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Bio-stimulatory effects of bull urine (BU), PRID and GnRH on estrus induction and ovarian activity in pubertal anestrus Sahiwal heifers

ABRAR AHMED[1], R P TIWARI[2] and G K MISHRA[3]

Chhattisgarh Kamdhenu Vishwavidyalaya, Anjora, Durg, Chhattisgarh 491 001 India

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ABSTRACT

The present study was designed to assess the bio-stimulatory effect of bull urine (BU) exposure, administration of progesterone impregnated intravaginal device (PRID) in combination with PGF2α and gonadotropin releasing hormone (GnRH), (PRID-PG-GnRH) and GnRH in combination with PGF2α (GnRH-PG-GnRH) on induction of estrus and resumption of ovarian activity in pubertal anestrus Sahiwal heifers. Thirty two pubertal anestrus Sahiwal heifers were randomly divided in to 4 groups viz., Gr.1, (n=8, BU), Gr.2, (n=8, PRID-PG-GnRH), Gr.3, (n=8, GnRH-PG-GnRH) and Gr. 4, (n=8, Control). Significantly lesser proportion of anestrus Sahiwal heifers were induced to estrus (25%) following BU exposure than PRID-PG-GnRH (100%) and GnRH-PG-GnRH (87.50%) treated heifers. However, there were no heifers induced to estrus in control group during the experiment. The time interval taken for onset of estrus (days) was shorter in PRID-PG-GnRH (8.00±1.51) and GnRH-PG-GnRH (8.00±1.25) than BU exposed heifers (23.00±2.41). On ultrasonographic imaging, no definite growth patterns of ovarian follicles were observed in BU exposed and control groups. However, definite growth pattern of ovarian follicles were recorded in PRID-PG-GnRH and GnRH-PG-GnRH treated heifers. Significant decrease in the number of small follicles from day 3 to 7 in PRID (14 vs 5) and in GnRH (13 vs 4) treated heifers. However, increase in the number of large follicles were observed in heifers treated with PRID-PG-GnRH (0 vs 1.87) and GnRH-PG-GnRH (0 vs 2) from day 0 to 7, respectively, as compared to control group. The overall conception rate in BU exposed heifer was lower (25%) than PRID (50%) and GnRH (50%) treated heifers. Bull urine (BU) mediated induction of estrus is significantly lower in pubertal anestrus Sahiwal heifers than PRID+PG and GnRH+PG treated heifers.

Key words: Anestrus, Bull urine, Follicular dynamics, Sahiwal heifers, Ultrasonography

Puberty is the age at which the genital organs become functional and reproduction may occur (Roberts 1972). Sahiwal heifers not showing any sign of estrus even after attaining 33 months of age may be classified as "pubertal anestrus animal". Various attempts have been made on the regulation and control of bovine reproduction through the use of hormones, drugs, chemicals, managemental practices and by application of biological agents (Martin 2009) and hormonal treatment appears to be highly effective (Patterson *et al.* 2003). The bio-stimulatory effect of bull urine is found to be mediated through pheromones by the release of a specific behaviour or physiological change in the recipient's endocrine or reproductive system (Rekwot *et al.* 2000a, b). Urine can convey to the external world much information, concerning the internal physiological state to the animal and thus provide a source of chemical signal to many species. Most of the investigations concerning bio-stimulatory effects of bull or BU on cow reproduction have been carried out in beef cattle and post partum cows; however, effects of bio-stimulation on reproductive characteristics in pubertal anestrus Sahiwal heifers have not been investigated. Therefore, this study was designed to examine the effect of bull urine exposure on induction of estrus and resumption of ovarian activity in pubertal anestrus Sahiwal heifers.

Present address: [1]Assistant Professor (abrar.vet@gmail.com), Department of Veterinary Gynaecology and Obstetrics, M.J.F. College of Veterinary and Animal Sciences, RAJUVAS, Rajasthan. [2]Professor and Head, (rptiwarivca@gmail.com), [3]Assistant Professor (drkodu@gmail.com), Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Sciences and Animal Husbandry.

MATERIALS AND METHODS

The experiment was conducted in 32 pubertal anestrus Sahiwal heifers of 35 months of age and randomly divided into 4 groups, viz. group 1 n= 8 (bull urine), group 2 n= 8 (PRID-PG-GnRH), group 3 n=8 (GnRH-PG-GnRH) and group 4 n=8 (control). The experimental animals were maintained under similar feeding and management conditions in Bull Mother Experimental Farm, College of Veterinary Science and Animal Husbandry, Anjora, Durg (C.G.). The animals of group 1 (n=8) were sprayed with bull urine (3 ml/animal/day) on the oronasal part of the heifers daily until onset of estrus or maximum of 30 days. The bull urine was collected on alternate days in a sterilized

glass beaker from different Sahiwal bulls (4 to 5 years age) maintained at Central Semen Station Anjora, Durg (C.G.). Immediately after collection, the urine was filtered through millipore (0.45 µm) membrane filter and stored in different aliquots at 4°C till use. In Gr. 2, the progesterone-releasing intravaginal devices (PRID) was inserted into the vagina and left *in situ* for 7 days. On day 7, PRID was removed and PGF2α analogue i.e. Cloprostenol 0.5 mg was administered intramuscularly. In Gr. 3, the synthetic analogue of GnRH i.e. Buserelin acetate 10 µg was administered intramuscularly on 0 day. On day 7, PGF2α analogue i.e. Cloprostenol 0.5 mg was administered intramuscularly. All the treated animals were observed visually for estrus signs twice daily after $PGF_2\alpha$ injection. All the treated animals were observed visually twice daily i.e. morning (7 AM) and evening (4 PM) after $PGF_2\alpha$ injection for estrus signs. The heifers in estrus were confirmed by per rectal examination. Forty-eight hours later (day 9), all animals were inseminated (fixed timed Artificial insemination, FTAI) with frozen-thawed semen from a superior-proven Sahiwal bull. Synthetic analogue of GnRH i.e. Buserelin acetate 10 µg was administered intramuscularly at the time of AI. The heifers in control group were not given any treatment except regular estrus detection like in treatment groups.

Ovarian follicular population of all the heifers in each treatment and control groups were monitored using a real-time, B-mode, diagnostic ultrasound scanner equipped with a trans-rectal 7.5 MHz, linear array transducer. Ultrasound examination was performed once daily i.e. day 0, 3, 7, in Groups 2 and 3. The ultrasonographic monitoring in heifer of Group 1 (bull urine) and Group 4 (control) was continued further on day 9, 12, 15, 18, 22, 26, 30. On each examination, the number and diameter of the visible follicle in each ovary were measured. The animals were palpated per rectum for confirmation of pregnancy 50–60 days post insemination. One way analysis of variance ANOVA was carried out with the help of computer software SPSS.

RESULTS AND DISCUSSION

Bio-stimulatory effects of bull urine (BU), PRID-PG-GnRH and GnRH-PG-GnRH on estrus response in pubertal anestrus Sahiwal heifers are presented in Table 1. Bull urine exposure for 30 days in heifers induced estrus in lower proportion than PRID-PG-GnRH and GnRH-PG-GnRH groups within 10 days of treatment. However, no heifer came into estrus throughout the study in control group. The time taken to onset of estrus (days) was found shorter in PRID-PG-GnRH and GnRH-PG-GnRH treated heifers than BU exposed heifers ($P<0.01$). Similarly, there was no significant difference in time interval (hr) taken to onset of estrus following PG injection in PRID and GnRH treated groups. The findings of BU are in agreement with the findings in cows (Fike *et al.* 1996). However, our results are contrary to the findings of Patra (2006). The variable results may be due to season (Patra 2006) and frequency of periodic stimulation (Tauck 2005). Immediate exposure of cows to bull urine induces a pheromonal activated trigger that stimulates hypothalamic release of LHRH and subsequently increased secretion of LH (Fernandez *et al.* 1996). Bull urine contains a pheromone that stimulates VNO neurons by the constant signal being reinforced by periodic and repeated exposure to cows in close proximity.

The estrus induction response in our study agrees with the results of Ozyurtlu *et al.* (2008). However, a slight lower estrus response (83.30%) was reported in Murrah buffaloes (Lakra *et al.* 2003). In present study, estrus induction response of PRID and GnRH is in close agreement with CIDR (100%, Savalia *et al.* 2014) and Ovsynch protocol (80%, Singh 2003) in buffaloes. Ozyurtlu *et al.* (2009) observed 80% of induce estrus after PRID removal in heifers. In contrary to present findings, much lower estrus induction response of 58.33% and 37.5% was recorded with CIDR protocol by Ravikumar *et al.* (2007) and Singh *et al.* (2010), respectively. The present finding of estrus induction interval following PGF2α is in close agreement with Savalia *et al.* (2014). Similarly, the estrus response (87.5%) from Ovsynch protocol in anestrus Sahiwal heifers is corroborated with the reports of Ingawale *et al.* (2007) in anestrus buffaloes (87%). However, Butani *et al.* (2009) and Khasatiya *et al.* (2008) found 100% estrus response following PGF2α treatment in Ovsynch protocol.

Table 1. Effects of bull urine, PRID-PG-GnRH, GnRH-PG-GnRH protocols and control on estrus and fertility response in pubertal anestrus Sahiwal heifers

Attributes	Treatment			
	Group 1 (Bull Urine)	Group 2 (PRID-PG-GnRH)	Group 3 (GnRH-PG-GnRH)	Group 4 (Control)
Total no. of animals	8	8	8	8
No. of heifers exhibiting estrus within treatment period	2(25)[a]	8(100)[b]	7(87.50)[b]	0(0)[c]
Time taken (days) for induction of estrus from beginning of treatment	23.00 ± 2.14	8.00 ± 1.51	8.00 ± 1.25	0
Time interval (hr) to onset of estrus following PG injection	-	50 ± 5.86	52.00 ± 4.15	-
Conception rate	2(25)	4(50)	4(50)	0(0)

Figures in parenthesis indicate percentage of animals. Values with different superscript (a,b,c) in same row differ significantly ($P<0.01$).

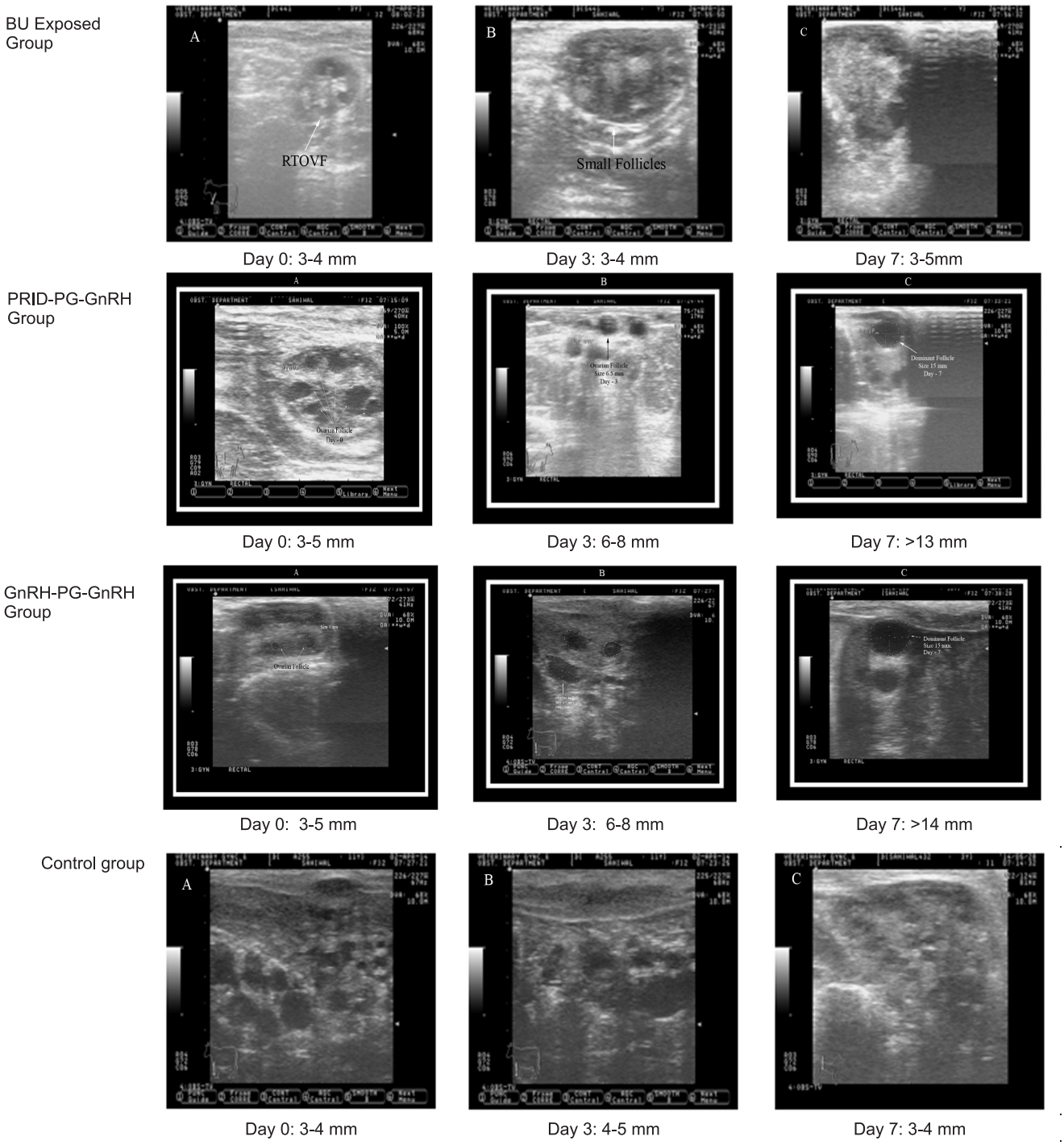

Fig. 1. Ultrasonographic imaging of ovarian follicles in treatment groups in pubertal Sahiwal heifers (8).

Bio-mechanics of ovarian follicles through ultrasonographic imaging of pubertal anestrus Sahiwal heifers in response to bull urine (BU), PRID-PG-GnRH, GnRH-PG-GnRH and control are presented (Table 2; Fig. 1). The mean total number of follicles at the beginning of experiment was within the range of 21–25 irrespective of treatment group. The mean number of total follicles differed significantly in PRID-PG-GnRH and GnRH-PG-GnRH treated groups as compared to BU- exposed and untreated control groups on day 7. Similarly on day 7, the mean number of small size (<5 mm) follicles was lesser in PRID-PG-GnRH and GnRH-PG-GnRH than those of BU exposed and untreated control group ($P<0.05$). However, the mean number of large follicles (>8 mm) in PRID-PG-GnRH and GnRH-PG-GnRH treated heifers were 1.87 and 2 respectively, on day 7 which was differed significantly from BU exposed and untreated control groups. This may be due to the exogenous progesterone provided from outside to PRID-PG-GnRH and GnRH-PG-GnRH treated heifers where several number of follicles reached more than >8

Table 2. Ultrasonographic imaging of ovarian follicles in treatment groups in pubertal Sahiwal heifers (8)

Groups	Particulars		Days		
			0	3	7
Group 1	Total no. of follicles		25	24^{ca}	23^{ca}
(BU)	Average no. of follicles/heifer (mm)	<5	19^{a}	16	16^{ab}
		5–8	6^{c}	8^{ab}	7
		> 8	0^{b}	0	0^{b}
Group II	Total no. of follicles		23^{ca}	17^{a}	11^{b}
(PRID-PG-GnRH)	Average no. of follicles/heifer (mm)	<5	18^{a}	14	5^{ba}
		5–8	5^{c}	3^{bc}	4
		> 8	0^{b}	0	1.87^{c}
Group III	Total no. of follicles		24^{ca}	19^{a}	9^{b}
(GnRH-PG-GnRH)	Average no. of follicles/heifer (mm)	<5	20^{a}	13	4^{ba}
		5–8	4^{c}	6^{bc}	3
		> 8	0^{b}	0	2^{c}
Group IV	Total no. of follicles		21	24^{cb}	26^{ca}
(Control)	Average no. of follicles/heifer (mm)	<5	17^{a}	16	18^{ab}
		5–8	4^{c}	8^{ab}	8
		> 8	0^{b}	0	0^{b}

Values with different superscript in same row and same column differ significantly ($P<0.05$).

mm in size on day 7 (Fig. 1). This is in agreement with the findings of Ali *et al*. (2008) on day 7 of PGF2α administration in buffaloes. However, the majority of follicles (<5 and 5–8 mm) throughout the course of experimental were more or less similar in number in BU exposed and untreated control groups. However, none of the follicle in BU treated and untreated control groups were >8mm in diameter which represents presence of anovulatory follicular wave, which indicates the state of true anestrus in these animals. These findings are consistent with the findings of Sahu (2002) who also reported that anestrus condition is characterized by presence of anovulatory follicular waves.

Anovulatory follicular waves in these anestrus heifers might be due to insufficiency of hypothalamic stimuli of GnRH to induce release of gonadotropins from anterior pituitary (Peters and Lamming 1984) rather than GnRH unresponsive follicles. The ultrasound monitoring of ovaries in pubertal anestrus Sahiwal heifers at 3 days interval for 30 days in BU and untreated control groups indicated the presence of anovulatory follicular waves in these heifers.

The present findings of overall conception rates at induced estrus (Table 1) in BU, PRID and GnRH treated animals are in close agreement with Tauck (2005) in bull urine exposed heifer than steer urine exposed heifers. Similarly our findings of overall conception rate at induced estrus in PRID-PG-GnRH and GnRH-PG-GnRH treated groups is in close agreement with Ali *et al.* (2012). However, Savalia *et al.* (2014) reported 50% overall conception rate in GnRH treated group. In contrast to our findings, Patra (2006) observed higher overall conception rate (72.73%) in bull urine (BU) exposed heifer. Khasatiya *et al.* (2008) observed higher overall conception rate (100%) among Ovsynch treated Surti buffaloes. However, Zulu *et al.* (2000) observed poor conception rates (28.6%) in anestrus cows after PRID treatment. These differences in pregnancy rates may be because of many factors such as fixed time artificial insemination (FTAI), low estrus intensity, breed, season, luteal activity and management factors.

From this study, it can be concluded that bio-stimulatory effect of bull urine (BU) for induction of estrus is significantly lower in pubertal anestrus Sahiwal heifers than PRID+PG and GnRH+PG treated heifers.

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