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DETERMINATION OF ESTRUS IN UMBLACHERY CATTLE (*Bos indicus*) BY SALIVARY FERN PATTERN

G.GNANAMUTHU AND K.RAMESHKUMAR*

Pheromone Research Lab
P.G. & Research Dept. of Zoology
Rajah Serfoji Govt. College (Autonomous), Thanjavur – 613 005
TamilNadu, INDIA
*e-mail: rameshnila1@rediffmail.com

The time of ovulation is a key element in reproductive management of cattle. Most of the infertility problems reported in healthy, normocyclic cattle are due to mistimed breeding. The lack of accurate, direct methods of determining ovulation in cattle may be one of the reasons for reproductive failure. Additionally, some females may have silent, fragmented or anovulatory cycles also contribute to reduce the fertility rate. To overcome these problems, in recent years a variety of small hand-held microscopes have been developed and marketed for the purpose of self-observing fern patterns in saliva. Theoretically, the fern (crystallization) pattern of saliva has some correlation with the estrous cycle and fertile period. Based on the above information, the present study is aimed to determine the day of ovulation by the salivary fern test. During the study, the following types of salivary fern patterns like none, dotted, branch-like, fir-like, fern-like and combinations of them were observed. The clear fern-like crystallization was shown during the ovulation period which was not appeared in all other phases of estrous cycle. Further, the results of this study are to confirm the presence and changes of salivary fern pattern in Umblachery cattle during estrous cycle. This salivary crystallization may be considered as a new parameter to aid in the diagnosis of effective estrus in Umblachery breed (*Bos indicus).* Further research is required to improve the use of the salivary fern test for the betterment of estrus detection by which the success rate of artificial insemination increased and improve the population of Umblachery breed.

Keywords: Saliva, fern pattern, estrus detection, Umblachery breed, artificial insemination

Introduction

Cattle evolved in the Indian sub continent and only spread to other parts of Asia. The nature of ancient cattle`s are wild. These wild cattle were large with big horns and powerful forequarters compared to domesticate cattle. The domesticated cattle were developed from wild cattle (*Bos primigenius*) in the Middle East, probably about 8000 – 10,000 years ago. The reason for domesticating the aurochs is not clear. Early domesticated cattle were

undoubtedly used for the production of milk, meat and other purposes (Phillips, 2001). Milk production and reproductive performance are two major determinants of dairy cow profitability. Milk production has dramatically increased over the last several years, but the reproductive performance of Umblachery breed cattle has declined worldwide (Lucy, 2001; Royal, 2000; Stevenson, 2001 and Gnanamuthu and Rameshkumar, 2014). Several attempts have been made to detect the time of ovulation through tail paint, chin-ball markers, video cameras, pedometers, radio telemetry and heat-mount detectors that have been developed over the years, but these methods are far from effective and also are too expensive. Most infertility problems reported in healthy, normal cyclic cattle are due to mistimed breeding (Zoldag, *et al.,* 1993, Johnston, *et al.,* 1994). The lack of accurate, direct methods of determining ovulation in bitches may be one reason for reproductive failure, especially when cattle present a long receptivity period or ovulation occurs either too early or too late. The bovine oestrous cycle refers to the physiological events that constitute the cyclical pattern of ovarían activity which allows the transition of the heifer from the status of non-sexual receptivity to that of sexual receptivity, leading to mating behaviour. The duration of a normal estrous cycle in cow is from 18 to 24 days, with an average of 21 days, and the cycle consists of two phases: the follicular phase and luteal phase (Senger, 2003; Forde, *et al.,* 2011).

Crystallisation (ferning) or arborisation of vaginal and cervical mucus was first described by (Papanicolau, 1942) in women. Garm and Skjerven, 1952 studied the crystallisation of cervico-vaginal mucus in cows, noted that crystals with fern morphology appeared in oestrus and disappeared in the luteal phase of the cycle. It was claimed that there were many advantages of salivary ferning test such as its high accuracy, low cost, convenience, reusable and easy to use for accurate estrus detection (Kirkman, 1997).

Heat and pregnancy detection are essential in successful management of cattle breeding but it is often quite expensive, time consuming and some needs veterinary assistance. Obtaining of saliva is very simple and it can be performed by a breeder. Crystallization, also called ferning or arborisation, was described in vaginal mucus (Noonan, *et al.,* 1975), nasal mucus (Peterson 1984), saliva (Pardo-Carmona, *et al.,* 2010), tears (Golding and Brennan, 1989), milk or colostrum (Zondek and Rozin, 1954). The typical fern crystallization is distinctive near the peak of follicular activity and around ovulation time

when oestrogens predominate. However, progesterone suppresses the crystallization (MacDonald, 1969; Linford, 1974).

In recent years, a variety of small hand-held microscopes have been developed and marketed for the purpose of self-observing ferning patterns in saliva. Theoretically, the fern pattern of saliva coincides with the female fertile period. The ferning is caused by NaCl (Roland, 1971; Zondek, 1959). In many cycles, ferning days were found throughout the cycle, and in other cycles, ferning was found only on 1 or 2 days. There was also no discernible beginning or end to the fertility cycle as determined by the salivary ferning test. Most of the records did not show the typical pattern that was reported in the literature (Guida, *et al.,* 1993),

Salivary ferning may be used as a new parameter to aid women to detect their fertile period. Later, there was an increase in the usage of salivary ferning test in different clinical settings such as to predict the ovulatory period (Guida, *et al.,* 1993), to detect the resumption of ovarian function in the postpartum period (Tommaselli, *et al.,* 2000). Therefore, further research should be conducted to improve the use of the salivary ferning test in special situations. There is some question regarding the theoretical basis of salivary ferning, in that ferning is also discovered in male saliva (Berardono, *et al.,* 1993) and can be found throughout the female cycle (Kellen, 1958).

The aims of the present study is to establish saliva fern pattern in Umblachery breed and to assess their usefulness as a tool for determining optimal mating time by which the success rate of artificial insemination increased and improve the population of Umblachery breed.

MATERIAL AND METHODS

Experimental animals

Bos indicus (Umblachery) breed is an excellent draught cattle of Tamil Nadu noted for its strength and sturdiness. They are distributed in eastern coastal districts such as Thiruvarur and Nagapattinam in Tamilnadu, India. It lies approximately between 10° 18’ and10° 54’ N and 79° 48’ E with an estimated total area of 3500 square kilometer. The elevation of the breeding tract ranges from 0 to 50 meters above the mean sea level. Sexually

matured female bovine (*Bos indicus*, Umblachery breed cattle) were selected for the present study. The animals were maintained at District Livestock Farm, Korukkai, Thiruthuraipoondi, Thiruvarur District, Tamil Nadu, India, and the experimental animals were fed with conventional diet (cultivated forage crops, rice straw, green fodder) and water *ad libitum.*

Estrus phase identification

The estrous cycle was determined with the help of the conventional estrus behaviours in female cow such as vaginal swelling, frequent intermittent urination, restlessness, flehmen, male mount with female, female mount with male, female - female mounting, male sniffing with female vulva and fern pattern (saliva and vaginal fluid) also were checked for the confirmation of estrus phase. The length of estrous cycle was assessed through observing vaginal smear in order to know the stage of cycle to collect the vaginal fluid samples. Vaginal smears were performed and analyzed by light microscopy for the proportion of three cell types' viz. leukocytes, epithelial and cornified cells. Pro-oestrus smear consists of predominance of nucleated epithelial cells, Estrus smear primarily consists of cornified cells, Met-estrus smear consists of the equal proportion of leukocytes and cornified cells and diestrus smear primarily consists of leukocytes (Archunan, 2009).

Salivary ferning

The saliva sample was collected daily before the meal in the morning using a clean dry finger placed sublingually. The tip of the tongue must be pressed to the palate. A drop of non-foamy saliva samples were consequently smeared on glass slide and air dried at room temperature. The samples were microscopically assessed once a week at magnification 100X. Crystals were classified according to a system adapted (Haberova, 2010) as none, dotted, branch-like, fir-like, fern like, mixed branch-like and fir-like, mixed branch-like and fern-like, mixed fir-like and fern-like, mixed branch-like, fir-like and fern-like, atypical. Then the fern formation was graded from (Roland, 1971). only one general type of crystallization was described in one sample. The entire areas of each saliva samples on glass slides were examined. Crystallization was observed and eventually photographed within 10 minutes after collection.

Results and discussion

The duration of a normal estrous cycle in cow is between 18 and 24 days with an average of 21 days. The time of ovulation can greatly aid in managing breeding and may help

to achieve the best conception rate. The presence of salivary crystallization in all tested animals and changes in types of crystallization during estrous cycle were none, dotted, branch-like, fir-like, fern like, mixed branch-like and fir-like, mixed branch-like and fern-like, mixed fir-like and fern-like, mixed branch-like, fir-like and fern-like, atypical showed in the fig.1. Similar studies of Haberova, 2010 showed that branch-like crystallization belonged among the most frequent types with overall incidence of 36.27%. Only two types of crystalline patterns were observed during insemination period, namely mixed branch-like (Noonan *et al.,* 1975). (Pardo-Carmona, *et al.,* 2010) determined the optimal mating time were highly variable, largely because saliva crystallisation appears during heat. (Skalova, *et al.,* 2013) reported that the fernlike and mixed branch-like and fern-like patterns occurred only after insemination with incidence of 1.1% and 0.4%, respectively. Both ferning and non-ferning features can be observed in the dried saliva when viewed through a normal microscope with a wide field of vision. The type of fern formations were graded, none fern (without fern formation) is was grade-IV, dotted fern is grade-III, branch like and mixed branch like ferns are grade-II, fern like fern formation grade-I were observed in the throughout Umblachery cattle estrous cycle. These grading patterns are more or less similar to Rolando grading patterns and supported my result (Roland,1971).

The salivary fern patterns of Umblachery cattle showed clear fern on 0-1days was observed and the fern was gradually disappeared showed in the fig.2. Similarly (Guida, *et al.,* 1993) reported that ferning days were found throughout the cycle, clear ferning was found only on 1 or 2 days and there was also no discernible beginning or end to the fertility cycle as determined by the salivary ferning test. (Braat, *et al.,* 1998) reported that the use of salivary ferning test to predict ovulation in 30 women with regular menstrual cycles. Pardo-Carmona, *et al.,* 2010 reported that the positive crystallisation patterns type 1 and 2, i.e. partial and total crystallisation appeared some days before and after the optimal time for breeding. Saliva crystallisation persisted for several days before and after the expected date of ovulation.

There are many different saliva ovulation predictors out on the market, both low-end devices such as fertility microscopes, or high-end devices, a hand-held computer monitor with the sensing device. Most saliva fertility monitoring systems can be used daily throughout estrous cycle. Most manufacturers recommend that you don’t use test until 2 to 3 hours after eating or drinking anything. Most quality saliva fertility predictors can produce results at an accuracy rate of over 95%, are hygienic and 100% safe. Saliva ovulation

tests allow tracking fertility and cycle changes. The observe a positive result (crystal/ferning patterns), ovulation is likely to occur within 24 to 72 hours. According to our results, the present study may be used as simple, non-invasive, sensitive and specific tests to detect accurate ovulation in Umblacherry cattle.

Conclusion

The present study concluded that there are sequence of changes occurred in saliva crystallization pattern of domestic cattle during estrous cycle. The clear fern pattern was observed during estrus day and not observed in other days of estrous cycle. Our result suggests that estrus detection by salivary fern patterns in cattle might be possible. Further, we also recommended that salivary ferning test in different purposes may have benefit and enhance the reproduction of Umblachery breed as well as others to increase the live stock production.

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References

Archunan, G. (2009). Vertebrate pheromones and their biological importance. *Ind. J. Exp. Biol.*, 2: 227–239.

Barbato, M., Pandolfi, A. and Guida M. (1993). A new diagnostic aid for natural family planning. *Adv Contracept.,* 9: 335-340.

Berardono, B., Melani, D., Ranaldi, F., Giachetti, E. and Vanni, P. (1993). Is the salivary "ferning" a reliable index of the fertile period? *Acta Eur Fertil*., 24: 61-65.

Braat, D., Smeenk, J., Manger, A., Thomas, C., Veersema, S. and Merkus, J. (1998). Saliva test as ovulator predictor. *Lancet.,* 352: 1283-1284.

Fehring, R.J. and Gaska, N. (1998). Evaluation of the Lady Free Biotester in determining the fertile period. *Contraception.,* 57: 325-328.

Forde, N., Beltman, M.E., Lonergan, P., Diskin, M., Roche, J.F. and Crowe, M.A. (2011). Oestrous cycles in *Bos taurus* cattle. *Anim. Reprod. Sci.* 124: 163-169.

Garm, O. and Skjerven, O. (1952). Investigation of cervikalslim for the diagnosis of early pregnancy and endocrine disorders contingent of sexual cycles with livestock. *Nordic. Vet. medicin.,* 4: 1098- 1103.

Gnanamuthu, G. and Rameshkumar, K. (2014). Biochemical and Fatty Acid Analysis of Faeces in Umblachery Cattle (*Bos indicus*) During Different Phases of Estrous Cycle. *Res. J. Animal, Veterinary and Fishery Sci.* 2: 1-5.

Golding, T.R. and Brennan, N.A. (1989). The basis of tear ferning. *Clin. Exp. Optom.*, 72: 102-112.

Golding, T.R. and Brennan, N.A. (1989). The basis of tear ferning. *Clinical and Experimental Optometry.*, 72: 102- 112.

Guida, M., Barbato, M., Bruno, P., Lauro, G. and Lampariello, C. (1993). Salivary ferning and the menstrual cycle in women. *Clin Exp Obstet Gynecol.,* 20: 48-54.

Haberova, T. (2010). A preliminary study of saliva crystallization in Bactrian camels (*Camelus bactrianus*). In Fernandez Cusimani, E. & Havrland, B. (Ed.): 4th Scientific Conference of Institute of Tropics and Subtropics. Sustainable Use of Natural Resources in Tropics and Subtropics. Prague: CULS Prague, p. 28.

Johnston, S.D., Olson, P.N. and Root, M.V. (1994). Clinical approach to infertility in bitches. *Seminars in Veterinary Medicine and Surgery (Small Animal)* 9: 2-6

Kellen, J. (1958). Spontaneous formation of fern-leaf patterns in human saliva. *J Clin Endocrinol Metab*., 18: 1434.

Kirkman, R.J. (1997). Approaches for incorporating ovulation detection devices and home kits into learning NFP - implications for service delivery. *Adv Contracept*., 13: 269-272.

Kullander, S. and Sonesson, B. (1965). Studies on saliva in menstruating, pregnant and post-menopausal women. *Acta Endocrinologica*., 48: 329-336.

Linford, E. (1974). Cervical mucus: an agent or a barrier to conception? *Journal of Reproduction and fertility*., 37: 239-250.

Lucy M.C. (2001). Reproductive loss in high-producing dairy cattle: where will it end?, *J Dairy Sci.,* 84: 1277–1293

MacDonald, R.R. (1969). Cyclic changes in cervical mucus. *Journal of Obstetrics and Gynecology of the British Common wealth* 76: 1090-1099.

Noonan, J.J., Schultze, A.B. and Ellington, E.F. (1975). Changes in bovine cervical and vaginal mucus during the estrous cycle and early pregnancy. *J. Anim Sci.* 41: 1084-1089.

Papanicalou, G.N. (1946). A general survey of the vaginal smear and its use in research and diagnosis. *Am J Obstet Gynecol*., 51: 316-328.

Pardo-Carmona, B., Moyano, M.R., Fernandez-Palacios, R. and Perez-Marin C.C. (2010). Saliva crystallisation as a means of determining optimal mating time in bitches. *Journal of Small Animal Practice*., 51: 437-442.

Phillips, C.J.C. (2001). Principle of cattle production. http://www.cabi.org, *CABI Publishing.*, 1

Roland, M. (1971). Can the fern test be graded? *Obstet Gynecol*., 37: 792-796.

Royal M.D., Darwash A.O., Flint A.P.F., Webb, R., Woolliaams J.A. and Lamming G.E. (2000). Declining fertility in dairy cattle: changes in traditional and endocrine parameters of fertility, *Anim Sci.*, 70: 487–502

Senger, P.L. (2003). *Pathways to Pregnancy and Parturition,* (Current Conceptions, New York). 34-35, 144-150.

Skalova, I., Fedorova, T. and Brandlova, K. (2013). Saliva Crystallization in Cattle: New Possibility for Early Pregnancy Diagnosis? *Agricultura Tropica Et Subtropica.,* 46 (3):102-104.

Stevenson J.S. (2001). Reproductive management of dairy cows in high milk-producing herds, *J. Dairy Sci.*, 84: 128–143

Tommaselli, G.A., Guida, M., Palomba, S., Barbato, M. and Nappi, C. (2000). Using complete breastfeeding and lactational amenorrhoea as birth spacing methods. *Contraception* 61: 253-257.

Zoldag, L., Kecskemethyl, S. and Nagy, P. (1993). Heat progesterone profiles of bitches with ovulation failure. *Journal of Reproduction and Fertility*., 1: 562-563.

Zondek, B. and Rozin, S. (1954). Cervical mucus arborization. Its use in the determination of corpus luteus function. *Obstet Gynecol.*, 3: 463-470.

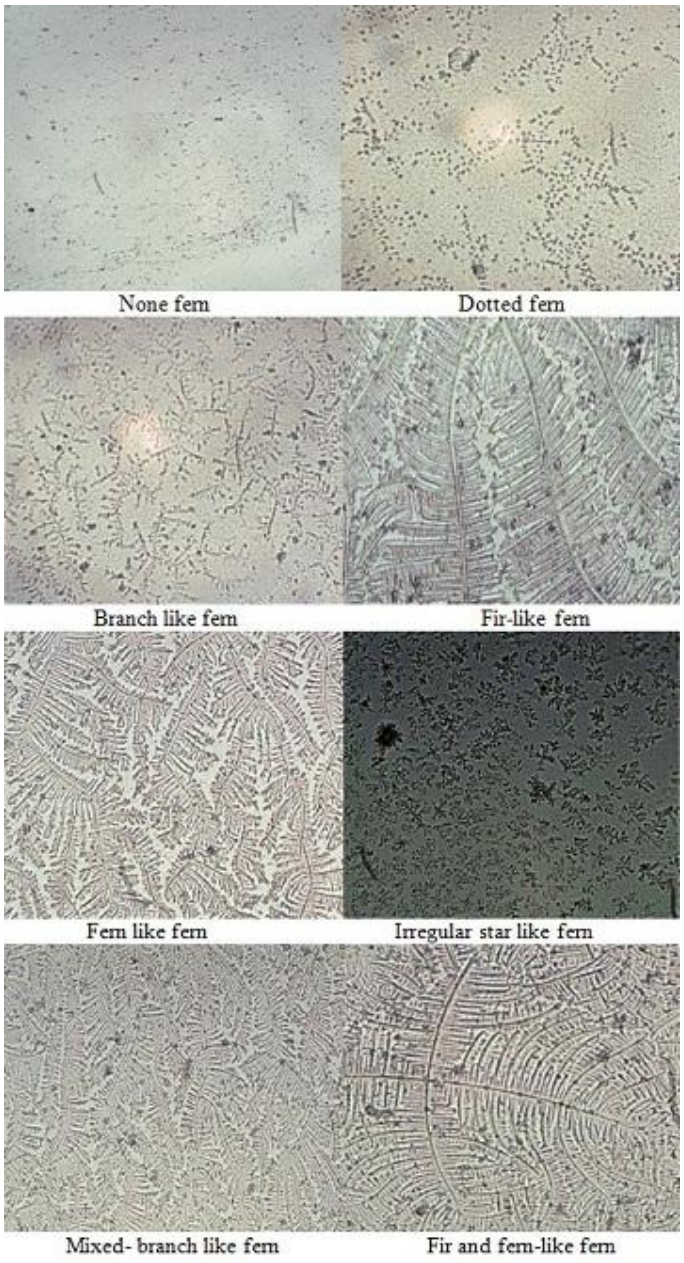

Fig.1 Types of salivary fern noticed in saliva of Umblachery breed cows

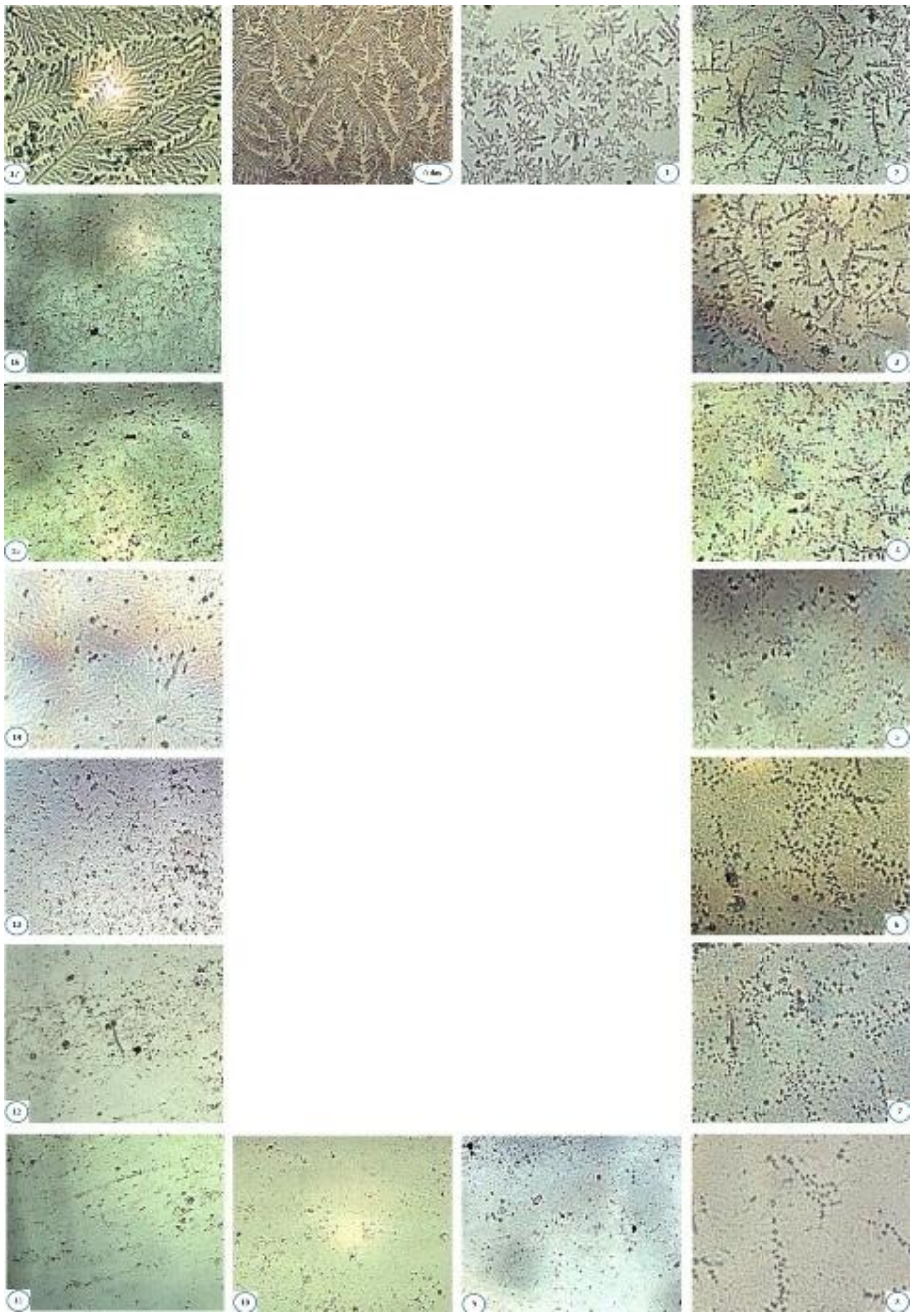

Fig.2 Salivary Fern patterns of Umblachery cow (*Bos indicus*) saliva throughout estrous cycle