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Aerobic Bacterial Flora of Semen and Stallion Reproductive Tract and its Relation to Fertility Under Field Conditions

By *L. Malmgren*[1], *E. Olsson Engvall*[2], *A. Engvall*[3] and *A. Albihn*[3]

[1]Department of Obstetrics and Gynaecology, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden. [2]Laboratory of Bacteriology, Naional Veterinary Institute, Uppsala, Sweden. [3]Division of Epizootiology, National Veterinary Institute, Uppsala, Sweden.

Malmgren L, Olsson Engvall E, Engvall A, Albihn A: Aerobic bacterial flora of semen and stallion reproductive tract and its relation to fertility under field conditions. Acta vet. scand. 1998, 39, 173-182. – This study was initiated in order to investigate the bacterial flora of the stallion genital tract by taking consecutive samples from normal stallions in regular use. The objective was to determine whether any growth of potential pathogens, particularly *P. aeruginosa* and *K. pneumoniae,* in fresh semen and urethra was associated with the presence of inflammatory cells in the semen and whether bacterial growth had any effect on sperm morphology and pregnancy results. Sixteen stallions, only used for A.I., housed at 3 different commercial stud farms, were used.

A wide variety of microorganisms was found in almost all samples from fresh semen (total 115 samples). *P. aeruginosa* was isolated from 46/115 (40%) of the samples and from 12 of the 16 stallions. *K. pneumoniae* was isolated from the semen of one stallion. Samples taken from the distal urethra after ejaculation contained fewer microorganisms than samples from fresh semen. No bacteria were found in 51% of the extended semen samples.

Most of the stallions had an acceptable sperm morphology, and very few of the ejaculates contained inflammatory cells. Pregnancy results among the stallions varied, but were acceptable for most of them. There was no correlation between the frequency of samples testing positive for *P. aeruginosa* in raw semen and pregnancy results.

bacterial growth, urethra, Pseudomonas aeruginosa, Klebsiella pneumoniae.

Introduction

The external genitalia of healthy stallions harbor many different microorganisms. The majority of them are generally considered to be non-pathogenic and are part of the normal microflora. However, there are also opportunistic or potentially pathogenic microorganisms capable of producing genital infection in susceptible mares (*Hughes et al.* 1967, *Burns et al.* 1975, *Bristol* 1991, *Klug and Sieme* 1992, *Clément et al.* 1993, *Danek et al.* 1993). The response of a mare to insemination or natural mating in cases where the semen contains potentially pathogenic microorganisms will depend on the status of the uterine defense mechanism (*Cheung et al.* 1985), as well as on the virulence and dosage of the introduced bacteria.

Taylorella equigenitalis, Pseudomonas aeruginosa, and *Klebsiella pneumoniae* are considered to be the most important potential venereal

pathogens, known to cause outbreaks of acute endometritis and lowered fertility in susceptible mares (*Couto & Hughes* 1993). Other bacteria isolated from the semen and genital tract of stallions and which are considered to be potential pathogens are β-hemolytic streptococci, *Staphylococcus aureus,* and *Escherichia coli* (*Klug & Sieme* 1992, *Rossdale & Ricketts* 1980).

However, the isolation of *P. aeruginosa* from the stallion genital tract does not necessarily indicate the presence of a venereal infection. In the study by *Hughes et al.* (1967), most stallions harboring *P. aeruginosa* had good conception rates, although some of the culture-positive stallions showed poor fertility. A number of strains of *P. aeruginosa* have been isolated, and differences in pathogenicity have been reported (*Atherton & Pitt* 1982, *Kenney et al.* 1992). Similarly, certain capsular serotypes of *K. pneumoniae,* such as K1, K2 and K5, have been reported as venereal pathogens, whereas the potential to cause metritis is considered to be much lower in others, such as K7 (*Atherton* 1975, *Kikuchi et al.* 1987). To judge pathogenicity simply on the basis of serotype, however, is an unreliable method, and according to others, every capsular serotype of *K. pneumoniae* should be regarded as a potential venereal pathogen (*Weiss et al.* 1976, *Rossdale & Rickets* 1980).

In Sweden, the regulation requires that stallions be tested for the presence of bacteria in their reproductive tract before being permitted to enter a breeding program. This rule is meant to prevent transmission of venereal diseases. It is, however, sometimes difficult to determine the significance of the bacteriological findings. To address this problem, we have investigated the bacterial flora of the genital tract by taking consecutive samples from normal stallions in regular use. Our intention was to determine whether the growth of potential pathogens, particularly *P. aeruginosa* and *K. pneumoniae,* in fresh semen or urethra was associated with the presence of inflammatory cells in the semen and if bacterial growth had any effect on sperm morphology or pregnancy results. In addition, different systems of transporting fresh sperm samples were compared in terms of their influence on bacterial growth.

Materials and methods

Stallions

Sixteen Standardbred stallions (A-P), aged 4-21 years, were included in the study, which was performed under field conditions. The stallions were housed at 3 different stud farms and were only used for A.I. The farms were well managed and had a generally high hygienic level, each farm having their own veterinarian who handled all bacteriological samplings. Two to 4 stallions were kept at each farm per year, and 5 of the stallions were sampled in both 1993 and 1994. Farm I kept stallions A-F, farm II stallions G-M, and farm III stallions N-P. During 1993, 10 of the stallions were sampled on 3 to 8 occasions each (sampling occasions 1-8), and during 1994, 11 of the stallions were sampled on 4 to 7 occasions (sampling occasions 9-15) (Table 2). All samples were taken during the breeding season and were collected, on almost all occassions, at 2-3 weeks intervals. In addition, all stallions were enrolled in the national control programme for Contagiuos equine metritis (CEM) and had been found negative (in this programme one swab is taken from the fossa urethralis and one from the urethra on one occasion).

Semen collection and vagina washing procedures

Semen was collected 3 times a week, according to the routine A.I. programme, by using an artificial vagina (Missouri model®, Phoenix, Med-

ical, USA, J. Kruuse, Odense, Danmark). Before collection, the penis of some of the stallions was occasionally washed using water and mild soap. Each stallion had its own artificial vagina, which was washed carefully after each collection with soap/detergent and water. At farm I, besides the initial washing, the vaginas were rinsed in 70% ethanol after each use, and at farm II, the vaginas were also soaked in Virkon® (Antec Int. Ltd, Suffolk, England) for 10 min before rinsing in hot tap water followed by 70% ethanol. At farm III, no further routine cleaning was performed; however, the vaginas were occasionally soaked in 70% ethanol.

Semen extender

Semen extenders used were Kenney's extender (*Kenney et al.* 1975) with 150 000 IU penicillin and 150 mg streptomycin/100 ml extender (farm I and III) or with 150 000 IU penicillin and 50 mg gentamicin/100 ml extender (farm II).

Bacteriological sampling procedure

On each bacteriological sampling occasion, one sterile vial with 2-3 ml of fresh semen and one swab (Culturette®, Copan, Bovezzo, Italy) soaked with fresh semen were collected. The second year, swabs were also taken on each occasion from the distal urethra after ejaculation. Samples from the extended semen were taken on 1 to 2 occasions (10 samples) the first year and on each sampling occasion the second year (57 samples). The extender was added to the semen 45-60 min before a swab was soaked in the mixture. The semen/extender ratio was 1+1 to 1+2 when the bacteriological samples were obtained. All samples were sent by regular mail and reached the laboratory within 24 h.

Bacteriology

Conventional methods for isolation and identification of microorganisms were used (*Quinn et al.* 1994). Samples were cultured on 5% horse blood agar, lactose bromocresol purple agar, and Cetrimide agar (Serva Fine Biochemical, Heidelberg, Germany) (selective for *Pseudomonas* spp.) in 37 °C. Each bacteriological culture was inspected and any bacterial growth registered after 24 and 48 h. Growth of *P. aeruginosa* and *K. pneumoniae* was always considered to be of significance and therefore interpreted as positive findings. Other bacterial isolates were typed if growth was moderate to abundant, pure, or dominating on the agar plate. In some cases, the growth of yeasts or molds was also recorded.

Semen evaluation

An evaluation of sperm morphology was made in 11 of the stallions twice during the second year, i.e. at the beginning of the breeding season and midway through the breeding season. Sperm head morphology was studied in smears stained with carbol-fuchsin according to the method described by *Williams* (1920). To search for proximal cytoplasmic droplets, abnormal acrosomes, detached heads, and abnormalities of the midpiece and tail (*Hancock* 1957), preparations of formol-saline fixed spermatozoa were examined under a phase-contrast microscope (1000×). The abnormalities were classified according to a system developed by *Bane* (1961) which is routinely employed in the semen laboratory at the Department of Obstetrics and Gynaecology. Two hundred spermatozoa were examined using each method, and the morphological abnormalities were recorded as a percentage of the total number of counted spermatozoa.

Smears for evaluation of inflammatory cells were made on the same occasions as the bacteriological samples were taken. The smears were stained according to *Papanicolaou* (1942), and numbers of leukocytes and abnormal spermatogenic cells were recorded.

Pregnancy evaluation

Pregnancy results were recorded as pregnancies per cycle and pregnancies per season. Numbers of covered mares ranged from 15 to 150 mares/stallion and season.

Statistical analysis

Statistical analyses were carried out using McNemar's test and Pearson's correlation (JMP, SAS Institute, Inc., Cary, NC, USA).

Results

Bacteriology

Fresh semen. A wide variety of microorganisms were found in almost all samples. There was only one sample from which no bacteria could be isolated. *P. aeruginosa* was isolated from 46/115 (40%) of the samples. Other commonly identified bacteria were *Bacillus* spp., *Enterobacter aerogenes,* other *Enterobacter* spp., and *Acinetobacter* spp. (Table 1).

From 12 of the 16 stallions, *P. aeruginosa* was isolated at least once (Table 2). Growth of *P. aeruginosa* was found in all samples from 3 of the stallions (B,F,I), in 50%-80% of the samples from 5 stallions (C,G,K,L,M), and in 18%-20% of the samples from 4 stallions (A,D,E,J). Thus there were only 4 stallions (H,N,O,P) from which no *P. aeruginosa* was ever isolated. Nine of the stallions harboring *P. aeruginosa* yielded negative samples on the first sampling occasion of the season, and another 5 were negative for this bacteria on the 2 first sampling occasions. The growth of *P. aeruginosa* was abundant in most cases: only 5 samples yielded sparse growth, and another 3 yielded moderate growth. *P. aeruginosa* was not isolated from cultures with semen from any of the stallions on farm III (Table 2).

K. pneumoniae was isolated from the semen of one stallion (stallion O, farm III). Approximately 30% of the samples from this stallion, distributed over the 2 seasons, were positive for *K. pneumoniae* (Table 2). In 1994, one isolate was serotyped at the Central Public Health Laboratory, London, and identified as serotype K3. *P. aeruginosa* or *K. pneumoniae* was isolated from 47 samples of semen that had been sent to the laboratory in a vial, whereas only 41 of the samples sent as swabs soaked in the semen yielded positive cultures. On 8 occasions, *P. aeruginosa* or *K. pneumoniae* was isolated from semen samples sent in a vial while the corresponding swabs were negative, and on 2 occasions, *P. aeruginosa* or *K. pneumoniae* was cultured from samples sent in as swabs, while the corresponding semen samples in vials were negative. The discrepancy between transport methods was mainly seen in 2 stallions, C and O, for which 3 out of 4 and 4 out of 5, respectively, of the positive culture samples were positive for the vial culture but not for the corresponding swab culture. In addition, there was often a rich growth of bacteria in the vial culture but only moderate growth in the corresponding swab culture. No statistically significant difference between the 2 methods was noted (McNemar's test).

Urethral swabs. Samples taken from the distal urethra after ejaculation contained fewer microorganisms than samples taken from fresh semen (Table 1). A sparse growth of *P. aeruginosa* was found in cultures of 2 samples (stallions F and G), and moderate to abundant growth of *K. pneumoniae* was noted in cultures from another 2 samples (stallion O, sampling occasions 10 and 13). *Acinetobacter* spp. was the bacteria most commonly isolated from urethral samples (43%).

Extended semen. No bacteria were isolated from 34 (51%) of the extended semen samples. Cultures of 3 extended semen samples contained a sparse growth of *P. aeruginosa.* No *K. pneumoniae* were isolated from any of the samples (Table 1). The occurrence of microorganisms in the extended semen samples was on al-

Table 1. Number of samples, in which microorganisms were found in A) fresh semen, year 1 (56 samples), B) fresh semen, year 2 (59 samples), C) urethra, year 2 (54 samples) and D) extended semen, years 1 and 2 (67 samples).

Microorganisms	A	B	C	D
No growth of bacteria	–	1	1	34
Sparse mixed culture	–	–	5	9
Medium–abundant mixed culture	–	–	2	1
Acinetobacter spp.	8	10	23	3
Aeromonas salmonicida	–	1	–	–
Bacillus spp.	20	11	2	1
Chryseomonas luteola	1	–	–	–
Comamonas testosteroni	1	–	–	1
Corynebacterium spp.	1	–	1	1
Enterobacter aerogenes	12	19	4	2
Enterobacter agglomerans	3	2	4	–
Enterobacter amnigenus	1	–	–	–
Enterobacter spp.[1]	4	–	–	–
Enterococcus spp.	3	4	–	2
Escherichia coli	3	4	2	–
Klebsiella pneumoniae	3	2	2	–
Kurthia spp.	–	–	1	–
Micrococcus spp.	3	–	–	–
Proteus spp.	1	1	–	–
Providentia stuartii	1	–	–	–
Pseudomonas aeruginosa	22	24	2	3
Pseudomonas spp.[2]	2	8	–	8
Coagulase – *Staphylococcus* spp.	3	8	8	2
Coagulase + *Staphylococcus* spp.	–	1	1	1
α–hemolytic *Streptococcus* spp.	6	4	1	–
Streptococcus equisimilis	–	4	1	–
Gramnegative rods	4	1	2	6
Xantomonas maltophilia	–	–	–	1
Candida spp.	–	–	–	4
Absidia spp.	–	1	–	–
Mucor spp.	–	–	1	–

[1] others than *E. aerogenes, E. agglomerans* and *E. amnigenus.*
[2] others than *P. aeruginosa.*

most all occasions found in the semen samples from farm III, but only occasionally in samples from farms I and II.

Sperm morphology

Five of the stallions had a satisfactory sperm morphology, i.e >70% morphologically normal spermatozoa (B,E,G,L, and O); four of the stallions had an acceptable sperm morphology, i.e. >50% morphologically normal spermatozoa (A,F,N, and P), and 2 had an unsatisfactory sperm morphology, i.e. <45% morphologically normal spermatozoa (K and M) (Table 3). The dominant abnormalities were abnormal head shapes (34% in stallion K), abnormal midpieces (48% in stallion M), and proximal

Table 2. Growth of *P. aeruginosa* (P.a.) and *K. pneumoniae* (K.p.) in raw semen, the number of mares inseminated, pregnancy per cycle and pregnancy per season for each stallion. The bacterial growth was in most cases abundant.

		Farm I (Stallion)						Farm II (Stallion)							Farm III (Stall.)		
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Year 1	Sample no																
	1	–	P.a.	–	–				–	P.a.	–				–	K.p.	
	2	P.a.	P.a.	P.a.	–			–	–	P.a.	–				–	K.p.	
	3	–	P.a	P.a.	–			–		P.a.	–				–	–	
	4	P.a.	P.a		P.a.			P.a.	–	P.a.	–				–	–	
	5	–	P.a.	P.a.				–		P.a.					–	K.p.	
	6				–										–	–	
	7	–	P.a.	P.a.				P.a.		P.a.	P.a.					–	
	8														–	–	
Year 2																	
	9	–	P.a.			P.a.		–				–	–	–	–	–	–
	10	–	P.a.			–	P.a.	–				P.a.	P.a.	–	–	K.p.	–
	11	–	P.a.			–	P.a.	P.a.				P.a.	P.a.	P.a.	–	–	–
	12	–	P.a.			–	P.a.	P.a.				P.a.	P.a.	P.a.	–	–	–
	13	–	P.a.			–	P.a.	P.a.				P.a.	P.a.	P.a.	–	–	–
	14														–	K.p.	–
	15															–	–
Year 1																	
Preg/cycle	%	67	75	0	60			60	40	49	52				55	51	44
Preg/season	%	90	89	0	86			88	68	77	78				85	91	85
No of mares		149	111	43	22			48	60	47	45				73	150	84
Year 2																	
Preg/cycle	%	68*	85*			70*	71*	55				56	63	56	29	59	
Preg/season	%	92	85			94	87	81				80	90	87	64	87	
No of mares		135	71			150	15	95				39	150	112	49	150	

* values calculated only from mares inseminated at the stud farm; shipped semen was not included.
– = no growth of P.a. or K.p.

droplets (33% in stallion N). The relationship between the frequency of morphologically normal spermatozoa and pregnancy results was r = 0.3, p = 0.37 (pregnancies/cycle) and r = 0.31, p = 0.36 (pregnancies/season), and between the frequency of morphologically normal spermatozoa and the frequency of samples testing positive for *P. aeruginosa* (r = –0.09, p = 0.77).

A low number of inflammatory cells (leukocytes) was found in 2 ejaculates that contained *K. pneumoniae* and were taken at the end of the sampling period from stallion O .

Pregnancy results

Pregnancy results varied among stallions. Most of the stallions had acceptable to good pregnancy results, i.e. >50% pregnancies/cycle and >75% pregnancies/season. One stallion (C) was excluded from further stud work since no mares inseminated with his sperm became pregnant. Infertility in this case was attributed to low sperm production and poor sperm morphology. The relationship between the frequency of semen samples testing positive for *P. aeruginosa* and pregnancy results was r = 0.51, p = 0.054

Table 3. The percentages of morphological normal spermatozoa in ejaculates collected from 11 stallions in year 2. Two ejaculates per stallion were examined, one in the beginning and one in the middle of the breeding season.

	Stallion										
	A	B	E	F	G	K	L	M	N	O	P
Ejac. 1 % normal	50	70	87	51	80	45	83	30	60	77	58
Ejac. 2 % normal	56	84	77	76	86	25	76	23	63	84	57

(pregnancies/cycle) and r = 0.17, p = 0.54 (pregnancies/season).

Discussion

In this study, where 16 stallions from 3 stud farms were examined, *P. aeruginosa* was commonly isolated from semen samples from stallions at 2 of the stud farms. This is in accordance with *Hughes et al.* (1967) who also found a high frequency of *P. aeruginosa* in stallion semen. However, the reported abundance of the various bacterial species/strains varies between investigations (*Klug & Sieme* 1992, *Clément et al.* 1993, *Madsen & Christensen* 1995). In the study by *Clément et al.* (1993) the most commonly found bacteria were streptococci of the Lancefield group C (*S. equisimilis, S. zooepidemicus*). *Madsen & Cristensen* (1995) found mostly coagulase-negative staphylococci and coryneforms, while *Pseudomonas* spp. (not *P. aeruginosa*) was the most common bacteria found in the study by *Klug & Sieme* (1992). However, it is difficult to compare studies since they can differ in terms of sampling techniques, sampling sites, and methods of transport.

Hughes et al. (1967) also found that the chance of obtaining a positive culture from a given stallion is higher if more than one culture is made. This is in agreement with the results of the present study, where a higher frequency of positive samples for *P. aeruginosa* was obtained when the stallions were cultured several times. Also, in our study, none of the samples from stallions from one of the farms tested positive for *P. aeruginosa,* whereas several samples from the other 2 farms yielded an abundant growth of *P. aeruginosa* upon culture. In another study, where *Malmgren et al.* (1996) investigated stallions from three other Swedish stud farms, no growth of *P. aeruginosa* was found in any of the total of 40 samples taken from 8 stallions during the breeding season. These remarkable differences in frequencies with which *P. aeruginosa* has been isolated between different stud farms could be due to an environmental source of inoculum. As suggested by *Kenney et al.* (1992), numerous parts of the horse's environment, such as standing water, artificial vaginas, and extenders, could be contaminated by *P. aeruginosa* and contribute to the development of a carrier state in the stallion. Under standard A.I. management systems, a cycle of contamination between stallion, artificial vagina and mares can become established. However, experience suggests that the *P. aeruginosa* strains and *K. pneumoniae* capsule types which originate from environmental contamination are less likely to produce true equine sexually-transmitted disease.

The growth of *P. aeruginosa* and *K. pneumoniae* in urethral samples was more sparse compared with the growth in semen samples, with *P. aeruginosa* or *K. pneumoniae* being isolated

from only 4 out of 54 urethral samples. Similar results were obtained in a study conducted by the National Veterinary Institute in Uppsala: out of 52 urethral swabs from 52 stallions examined, *P. aeruginosa* was only isolated from one sample, where it showed sparse growth (unpublished). This indicates that it is primarily the surface of the penis and prepuce that becomes colonized by *P. aeruginosa* and that the semen becomes secondarily contaminated at ejaculation. In cases where the internal genital organs are infected, this condition is usually associated with large numbers of bacteria, leukocytes, and purulent debris in the semen and bacteria from post-ejaculation swabs of the urethra (*Blanchard et al.* 1988). Stallions in this study showed no clinical symptoms associated with an infection in the internal genitalia, except for one stallion which had a few leukocytes in the semen on 2 occasions. However, this stallion did not show any other signs of disease, and its fertility was satisfactory. Nor could *Hughes et al.* (1967) find any clinical symptoms in the stallions harboring *P. aeruginosa.*

After incubation of semen with an extender containing antibiotics, the number of bacteria was markedly reduced. This is in agreement with the findings of *Burns et al.* (1975) and *Squires et al.* (1981). However, bacteria were isolated from approximately half of the samples in the present study, with semen samples from farm III accounting for most of the positive tests. On farm II, gentamicin was used routinely. This antibiotic has been shown to reduce the growth of *P. aeruginosa* and *K. pneumoniae* at a concentration of 0.1 mg/ml and 1 mg/ml, respectively (*Squires et al.* 1981). *Clément et al.* (1993) suggested that a concentration of gentamicin below 0.05 mg/ml should be safe for use in artificial insemination. However, on one occasion there was a sparse growth of *P. aeruginosa* in extended semen from farm II. Although only penicillin and streptomycin were used in the extender on farms I and III, there was still a marked reduction in the growth of *P. aeruginosa* and *K. pneumoniae* on all but 2 occasions. This was surprising since in vivo penicillin has no effect on *P. aeruginosa* or *K. pneumoniae,* and streptomycin has only a slight effect. Nevertheless, concentrations of streptomycin and penicillin in the present study seemed to be high enough to reduce the *in vitro* growth of *P. aeruginosa* and *K. pneumoniae.*

Whether or not opportunistic bacteria can affect semen quality has been a subject of controversy. *Pickett* (1993) did not find any adverse effects on seminal characteristics in stallions with potentially pathogenic bacteria in genital samples, whereas *Rideout et al.* (1982) found that metabolites produced by certain bacteria suppressed the motility of equine spermatozoa. *Hughes et al.* (1967) suggested that stallions carrying large numbers of potentially pathogenic organisms had a low sperm survival time. *Danek et al.* (1993) found that semen from which potentially pathogenic β-hemolytic streptococci had been isolated was of lower quality (i.e. lower motility, higher numbers of leukocytes in the semen and more agglutination) compared with pathogen-free semen. In the present study, β-hemolytic *Streptococcus equisimilis* was isolated from raw semen in stallion E on 4 occasions; yet semen morphology and fertility were satisfactory. Further, in our study, the presence of potentially pathogenic bacteria in a stallion's semen did not seem to affect the stallion's semen quality (i.e. sperm morphology).

In the literature, some confusion exists concerning the significance of certain serotypes of *P. aeruginosa* in relation to fertility. However, *Atherton & Pitt* (1982) concluded that all serotypes have pathogenic potential since they found no correlation between pathogenicity and serological or phage types in their study of endometritis outbreaks caused by *P. aeruginosa.*

Serotyping offers a valuable tool in work aimed at gaining a better understanding of the epidemiology of *P. aeruginosa* infections, but could not be used for predicting a pathogenic potential. In the present study, the *P. aeruginosa* isolates were not serotyped, and although there was a high frequency of samples of fresh semen containing *P. aeruginosa,* pregnancy rates for most of the stallions were satisfactory. It should be noted that all stallions in this study were used only for A.I., and that the semen was diluted with an antibiotic-containing extender prior to use. As seen in this study, the bactericidal effect of this treatment varied, which is noteworthy. However, other factors, such as the mare's uterine defense mechanisms are higly critical in determining wheter bacterial colonization and concurrent disease will follow.

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Sammandrag

Aerob bakterieflora i sperma och könsvägar hos hingst, och dess relation till fertilitet.

Syftet med studien var att undersöka bakteriefloran i prov från hingstars sperma och yttre genitalia och se om växt av potentiella patogener, med tyngdpunkt på *P. aeruginosa* och *K. pneumoniae,* i färsk sperma och urethra var kopplat till fynd av inflammatoriska celler i sperman. Vidare att undersöka om bakterieväxten hade någon effekt på spermiemorfologin och dräktighetsresultatet. Sexton hingstar, uppstallade på tre olika stuterier (samtliga använde A.I.teknik), användes i studien. Prover togs regelbundet från varje hingst under sexualsäsongen.

En stor variation av olika mikroorganismer sågs i så gott som samtliga prover från färsk sperma (totalt 115 prover). *P. aeruginosa* isolerades från 45/115 (40%) av proverna och 12 av de 16 hingstarna uppvisade ett eller flera positiva prover avseende växt av *P. aeruginosa.* Prover tagna från distala urethra efter ejakulation innehöll färre mikroorganismer jämfört med proverna tagna från färsk sperma. I 51% av proverna från spädd sperma sågs ingen bakterieväxt. De flesta hingstarna hade en acceptabel spermiemorfologi och endast ett fåtal av ejakulaten innehöll inflammatoriska celler. Dräktighetsresultet varierade mellan hingstarna, men majoriteten hade en tillfredställande fruktsamhet. Ingen korrelation sågs mellan frekvensen *P. aeruginosa* positiva prover och dräktighetsresultatet.

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Reprints may be obtained from: L. Malmgren, Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, PO Box 7039, S-750 07 Uppsala, Sweden.