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Microbial Quality of Fresh Semen of Tunisian Arab Stallions

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Abstract: The aim of this study was to determine the quantitative and qualitative bacterial contamination of fresh semen of Tunisian Arab stallions. Semen of 5 stallions (total ejaculates = 21), aged between 11 to 15 years, was collected by an artificial vagina (AV) and analysed for seminal parameters. Sexual behaviour parameters such us collect duration and number of mounts in AV were investigated during semen collection. Then, bacterial flora concentration of fresh semen was determined and analysed to seek for potentially pathogenic bacteria. ANOVA was carried using a software SAS (1997) to study factors influencing bacterial concentration in fresh semen. We found that bacterial concentration in fresh semen varied from $1,9.10^5$ to $2,1.10^6$ CFU/ml according to stallions ($p < 0.05$) and according to ejaculates in the same stallion ($p < 0.05$). Two pathogenic bacteria were detected in ejaculates. *Pseudomonas aeruginosa* was found in 4 stallions, whereas *Staphylococcus aureus* was found only in 2 stallions. The study showed lower correlation between sexual behaviour parameters and bacterial flora concentration which varied from $r = 0.15$ for collect duration to $r = 0.25$ for number of mounts. Lower correlations between semen parameters and bacterial flora concentration were also proved and varied from $r = 0.05$ to $r = 0.31$. Our study showed that was a great variation of bacteria flora concentration between stallions and between ejaculates of the stallion. In conclusion, none of the sexual behaviour and semen parameters was correlated significantly with the bacteria flora concentration.

Key words: Semen · Bacteria flora · Pathogenic bacteria · Arab stallions

INTRODUCTION

Prepucial mucosa and urethra are home to many bacterial species that contaminate semen during ejaculation in natural breed or in artificial insemination (AI). A high semen bacterial contamination is detrimental to sperm survival [1]. In addition, some bacteria are pathogenic and contribute to aging of the endometrium in mares [2].

The purpose of this study was to evaluate quantitative and qualitative bacterial contamination of fresh semen of Tunisian Arab stallions and their relationships with sexual behaviour parameters during collection and with semen parameters.

MATERIALS AND METHODS

General: Experience took place in the National Stud Farm of Sidi Thabet, situated in the north of Tunisia, during the months of January and February 2010. Semen was collected from 5 Arab stallions named A, B, C, D and E (total ejaculates = 21; 4 ejaculates for each of the 4 stallions and 5 ejaculates for the 5th stallion) aged between 11 to 15 years. The bacteriology examination was carried in the department of microbiology-immunologie of the veterinary medicine school of Sidi Thabet.

Sexual Behaviour and Semen Evaluation Parameters: Ejaculates were collected using a Missouri artificial vagina (AV) (IMV, France) and an estrous mare.

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During each collection, the number of mount in the AV and the collection duration were determined as described by Clément *et al.* [3] and Haras Nationaux [4].

After collection, the ejaculate was immediately filtered using a suitable filter as gauzes with hidrophilics to remove the gel fraction and sperm volume was determined. Sperm concentration, percentage of mobile spermatozoa, percentage of dead spermatozoa and percentage of abnormal spermatozoa were determined as described by Haras Nationaux [4].

Microbial Quality of Semen: A sample of 1 ml of each fresh ejaculate was diluted with buffered peptone sterile water with the rate of 10^{-1}. Then, 100µl of this suspension were inoculated on Petri dish of blood agar, blood agar colistin nalidixic acid, cetrimide agar, XLD agar and Chapman agar. Petri dish were incubated for 48 hours at 37°C in aerobic conditions for cetrimide, XLD and Chapman agar and in anaerobics conditions (7% Co_2) for blood agar and blood agar colistin nalidixc. Then, colonies were counted on Petri dishes and the number founded was multiplied by 1000 to indicate the number of bacteria per ml (CFU/ml) [5]. To identify the bacteria, colony was removed using the platinum loop and suspended in 0.5 ml of brain heart infusion after performing a Gram stain. Seeding is done on a proper gallery: Hajna-Kliger, Mannitol-mobility and urea-indole medium for Gram-negative, DNA agar and rabbit plasma for staphylococci. Final identification of the bacteria was performed by automated biochemical and enzymatic essays (mini API system, BioMérieux, France): API 20E for Enterobacteriaceae, API 20NE for Gram-negative non Enterobacteriaceae and API STREP for streptococci.

Statistical Analysis: Data were analysed using a software SAS (version 1997). Bacterial concentration of fresh semen was transformed into decimal logarithm and analysed. A general linear model was used to study the influence of sexual behaviour and semen parameters on the bacterial concentration of the fresh semen. Correlations between sexual behaviour parameters, semen parameters and bacterial concentration were established. The threshold of significance was fixed at 5 %.

RESULTS

Bacterial flora concentration in fresh semen varied from $1.9\,10^5$ to $2.1\,10^6$ CFU/ml according to stallions ($p < 0.05$) (Figure 1). Results showed a significant effect of ejaculates in the same stallion on bacterial flora concentration ($p < 0.05$).

Two pathogenic bacteria among the four searched were founded in the fresh semen (Table 1). *Pseudomonas aeruginosa* was founded in 4 stallions. However, *Staphylococcus aureus* was founded just in 2 stallions. The percentage of contaminated ejaculates with *Pseudomonas aeruginosa* varied from 20 to 75% according stallions. The percentage of contaminated ejaculates with *Staphylococcus aureus* varied from 20 to 25%.

The percentage of contaminated ejaculates with pathogenic bacteria varied from 28% for those obtained from the 1st mount in the AV to 67% for those obtained from the 3rd mount in the AV (Figure 2).

Bacterial concentration seemed to vary according to the duration of collection and the presence or absence of the gel fraction (Table 2), however the statistical analysis

Table 1: Percentage of contaminated ejaculates with pathogenic bacteria

	Percentage of contaminated ejaculates			
Stallions	*Streptococcus zooepidemicus*	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
A (n= 4)	0 %	0 %	0 %	25 %
B (n= 4)	0 %	0 %	0 %	0 %
C (n= 4)	0 %	25 %	0 %	50 %
D (n= 4)	0 %	0 %	0 %	75 %
E (n= 5)	0 %	20 %	0 %	20 %

Table 2: Variation of bacterial concentration with duration collection and presence of gel fraction in the ejaculate

	Duration collection < 100 sec	Duration collection ≥ 100 sec	Ejaculates with gel fraction	Ejaculates without gel fraction
Means (cfu/ml)	$1.2\,10^6$	$7\,10^5$	$1.5\,10^6$	$7.7\,10^5$
Sem	$4.7\,10^5$	$3\,10^5$	$6.7\,10^5$	$2.9\,10^5$
r^2	-0.15		0.09	

Table 3: Variation of bacterial concentration with semen parameters

	Sperm concentration (C)		Percentage of mobile spermatozoa		Percentage of dead spermatozoa		Percentage of abnormal spermatozoa	
	$C < 200\ 10^6$ spermatozoa/ml	$C \geq 200\ 10^6$ spermatozoa/ml	< 70%	≥ 70%	≤ 40%	> 40%	≤ 40%	> 40%
Means (cfu/ml)	$1.3\ 10^6$	$7.9\ 10^5$	$1.3\ 10^6$	$9.3\ 10^5$	$1.2\ 10^6$	$1.3\ 10^6$	$9.1\ 10^5$	$1.1\ 10^6$
Sem	$6\ 10^5$	$3.2\ 10^5$	$6.8\ 10^5$	$3.4\ 10^5$	$4.4\ 10^5$	$7.1\ 10^5$	$2.9\ 10^5$	$5.1\ 10^5$
r^2	-0,27		-0,31		0,12		0,15	

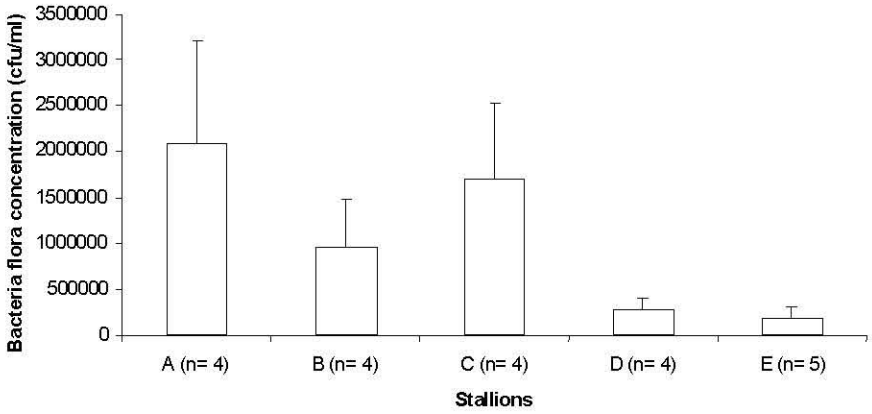

Fig. 1: Bacterial flora concentration in the fresh semen of Arab stallions (means ± sem)

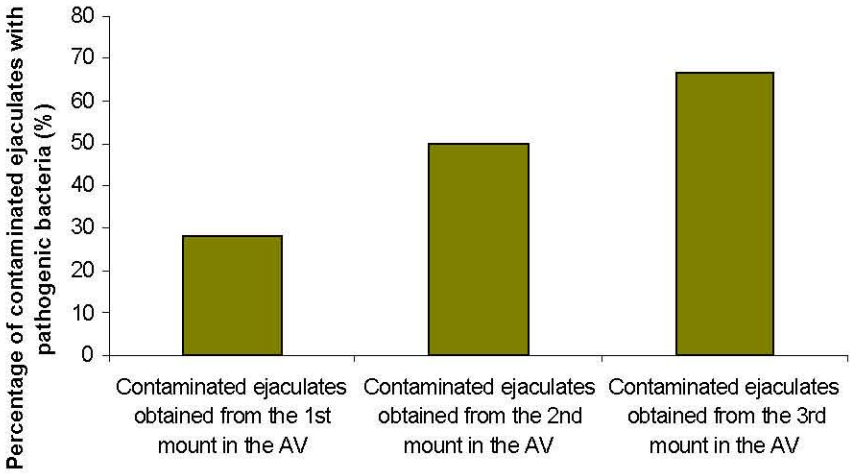

Fig. 2: Percentage of contaminated ejaculates with pathogenic bacteria according to the number of mounts in the AV

didn't show any significant effect. Lower correlation of bacterial concentration with duration collection and presence of gel fraction in the ejaculate were found.

Statistical analysis showed also no significant effects of semen parameters on the bacterial concentration flora (Table 3). Lower correlations was also found between each semen parameter and bacterial concentration and varied from $r^2 = -0.31$ between sperm concentration and bacterial concentration to $r^2 = 0.15$ between percentage of abnormal spermatozoa and bacterial concentration (Table 3).

DISCUSSION

The variability of the bacterial flora concentration founded among stallions and among ejaculates of the same stallion is in agreement with Clément *et al.* [5]. These authors [5] also indicated that the variation between ejaculates of the same stallion still low when the ejaculates where collected at 24 hours intervals. In our study, ejaculates of the stallion were collected spaced by an interval time of 48 hours. This variability could be attributed to the collection

conditions and to the hygiene state of the stallion penis or to the handling conditions of the semen in laboratory.

Our results showed an increase of the percentage of contaminated ejaculates when the number of mount in the AV exceed one mount. This is in agreement with [5]. Moreover, we found that *Pseudomonas aeruginosa* had a high occurrence frequency in ejaculates. Madsen and Christensen [6] reported that the frequency of contamination with pathogenic bacteria increased when collection was performed with an open AV, which is the case in our study.

The study showed also no significant correlation between semen parameters and bacterial flora concentration, especially for the percentage of abnormal sperm. Malmgren *et al.* [7] in their study didn't show any correlation between the frequencies of *Pseudomonas aeruginosa* occurrence and sperm morphology features. They also found that stallions having these pathogenic bacteria had acceptable fertility per cycle. In addition, Katila *et al.* [8] found no correlation between bacterial flora concentration and the rate of foaling. However, Rideout *et al.* [1] cited that bacterial secretion influence the sperm motility. In our case, we can say that bacterial flora concentration is independent from semen parameters studied.

In conclusion, our study showed that *Pseudomonas aeruginosa* is the most bacteria that have a high occurrence in ejaculates. Besides, there are any relationships between sexual behaviour and semen parameters with bacterial flora concentration of fresh semen of Arab stallions. Instead of these results we can conclude that quality of fresh semen was not affected by his bacterial flora concentration.

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