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# Effect of clitoral stimulation after artificial insemination on conception and hormonal profile in the Murrah buffalo

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ABSTRACT

Clitoral stimulation around the time of artificial insemination (AI), which is a simple managerial tool, improves the conception rate (CR) in cattle. Accordingly, an experiment was done to study the effect of clitoral stimulation post-insemination on the modulation of luteinizing hormone (LH) levels and conception rate in the Murrah buffalo. Buffaloes (92) comprising parous (68) and heifers (24) were equally divided into treatment and control groups. Buffaloes in the treatment group were given 30 sec of clitoral stimulation (massage) after AI while no such stimulation was given in the control. There was a significant increase in the pooled LH concentration in the treatment group (6.405±2.64 ng/ml) as compared to control (2.37± 0.838 ng/ml) irrespective of the sampling time. LH level in the treatment group was higher and maximum concentration was observed within 5 h of stimulation. Neither clitoral stimulation nor sampling time significantly affected the levels of serum estradiol. No significant difference was observed between the groups on different sampling days, except day 18 when significantly high levels of progesterone was detected in the treatment group as compared to control. Overall, the CR in the clitoral stimulation group was 1.9 times higher than that of control. Similarly, it was significantly higher in the treated parous buffaloes (67.65%) as compared to control (32.35%).

**Key words:** Artificial insemination, Buffalo, Clitoral stimulation, Conception rate, LH

Buffalo is one of the most versatile dairy animals of tropical and sub-tropical countries. However, the reproductive efficiency is low owing to delayed onset of puberty, seasonal breeding, poor estrus expression, prolonged inter-calving intervals and poor conception rate (CR) with AI. To overcome these inherent limitations, various approaches are being explored. For instance, we reported that subcutaneous melatonin injection at 18 mg/ 50kg body weight could be an effective approach for breaking summer anoestrus and amelioration of heat stress in the Murrah buffalo (Kumar 2014). Further, resumption of ovarian cyclicity and fertility response in bull-exposed postpartum buffaloes (Gokuldas *et al.* 2010), use of various hormones at the time of AI and the use of various estrus and ovulation synchronization protocols (Rensis 2007) have been devised to optimize the reproductive potential of buffaloes.

An increase in the uterine motility in cows has been found, when exposed to the presence of the bull, nuzzling by the bull, non-copulatory mounting and mounting with copulation (VanDemark and Hays 1952). Release of hypophyseal oxytocin following suckling is a well-established neurohumoral reflex in most mammals. Similarly, copulatory stimulus or mechanical stimulation of vagina induces LH surge and ovulation in rabbit, cat and camel. Though genital stimulation is not essential for preovulatory LH surge in the cattle and buffalo, it may have a favourable effect on the conception by synchronizing the time of AI and ovulation or potentiating the LH surge. Massaging the clitoris in the cows enlarges the cervical lumen and allows easier passage of sperm (Pointner 1986). It also stimulates the release of oxytocin causing increased contractions of the uterus towards the oviduct (Cooper and Foote 1986, Coyan and Tekeli 1996). Clitoris is composed of erectile tissue covered by stratified squamous epithelium and well-supplied with sensory nerve endings. Clitoral massage positively affects the release of certain protein hormones, such as oxytocin and LH (Randel *et al.* 1975), which is essential for ovulation.

The general excitability of buffalo upon seeing a stranger or unusual stimulus is high as compared to cattle. Unlike exotic dairy cow, weaning the calf at birth is impossible in the buffalo owing to its ability to withhold milk and consequent drop in the milk production. In fact, it is a common practice by buffalo rearing farmers to create a phantom calf if the newborn dies within a month of calving. Domestication of buffalo is a recent event as compared to cattle (Cockril 1974). Thus, it is likely that buffalo may respond for clitoral stimulation better than cow because of inherently strong neurohumoral mechanism. To the best of

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our knowledge, this is the first report on the effect of clitoral stimulation at AI on the hormonal profile and CR in the riverine buffalo; thus, the present study was carried out.

## MATERIALS AND METHODS

Apparently healthy female Murrah buffalo maintained at the cattle and buffalo farm, LPM Section, IVRI, Izatnagar, were utilized for this trial during the breeding season (September 2014 - February 2015). The animals were maintained under isomanagerial conditions of management, feeding with intensive system and housed in a well ventilated brick cemented house with non-slippery floor open byre and offered standard ration having green and dry fodder along with concentrate. Estrus detection was done by a teaser bull parading twice a day and observing the overt signs of heat. Estrus animals were inseminated as per AM-PM schedule using good quality frozen semen. Experimental buffaloes (2) were divided into parous (68) and heifer (24) categories, to examine the effect of parity. Further, parous animals were subdivided into treatment (34) and control (34) groups. Similarly, heifers were subdivided into treatment (12) and control (12) groups. Animals in the treatment group were given 30 sec of clitoral stimulation (massage) after AI while no such stimulation was given in the control group. Buffaloes that failed to settle in the first AI were not used subsequently.

Clitoral stimulation was done by placing the forefinger and thumb on the embedded portion of clitoris located at the ventral commissure of vulva and applying gentle massage for 30 sec. To study the effect of clitoral stimulation on LH and estradiol, three buffaloes were randomly selected from each group on the day of estrus. First blood sample was collected 1 h before AI and the remaining seven samples at hourly interval. Blood was collected by jugular venipuncture followed by serum separation and stored at –20°C till use. Progesterone was estimated on day 0, 6, 12 and 18 post AI (6/group). LH was estimated by Bovine LH ELISA test kit, that had a minimal detectable limit of 0.1 ng/ml. The ovarian estradiol and progesterone were assayed by RIA kits. Pregnancy diagnosis was done on day 75–90 of breeding in the non-return buffaloes by rectal palpation. CR was recorded as the number of pregnant animals divided by the total number of inseminated animals in a group and expressed as percentage. Repeat-measure ANOVA was applied for the data on the hormones. Chi-square test was used for finding the association between clitoral stimulation and CR in the Murrah buffalo. Odds of pregnancy following the clitoral massage in relation to control were also calculated. SAS version 9.3 was used for data analysis.

## RESULTS AND DISCUSSION

*Luteinizing hormone:* Mean serum LH concentration (ng/ml) on day of estrus was in the range of 0.43–6.58 and 0.36 –15.83 ng/ml in the control and clitoral stimulation groups, respectively. Highest serum LH concentration (ng/ml) of 6.58±5.64 was recorded in the control at 5 h post-AI. In contrast, highest value of 15.83±14.57 ng/ml was observed within 1 h of AI in the buffaloes that received clitoral stimulation. Pooled mean LH concentration in the control and treatment group was 2.327± 0.84 and 6.405±2.64 ng/ml, respectively (Fig. 1, Table 4). There was no significant effect of time on the LH level either in the control or treatment group. Similarly, the interaction between the clitoral stimulation treatment and time was not significant. In contrast, there was a significant ($P<0.05$; F, 9.948) increase in the overall mean LH level in the treatment group as compared to control irrespective of the sampling time. This is due to the fact at every time point tested, clitoral stimulation had numerically higher values of LH than that of control except 4 and 6 h post-AI. LH concentration of the buffaloes in the treatment group was higher and maximum values were observed within 5 h of stimulation (Fig. 1) presumably resulting in the better synchrony among insemination-ovulation-fertilization. The LH concentration was at basal levels till fifth hour in the control group and the highest value was recorded at 6 h post-AI. Clitoral cauterization applied under epidural anaesthesia in healthy dairy cows did not affect the mean LH concentration, peak concentration of LH and mean duration of LH surge indicating that clitoris is not inevitable for LH surge (Yüksel and Deveci 2011). However, clitoral stimulation at the time of AI can have an additive effect on the quality of LH pulses in the buffalo and thus might have improved CR in the present study. Peak value of LH in buffaloes at estrus ranges between 20–35 ng/ml (Batra and Pandey 1982). Peak LH levels observed in the control group was 6.58±5.64 at 6 h which is lower than the earlier report (Heranjal *et al.* 1976) where it ranged from 14.03 to 38.5 ng/ml at estrus in the

Table 1. Effect of clitoral stimulation on the conception rate (CR) in the parous Murrah buffalo.

| Group | CR (%) | Chi-Square & P value | Odds ratio (with 95% CI) |
|---|---|---|---|
| Treatment (n=34) | 67.65 (23/34)* | $\chi^2 df_1 = 8.471$ | 4.372 |
| Control (n=34) | 32.35 (11/34)* | P = 0.0035 | (1.582 to 12.08) |

*Figures in the parenthesis indicate the number of buffaloes pregnant out of total inseminations

Table 2. Effect of clitoral stimulation on the conception rate in the Murrah heifers.

| Group | CR (%) | Chi-Square & P value | Odds ratio (with 95% CI) |
|---|---|---|---|
| Treatment (n=12) | (66.67) (8/12)* | $\chi^2 df_1 =$ 1.510 | 2.80 (0.5318 to 14.74) |
| Control (n=12) | 41.67 (5/12)* | P = 0.2191 | |

*Figures in the parenthesis indicate the number of buffaloes pregnant out of total inseminations

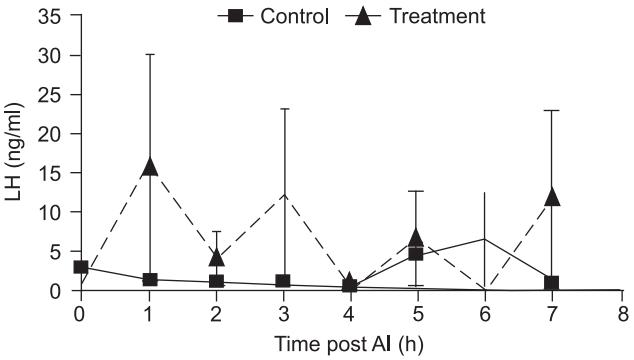


Fig. 1. Effect of clitoral stimulation on mean serum LH (ng/ml) on the day of estrus in the Murrah buffalo (Mean ± SE). Zero hour represents time of AI. Data were analyzed by repeat measure ANOVA using SAS version 9.3. There was no significant effect of group or time; however, overall LH concentration was significantly high in the treatment group (P<0.05).

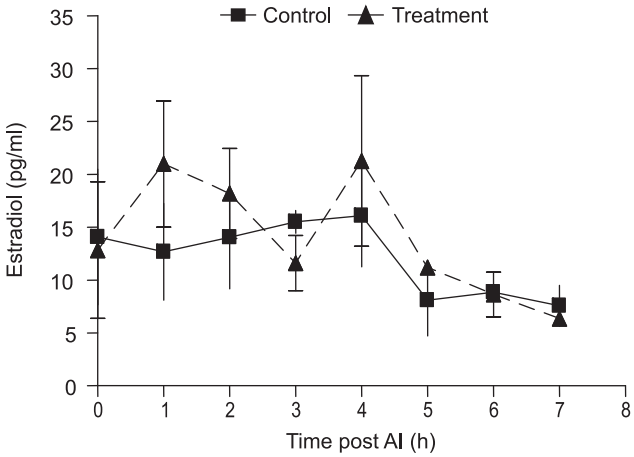


Fig. 2. Effect of clitoral stimulation on serum estradiol concentration (pg/ml) in the Murrah buffalo (Mean±SE). Data were analyzed using repeat measure ANOVA using SAS version 9.3. Neither treatment nor time had a significant effect on the estradiol (P>0.05).

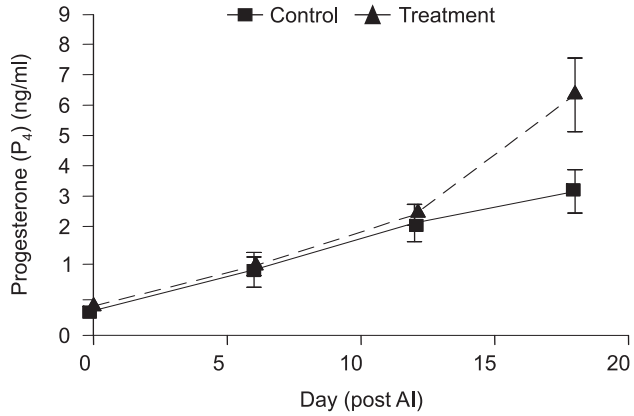


Fig. 3. Effect of clitoral stimulation on serum progesterone concentration (ng/ml) in the Murrah buffalo (Mean±SE). Data were analyzed by repeat measure ANOVA using SAS version 9.3. No significant difference was observed between the groups on different sampling days, except day 18 when significantly high concentration of $P_4$ was detected in the treatment group as compared to control (P<0.05).

Murrah buffalo. However, following clitoral stimulation, the levels ranged from 18.64 to 45 ng/ml, which is considerably higher than that of Heranjal *et al.* (1976) and others (Kaker *et al.* 1980, Avenell *et al.* 1985) indicating the positive effect of treatment in same breed of animals.

Table 3. Effect of clitoral stimulation on the overall conception rate in the Murrah Buffalo

| Group | CR (%) | Chi-Square & P value | Odds ratio (with 95% CI) |
|---|---|---|---|
| Treatment (n=46) | 67.39* | $\chi^2 df_1 = 9.787$ P= 0.0018 | 3.875 (1.631 to 9.205) |
| Control (n=46) | 34.78 (16/46)* | | |

*Figures in the parenthesis indicate the number of buffaloes pregnant out of total inseminations

Table 4. Effect of clitoral stimulation on mean serum LH (ng/ml) on the day of estrus in the Murrah buffalo (Mean±SE). Zero hour represents time of AI.

| Time (h) | Control | Treatment |
|---|---|---|
| 0 | 2.74 ± 1.25 | 0.67 ± 0.08 |
| 1 | 1.36 ± 0.30 | 15.83 ± 14.578 |
| 2 | 0.87 ± 0.25 | 3.91 ± 3.43 |
| 3 | 0.48 ± 0.097 | 11.79 ± 11.28 |
| 4 | 0.43 ± 0.19 | 0.39 ± 0.035 |
| 5 | 4.41 ± 3.61 | 6.51 ± 6.06 |
| 6 | 6.58 ± 5.64 | 0.367 ± 0.046 |
| 7 | 1.72 ± 1.23 | 11.74 ± 11.13 |
| Mean | 2.327 ± 0.838 | 6.405 ± 2.64 |

Table 5. Effect of clitoral stimulation on serum estradiol concentration (pg/ml) in the Murrah buffalo (Mean±SE).

| Time (h) | Control | Treatment |
|---|---|---|
| 0 | 14.07 ± 6.40 | 12.83 ± 6.46 |
| 1 | 12.69 ± 4.56 | 20.97 ± 5.95 |
| 2 | 14.04 ± 4.87 | 18.19 ± 4.26 |
| 3 | 15.50 ± 1.06 | 11.60 ± 2.60 |
| 4 | 16.08 ± 4.84 | 21.26 ± 8.07 |
| 5 | 8.09 ± 3.38 | 11.19 ± 0.55 |
| 6 | 10.17 ± 0.79 | 8.64 ± 2.11 |
| 7 | 10.89 ± 1.89 | 6.35 ± 0.40 |
| Mean | 12.693 ± 1.28 | 13.882 ± 1.75 |

Table 6. Effect of clitoral stimulation on serum progesterone concentration (ng/ml) in the Murrah buffalo (Mean±SE).

| Day | Control | Treatment |
|---|---|---|
| 0 | 0.690 ± 0.10 | 0.823 ± 0.10 |
| 6 | 1.84 ± 0.49 | 1.94 ± 4.34 |
| 12 | 3.15 ± 0.53 | 3.38 ± 0.29 |
| 18 | 4.04 ± 0.61 | 6.75 ± 1.04 |

The LH values after clitoral stimulation in our study are higher than that reported in the cattle (Randel 1973). Mechanical stimulation of the reproductive tract tends to hasten the LH surge and thus, shortens the interval from estrus to ovulation (Randel 1973). Actual LH surge could not be predicted due to difficulty of detecting exact time of onset of heat in the buffalo because of twice a day heat detection program.

*Serum estradiol (E2):* Mean serum E2 concentration (pg/ml) on the day of estrus ranged between 6.04–8.09 and 6.35–21.26 in the control and treatment groups, respectively. Mean serum E2 in the control and treatment group was 12.693±1.28 and 13.882±1.75 pg/ml, respectively (Fig. 2 and Table 5). Neither treatment nor time had a significant effect on the E2 concentrations between the control and treatment groups (P>0.05). Serum E2 (Fig. 2 and Table 5) are in accordance with earlier reports which demonstrated non-significant increase in E2 concentration after clitoral massage in the dairy cattle (Bozkurt 2007). The pattern of serum E2 and occurrence of maximum concentration around estrus (16.08±4.84 pg/ml) in the control group in the present study is in accordance with the earlier reports (Malfatti 2003, Singh *et al.* 2001). However, peak concentrations of E2 (30–35 pg/ml) were detected on the day of estrus or a day before (Batra and Pandey 1982), followed by a decline to 5–10 pg/ml within two days which is indicative of enhanced E2 production by the preovulatory follicle during pro-estrus. The serum concentration of E2 in buffalo during the follicular phase of the estrous cycle appears to be relatively lesser than that in cattle (Avenell 1985).

*Serum progesterone (P4):* Serum P4 concentration (ng/ml) in the control and treatment group on day 0, 6,12 and 18 post-AI ranged between 0.69–4.04 and 0.823–6.75, respectively. Lowest values were recorded on the day of estrus in either group. No significant difference was observed between the groups on different sampling days, except day 18 when significantly high levels of P4 was detected in the treatment group as compared to control (P>0.05). Progesterone showed a significant time effect on day 12 and 18 post-breeding in the control and treatment groups, which is due to the effect of luteal phase. The interaction between clitoral stimulation treatment and time was not significant (Fig. 3 and Table 6). Progesterone profile in the buffalo followed the same pattern as that of cattle during the estrus cycle (Mondal and Prakash 2002, Terzano *et al.* 2012). In our study, a significant treatment effect was seen on P4 level on day 18 post-AI (Fig. 3 and Table 6). Retrospective analysis of the data revealed that more number of buffaloes became pregnant in the treatment group (5/6) than that of control (3/6) which might be the most likely reason for elevated P4 levels in the former group. The results obtained in the control group were in accordance with other workers (Pahwa and Pandey 1983) that P4 varies from 0–6 ng/ml during the cycle, being at basal levels during estrus and rises thereafter. The changes in the concentration of P4 in blood and milk during estrous cycle are similar to those in cattle, but the peak concentration is relatively less (Dobson and Kamonpatana 1986, Singh *et al.* 2001).

*Conception rate (CR):* In the present study, the effect of clitoral stimulation post-AI on the CR of the Murrah buffalo was examined. In parous category, the CR following clitoral stimulation was 67.65%, which was significantly higher than that of 32.35% recorded in the control (P<0.01). Heifers that received clitoral stimulation had an increased CR by 25%; but, the difference was not statistically significant (P>0.05). However, the pooled CR following clitoral stimulation was 67.39%, which was significantly (P<0.01) higher than 34.78% recorded in the control group (Tables 1–3).

It is evident that the effect of clitoral stimulation on the CR to first AI was highly significant in the parous buffalo (Table 1). The CR was non-significantly high in the treated heifers (Table 2). The overall CR was also highly significant in the group of buffaloes that received clitoral massage at the time of AI (Table 3). The results are supported by the fact that the overall odds of conception in a buffalo that received clitoral massage at the time of first AI was 3.875 times higher as that of unstimulated control. The odds of getting pregnant after clitoral stimulation in the parous buffalo was 4.37 (95% CI: 1.582–12.08) as compared to parous buffaloes without clitoral stimulation and the odds of getting pregnant after clitoral stimulation in the heifers was 2.80 (95% CI: 0.531–14.74) as compared to the heifers without clitoral stimulation. It is tempting to speculate that the significant increase in the pooled LH concentration following clitoral stimulation could be one of the reasons behind the significant increase in the CR though a time effect was not found.

Results on the CR is in accordance with earlier work in the cattle (Randel 1975) which demonstrated that clitoral stimulation should be used as a routine procedure following AI in the beef cattle. Clitoral stimulation significantly increased the pregnancy rate in cattle heifers (Cooper *et al.* 1985, Rodriguez *et al.* 1980, Arbeiter *et al.* 1985). The failure to obtain a statistical significance to clitoral massage on CR in the heifers might be due to small sample size. Glauber (1989) also found that the CR was higher in the cows that received clitoral massage for 10 seconds (65.08%) than that of control (51.43%). Clitoral stimulation at the time of AI was reported as an effective means of increasing the pregnancy rate in the dairy cow (Lunstra 1983). However, Rodriguez *et al.* (1980) showed that the pregnancy rate was higher in animals not receiving clitoral massage than in those receiving it. Clitoral massage shortens the estrus duration in crossbred cattle herds with prolonged estrus, in terms of number of days of expression of estrus signs. This in turn reduces the necessity for double AI and improves the CR of single AI, lowering number of services per-conception (Kutty 2006). Hastening the follicular growth and ovulation in clitoral massage group has been observed as compared with the control (Arbeiter *et al.* 1985). Overall, these facts support our study that clitoral stimulation following AI would increase CR in the riverine buffalo.

From our study, we did not exactly elucidate the mechanism behind the increase in CR in the buffalo receiving clitoral stimulation following AI, except finding a positive effect of stimulation on the endogenous LH release. Various neural pathways exist between the reproductive system and hypothalamic-pituitary axis. Stimulation of the reproductive tract at AI hastened the LH surge and ovulation in the cow (Randel 1973). Sperm transport may be the point of greatest action as stimulation of the reproductive tract has been shown to affect uterine motility (VanDenmark and Hays 1952, Hays and VanDenmark 1953a,b). Clitoral stimulation at the time of AI causes oxytocin release from the posterior pituitary gland, which has a positive effect on the sperm transported into female genital tract (Bozkurt 2007). Oxytocin was used parenteral to increase CR by improving the sperm transport in the female reproductive tract of several species (Sayre and Lewis 1997, Yildiz 2005).

Considering the increased LH level and CR following clitoral stimulation, it appears that there is a neural circuit between the sensory afferents from the clitoris to the GnRH centre in the buffalo. It is concluded that clitoral stimulation immediately after AI would enhance the CR at first AI in the Murrah buffalo that needs to be validated using large sample size.

The effect of clitoral stimulation for 30 sec at first AI was studied on the levels of LH, estradiol over a period of 8 h in the Murrah buffalo using unstimulated group as control (3/group). Based on confirmed pregnancy, CR was recorded (92). Pooled LH concentration in the treatment group was significantly higher (4.035 ng/ml) than the control ($P<0.05$) and maxium level was recorded within 5 h of stimulation. Neither clitoral stimulation nor sampling time significantly affected the levels of serum estradiol. Clitoral stimulation significantly increased the CR by 35.3% in the parous buffalo with respect to control ($P<0.05$). Though the CR in the heifer was 66.67% following clitoral stimulation, it was approaching significance as compared to 41.67% observed in the control. The pooled CR was 67.39% in the treatment group which was significantly ($P<0.01$) higher than that of 34.78% recorded in the control group. The results suggested that the clitoral stimulation at first AI has the potential to increase the fertility in the Murrah buffalo.

## REFERENCES


Arbeiter K, Pohl W and Rumpf R. 1985. Physical methods for inducing ovulation in heifers. *Tierärztl. Umschau* **40**: 442–50.

Avenell J A, Saepudin Y and Fletcher I C. 1985. Concentration of LH, estradiol–17h and progesterone in the peripheral plasma of Swamp buffalo. *Journal of Reproductive Fertility* **74**: 419–24.

Batra S K and Pandey R S. 1982. Luteinizing hormone and oestradiol-17ß in blood plasma and milk during oestrous cycle and early pregnancy in Murrah buffaloes. *Animal Reproduction Science* **5**: 247–57.

Bozkurt T, Turk G and Gur S. 2007. Effect of clitorial massage on levels of estradiol, testosterone, dehydroepiandrosterone sulfate and pregnancy rates in cows. *Veterinarski Arhiv.* **77**(1): 59–67.

Cockrill W R (ed.) 1974. The husbandry and health of the domestic buffalo. Food and Agriculture Organization of the United Nations, Rome.

Cooper M D and Foote R H. 1986. Effect of oxytocin, prostaglandin F2á and reproductive tract manuplations on uterine contractility in holstein cows on days 0 and 7 of the estrous cycle. *Journal of Animal Science* **63**: 151–61.

Cooper M D, Newman S K, Schermerhorn E C and Foote R H. 1985. Uterine contractions and fertility following clitoral massage of dairy cattle in estrus. *Journal of Dairy Science* **68**: 703–08.

Coyan K and Tekeli. 1996. Ýneklerde Suni Tohumlama. Konya-Türkiye, Birinci Basým, Bahçývanlar *Basým Þirketi.* 46–51

Custer E E, Berardinelli J G, Short R E, Wehman M and Adair R. 1990. Postpartum interval to estrus and patterns of LH and progesterone in first-calf suckled beef cows exposed to mature bulls. *Journal of Animal Science* **68**: 1370–77.

Dobson H and Kamonpatana M. 1986. A review of female cattle reproduction with special reference to a comparison between buffaloes, cows and zebu. *Journal of Reproductive Fertility* **77**: 1–36.

Glauber C E. 1989. Effect of clitoral massage after artificial insemination on conception rate in beef cows. *Veterinaria Argentina* **6**: 438–39.

Gokuldas P P, Yadav M C, Kumar H, Singh G, Mahmooda S, Tomar A K S. 2010. Resumption of ovarian cyclicity and fertility response in bull-exposed postpartum buffaloes. *Animal Reproduction Science* **121**: 236–41.

Hays R L and VanDemark N L. 1953a. Effect of stimulation of the reproductive organs of the cow on the release of an oxytocin-like substance. *Endocrinology* **52**: 634.

Hays R L and VanDemark N L. 1953b. Effects of oxytocin and epinephrine on uterine motility in the bovine. *American Physiol*ogy **172**: 557.

Heranjal D D, Sheth A R, Moodbidri S B, Desal R and Rao S S. 1976. A note on LH during estrous cycle and early pregnancy in Indian buffaloes. *Indian Journal of Animal Sciences* **46**: 553–55.

Kamboj M and Prakash B S. 1993. Relationship of progesterone in plasma and whole milk of buffaloes during cyclicity and early pregnancy. *Tropical Animal Health and Production* **25**: 185–92.

Kaker M L, Razdan M N and Galhotra M M. 1980. Serum LH concentrations in cyclic buffalo (*Bubalus bubalis*). *Journal of Reproductive Fertility* **60**(2): 419–24.

Kumar A. 2014. 'Effect of melatonin administration on restoration of fertility in summer anestrus buffalo'. Ph. D. Thesis, IVRI, Izatnagar, India, pp 69–85.

Kutty C I. 2006. Effect of post-insemination clitoris massage on conception rate and duration of oestrus in crossbred cows with prolonged oestrus. *Indian Journal of Animal Sciences* **76** (1): 10–13.

Lunstra D D, Hays W G, Bellows R A and Laster D B. 1983. Clitoral stimulation and the effect of age, breed, technician, and postpartum interval on pregnancy rate to artificial insemination in beef cattle. *Theriogenology* **19**: 555–63.

Malfatti A. 2003. Recent advances in buffalo endocrinology. Atti Secondo Congresso Nazionale sull'Allevamento del Bufalo, Monterotondo. Roma; pp. 161–176.

Mondal S and Prakash B S. 2002. Peripheral plasma progesterone concentrations in relation to oestrus expression in Sahiwal cows and Murrah buffaloes. *Reproduction* **28**: 29–30.

Pahwa G S and Pandey R S. 1983. Gonadal steroid hormone concentration in blood plasma and milk of primiparous and multiparous pregnant and non-pregnant buffaloes. *Theriogenology* **19**: 491–505.

Pointner J. 1986. Clitoral massage as a supporting measure in manipulation of the bovine uterus. *Tierärztl. Praxis* **14**: 217–18.

Randel R D, Short R E, Christensen D S and Bellows R A. 1973. Effects of various mating stimuli on the LH surge and ovulation time following synchronization of estrus in the bovine. *Journal of Animal Science* **37**: 128.

Randel R D, Short R E, Christensen D S and Bellows R A. 1975. Effect of clitoral massage after artificial insemination on conception in the bovine. *Journal of Animal Science* **40**: 1119–23.

Rensis F De and Lo´pez-Gatius F. 2007. Protocols for synchronizing estrus and ovulation in buffalo (*Bubalus bubalis*): A review. *Theriogenology* **67**: 209–16.

Robertson H A and Rakha A M. 1965. The timing of the neural stimulus which leads to ovulation in the sheep. *Journal of Endocrinology* **32**: 383–86.

Rodriguez T, Verde O and Espinoza J. 1980. Effect of time of insemination, clitoral massage, season, breed and other factors on fertility in cattle. In: 9th International Congress on Animal Reproduction and Artificial Insemination, Madrid, Spain. pp 205.

Rodriguez-Martinez H, Mckenna D, Weston P G, Gustafsson B K and Whitomore H L. 1987. Uterine motility in the cow during the estrous cycle. II. Effect of oxytocin, xilazine, and adrenoceptor blockers. *Theriogenology* **27**: 359–68.

Sayre B L and Lewis G S. 1997. Fertility and ovum fertilization rate after laparoskopik or transcervical intrauterine artificial insemination of oxytocin-treated ewes. *Theriogenology* **48**: 267–75.

Segura C V M and Rodriguez R O L. 1994. Effect of clitoral stimulation after artificial insemination on conception in zebu-crossbred heifers in the tropics. *Theriogenology* **42**: 781–87.

Short R E, Carr J B, Graves N W, Mimline W L and Bellows R A. 1979. Effect of clitoral stimulation and length of time to complete AI on pregnancy rates in beef cattle. *Journal of Animal Science* **49**: 647–50.

Singh B, Dixit V D, Singh P, Georgie G C and Dixit V P. 2001. Plasma inhibin levels in relation to steroids and gonadotrophins during the oestrus cycle in buffalo. *Reproduction in Domestic Animals* **36**(3–4): 163–67.

Terzano G M, Barile V L and Borghese A. 2012. Overview on reproductive endocrine aspects in buffalo. *Journal of Buffalo Science* **1**: 126–38.

VanDemark N L and Hays R L. 1952. Uterine motility responses of mating. *American Journal of Physiology* **170**: 518.

Yildiz A. 2005. Effect of oxytocin on conception rate in cows. *Journal of Firat University of Health Science* 19: 75–78.

Yüksel M and Deveci H. 2011. The efficiency of cauterization of clitoris on serum LH Levels in cows. *Fýrat University Medical Journal of Health Sciences* **25**(2): 57–60.