Neutrophil prevalence in dismount semen samples of Thoroughbred stallions*

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Introduction

Semen dismount samples from Thoroughbred stallions are routinely monitored after natural mating.[1,2] Due to the relative ease of obtaining the sample, it is tempting to use features such as neutrophil presence to predict fertility.[3-6] There are reports that neutrophils in a dismount sample from a stallion may indicate an inflammatory or infectious process in the reproductive tract of that stallion, or that they may be from the mare with an inflamed, and possibly infected reproductive tract with which the stallion has mated.[2] The presence of neutrophils in dismount samples has been reported to decrease the odds of pregnancy.[7,8] However, neutrophil prevalence in dismount samples of stallions with no reported fertility problems is unknown.

The aim of this study was to investigate the prevalence of neutrophils in stallion dismount samples from stallions with no perceived fertility problems. The study group consisted of commercial Thoroughbred stallions on a breeding farm in Eastern Australia.

Keywords: Stallion, dismount semen sample, neutrophils

Materials and methods

Study design

The seven Thoroughbred stallions chosen for the study were all used commercially for the full length of the 2009 southern hemisphere breeding season. No abnormalities of the reproductive tracts of any of the stallions were detected. The stallions all resided on the same farm.

The sampling strategy was convenience sampling. Dismount semen samples from the distal penis and urethra were collected after natural mating from as many matings as possible, Monday through Friday from 10 September to 7 December 2009 (Figure 1). The date, the time of collection and the stallion were recorded for each sample.

Sample processing and assessment

The samples were examined within 24 hours of collection. After agitating the sample, a 25 microliter drop was smeared across a micrscope slide, air dried and stained with modified Wright Giemsa stain according to the procedure described by Schumacher and Moll[9] (Figures 2 and 3).

The length of the slide was scanned initially at 100x magnification, then more critically evaluated at 1000x magnification with an oil immersion objective. Numbers of neutrophils were counted in each field and the sample was categorized according to Table 1. The number of slides falling into each category was counted.

Category	Number of neutrophils per high power field (HPF)
0	0
1	<1 per 5 HPF
2	1 per 5 HPF - <1 per 3 HPF
3	>1 per 3 HPF- <1 per HPF
4	1-2 per HPF
5	>2 per HPF

Table 1. Sample category according to number of neutrophils in stallion semen dismount sample.

*Data presented at Australian College of Veterinary Scientists Science Week, 1-3 July 2010 and Association of Applied Animal Andrology 7th Biennial Meeting, Sydney, Australia, 28-29 August 2010.

Figure 1. Collection of a dismount sample from the penis of a stallion.

Analysis

The prevalence of neutrophils in each category was calculated, with a 95% confidence interval. The number of samples collected from each stallion was compared to expected numbers (chi-square test), to determine that a particular stallion was not under- or over-represented by the sampling method. The number of samples containing neutrophils for each stallion was also compared to expected numbers (chi-square test), to investigate whether samples with neutrophils were more likely to come from one stallion than another.

Data on the number of services for each stallion per week were not available. However, the number of samples collected each week and the number of samples with neutrophils each week was examined, to ascertain that the samples had been taken over the entire season, and to investigate whether neutrophil presence could be associated with a particular time of season.

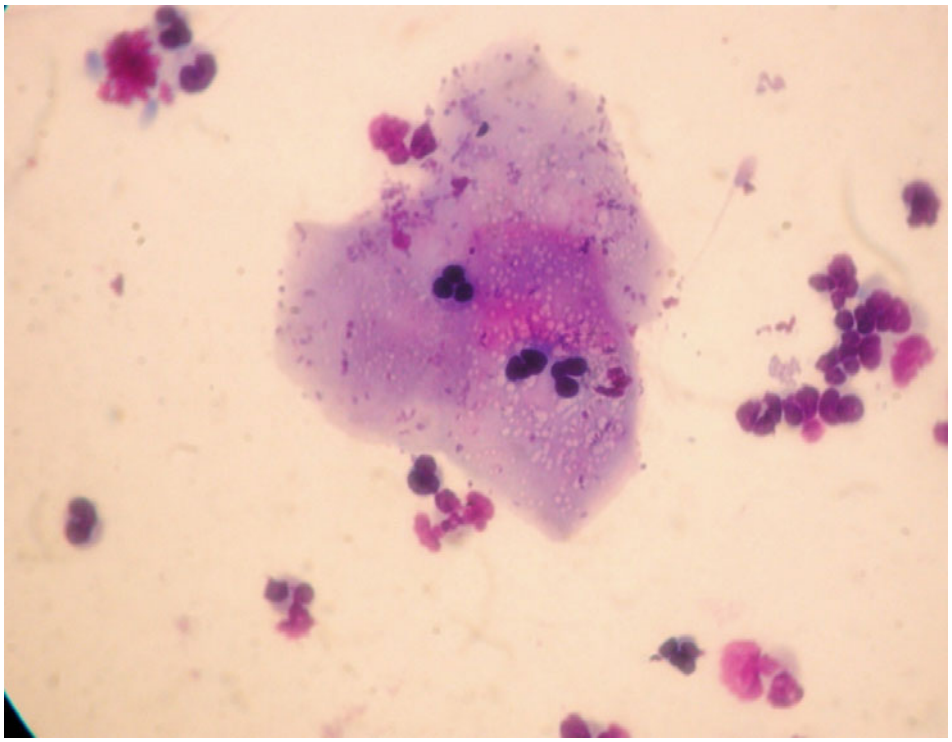

Figure 2. Neutrophils, stratified squamous cells and a spermatozoan.

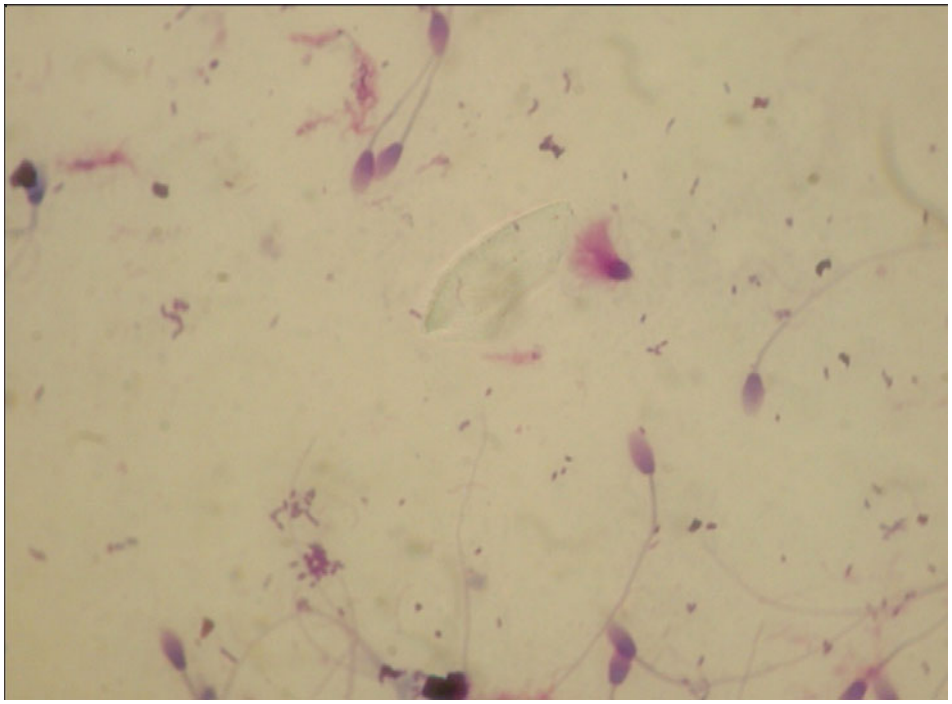

Figure 3. Spermatozoa and ciliated epithelial cell.

Results

Four hundred eighteen samples were collected (Table 2).

Stallion	Total services (n)	Samples (n)	Samples with neutrophils (n)
1	257	91	27
2	167	63	8
3	96	38	9
4	210	93	20
5	115	42	8
6	115	40	6
7	148	51	6
Total	1108	418	84
P value of x^2 statistic		P=0.71	P=0.20

Table 2. Total number of services, number of samples collected and number of samples containing neutrophils, by stallion.

The samples were categorized according to neutrophil presence, and the prevalence of neutrophils calculated for categories 1 to 5 (Table 3).

Category	Number of Samples	Percent of Total	Prevalence of Neutrophils (95% confidence interval)
0	334	80.0	
1	42	10.0	0.10 (0.16, 0.24)
2	11	2.6	0.03 (0.01, 0.04)
3	9	2.1	0.02 (0.01, 0.04)
4	12	2.9	0.03 (0.01, 0.04)
5	10	2.4	0.02 (0.01, 0.04)
Total	418	100	0.20 (0.16, 0.24)

Table 3. Number and percent of stallion semen dismount samples in each neutrophil score category.

A graph was plotted of the number of samples collected weekly and the number of samples with neutrophils (categories 1-5) over time (Figure 4).

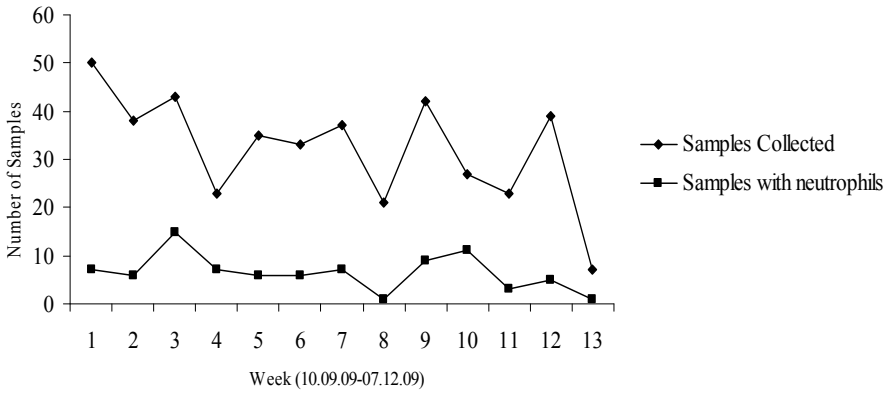

Figure 4. Graph showing the weekly number of stallion semen dismount samples collected and the number of samples with neutrophils.

Discussion

The results of this study show that neutrophils are present in 20% (95% confidence interval, 16%-24%) of dismount semen samples from this population of Thoroughbred stallions with no detected fertility problems from a commercial breeding operation in Australia.

The sampling strategy (convenience) did not significantly over- or under-represent any of the stallions ($p>0.05$). The number of samples containing neutrophils was not significantly different between each stallion ($p>0.05$).

The sample collection appears to be spread over the entire breeding season. Samples with neutrophils are also evenly distributed over time, and appear to be of similar proportion to number of samples collected each week (Figure 1), indicating that neutrophil presence in stallion dismount samples is a consistent feature.

The origin of the neutrophils was not determined in this study, and further work is required to establish whether they are from the mare or the stallion, or both. Further work is also required to determine the outcome of individual matings where neutrophils are present compared to those without neutrophils.

Conclusion

In this study, approximately 20% of dismount samples from Thoroughbred stallions contained neutrophils. In the authors' opinion, neutrophil presence in a dismount semen sample should not be used as an indicator of stallion fertility.

Acknowledgements

The authors thank John Chopin for advice in preparing the manuscript.

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