Indian J Anim Health (2022), 61(1): 60- 64
DOI: https://doi.org/10.36062/ijah.2022.09221

Research Article

Relationship between degree of intrauterine fluid, cytology and bacterial isolates on pregnancy rate in mares during foal heat

B. R. Suchitra[1*], N. M. Dinesh[2], B. R. Sumathi[3], H. M. Yathish[4], M. C. Anil Kumar[5] and C. Murthy[6]

[1]Department of Veterinary Gynaecology and Obstetrics, Veterinary College, Hebbal, Bengaluru-560 024, Karnataka, India; [2]Kunigal stud farm, Kunigal - 572 130, Karnataka, India; [3]Institute of Animal Health & Veterinary Biologicals, Hebbal, Bengaluru - 560 024, Karnataka, India; [4]Department of Animal Genetics & Breeding, Veterinary College, Hebbal, Bengaluru - 560 024, Karnataka, India; [5]Department of Veterinary Clinical Complex, Veterinary College, Hebbal, Bengaluru - 560 024, Karnataka, India; [6]Department of Livestock Farm Complex, Veterinary College, Hebbal, Bengaluru - 560 024, Karnataka, India

Abstract

This study was conducted to investigate the effect of intra uterine fluid, cytology and bacterial isolates obtained in foal heat mares on the pregnancy rate. Postpartum mares in foal heat were subjected to ultrasonography, cytology and bacteriological studies. Mares with U+ intrauterine fluid and negative for both endometrial cytology and bacteriology showed 85.71 per cent pregnancy rate. The pregnancy rate deteriorated substantially in mares with U+, positive for cytology and bacterial culture, while it was found to be nil in mares with U++, positive for cytology and bacterial culture. Hence, it is evident from the current study that mares with the presence of moderate to severe intrauterine fluids, positive for endometrial cytology and bacteriology, showed decreased pregnancy rate.

Key words: Bacteriology, Cytology, Foal heat mares, Intrauterine fluid, Pregnancy

Highlights

- Endometrial cytology, bacteriology and ultrasonography provide insights into uterine health status in postpartum mares.
- Subjecting postpartum mares to uterine health checks using different diagnostic approaches in combination would be beneficial to identify mares at risk of early embryonic death.

INTRODUCTION

The primary requirement for efficient and economically viable broodmare management is the production of a foal per year. Exploiting the first fertile estrus (foal heat) which occurs within 6 to 20 days of foaling would be commercially beneficial for the equine industry (Ginther, 1992). But the rate of fertility during first postpartum heat is considered inferior due to delayed uterine involution, subclinical endometritis and the presence of intrauterine fluid (Baranski *et al*., 2003; Malschitzky *et al.*, 2003). To obtain optimum fertility in mares during postpartum, diagnosis of uterine health status should include gynaeco-clinical inspection, rectal examination, examination per vaginum, ultrasonography, uterine bacteriology, endometrial cytology and endometrial biopsy (Reiswig *et al*., 1993; Bourke *et al*., 1997; Dascanio *et al*., 1997; Card, 2005; Aguilar *et al*., 2006; Davies Morel *et al*., 2013). The combination of cytological and bacteriological examination gives more reliable diagnosis of inflammatory processes in the uterine endometrium (Nielsen, 2005; Aguilar *et al*., 2006; Davies Morel *et al*., 2013). Mares with ultrasonographically detectable uterine fluid, positive for cytology and bacteriology prebreeding had significantly

*Corresponding Author, E Mail: suchitraanilkumar2@gmail.com

lowered pregnancy rate than mares without intrauterine fluid (Squires *et al*., 1989; Pycock and Newcombe, 1996; Ozgen *et al*., 2002; Baranski *et al*., 2003; Blanchard *et al*., 2004; Benko *et al*., 2015). Previous studies have demonstrated/ compared cytology and bacteriology as a means to diagnose postpartum endometritis in mares. The combined effect of intrauterine fluid, PMN cells and bacterial culture either present or absent *in uetro* has not been elucidated previously. To attend to this research gap, we performed cytology, bacteriology and ultrasonography of endometrium and results were compared with the pregnancy rates in postpartum mares. In this context, the present study was undertaken to evaluate the degree of intrauterine fluid present, bacteriological and cytological examinations, status of health of the mares' uterus during foal heat as well as the pregnancy rate.

MATERIALS AND METHODS

Animals: The present research work was carried out in a Thoroughbred Stud Farm located in the southern part of India at latitude 13.0232 N and longitude 77.0252 E with an average altitude of 2536 feet. A total of 21 mares were kept in an open shelter and outdoor paddock, fed daily ration according to their reproductive status, as practised in Indian stud farms (Lucerne/hay and pasture grass ad libitum plus 6 kg of concentrate divided thrice a day). The mares with the history of normal parturition and fetal membranes expelled within 3 hours postpartum were considered for this investigation.

Teasing: The mares were teased in the early morning from 6th day onwards post foaling. The signs of first postpartum estrus were detected with visual external symptoms like urination, winking of the vulva, vaginal discharge, relaxation of vulva and reddening of the vaginal mucosa, lack of resistance by the mare in the presence of a stallion as well as allowing stallion to mount.

Transrectal ultrasonography: Ultrasound examination was done with a real-time, B-mode scanner operational with a 3.5 or 7.5 MHz linear-array transducer- Aloka-SSO- 500. The linear transducer was placed over the uterus and the uterine horns to ascertain involutory process in the uterus and to assess the presence of intrauterine fluid and scored from the viewing screen at the time of examination. Intrauterine fluid (IUF) was graded as U0: Absence, U: Very small amount, U+: Mild quantity, U++: Moderate quantity, U+++: Excess quantity.

Endometrial cytology: Two days before breeding, the samples for bacteriology and cytology were collected. Before collecting samples from the uterus, tail was bandaged and perineum and vulva were aseptically cleaned with paper towels and an iodine solution was applied to reduce contamination of the internal genital tract. Sample from the uterus was collected using double guarded Cytobrush (Cytology Brush, Minitüb, Tiefenbach, Germany) (Plate 1). Immediately, the swab was rolled on a grease-free clean slide, fixed with methanol (acetone-free, NICE chemicals) for cytological studies and later stored in a 0.9 per cent normal saline maintained at 4°C until bacteriological examination.

The fixed slides were stained within four hours using 9:1 diluted Geimsa stain (HiMedia). The presence of polymorpho-nuclear cells and epithelial cells was examined with light microscopy (Labomed) under oil immersion (1000X). Ten fields were examined, and the total numbers of PMN cells were counted (Riddle *et al*., 2007).

Endometrial bacteriology: The endometrial swabs collected from the mares during foal heat using double guarded cytobrush were subjected for aerobic bacterial studies in order to identify whether the uterus was bacteriologically sterile or infected. The analysis for bacteriology was done according to the standard method as described by Cruickshank *et al*. (1980).

For this bacteriology studies ready to use Agar plates- Nutrient agar, Brain Heart Infusion agar, Mannitol salt agar, MacConkey agar, Sheep Blood agar, EMB agar and Gram's staining kit were used.

Breeding: In this study, the 'In-hand breeding technique' was followed to cover the mares in

heat after checking the presence of pre-ovulatory follicle. The breeding of mares was done with a proven stallion as per standard procedure with appropriate aseptic measures.

Pregnancy diagnosis: The pregnancy was diagnosed by ultrasonography on day 14 post covering (Plate 2). The next confirmation was done at 18th day, 21st day and 28th day post covering. The resorption, abortion and birth of still born, if any were recorded throughout the pregnancy period.

The data was subjected for statistical analyses using SPSS software. To compare percentages, a Chi-squared test was used. Means are presented ± standard error.

RESULTS

All the examined mares had enlarged and asymmetric uterine horns with different degrees of uterine fluid during foal heat (Fig.1, Plates 3 & 4). The intrauterine fluid (IUF) score was U+ in 61.91 per cent (13/21) of mares, U++ in 33.33 per cent (7/21) and U+++ in 4.76 per cent (01/21) of mares in foal heat. The rate of pregnancy in mares with IUF score of U+, U++ and U+++ was 76.92 per cent, 14.29 per cent and 0.00 per cent, respectively.

Cytological examination of endometrium revealed the presence of PMNs and epithelial cells (Fig. 1, Plates 5 & 6) (2-5 PMN cells/field) in 66.7 per cent (14/21) in foal heat mares and 33.3 per cent (7/21) mares had less than 2 PMNs cells in the endometrial smears. The rate of pregnancy in cytologically positive mares was 35.71 per cent, while it was 85.71 per cent in cytologically negative mares.

Likewise, the bacteriological examinations of the endometrium of mares revealed the presence of bacteria in 33.33 per cent (7/21) mares in foal heat and 66.66 (14/21) per cent mares had no bacteria in the culture examination (Fig. 1). The rate of pregnancy was 14.29% in mares who were found to be positive for endometrial culture, and it was 71.43% in mares free from bacterial infection. In 42.86 per cent (3/7) of mare's endometrial cultures, *E coli* was isolated, and 28.57 per cent (2/7) of mares had β haemolytic Streptococcus. Similarly, 14.29 per cent of mares had Staphylococci organisms (1/7) and interestingly, 14.29 per cent of mares (1/7) had mixed infection of E-coli, β haemolytic Streptococcus and fungal due to urine pooling.

The rate of fertility in foal heat mares with subclinical disorders of the uterus was higher than in mares with pathological changes. Pregnancy was diagnosed in 85.71 per cent (6/7) of mares with the healthy uterus (U+ IUF, negative for cytology and culture), while the pregnancy rate was 75 per cent (3/4) in mares with U+ IUF, 2-5 PMN cells/field and negative for culture, 50 per cent (1/2) in mares with U+ IUF, 2-5 PMN cells/field and positive for culture, 33.33 per cent (1/3) in mares with U++ IUF, 2-5 PMN cells/field and positive for culture. The grave scenario was observed in mares with U++ IUF, positive for bacteriological and cytological examinations. In this case, none of the mares could achieve conception.

DISCUSSION

All the mares considered for the current study showed the presence of intrauterine fluid at different degrees, while 64.9 per cent (Malschitzky *et al*., 2003), 12 per cent (Baranski *et al*., 2003), 70 per cent (Mahal *et al*., 2020) of mares were reported to have intrauterine fluids in foal heat, which is different when compared to the current study. The pregnancy rate in mares with moderate to severe uterine fluids (U++ and U+++) was found to be extremely poorer when compared to mares with mild IUF (U+) and is found to be in accordance Ozgen *et al*. (2002) and Baranski *et al*. (2003). Reduced pregnancy rate may be attributed to the fact that the presence of fluid in the postpartum uterus could be spermicidal (Katila *et al*., 1997), causing premature luteolysis or embryonic death (Malschitzky *et al*., 2003), makes uterus susceptible to endometritis (Allen and Pycock, 1988), abnormalities in myometrial electrical pattern and exhaustion of the uterine smooth muscle (Troedsson and Liu, 1991).

The most popular routine sampling technique is endometrial cytology using a guarded swab which yields an accurate diagnosis of uterine health status (Aguilar *et al*., 2006). Cytological examination of endometrium revealed the presence of PMNs and epithelial cells (2-5 PMN cells/field) in 66.7% of foal heat mares in the current study which is in agreement with 66.66 per cent (Baranski *et al*., 2003). Reduced rate of pregnancy in mares which are positive for cytology was reported by Baranski *et al*. (2003) and Riddle *et al*. (2007), which is in harmony with the present study. It is assumed that the presence of these cells is associated with the involution of the uterus and/ or the presence of infectious or inflammatory factors *in utero* (Riddle *et al*., 2007).

Bacteriological examination of endometrium in foal heat mares was positive in 33.33 per cent (7/21), which is lower when compared to 66.2 per cent (Baranski *et al*., 2003), 63.60 per cent (Albihn *et al*., 2003), 49 per cent (Frontoso *et al*., 2008) and 69.7 per cent (Benko *et al*., 2015). The reason for such lower incidence of uterine infection in our experimental animals could be attributed to maintenance of proper hygiene in the foaling barn, higher local and systemic resistance of the mares, as well as lesser possibility of infections from bedding and hay during parturition and then after. In accordance with Albihn *et al*. (2003), *E. coli* was isolated majorly followed by ß-hemolytic Streptococci among uterine microbial isolates, but in contrast to Frontoso *et al*. (2008) and Köhne *et al*. (2020) who demonstrated the overall dominance of β-haemolytic Streptococci relative to *E. coli* in uterine swab samples. These contrasting data may be due to the different study areas and different horse populations.

The poor results were seen in mares with only positive bacteriology and positive for both bacteriology and cytological examinations and pregnancy was diagnosed only in 14.28 per cent in each group, which is lower when compared to 18.2 per cent (Baranski *et al*., 2003) and 36.4 per cent (Barber *et al*., 2012). The mares with moderate to severe IUF, severe inflammation combined with bacteria were found negative for pregnancy.

All three parameters like positive for cytology, positive for bacterial isolates and presence of moderate to severe IUF in combination, were equally indicative of uterine health. The pregnancy rate in postpartum mares was poor in mares with the immunologically compromised uterus. Our data also showed that the combination of a cytological and a bacteriological examination of endometrial sample taken with the double guarded cytobrush might be the most promising approach to diagnose postpartum endometritis in mares.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: NMD and CM: Designed the present study; BRS, NMD, BRS and MCAK: Executed the work; HMY: Data analysed; BRS and HMY: Prepared the manuscript with inputs.

Ethical animal research: All experimental procedures were performed with prior approval of the institute's Animal Ethics Committee, Veterinary College, Hebbal, Bengaluru.

ACKNOWLEDGEMENT

The research was a part of Ph.D thesis of 1st Author and the Authors are grateful to Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karanataka, India. We thank the participating stud farm and clinicians at Kunigal Stud Farm, Kunigal, Karnataka, India for permiting us to execute this research study in the farm.

REFERENCES

Aguilar J, Hanks M, Shaw DJ, Else R and Watson E, 2006. Importance of using guarded techniques for the preparation of endometrial cytology smears in mares. Theriogenology, 66(2): 423-430, doi: 10.1016/j.theriogenology.2005.12.007

Albihn A, Baverud V and Magnusson U, 2003. Uterine micro-biology and antimicrobial susceptibility in isolated bac-teria from mares with fertility problems. Acta Vet Scand, 44(3-4): 121-129, doi:10.1186/1751-0147-44-121

Allen WE and Pycock JF, 1988. Cyclical accumulation of uterine fluid in mares with lowered resistance to endometritis. Vet Rec, 122(20): 489-490, doi: 10.1136/vr.122.20.489

Baranski W, Janowski T, Ras A, Podhalicz-Dzielgielewska M and Strzezek R, 2003. Relationship between bacteriological and cytological examination of the mare's uterus during foal heat and fertility rate. Bull Vet Inst Pulawy, 47: 427-433

Barber BK, Bradecamp EA, Howard BS and Mollenkopf BS, 2012. Net effect of foregoing uterine culture and cytology in first-cover foaling mares. AAEP Proceedings, 58, pp 526-527

Benko T, Boldizar F, Novotny V, Hura I, Valocky K *et al*., 2015. Incidence of bacterial pathogens in equine uterine swabs, their antibiotic resistance patterns, and selected reproductive indices in English thoroughbred mares during the foal heat cycle. Vet Med, 60(11): 613-620, doi: 10.17221/8529-VETMED

Blanchard TL, Thompson JA, Brinsko SP, Stich KL, Wendt KM *et al*., 2004. Mating mares on foal heat: A five year retrospective study. Proceedings of the 50th annual convention of the American Association of the Equine Practitioners, Denver, Colorado, USA, pp 525-530

Bourke M, Mills JN and Barnes AL, 1997. Collection of endometrial cells in the mare. Aust Vet J, 75(10): 755-758, doi: 10.1111/j.1751-0813.1997.tb 12263.x

Card C, 2005. Post-breeding inflammation and endometrial cytology in mares. Theriogenology, 64(3): 580-588, doi: 10.1016/j.theriogenology. 2005.05.041

Cruickshank R, Duguid JP, Marimo BR and Swain RH, 1980. Medical Microbiology. 2nd Edn., 11, Livingston London, New York

Dascanio J, Ley WB and Bowen JM, 1997. How to perform and interpret uterine cytology. AAEP Proceedings, 43: 182-186

Davies Morel MCG, Lawlo O and Nash DM, 2013. Equine endometrial cytology and bacteriology: effectiveness for predicting live foaling rates. Vet J, 198(1): 206-211, doi: 10.1016/j.tvjl. 2013.08.002

Frontoso R, De Carlo E, Pasolini MP, Van Der Meulen K, Pagnini U *et al*., 2008. Retrospective study of bacterial isolates and their antimicrobial suscep-tibilities in equine uteri during fertility problems. Vet Sci Res J, 8: 1-6, doi: 10.1016/j.rvsc.2007.02.008

Ginther OJ, 1992. Reproductive biology of the mare : basic and applied aspects. 2nd Edn., Cross Plains, Wis.: Equiservices, 1992

Katila T, Reilas T, Makela O, Huhtinen M and Koskinen E, 1997. Intrauterine fluid accumulation in oestrous mares in: Havemeyer Foundation Int. Workshop on uterine defense mechanisms in the mare: aspects of physical clearance. Gainesville, Kongr.ber

Köhne M, Kuhlmann M, Tönißen A, Martinsson G and Sieme, H, 2020. Diagnostic and treatment practices of equine endometritis- A questionnaire. Front Vet Sci, 7: 547, doi: 10.3389/fvets.2020.00547

Mahal JS, Honparkhe M, Kumar A and Singh P, 2020. Conception rates following breeding at foal heat versus induced heat in postpartum indigenous mares. Int J Curr Microbiol App Sci, 9(4): 1904-1907, doi: 10.20546/ijcmas.2020.904.225

Malschitzky E, Schilela A, Mattos ALG, Garbade P, Gregory RM *et al*., 2003. Intrauterine fluid accumulation during foal heat increases embryonic death. Pferdeheilkunde**,** 19(6): 246-249

Nielsen JM, 2005. Endometritis in the mare: A diagnostic study comparing cultures from swab and biopsy. Theriogenology, 64(3): 510-518, doi: 10.1016/j.theriogenology.2005.05.034

Ozgen S, Schoon HA, Aupperle H, Sieme H and Lug E, 2002. Etiopathogenesis of equine intrauterine fluid accumulation. Pferdeheilkunde, 18(6): 594-599, doi: 10.21836/PEM20020614

Pycock JF and Newcombe JR, 1996. Assessment of the effect of three treatments to remove intrauterine fluid on pregnancy rate in the mare. Vet Rec, 138(14): 320-323, doi: 10.1136/vr.138.14.320

Reiswig JD, Threlfall WR and Rosol TJ, 1993. A comparison of endometrial biopsy, culture and cytology during oestrus and dioestrus in the horse. Equine Vet J, 25(3): 240-241, doi: 10.1111/j.2042-3306.1993.tb02952.x

Riddle WT, LeBlanc MM and Stromberg AJ, 2007. Relationship between uterine culture, cytology and pregnancy rates in a thoroughbred practice. Theriogenology, 68(3): 395-402, doi: 10.1016/j.theriogenology.2007.05.050

Squires EL, Barnes CK, Rowley HS, Mckinnon AO, Pickett BW *et al*., 1989. Effect of uterine fluid and volume of extender on fertility. Proc 35th Ann Conv Am Assoc Equine Practitioners, USA, pp 25-30

Troedsson MHT and Liu IKM, 1991.Uterine clearance of non-antigenic markers in response to a bacterial challenge in mares potencially susceptible and resistant to chronic uterine infections. J Reprod Fertil, 44: 283-288

Received – 02.11.2021, Accepted – 25.03.2022, Published - 15.05.2022 (Online), 01.06.2022 (Print)
Section Editor: Prof. S. K. Nandi, Associate Editor