for artificial insemination during standing estrus. Further, clear cervical mucus discharge during artificial insemination (AI) was positively associated with increased first service conception rate (Loeffler *et al.* 1999).

The average age at first calving was significantly ($P \leq 0.05$) lower in FBE (30.20±0.73) and FBE+DBE (29.90±0.44) groups as compared to NBE (34.29±0.53) group. Our results in present study were lower than observed by Rehman *et al.* (2008) and Bansal *et al.* (2017). These results may be attributed due to the priming of heifers by bull pheromones which have resulted in early onset of puberty and proper estrus detection in all 3 groups have led to insemination of heifers during standing estrus.

Cervical mucus characteristics

Colour and consistency: The proportion of clean cervical mucus in FBE and FBE+DBE heifers was higher (85.72%) than in NBE heifers (66.67%) whereas cloudy mucus was in 33.33% of NBE heifers and only 14.28% heifers exhibited cloudy mucus in FBE and FBE+DBE heifers. The results of present study in FBE and FBE+DBE group heifers corroborate with findings of Gunasekaran *et al.* (2008) and Verma *et al.* (2014) in buffaloes. More than two-thirds of heifers in FBE and FBE+DBE groups displayed thin consistency of cervical mucus and only 33.34% heifers in NBE group showed thin consistency of cervical mucus. Around 14.28% heifers of FBE and FBE+DBE exhibited medium and thick consistency of cervical mucus and in NBE 33.34% of heifers had medium and thick consistency (Fig. 1). The higher clean and thin cervical mucus in present study may be attributed to increase level of estrogen, whereas, cloudy mucus and medium to thick consistency of mucus possibly have occurred due to increase level of progesterone hormones (Hansel and Snook 1970). This increased level of estrogen and progesterone in our study may be attributed to bull effect.

Hornbuckle *et al.* (1995) and Fike *et al.* (1996) further support our hypothesis. The present study revealed no animal with dirty discharge as reported by previous workers (Deo and Roy 1971, Verma 2012). This may be due to the collection procedure of the cervical mucus. The collection method followed in present study may avoid the contamination from the vagina and external genitalia during the collection may impart the dirty appearance to the mucus. Further, presence of bull aided proper estrus detection might be the reason for our findings of getting clear cervico-vaginal discharge which increased the estrus detection efficiency.

Arborization / Fern pattern: Mucus samples of 85.72%

heifers in FBE and FBE+DBE groups showed typical fern pattern with primary, secondary and tertiary venation and rest of heifers exhibited atypical fern pattern. Whereas, two-thirds of heifers in NBE group exhibited atypical fern pattern and 33.34% heifers displayed typical fern pattern (Fig. 2). Results of present study are in agreement with the results reported by Verma (2012). This higher proportion of mucus samples in FBE and FBE+DBE groups displaying typical fern pattern at the time of artificial insemination was attributed to the presentation of animal for AI during standing estrus during which estrogen level is at peak whereas, on the other side in NBE group, the estrus detection was poor due to weak estrus intensity and therefore, the heifers had atypical arborization pattern (Tsiligianni *et al.* 2001).

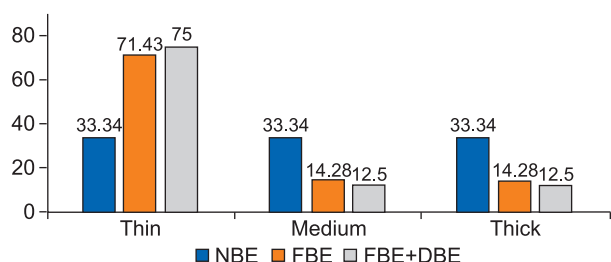


Fig. 1. Consistency of cervical mucus at the time of AI in 3 groups of Sahiwal heifers observed in estrus.

Ovarian cyclicity using Ultrasonography: On the day of start of experiment, the mean total number of follicles in NBE, FBE and FBE+DBE group heifers were 6, 7 and 7 respectively (Table 3) and were not significantly different from each other. The average number of small sized (<5 mm) follicle in NBE group (5.51) were significantly ($P<0.05$) lower than FBE and FBE+DBE groups (10.24 and 11.64), respectively. However, mean number of medium and large follicle in diameter were significantly higher in FBE (3.38 and 1.15) and FBE+DBE (2.89 and 1.09) heifers than NBE (1.85 and 0.6) heifers respectively. The number of medium and large sized (>8 mm) follicle increased from

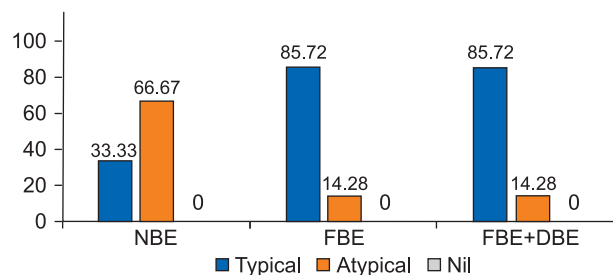


Fig. 2. Fern pattern of cervical mucus at the time of AI in 3 groups of Sahiwal heifers observed in estrus

the fifth fortnight onwards in the FBE and FBE+DBE group which indicated the initiation of ovarian activity in treatment groups. However, only negligible number of follicles in control were large sized which indicated the presence of anovulatory follicular wave, which might be responsible for anestrus in these animals. Sahu (2002) also reported that anestrus condition is characterized by presence of anovulatory follicular waves. Small size follicles in these pubertal heifers might be due to insufficiency of hypothalamic stimuli of GnRH to induce release of gonadotropins from anterior pituitary rather than not having a GnRH responsive follicle. Therefore, bull exposure promoted ovarian follicular growth rate in prepubertal Brahman heifers (Bastidas *et al.* 1999). The findings of the present study were in agreement with the findings of Fiol *et al.* (2016).

Serum progesterone concentration: Per-rectal and ultrasonographical examination revealed that heifers that stood to be mounted had apparently functional CL on their ovaries 10 days post-estrus. The mean concentration of progesterone in FBE group was 2.14 ± 0.02 ng/ml and in FBE+DBE group was 2.08 ± 0.02 ng/ml while the serum progesterone concentration in NBE group was below 1 ng/ml (0.96 ± 0.13). Progesterone concentrations in FBE and FBE+DBE groups of heifers started increasing from the fifth month onwards from the start of experiment, whereas in NBE it increased from sixth month onwards. The increase

Table 3. Average number of different size of follicles in Sahiwal heifers

Fortnight	NBE				FBE				FBE+DBE			
	Small	Medium	Large	Total	Small	Medium	Large	Total	Small	Medium	Large	Total
1	5	1	—	6	5	1	0.2	7	5	2	0.2	7
2	5.60	0.8	—	6	11.2	2.2	0.6	14	12.2	2.6	0.6	15
3	4.40	1.6	—	6	10.6	2.2	0.8	14	11.4	1.2	0.8	13
4	3.80	1.4	—	5	10.2	2.6	1	14	13.4	2	1	16
5	5.00	2.6	—	8	9.8	4.2	1.3	15	10.2	3.2	1.2	15
6	5.00	2.4	—	8	10.2	3.2	1.46	15	12.6	2	1.2	16
7	6.60	2.4	—	9	10.4	3.4	1.4	15	13.4	3.8	1.4	19
8	5.60	2.4	—	8	12.2	4	1.56	18	13.4	5.2	1.34	20
9	6.40	2.4	—	9	11.2	3.4	1	16	13.6	3.8	1	18
10	6.80	1.6	0.2	9	10.6	4.4	1.6	17	11.6	2.2	1.56	15
11	6.80	1.6	0.4	9	11.2	6	1.8	19	11.2	4.4	1.78	17
Overall	5.51 ^a	1.85 ^c	0.6 ^e		10.24 ^b	3.38 ^d	1.15 ^f		11.64 ^b	2.89 ^d	1.09 ^f	

*Values with different superscript (Small ^{a,b}), (Medium ^{c,d}) and (Large ^{e,f}) in same row differ significantly ($P<0.05$).

in progesterone concentration was due to the formation of corpus luteum on ovary which indicated first ovulation and thus the onset of puberty. Our results pertaining to progesterone concentration in FBE and FBE+DBE heifers are in agreement with findings of Fike *et al.* (1996). However, Patra (2006) reported no difference in progesterone concentration between bull-exposed and non-exposed post-partum cows which might be due to different duration of experimental study and breed difference.

The biostimulation of Sahiwal heifers through fenceline and fenceline + direct contact of a mature bull during growing period hastened the age at puberty and improved the reproductive performance in comparison to non-bull exposed heifers. The fenceline bull contact along with direct bull contact in the paddock of these heifers had no additional advantage in further hastening the age at puberty and in improving reproductive performance.

ACKNOWLEDGEMENTS

The authors appreciatively acknowledge the Director, National Dairy Research Institute, Karnal and the In-charge, Livestock Research Center of NDRI, Karnal for providing the necessary facilities for completion of this work.

REFERENCES

- Abeni F, Calamari L, Stefanini L and Pirlo G. 2000. Effects of daily gain in pre and post pubertal replacement dairy heifers on body condition score, body size, metabolic profile and future milk production. *Journal of Dairy Science* **83**(7): 1468–78.
- Abeygunawardena H and Dematawewa C M B. 2004. Pre-pubertal and postpartum anestrus in tropical Zebu cattle. *Animal Reproduction Science* **82–83**: 373–87.
- Bansal S K, Sinha A K, Gokhale S B, Bhagat R L. 2017. Factors affecting age at first calving in Sahiwal cattle under Jharkhand rural conditions. *International Research Journal Natural and Applied Science* **4**(7).
- Bashir M K. 2006. 'Genetic and phenotypic aspects of some performance traits of Nili-Ravi buffaloes in Pakistan'. PhD Thesis University of Agriculture, Faisalabad, Pakistan.
- Berardinelli J G and Tauck S A. 2007. Intensity of the biostimulatory effect of bulls on resumption of ovulatory activity in primiparous, suckled, beef cows. *Animal Reproduction Science* **99**: 24–33.
- Bhatti S A, Sarwar M, Khan M S and Hussain S M I. 2007. Reducing the age at first calving through nutritional manipulations in dairy buffaloes and cows: a review. *Pakistan Veterinary Journal* **27**(1): 42–47.
- Bureau of Indian Standards. 1987. Indian standard recommendations for loose housing system for animals. BIS 12237.
- Bastidas P, Ruiz J, Manzo M and Silva O. 1999. Bull exposure increases ovarian cyclicity in Brahman heifers. *Journal of Reproduction and Fertility* (Supplementary) **54**: 506.
- Deo S and Roy D J. 1971. Investigations on repeat breeding cows and buffaloes-Studies on physical properties of cervical mucus. *Indian Veterinary Journal* **48**: 479–84.
- Fernandez D L, Berardinelli J G, Short R E and Adair R. 1996. Acute and chronic changes in luteinizing hormone secretion and postpartum interval to estrus in firstcalf suckled beef cows exposed continuously or intermittently to mature bulls. *Journal of Animal Science* **74**: 1098–1103.
- Fike K E, Bergfeld E G, Cupp A S, Kojima F N, Mariscal V, Sanchez T S, Wehrman M E and Kinder J E. 1996. Influence of fence line bull exposure on duration of anestrus and pregnancy rate in beef cows. *Animal Reproduction Science* **41**: 161–16.
- Filho M L S, Bezerra L R, Ferreira-Silva Souto F M P P, Oliveira N R P, De Lima P F, Bartholomew C C and de Oliveira M A L. 2015. Influence of biostimulation and temporary weaning on follicular dynamics and pregnancy rates in Nellore cows (*Bos taurus indicus*). *Tropical Animal Health Production* **47**: 1285–91.
- Fiol C and Ungerfeld R. 2012. Biostimulation in cattle: Stimulation pathways and mechanisms of response. *Tropical and Subtropical Agroecosystems* **15**(1): 29–45.
- Fiol C and Ungerfeld R. 2016. Positive effects of biostimulation on luteinizing hormone concentration and follicular development in anestrus beef heifers. *Journal of Animal Science* **94**: 971–77.
- Gunasekaran M, Singh C and Gupta A K. 2008. Effect of estrus behaviour on fertility in crossbred cattle. *Indian Veterinary Journal* **85**: 159–63.
- Hornbuckle T I I, Ott R S, Oh I M W, Zinn G M, Weston P G and Hixon J E. 1995. Effects of bull exposure on the cyclic activity of beef cows. *Theriogenology* **43**: 411–18.
- Izard M K and Vandenberg J G. 1982. The effect of bull urine on puberty and calving date in crossbred beef heifers. *Journal of Animal Science* **55**: 116–18.
- Izard M K. 1983. Pheromones and reproduction in domestic animals. (Ed.) Vandenberg J G. *Pheromones and Reproduction in Mammals*. Academic Press, New York. pp 253–285.
- ICAR. 2013. Nutrient requirement of cattle and buffaloes. *Nutrient Requirement of Animals*. Indian Council of Agricultural Research, New Delhi.
- Hernández A J L. 2004. Effect of biostimulation on uterine involution, early ovarian activity and first postpartum estrous cycle in beef cows. *Theriogenology* **61**: 1521–32.
- Kinde J E, Roberson M S, Wolfe M W and Stumpf T T. 1994. Management factors affecting puberty in the heifer. CRC Press, Boca Raton, Florida.
- Luktuke S N and Roy D J. 1967. Studies on the cervical mucus pattern in relation to fertility in bovine. *Indian Journal of Veterinary Science and Animal Husbandry* **37**: 26–31.
- MacMillan K L, Allison A J and Struthers G A. 1979. Some effects of running bulls with suckling cows or heifers during the premating period. *New Zealand Journal of Experimental Agriculture* **7**: 121–24.
- Madgwick S, Evans A C and Beard A P. 2005. Treating heifers with GnRH from 4 to 8 weeks of age, advance growth and the age at puberty. *Theriogenology* **6**(8): 2323–33.
- Martin G B, Milton J T B, Davidson R H, Banchero H G E, Lindsay D R and Blache D. 2004. Natural methods for increasing reproductive efficiency in small ruminants. *Animal Reproduction Science* **82**(83): 231–45.
- Narvariya D S. 2014. 'Effect of supplementary feeding and GnRH administration on sexual maturity in winter born Sahiwal heifers'. MVSc Thesis, ICAR-National Dairy Research Institute Karnal, Haryana, India.
- Oliveira C M G, Oliveira, Filho B D, Gambarini M L, Viu M A O, Lopes D T and Sousa A P F. 2009. Effects of biostimulation

- and nutritional supplementation on pubertal age and pregnancy rates of Nellore heifers (*Bos indicus*) in a tropical environment. *Animal Reproduction Science* **113**: 38–43.
- Patra M K, Barman P and Kumar H. 2012. Potential application of pheromones in reproduction of farm Animals—A review. *Agriculture Reviews* **33**(1): 82–86.
- Rehman Z U, Khan S M, Bhatti S A and Iqbal J. 2008. Factors affecting first lactation performance of Sahiwal cattle in Pakistan. *Archives Animal Breeding* **51**(4): 305–17.
- Rekwot P I, Ogwu D, Oyedipe E O and Sekoni V O. 2000. Effects of bull exposure and body growth on onset of puberty in Bunaji and Friesian Bunaji heifers. *Reproduction Nutrition Development* **40**: 1–9.
- Rekwot P I, Ogwu D, Oyedipe E O and Sekoni V O. 2001. The role of pheromones and biostimulation in animal reproduction. *Animal Reproduction Science* **65**: 157–70.
- Rekwot P I, Akinpelumi O P, Sekoni V O, Eduve L O and Oyedipe E O. 2004. Effects of nutritional supplementation and exposure to bulls on resumption of post-partum ovarian activity in Bunaji (*Bos indicus*) cattle. *Veterinary Journal* **167**: 67–71.
- Roberson M S, Wolfe M W, Stumpf T T, Werth L A, Cupp A S, Kojima N D, Wolfe P L, Kittock R J and Kinder J E. 1991. Influence of growth rate and exposure to bulls on age at puberty in beef heifers. *Journal of Animal Science* **69**: 292–98.
- Roelofs J B, Soede N M, Dieleman S J, Voskamp-Harkema W and Kemp B. 2007. The acute effect of bull presence on plasma profiles of luteinizing hormone in postpartum, anoestrus dairy cows. *Theriogenology* **68**: 902–07.
- Sahu. 2002. 'Ovarian activity and vaginal resistance in pubertal and postpartum anestrus sahiwal cattle'. MVSc. Thesis, IGKV, Raipur, Chhatisgarh.
- Snedecor G W and Cochran W G. 1994. *Statistical Methods*. 8th edn. Iowa State University Press, Ames, Iowa.
- Tsiligianni T, Karagiannidis A, Brikas P and Saratsis P. 2001. Physical properties of bovine cervical mucus during normal and induced (progesterone and/or PGF2 α) estrus. *Theriogenology* **55**: 629–40.
- Ungerfeld R. 2009. Short-term exposure of high body weight heifers to testosterone treated steers increases pregnancy rate during early winter bull breeding. *Animal Reproduction Science* **6**(3): 446–49.
- Verma K K. 2012. 'Managerial interventions to improve heat detection accuracy, efficiency and conception rate in Murrah buffaloes'. MVSc. Thesis, NDRI, Karnal.