J. Animal and Poultry Prod., Mansoura Univ., Vol.8 (9): 363 - 365, 2017

The Value of Cyclical Saliva Crystallization Patterns for the Prediction of Buffaloes Pregnancy Status.

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ABSTRACT

Prediction of reproductive status by a simple, non-invasive and applicable procedure is greatly desirable. Saliva sampling is a simple and low-cost process. The purpose of this study was to assess the value of saliva crystallization patterns (SCP) for the prediction of pregnancy in buffalo cows. A total number of 160 saliva samples were collected from twenty buffaloes from Day 0 to 45 post-breeding. The following types of crystallization have been discriminated; none, dotted, branch-like, fir-like, fern-like and mixtures of them. Pregnancy was affirmed ultrasonographically at 25-30 days after breeding. Accordingly, the animals were allotted into pregnant (n=12) and non-pregnant (n=8) groups. Saliva crystallization patterns varied during the post-insemination period between pregnant and non-pregnant animals. The peak incidence of the saliva ferning patterns mainly branch-like pattern at Days 20-25 and 28, and the fern-like pattern at days 15-28 post-breeding could be used as an indicator for pregnant and non-pregnant buffaloes, respectively. In conclusion, SCP is a non-invasive promising diagnostic technique could help to verify early pregnancy at 15-28 days and to reduce the number of the opened buffalo cows.

Keywords: Arborisation, crystalline patterns, ferning, pregnancy Diagnosis, reproductive cycle.

INTRODUCTION

The identification of animal reproductive status e.g. estrus and pregnancy are necessary for the successful management of buffalo breeding. Methods of pregnancy diagnosis are quite expensive, time uncontrollable and need veterinary assistance. On the other hand, obtaining the saliva is very simple and can be performed by the breeder.

Crystallization, also called ferning or arborisation, has been described in vaginal mucus (Noonan *et al*., 1975), nasal mucus (Peterson, 1984), saliva (Skalova *et al*., 2013), tears (Golding and Brennan, 1989), and milk or colostrum (Zondek and Rozin, 1954). The typical fern crystallization in vaginal mucus is distinctive near the peak of follicular activity and around ovulation time when estrogens predominate and progesterone diminish (MacDonald, 1969; Linford, 1974).

Saliva crystallization has been studied in Bactrian camels (Haberová, 2010), beagle bitches (Pardo‐Carmona *et al*., 2010) and women (Berardono *et al*., 1992). For authors knowledge, there are no reports concerned saliva fern patterns in buffaloes. Therefore, the aim of the present research was to characterize the changes in saliva crystallization patterns in buffaloes during a synchronized estrous cycle and early pregnancy.

MATERIALS AND METHODS

1. Experimental animals

This study was carried out at Buffaloes farm belongs to Department of Animal Production, Faculty of Agriculture, Al-Azhar University, at Mostorod, Qalyubia Governorate, Egypt, during the period from early February to May 2016. Twenty cyclic buffaloes, 4-7 years of age, weighing 470–540 kg, reared in an open yard barn (30 × 70 m2) under natural conditions of temperature and light-dark ratio, were used in this study. The animals were daily fed a mixed ration (16% CP) consisted of 50–55% forage and 45–50% concentrate. Tap Water and green fodder and straw were provided ad libitum as a bulky material.

2. Estrous synchronization and animal breeding

Animals were synchronized according to regimen described by Kelley *et al*. (2016). Briefly, controlled internal drug release (CIDR) insert (EAZIBREED, inter Age, Hamilton, New Zealand) impregnated with 1.38 mg progesterone was placed in the vagina for seven days. Cloprostenol sodium (Estrumate®, a synthetic prostaglandin analogue, MSD Animal Health New Zealand) was given IM at a dose of 500 µg (equivalent to 2.0 ml of cloprostenol sodium) on the 6th day after CIDR insert. Females were naturally bred upon estrus detection on 3-5 days after CIDR withdrawal (Day 7).

3. Salivary Crystallization or ferning

A total number of 160 saliva samples were collected. Samples were collected weekly in the morning before the meal using a cotton swab. A drop of non-foamy saliva sample was consequently smeared on a glass slide and air dried at room temperature. The entire saliva glass slides were microscopically assessed at magnification (100×) and eventually photographed. Crystallization patterns were classified as branch-like (BL), fir-like (FIL), fern-like (FL) and mixed patterns according to Haberová (2010).

4. Ultrasonographic examinations

Transrectal ultrasonographic examinations were accomplished to detect pregnancy status between 25-30 days post-breeding using real-time, B-mode, diagnostic ultrasound SonoAce R3 (Samsung, Medison, South Korea) equipped with high frequency (12 MHz) endorectal transducer according to Awasthi *et al*. (2011).

5. Statistical analysis

Chi-square test analysis was used to check the statistical differences in the incidence of saliva crystallization pattern between pregnant and non-pregnant buffalo groups($P < 005$) defines the statistical significance.

RESULTS

Representative micrographs of salivary crystallization patterns in buffaloes are presented in Fig. (1).

The incidence of a single pattern of salivary crystallization; BL, FIL and FEL was 39.4% and 14.3%, 8.1% and 9.5%, and 9.1% and 23.8% in pregnant and non-pregnant buffaloes, respectively (Fig. 2). The incidence of FIL+FEL, BL+FIL and BL+FIL+FEL patterns in the saliva were 12.1, 13.1 and 4.0 % in pregnant group, and 12.7, 20.6 and 6.3% in the non-pregnant group, respectively.

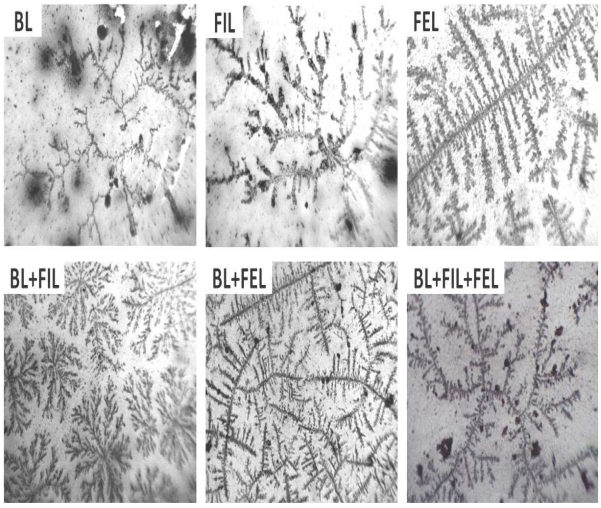

Fig. 1. Typical types of crystallization noticed in saliva of buffaloes (BL = branch-like, FIL = fir-like, FEL = fern-like). (Magnification × 100).

Chi-square test analysis of the incidence of salivary crystallization patterns verified a significant difference in BL ($p<0.05$) and FEL ($p<0.005$) patterns between pregnant and non-pregnant groups. Other examined patterns did not show significant ($p>0.05$) dissimilarities between the examined buffaloes.

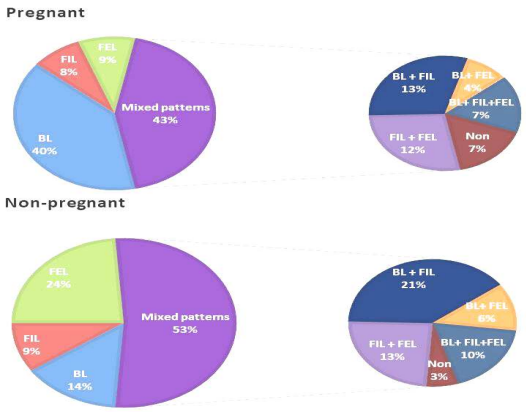

Fig. 2. the incidence rate of saliva crystallization patterns in pregnant and non-pregnant buffaloes during early gestation period. BL = branch-like, FIL = fir-like, FEL = fern-like.

Branch-like pattern obviously characterized the pregnant samples on days 20-25 and 28 post-breeding, meanwhile, the FEL pattern peaked in non-pregnant buffalo's samples on days 15-28 days after heat (Fig. 3).

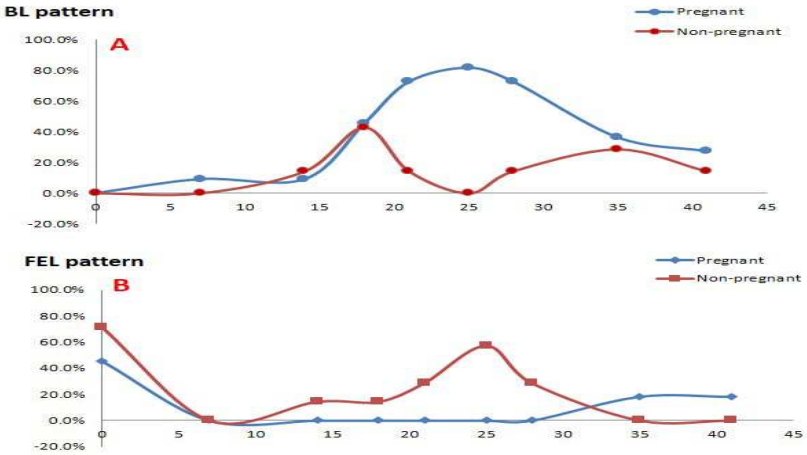

Fig. 3. Distribution of saliva crystallization branch like (A) and Fern like (B) patterns in pregnant and non-pregnant buffaloes during the post-breeding period. BL = branch-like, FEL = fern-like.

DISCUSSION

Finding a simple reliable non-invasive method for defining the animal reproductive status is mandatory for dairy herd management. Salivary ferning provides a clue for the ovulation status to reduce the estrus detection problem in buffaloes (Ravinder *et al*., 2016), and cows (Gnanamuthu and Rameshkumar, 2015). The proportion of estrus determination (0.84) based on salivary fern patterns was considerably ($P<0.01$) higher than the proportion of heat detection (0.50) in the field condition (Ravinder *et al*., 2016). The current data brings additional values for salivary arborisation in the detection of pregnancy. This would practically help to reduce the number of opened animals. Different saliva crystallization is substantially a reflection to alteration in steroid hormonal levels in the saliva and blood stream that is correlated with ovarian functional activity. A significant increase in salivary steroid hormones in association with reproductive status of buffaloes with special emphasis to progesterone during pregnancy (Lasheen *et al*., 2017), estradiol concentration levels and estrogen/ progesterone ratio at the estrus (Ravinder *et al*., 2016). It was found that progesterone suppresses the crystallization configuration (MacDonald, 1969; Linford, 1974), perhaps through the decrease of salivary pH and buffer capacity during pregnancy. During pregnancy, progesterone is known to lower plasma bicarbonate concentrations (Newman, 1957). Bicarbonate anion (HCO3) is mainly responsible for the buffer effect in saliva (Söderling *et al*., 1984). Similar trend was reported by Skalova *et al*. (2013), verified the absence of significant differences in the type of crystallization between pregnant and non-pregnant cows in periods between 1-19 and 30-34 days after AI, though the type of crystallization was significantly different between 20 and 29 days of pregnancy. Noonan *et al*. (1975) examined the crystallization of vaginal mucus during estrus in bovines

and found that FEL and BL+FEL patterns occurred only after insemination with an incidence of 1.1% and 0.4%, respectively. The saliva on drying showed a typical fern pattern at the estrus phase in buffalo, and this pattern had significantly ($P < 0.05$) lower fractal dimension value than the other days of the estrous cycle. Haberová (2010) found BL crystallization among the most frequent types of saliva ferning with an overall incidence of 36.27% in camels.

CONCLUSION

It could be concluded that saliva ferning patterns mainly BL pattern on Days 20-25 and 28, and the FEL pattern on days 15-28 days post-breeding would identify the pregnant and non-pregnant buffaloes, respectively. Therefore, the investigation of saliva ferning pattern from Day 15 to 28 after breeding is suggested as a non-invasive accessible diagnostic method to distinguish between pregnant or non-pregnant buffaloes.

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القيمة التشخيصية للتغيرات الدورية في أنماط تبلور اللعاب لحالة العُشر في الجاموس

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التنبؤ بالحالة التناسلية و خاصة العُشر فى الحيوان عن طريق فحص عينة بسيطة غير تداخلية وقابلة للتطبيق هو أمر مرغوب فيه إلى حد كبير. إن تقنية تجميع و فحص عينات من لعاب الحيوان هو عملية سهلة ومنخفضة التكلفة. هدفت هذه الدراسة إلى تقييم التغير الدورى فى أنماط تبلور اللعاب للتنبؤ بالعُشر في الجاموس. تم تجمع مائة و ستون عينة لعاب لعشرين من إناث الجاموس بين اليوم الاول والخامس و الاربعين بعد التزاوج. وقد تم توصيف أنماط تبلور اللعاب كالتالى :لا شيء، منقط، متفرع مثل التنوب، السرخس، والمزيج بينهم. كما تم تاكيد العُشر فى الحيوانات باستخدام الموجات فوق الصوتية عند اليوم 25-30 بعد التزواج. و وفقا لذلك تم تقسيم الحيوانات الى مجموعة الحيوانات العِشار (ن = 12) والغير عِشار (ن = 8). اظهرت النتائج تفاوت أنماط تبلور اللعاب خلال فترة ما بعد التلقيح بين الحيوانات العِشار وغير العِشار. وكان نمط اللعاب المتفرع موشر قويا للتنبؤ بالعُشر فى الفترة من اليوم العشرين الى الثامن و العشرين بعد التزاوج، بينما كان نمط السرخس (المتعامد على المحور) موشرا لبدء دورة شبق جديدة فى الحيوانات غير العِشار فى الفترة من اليوم الخامس عشر الى الثامن و العشرين بعد التزاوج. من هنا يمكن ان نستخلص أن نمط تبلور اللعاب يعتبر تقنية حقلية واعدة لتشخيص حالة العُشر مبكراً، وبالتالي تعمل على خفض الفترة الفاصلة بين الولادة والعُشر في الجاموس.