Journal of Equine Veterinary Science 74 (2019) 65–67

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Short Communication

Endometrial Histology of Mares from a Semi-Feral Pony Herd of Known Lifelong High Fertility and Fecundity

Kristina M. Janson Whitesell, Patricia L. Sertich, Sue M. McDonnell*

Department of Clinical Studies-New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA

ARTICLE INFO

Article history:
Received 15 November 2018
Received in revised form
20 December 2018
Accepted 21 December 2018
Available online 23 December 2018

Keywords:
Endometrial histology
Semi-feral pony herd
Equine

ABSTRACT

This study was conducted to better understand factors contributing to lifelong high fertility and fecundity in a herd of ponies kept under natural social organization and environmental conditions, where most fillies begin foaling as 2-year-olds and continue to foal annually as long as they remain in the herd. Endometrial biopsy samples were procured from 23 mares on Day 6 postpartum for histologic evaluation. Kenney classification as well as degrees of lymphocytic endometritis and periglandular fibrosis of mares aged 2–9 years (after one to eight pregnancies, n = 14) were compared with those of mares aged 10 years and older (after 9–14 pregnancies, n = 9). Periglandular fibrosis ranged from none to moderate. As expected postpartum, lymphocytic endometritis was reported as moderate for 22 mares. The one exception was reported as moderate to severe. Differences in the proportion of younger versus older mares with classifications of I, IIA, and IIB, the proportion of mares with greater than mild lymphocytic endometritis, and the proportion of mares with greater than mild periglandular fibrosis were not significant (Fisher's exact tests, $P > .10$).

1. Introduction

Since 1994, a herd of Shetland-sized ponies established initially with 13 mares and 13 stallions has been maintained for study of reproductive physiology and behavior under natural social and environmental conditions. Management includes supplemental hay in winter and removal of equal numbers of males and females annually to maintain 50–100 animals. Of 85 fillies born and kept in the herd through maturity, 79 were first observed in estrus and confirmed pregnant at 10–16 months of age to deliver foals at 22–27 months of age. The remaining six were first confirmed pregnant as 2-year-olds and foaled as 3-year-olds. Mares kept in the herd have typically produced foals annually, consistent with conception at the first, or less often second, postpartum estrus. Annual foaling has continued for mares that were kept until natural death in their 30s. This sustained high fertility within this semi-feral herd is in contrast to what is accepted as generally declining reproductive efficiency with age and parity in domestically managed mares [1–3]. Work by Bosh et al and Allen et al summarized in a 2015 review on the effect of age on fertility, pregnancy, and offspring vigor in Thoroughbred broodmares showed that Day 40–42 per cycle pregnancy rates began to decline at as early as 9 years of age [4]. In domestically managed mares, endometrial pathology has been accepted as a key factor in age and parity-related decline in reproductive efficiency [2,3,5]. The objective of this study was to characterize the endometrial histology among mares of this semi-feral pony herd, specifically comparing younger (lower parity) versus older (higher parity) mares.

2. Materials and Methods

This research protocol was approved by the University of Pennsylvania Institutional Animal Care and Use Committee, according to all applicable humane guidelines. Twenty-three mares (aged 2–16 years) from this semi-feral herd were included. Age and associated reproductive histories for individuals are summarized in Table 1a. The day of examination and biopsy procurement was set at Day 6 postpartum to target a window of opportunity before postpartum estrus, breeding, and subsequent conception but

Animal welfare/ethical statement: This research protocol was approved by the University of Pennsylvania Institutional Animal Care and Use Committee, according to all applicable humane guidelines. This article reports the results of original work that has not been previously published except in abstract form.

Conflict of interest statement: The authors declare no conflicts of interest.

* Corresponding author at: Sue M. McDonnell, Department of Clinical Studies-New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA.

E-mail address: suemcd@vet.upenn.edu (S.M. McDonnell).

https://doi.org/10.1016/j.jevs.2018.12.021
0737-0806/

Table 1
Mare histories, endometrial biopsy findings, and subsequent fertility.

a. Mares and History			b. Endometrial Biopsy Findings			c. Subsequent Fertility	
2013 Age (y)	Age at First Parturition (mo)	Number of Pregnancies	Kenney Category	Periglandular Fibrosis	Endometritis-Lymphocytic	Days Open After Biopsy (mo/d of Parturition)	Subsequent foals/years in the Herd (as of 2018)
Younger mares (n = 14)							
2	25	1	I	None	Mild-moderate	23 (5/12)	1/1
3	27	2	IIA[a]	Mild	Mild	30 (5/17)	1/1
5	23	4	IIA	Mild	Mild	14 (7/20)	5/5
6	22	5	IIA	Mild	Moderate	7 (7/9)	5/5
6	25	5	IIA	Mild	Moderate	15 (5/12)	2/2
6	25	5	IIA	Mild	Moderate	14 (5/11)	5/5
7	25	6	IIA[a]	Mild	Moderate	56 (3/17)	2/2
7	24	6	IIA[a]	Mild	Moderate	57 (3/19)	5/5
7	35	5	IIA	Mild	Moderate	11 (4/29)	2/2
7	26	5	I	None	Mild	10 (5/25)	3/3
8	24	6	IIA[a]	Mild	Mild	40 (3/27)	3/3
8	35	6	IIB[a]	Mild	Moderate	23 (5/5)	5/5
9	24	8	I	Mild	Mild	42 (3/17)	5/5
9	24	8	IIA[a]	Mild	Mild	7 (4/21)	5/5
Older mares (n = 9)							
10	24	9	IIA	Mild	Mild	34 (3/22)	5/5
10	25	8	IIA	Mild	Mild	20 (4/22)	5/5
11	22	10	IIB[a]	Mild	Moderate-severe	21 (4/21)	4/5
11	25	10	IIA	Mild	Moderate	17 (5/11)	5/5
12	22	11	IIA[a]	None	Mild	30 (4/11)	5/5
12	23	11	IIA	Mild	Mild	19 (3/15)	4/4
12	32	10	IIA	Mild	Mild	23 (4/14)	5/5
15	35	13	IIA	Mild	Mild-moderate	21 (4/23)	5/5
16	25	14	IIA[a]	Moderate	Mild-moderate	18 (5/12)	5/5

[a] Histology report comment "category primarily influenced by inflammation which, if resolves, would improve."

delayed enough to minimize effect of inflammation associated with histologic uterine involution [6]. The harem family of each mare was first separated from the herd by luring them into a sub-enclosure within their pasture environment, allowing the mare to then be further separated with her foal into a smaller pen. The mare was haltered and held in hand for the procedure. In a few instances, xylazine HCl (1 mg/kg IV) was administered to facilitate examination. The tail was wrapped in a clean plastic bag and held off the perineum with a cord secured around the neck. External genitalia were visually evaluated for signs of foaling trauma. Palpation per rectum was performed to evaluate ovarian activity and estimate gross uterine involution based on diameter of the uterine horns, uterine tone, and presence of palpable fluid. The perineum was washed with povidone scrub and rinsed with water. The endometrial biopsy sample was procured using standard technique with a 48 cm alligator biopsy forceps [7]. A minimum 2 cm sample of endometrium was placed in Bouin's fixative and submitted for slide preparation to the Pennsylvania Animal Diagnostic Laboratory System Large Animal Pathology Service at New Bolton Center. Slides were prepared with hematoxylin and eosin and trichrome staining [8]. Histologic evaluation following the Kenney protocol [7,9] was performed by a single board–certified theriogenologist (P.L.S.). This evaluator was blind to age and reproductive histories but aware of Day 6 postpartum status.

2.1. Statistics

For comparison, mares were grouped as either younger (2–9 years, one to eight pregnancies, n = 14) or older (10–16 years, 9–14 pregnancies, n = 9). The proportions of younger and older mares whose endometrial sample was classified as Categories I, IIA, and IIB were compared using Fisher's exact test. Proportions with greater than mild lymphocytic endometritis and proportions with greater than mild periglandular fibrosis were similarly compared.

3. Results

No signs of foaling trauma were evident in any mare. Gross uterine involution and ovarian activity were in all cases consistent with Day 6 postpartum. The degree of inflammation and periglandular fibrosis, along with category assigned are listed in Table 1b. Periglandular fibrosis ranged from none to moderate. As expected postpartum, 22 of the 23 mares had mild to moderate lymphocytic endometritis. The one exception had moderate-severe lymphocytic endometritis. Differences in the proportion of younger versus older mares with classifications of I, IIA, and IIB, the proportion of mares with greater than mild lymphocytic endometritis, and the proportion of mares with greater than mild periglandular fibrosis were not significant (Fisher's exact tests, $P > .10$). For 10 of the 23 samples, the evaluator's assessment was that endometrial biopsy category would improve should postparturient inflammation resolves with involution. At the time of writing 22 of the 23 sampled mares continued to foal annually for the 1–5 years following the year of biopsy procurement that they remained in the herd (Table 1c). The one mare with a Category IIB biopsy as a result of moderate to severe lymphocytic endometritis foaled annually for each of the following 4 years but not the fifth.

4. Discussion

Two differentiating factors emerge when comparing this semi-feral population to previously referenced broodmare populations. First, the domestically managed mares are unlikely to have become pregnant as yearlings, as breeding is most often delayed until 3 years old or later. Secondly, domestically managed broodmares may not continue to foal annually either electively or as a consequence of fertility challenges [10]. Endometrial aging has been demonstrated in maiden mares; however, it has been hypothesized that factors associated with pregnancy and parturition hasten the onset of degenerative changes [2]. Within this semi-feral herd, any

"aging" effect associated with pregnancy and parturition appears to be minimally consequential as the histologic character of these mares' endometria was consistent with high lifetime reproductive efficiency. Degree of periglandular fibrosis ranged from none to moderate and was not greater in older mares (aged 10–16 years, following 9–14 pregnancies). The degree of lymphocytic endometritis was consistent with Day 6 postpartum and typical of the inflammation normally associated with involution of the microcaruncle [6]. Although we did not evaluate vascular integrity in this study, previous work has shown a high correlation between vascular degeneration and parity, as well as a positive correlation between endometrial grade and vascular degeneration, which was not observed in age-matched nulliparous mares [11]. One would expect to see some degree of reduced fertility secondary to vascular degeneration and compromised myometrial function within a high parity population. However, we have not observed parity-associated decline in fertility within this semi-feral population. A follow-up study would be required to explore this further.

Several aspects of semi-feral natural social organization and environmental conditions could be expected to result in fewer endometrial changes due to age and parity compared with domestic management conditions. Parturition, including expulsion of fetal membranes, typically occurs well under the generally accepted "normal" time frames for mares foaling under domestic conditions. Complications, including fetal membrane retention, have been extremely rare in this semi-feral herd. In 23 years, there have been a total of 379 foalings. Approximately, 35 were either observed or the foal was found minutes after passage (estimated based on mare and foal recumbency, amnion draping foal with or without cord attachment, or foal wet with fetal fluids). For those, fetal membranes were observed to pass within the first half an hour. In cases where foals were found more than an estimated hour after parturition, for all but one, the fetal membranes had already passed. For the one exception, fetal membranes passed between an estimated 5 and 8 hours after parturition. Observed foalings in this herd have been extremely rapid compared with those observed in domestically managed mares, with typically <10 minutes from the first sign of labor to full expulsion of the foal. Rapid uncomplicated parturition likely limits uterine contamination, inflammation, and endometrial damage. In addition, during estrus and breeding, uterine clearance is likely aided by cervical dilation during frequent copulation and oxytocin release as a result of frequent precopulatory interaction with the stallion [12]. Furthermore, suckling of the neonate, yearling, and even occasional suckling by an older offspring still in the natal band will stimulate uterine contractions by the frequent and repeated release of oxytocin. In addition, mares living under natural social and foraging conditions are generally much more active following parturition compared with domestically managed mares. Foraging, tending to their neonate, along with fastidious herding and tending by their harem stallion keeps mares in this semi-feral herd moving almost continuously (McDonnell unpublished observations). Similarly, under semi-feral social conditions, exercise is much greater during estrus and following ovulation. During estrus, the typically vigilant harem stallion herds his harem away from potential sneak breeders. For as long as 48 hours after ovulation, mares remain attractive but nonreceptive to the stallion. This results in continued vigilant herding along with precopulatory interaction without copulation many times per hour. Frequent oxytocin release stimulated by these interactions with the stallion [12] and associated exercise would be expected to promote uterine clearance.

Other aspects of natural social organization and environmental conditions may contribute to high fertility and fecundity. Data suggest that natural continuous foraging may enhance reproductive efficiency, presumably by avoiding stress [13]. Furthermore, social stability of established harem families protected by a stallion inherent to natural social organization may be less stressful for mares than typical domestic conditions of arbitrary and changing social groupings and/or isolation, particularly during breeding and for foaling.

Acknowledgments

This was a Dorothy Russell Havemeyer Foundation Project.

Authors' contributions: All authors were actively involved in all aspects of study design, data collection, and writing of this report.

References

[1] Vanderwall DK, Peyrot LM, Weber JA, Woods GL. Reproductive efficiency of the aged mare. Proc Annu Meet Soc Theriogenol 1989:153–6.
[2] Ricketts SW, Alonso S. The effect of age and parity on the development of equine chronic endometrial disease. Equine Vet J 1991;23:189–92.
[3] Carnevale EM, Ginther OJ. Relationship of age to uterine function and reproductive efficiency in mares. Theriogenology 1992;37:1101–15.
[4] Scoggin CF. Not just a number: effect of age on fertility, pregnancy and offspring vigour in thoroughbred brood-mares. Reprod Fertil Dev 2015;27:872–9.
[5] Doig PA, McKnight JD, Miller RB. The use of endometrial biopsy in the infertile mare. Can Vet J 1981;22:72–6.
[6] Sertich PL, Hinrichs K, Kenney RM. Histological aspects of uterine involution in the post parturient, ovariectomized embryo-recipient mare: a model for the study of involution. Equine Vet J 1989;21:56–8.
[7] Kenney RM. Cyclic and pathologic changes of the mare endometrium as detected by biopsy, with a note on early embryonic death. J Am Vet Med Assoc 1978;172:241–62.
[8] Blanchard TL, Garcia MC, Kintner LD, Kenney RM. Investigation of the representativeness of a single endometrial sample and the use of trichrome staining to aid in the detection of endometrial fibrosis in the mare. Theriogenology 1987;28:445–50.
[9] Kenney RM, Doig PA. Equine endometrial biopsy. In: Morrow DA, editor. Current therapy in theriogenology. 2nd ed. Philadelphia: Saunders; 1986. p. 723–9.
[10] Bosh KA, Powell D, Neibergs JS, Shelton B, Zent W. Impact of reproductive efficiency over time and mare financial value on economic returns among Thoroughbred mares in central Kentucky. Equine Vet J 2009;41:889–94.
[11] Esteller-Vico A, Liu IK, Couto S. Uterine vascular degeneration is present throughout the uterus wall of multiparous mares. Colinearity between elastosis, endometrial grade, age and parity. Theriogenology 2012;78:1078–84.
[12] Madill S, Troedsson MH, Alexander SL, Shand N, Santschi EM, Irvine CH. Simultaneous recording of pituitary oxytocin secretion and myometrial activity in oestrous mares exposed to various breeding stimuli. J Reprod Fertil Suppl 2000;56:351–61.
[13] Benhajali H, Ezzaouia M, Lunel C, Charfi F, Hausberger M. Temporal feeding pattern may influence reproduction efficiency, the example of breeding mares. PLoS One 2013;8:e73858.