The Pharma Innovation Journal 2022; SP-11(10): 2061-2065

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(10): 2061-2065

www.thepharmajournal.com
Received: 15-07-2022
Accepted: 19-08-2022

Md. Zakiuddin
Department of Veterinary Gynaecology and Obstetrics, Veterinary College, Bidar, Karnataka, India

MK Tandle
Professor and Director of Instruction (PGS), KVAFSU, Bidar, Karnataka, India

SM Usturge
Retired Dean, Veterinary College, Bidar, Karnataka, India

NA Patil
Director of Extension, KVAFSU, Bidar, Karnataka, India

D Dilip Kumar
Dean, Veterinary College, Bidar, Karnataka, India

VR Kasaralikar
Professor and Head, Department of Veterinary Medicine, Veterinary College, Bidar, Karnataka, India

MD Suranagi
Director of Student Welfare, KVAFSU, Bidar, Karnataka, India

Corresponding Author:
Md. Zakiuddin
Department of Veterinary Gynaecology and Obstetrics, Veterinary College, Bidar, Karnataka, India

Therapeutic management of non-infectious repeat breeder buffaloes using GNRH analogue

Md. Zakiuddin, MK Tandle, SM Usturge, NA Patil, D Dilip Kumar, VR Kasaralikar and MD Suranagi

Abstract
The study was conducted to determine the effect of GnRH (Buserelin Acetate) administration as a therapy for repeat breeding syndrome in 30 buffaloes. White side test was carried out to exclude the possibility of sub-clinical genital infection in these animals. The repeat breeder buffaloes which were found to be negative for White side test were divided randomly into three equal groups (n=10). The Group I buffaloes received GnRH analogue 10 µg at the time of insemination, Group II received GnRH analogue 10 µg at the time of insemination and another dose of GnRH analogue 10 µg on 12th day post insemination. Group III kept as control. The Vaginal Electrical Resistance (VER) values of pregnant (242±6.27) and non-pregnant buffaloes (262.04±7.07) differ significantly (p<0.05). The color of estrual mucus in repeat breeder buffaloes was 62.50% transparent, 37.50% translucent and 00.00% whitish. 16.67%, 45.83% and 37.50% of repeat breeder buffaloes were having thick, viscous and thin consistency of estrual mucus. The mean pH value of estrual mucus was 7.37±0.04. Conception rate in buffaloes is more when cervico-vaginal mucus on the day of estrus is transparent, viscous, with typical fern pattern, spinnbarkeit value more than 10 cm and pH ranges 7-8. Conception rate in repeat breeder buffaloes was 90%, 70% and 10% in Group II, I and III respectively.

Keywords: Repeat breeder buffaloes, GnRH analogue, vaginal electrical resistance (VER), estrual mucus

Introduction
Buffaloes are called as black gold of Indian farmers because of their adoptive nature in harsh climatic conditions, endowed tolerance to diseases and better survival under poor feeding and management practices. Repeat breeding is a major problem adversely affecting the productive and reproductive performance in buffaloes. The repeat breeding syndrome is defined as a condition in which dairy animal have a regular estrus cycle and appear normal on superficial clinical examination but fail to become pregnant following three or more breeding (Bartlett *et al*., 1986) [7]. Repeat breeding results in delayed conception and longer inter-calving period and thus, reduces the economy of the dairy industry. The incidence of repeat breeding varies from 15-32% and seems to be lower in buffaloes kept individually on small-holdings than in large herds (Dhami *et al*., 2009) [11]. Previous work has indicated that, delayed ovulation (Erb *et al*., 1976) [14], inadequate luteal function (Kimura *et al*., 1987) [18], embryonic death (Ayalon, 1984) [6], management errors (O'Farell *et al*., 1983) [33] and non-specific uterine infection (Samad *et al*., 1984) [43] are among some possible causes of repeat breeding. However, failure of fertilisation and early embryonic death are the two major groups responsible for repeat breeding (Tanabe and Casida, 1949) [51].
One of the major factors of repeat breeding in bovines may be delayed ovulation (Erb *et al*., 1976) [14]. Luteal dysfunction leading to inadequate progesterone production during early luteal phase of the cycle could be a cause of early embryonic mortality (Pandey *et al*., 2013) [34] leading to repeat breeding. Ahmad *et al*. (2002) [3] stated that GnRH analogue improved the conception rate in Nili Ravi buffaloes, when administered as a single dose at the time of AI rather than using in split doses. Mann *et al*. (1995) [25] reported that, GnRH administered at mid luteal phase suppressed the small pulses of PGF_2 α occurring from day 12 onwards and thus, reduced the strength of luteolytic drive. Therefore, the present investigation was aimed to study Vaginal Electrical Resistance (VER), physical characteristics of estrual mucus including colour, consistency, pH, arborization, spinnbarkeit value and white side test in repeat breeder buffaloes. Further, it was planned to assess the therapeutic efficacy of different line of GnRH (Buserelin Acetate) treatments in treating repeat breeder buffaloes.

Materials and Methods

The study was carried out in 30 repeat breeder buffaloes belonging to members of Bidar-Gulbarga Milk Producers Union, buffaloes presented to VGO-OPD, Veterinary College, Bidar and APMC Veterinary Hospital Bidar. The buffaloes, which failed to conceive to 2 to 3 or more insemination using good quality frozen semen / served by healthy fertile buffalo bull at regular cycling estrus without any reproductive tract abnormalities were selected for the study. These buffaloes were diagnosed for repeat breeding on the basis of history obtained by the animal owner (age, parity, number of AI / natural service done, history of dystocia / abortion / RFM, regularity of estrous cycle), per rectal examination, white side test and records.

Vaginal electrical resistance (VER)

VER was tested by estrus detector[1] on the day of estrus in all the repeat breeding buffaloes and values were recorded.

Physical characteristics of estrual mucus

Following confirmation of estrus, the cervico-vaginal mucus from buffaloes was collected aseptically in a sterile vial for studying various physical parameters / tests viz. color, consistency, pH, spinnbarkeit, arborization and white side test.

1. **Color:** Color of cervico-vaginal mucus was observed and graded as transparent, translucent and whitish.
2. **Consistency:** Consistency of cervico-vaginal mucus was observed and graded as viscous, thin and thick.
3. **pH:** The pH of cervico-vaginal mucus was recorded using universal indicator pH paper.
4. **Spinnbarkeit value:** A drop of cervico-vaginal mucus was spread on a clean glass slide and was lifted with the help of another glass slide along the side of a scale fixed vertically. The point of breaking up of mucus while lifting is the spinnbarkeit value recorded in centimeters.
5. **Arborization pattern:** A drop of cervico-vaginal mucus was spread on a clean glass slide and air dried then observed under low power objective under microscope for appearance and presentation of crystallization pattern. The type of arborization was classified as per Vadodaria and Prabhu, (1989) [54].
6. **White side test:** One ml of cervico-vaginal mucus was mixed with equal volume of 5% Sodium hydroxide (NaOH) solution and heated up to the boiling point in a water bath for two minutes as described by Pateria and Rawal (1990) [36].

Study model

The study was carried out in 30 repeat breeder buffaloes. These buffaloes were categorized into treatment (n=20) and control (n=10) groups.

The treatment buffaloes (n=20) were sub-divided into 2 groups i.e. Group-I, and II. The Group I buffaloes received GnRH analogue 10 µg at the time of insemination, Group II received GnRH analogue 10 µg at the time of insemination + another dose of GnRH analogue 10 µg on 12th day post insemination and Group III kept as control.

Follow up

All the buffaloes were inseminated in the subsequent 1st, 2nd and 3rd estrus periods if repeated after treatment. The pregnancy was diagnosed per rectally after 60 days of A.I.

Statistical analyses

The data obtained was subjected for statistical analyses as per methods described by Snedecor and Cochran (1980) [49]. Statistical analysis of experimental data was carried out by employing paired 't' test and ANOVA, using the "SAS 9.3" software which was procured from Indian Council of Agriculture Research under the NAIP project "Strengthening statistical computing for NARS".

Results and Discussion

Vaginal electrical resistance (VER)

The mean value of VER in non-infectious repeat breeder buffaloes was 249.13±6.93. The mean value of VER in pregnant repeat breeder buffaloes was 242±6.27 and in non-pregnant repeat breeder buffaloes was 262.04±7.07. The difference between the VER values of pregnant and non-pregnant animals was statistically significant (p<0.05). The conception rate is more when VER values are less on the day of estrus in repeat breeder buffaloes. Sharma *et al.* (2004) [45] found significant association (p<0.01) between VER and non-return rates as well as with pregnancy rate (p<0.05). This result is in agreement with the findings of Meena *et al.* (2001) [29].

Physical characteristics of estrual mucus

1. **Color:** Non-infectious repeat breeder buffaloes exhibited 0.00% whitish, 37.5% translucent and 62.5% transparent color. Mehta (1986) [30] reported that, 54.17% of repeat breeder animals had clean and transparent cervical mucus. Samad *et al.* (2002) [44] reported that, estrus mucus was transparent in 55.00%, translucent in 38.33% and whitish in 6.67% repeat breeder buffaloes. Enkhia and Kohli (1982) [13] found 50.00% transparent, 30.00% translucent and 20.00% yellowish in repeat breeder cows.
2. **Consistency:** The estrual mucus consistency was thick in 16.66%, viscous in 45.83% and thin in 37.5% non-infectious buffaloes. Higher pregnancy rate of 59.09% was recorded in buffaloes having viscous type of estrual mucus discharge followed by thick (27.72%) then followed by 18.18% in thin mucus discharge. These results are in agreement with Vadodaria and Prabhu (1990) [55]. In contrast, Sharma *et al.* (2011) [48] recorded 44.44% in viscous, 33.33% in thick and 22.33% conception rate in thin vaginal mucus. Deo and Roy (1971) [10] reported 59.10% and 32.90% of conception rates in buffaloes with thin and thick cervical mucus respectively. Gebhard and Schumacher (1970) [15] reported that, profuse, watery and clear cervical mucus was favourable for sperm penetration and thick, scanty and opaque cervical mucus was unfavourable for sperm penetration.
3. **pH:** The mean value of pH was 8.45±0.10 in infectious and was 7.375±0.04 in non-infectious repeat breeder buffaloes. The pH values are towards alkaline side. This is in agreement with Vadodaria and Prabhu (1990) [55], Salphale *et al.* (1993) [42], Samad *et al.* (2002) [44], Kumar *et al.* (2011) [19] and Ramsingh *et al.* (2013) [38] but Tsiligianni *et al.* (2001) [53] reported slightly acidic i.e. 6.5-6.7. Out of all repeat breeder buffaloes 72.72% conceived when the estrual mucus pH ranged from 7 to 8 followed by 13.63% conception rate in pH of both 7 and 8-9.

[1] Draminski Estrous Detector®, Electronics in Agriculture, Owocowa

4. **Spinnbarkeit value:** The mean spinnbarkeit value of non-infectious repeat breeder buffaloes was 9.87±0.88 cm. Sharma *et al.* (2011) [48] reported average spinnbarkeit value of mucus was 9.35±0.66 cm which is in close conformity with Rangnekar *et al.* (2002) [40]. Dodamani (2000) [12] reported higher spinnbarkeit value (24.67±1.32) in repeat breeding cows this value was in conformity of with Mohanthy *et al.* (1996) [31] and Choudri *et al.* (1997) [9]. Among all experimental repeat breeder buffaloes, 54.54% conceived when the spinnbarkeit value was more than 10 cm followed by 27.27% conception rate in spinnbarkeit value ranged from 6-10 cm and 18.18% conception rate in spinnbarkeit value from 1-5 cm respectively. Patil (1987) [37] and Sharma *et al.* (2011) [48] reported that, buffaloes that conceived had significantly higher (p<0.01) mean spinnbarkeit value (12.94±0.81 vs 7.91±0.96 cm) as compared to those that did not conceive. But Jadhav (1996) [17] and Bennur (2004) [8] did not find such variation in fertile and infertile estruses and conceiving and non-conceiving cows (7.38±5.60 and 8.05±1.33 cm).
5. **Arborization pattern:** Out of all experimental buffaloes, 45.00% typical, 55.00% atypical and 00.00% nil arborization pattern was observed. Luktuke and Roy (1967) [22] reported the cervical mucus fern pattern as typical (72.7%), atypical (18.2%) and nil type (9.1%). Pandya *et al.* (1987) [35] reported 35.71% typical, 44.24% atypical and 19.05% nil type of fern pattern in buffaloes. Kumaresan *et al.* (2001) [20] reported that, out of 69 buffaloes, 40, 16 and 13 showed typical, atypical and nil fern pattern. Conception rate of 57.5% and 18.57% were obtained in buffaloes with typical and atypical fern pattern. Samad *et al.* (2002) [44] indicated that, fern pattern was typical in 55% atypical in 45.0% and 0% nil in repeat breeder buffaloes. Sharma *et al.* (2011) [48] reported typical, atypical and nil fern pattern of mucus was observed in 39.34%, 42.63% and 18.03% buffaloes.

It is concluded that higher conception rate in repeat breeder buffaloes was noticed when cervico-vaginal mucus was transparent, viscous with typical fern pattern, spinnbarkeit value more than 10 cm and with pH range from 7-8.

Efficacy of GnRH analogue

The effects of GnRH treatment at A.I. on fertilization rates and embryonic mortality may be related to timing of ovulation and progesterone secretion by corpus luteum. McDougall *et al.* (1995) [27] reported that, an LH surge could be induced by treatment with GnRH. Several studies have reported that GnRH administration has positive effect on follicular recruitment, ovulation and development of large luteal cells of the corpus luteum (Mee *et al.*, 1993, Morgan and Lean, 1993 and Troxel, 1993) [28, 32, 52]. Aminu deen *et al.* (1991) stated that, GnRH has the properties of inducing a gonadotrophin surge which mimics to a pre-ovulatory endocrine surge. Sreenan and Diskin (1983) [50] concluded that, GnRH / hCG and progesterone have been successful to induce timely ovulation, sustain early pregnancy and improve conception rate in repeat breeding bovines.

An average of 70.00% repeat breeder buffaloes conceived with 2 services per conception in group III (GnRH on day of estrus). The present results are in accordance with the findings of Mandal *et al.* (2004) [24], Sah and Nakao (2006) [41], Honparkhe *et al.* (2009) [16] and Ahmed *et al.* (2010) [4]. The present results are better than Rao and Rao (1984) [39], Ahmad *et al.* (2002) [3], Samad *et al.* (2002) [44] and Dhami *et al.* (2009) [11] and lesser than the findings of Sharma and Dhami (2008) [46], Markandeya and Patil (2008) [26] and Sharma *et al.* (2008) [47].

An average of 90.00% repeat breeder buffaloes conceived with 1.6 services per conception in group IV (GnRH on day of estrus + GnRH on day 12 post insemination). The present results are in accordance with the findings of Dodamani (2013) [34], Mandal *et al.* (2004) [24] and better than Pandey *et al.* (2013) [34]. MaCMillan *et al.* (1985) [23] suggested that, GnRH analogue has both luteotropic and luteoprotective effect which helps in maternal recognition of pregnancy, also reported better conception rate when GnRH was administered on day 12 than that administered on day 7. Mann *et al.* (1995) [25] reported that, GnRH administered at mid luteal phase suppressed the small pulses of $PGF_2\alpha$ occurring from day 12 onwards and thus, reduces the strength of luteolytic drive. This resulted in prolonged life of CL resulting into secretion of sufficient progesterone required for pregnancy maintenance. Lopez-Gatius *et al.* (2006) [21] has demonstrated that, GnRH treatment at the time of insemination and 12 days later increases conception rate (35.4%) more than single injection at AI. Administration of buserelin acetate or hCG on day 12 post-ovulation has beneficial impact on conception rate in buffaloes through an improvement in the post-treatment luteal profile as revealed by better development of spontaneous CL, formation of accessory CL and an increase in plasma progesterone (Pandey *et al.*, 2013) [34].

It may be concluded from the present study that, GnRH analogue improved the conception rate in non-infectious repeat breeder buffaloes, when administered at the time of AI and day 12 after insemination rather than using GnRH once at the time of AI.

References

1. Aboul-ELA MB. Proc. Buffalo seminar on reproduction and meat production. Tanuku (A.P.), India w.e.f. 15-17th; 1982. p. 529.
2. Aboul-Ela MB, Topps JH, Macdonald DC. Relationships between intravaginal electrical resistance, cervicovaginal mucus characteristics and blood progesterone and LH. Animal Reproduction Science. 1983 May 1;5(4):259-73.
3. Ahmad G, Saeed MA, Bashir IN. Use of GnRH to improve conception rate in repeat breeder buffaloes during the low breeding season. Pakistan Veterinary Journal. 2002;22(1):42-44.
4. Ahmed WM, EL-khadrawy HH, Hanafi EM, Ali AH, Shalaby SA. Clinical perspective of repeat breeding syndrome in buffaloes. Journal of American Science. 2010;6(11):661-666.
5. Aminu Deen, Khar SK, Khrana NK, Galhotra MM, Dixit VP. Effect of GnRH on postpartum ovarian activity. Paper presented in 9th Ann. Conv. Of ISSAR, HAU, Hissar, 1991 Feb:6-8.
6. Ayalon N. The repeat breeder problems. Prec. 10th Int. congr. Anim. Reprod. and A.I. 1984;4:1.
7. Bartlett PC, Kirk JH, Mather EC. Repeated insemination in Michigan Holstein-Friesian cattle: Incidence, descriptive epidemiology and estimated economic impact. Theriogenology. 1986 Sep 1;26(3):309-22.
8. Bennur PC, Honnappagol SS, Tandle MK. Effect of physico-chemical properties of cervico-vaginal mucus on fertility in cows. Indian veterinary journal.

2004;81(9):1069.
9. Choudri SC, Arali GS, Honnappagol SS. Incidence of various reproductive disorders in cows and buffaloes of Bidar. XIV ISSAR- National Symposium on recent advances for enhancement of reproductive efficiency in farm animals; c1997. p. 104.
10. Deo S, Roy DJ. Investigations on repeat breeding cows and bullaloes--studies on physical properties of cervical mucus. Indian veterinary journal. 1971;48(5):479-484.
11. Dhami AJ, Butani MG, Sharma SK. Therapeutic management of repeat breeding buffaloes with hormones and antibiotics under field conditions. Intas Polivet. 2009;10(1):39-44.
12. Dodamani MS. Therapuetic management of repeat breeding in bovines using gonadotrophin releasing hormone. M.V.Sc. Thesis, University of Agricultural Sciences, Dharwad, India; c2000.
13. Enkhia KL, Kohli IS. Note on the physical properties of cervico-vaginal mucus during oestrus in normal and repeat-breeding Rathi cows. Indian journal of animal sciences. 1982;52:1239-1240.
14. Erb RE, Garverick HA, Randel RD, Brown BL, Callahan CJ. Profiles of reproductive hormones associated with fertile and nonfertile inseminations of dairy cows. Theriogenology. 1976 May 1;5(5):227-42.
15. Gebhard FB, Schumacher MD. Biochemistry of cervical mucous. Fertil. Steril. 1970;21(10):967.
16. Honparkhe M, Ghuman SP, Dadarwal D, Jagir S, Dhaliwal GS. Embryonic mortality and luteal profile in buffaloes administered GnRH at the onset of estrus or hCG 5 days after AI. Indian Journal of Animal Sciences. 2009;79(8):778-80.
17. Jadhav RS. Effect of single/double insemination with or without exogenous oxytocin. M.V.Sc. thesis submitted to Konkan Krishi Vidyapeeth, Dapoli, India; 1996.
18. Kimura M, Nakao T, Moriyoshi M, Kawata K. Luteal phase deficiency as a possible cause of repeat breeding in dairy cows. British Veterinary Journal. 1987 Nov 1;143(6):560-6.
19. Kumar R, Kumar D, Roy B. Studies on repeat breeding of buffaloes. Buffalo Bulletin. 2011 Sep 1;30(3):177-87.
20. Kumaresan A, Ansari MR, Rawal CV, Purbey LN, Sanwal PC. Influence of plasma progesterone level and cervical mucus fern pattern on conception rate in bovines. Indian Journal of Animal Reproduction. 2001;1:83-84.
21. López-Gatius F, Santolaria P, Martino A, Delétang F, De Rensis F. The effects of GnRH treatment at the time of AI and 12 days later on reproductive performance of high producing dairy cows during the warm season in northeastern Spain. Theriogenology. 2006 Mar 1;65(4):820-830.
22. Luktuke SN, Roy DJ. Studies on cervical mucus pattern in relation to fertility in bovine. Indian J Vet. Sci. 1967;37:26-31.
23. Macmillan KL, Day AM, Taufa VK, Gibb M, Pearce MG. Effects of an agonist of gonadotrophin releasing hormone in cattle. I. Hormone concentrations and oestrous cycle length. Animal Reproduction Science. 1985 Apr 1;8(3):203-212.
24. Mandal DD, Srivastava SK, Kumar P. Effect of GnRH administratiion on conception rate in buffaloes. Indian Journal of Animal Sciences (India). 2004;74(12):1189-1191.
25. Mann GE, Lamming GE, Fray MD. Plasma oestradiol and progesterone during early pregnancy in the cow and the effects of treatment with buserelin. Animal Reproduction Science. 1995 Jan 1;37(2):121-131.
26. Markandeya NM, Patil AD. Corpus luteum development and efficacy of GnRH treatment in non-infectiouscyclic non-breeder animals. Indian Journal of Animal Sciences (India). 2008;78(2):170-171.
27. McDougall S, Williamson NB, Macmillan KL. GnRH induces ovulation of a dominant follicle in primiparous dairy cows undergoing anovulatory follicle turnover. Animal Reproduction Science. 1995 Aug 1;39(3):205-214.
28. Mee MO, Stevenson JS, Alexander BM, Sasser RG. Administration of GnRH at estrus influences pregnancy rates, serum concentrations of LH, FSH, estradiol-17 beta, pregnancy-specific protein B, and progesterone, proportion of luteal cell types, and in vitro production of progesterone in dairy cows. Journal of Dairy science. 1993 Jan 1;78:1470-1476.
29. Meena RS, Sharma SS, Purohit GN. Efficiency of vaginal electrical resistance measurement foe estrus detection and insemination in Rathi cows. XVIIth Annual convention and national seminar on fertility management of farm animals under adverse agro-climatic conditions. Oct. 6-8th 2001. Jodhpur, Abstr. Souv; c2001. p. 26-27
30. Mehta GB. A study on repeat breeding conditions in crossbred (K X J and K X HF) cattle with special reference to cervical mucous. Indian J. Anim. Reprod. 1986;7(2):102-103.
31. Mohanthy BN, Dash RN, Mohanthy DN, Giri GC. Physiochemical properties of cervical mucus in normal and repeat breeding cows, Abstract from XIII National convention of ISSAR and National symposium on Animal Biotechnology. Dec. 4 to 6, 1996. Panthnagar, India; c1996.
32. Morgan WF, Lean IJ. Gonadotrophin-releasing hormone treatment in cattle: a meta-analysis of the effects on conception at the time of insemination. Australian veterinary journal. 1993 Jun;70(6):205-206.
33. O'Farrell KJ, Langley OH, Hartigan PJ, Sreenan JM. Fertilisation and embryonic survival rates in dairy cows culled as repeat breeders. The Veterinary Record. 1983 Jan 1;112(5):95-97.
34. Pandey AK, Ghuman SP, Dhaliwal GS, Kumar A, Agarwal SK. Impact of buserelin acetate or hCG administration on day 12 post-ovulation on subsequent luteal profile and conception rate in buffalo (*Bubalus bubalis*). Animal reproduction science. 2013 Jan 30;136(4):260-267.
35. Pandya V, Dhami A, Derashri H, Kodagali S. Arborization pattern of cervical-mucus and ovulation in clomiphene citrate induced oestrum in anestrous buffalos. Indian veterinary journal. 1987 Nov 1;64(11):940-943.
36. Pateria AK, Rawal CV. White side test for subclinical metritis in buffaloes. Indian J Anim. Reprod. 1990;11(2):142-144.
37. Patil MS. A study on pH, spinnbarkeit and electrical resistance of cervical mucus in normal and repeat breeder cows with special reference to intrauterine antibiotic treatment. M. V. Sc. Thesis submitted to Konkan Krishi Vidyapeeth, Dapoli, India; c1987.
38. Ramsingh L, Sadasiva Rao K, Muralimohan K. Therapeutic management of repeat breeding in bovines. Int. J Agrl. Sc. & Vet. Med. 2013;1(1):1-3.

39. Rao AR, Rao KS. Improved conception rate in buffaloes after administration of receptal. Indian Veterinary Journal. 1984;61:813
40. Rangnekar MN, Dhobli R, Gacche MG, Ingawale MV, Sawale AG, Jadhav JM. Physical properties of estrual cervical mucus in repeat breeding crossbred (Holstein-Friesian) cows with reference to fertility. The Indian Journal of Animal Sciences. 2002 Dec 1;72:1122-1124.
41. Sah SK, Nakao T. Characteristics of repeat breeding buffaloes in Nepal. J Reprod. Dev. 2006;52:335-341.
42. Salphale GV, Kadu MM, Fasihuddin M, Kadu MS. Study of some physical properties of estrual cervical mucus in synchronized normal and repeat breeder crossbred cows with reference to fertility. Indian J Anim. Reprod. 1993;14(2):77.
43. Samad HA, Ali CS, Ahmad KM, Rehman NU. Reproductive diseases of water buffaloes. 10th Int. congr. Anim. Reprod. A.I. Urbana; c1984.
44. Samad HA, Iqbal Shah SM, Nazir Ahmad, Nafees Akhtar. Physical characteristics of oestrus mucus and conception rates in repeat breeder buffaloes. Pakistan. Veterinary Journal., 2002;22(1):31-34.
45. Sharma P, Tiwari RP, Singh M, Sahu SK, Yadav SK. Vaginal Mucus Impedance (VMI) as an aid for prediction of optimum time for breeding during estrus in cows. Indian J Anim. Reprod. 2004;25(2):104-106.
46. Sharma S, Dhami AJ. Effect of postinsemination antibiotics and hormone therapy on fertility in relation to macrominerals profile in repeat breeding animals. Indian J Field Vets. 2008;3(3):1-6.
47. Sharma S, Dhami AJ, Sharma HC. Whether post insemination antibiotics and hormone therapy can modulate blood biochemical profile in repeat breeder buffaloes. Indian Journal of Animal Sciences. 2008;78(9):914-918.
48. Sharma HC, Dhami AJ, Kavani FS. Properties of estrual cervical mucus in relation to plasma progesterone and conception rates in buffaloes. Indian Journal of Animal Reproduction. 2011;32(2):8-11.
49. Snedecor GW, Cochran WG. Statistical methods, 7th Edn, Oxford and IBH publishing company, 17 park street, Calcutta; c1980.
50. Sreenan JM, Diskin MG. Early embryonic mortality in the cow: its relationship with progesterone concentration. The Veterinary Record. 1983 May 1;112(22):517-21.
51. Tanabe TY, Casida LE. The nature of reproductive failures in cows of low fertility. Journal of Dairy Science. 1949 Mar 1;32(3):237-46.
52. Troxel TR, Cruz LC, Ott RS, Kesler DJ. Norgestomet and gonadotropin-releasing hormone enhance corpus luteum function and fertility of postpartum suckled beef cows. Journal of animal science. 1993 Oct 1;71(10):2579-85.
53. Tsiligianni TH, Karagiannidis A, Brikas P, Saratsis PH. Physical properties of bovine cervical mucus during normal and induced (progesterone and/or PGF2α) estrus. Theriogenology. 2001 Jan 15;55(2):629-640.
54. Vadodaria VP, Prabhu GA. Physico-biochenical profile of estrual mucus in conceived and non conceived groups of mehasani buffaloes. National Symposium on applied reproduction in farm animals and VIIIth national convention of ISSAR 10-12th Novenber, College of Veterinary Science and ah. Gujarat Agricultural University, Anand, India; 1989.
55. Vadodaria VP, Prabhu GA. Volume and pH of oestrual cervical mucus congenial for conception in Mehsani buffaloes and heifers. Indian Journal of Animal Sciences. 1990;60(4):406-410.