The Distribution of Mycoplasmas and Ureaplasmas in the Genital Tract of Normal Artificial Insemination Bulls

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ABSTRACT

Bull semen is commonly contaminated with mycoplasmas. To determine the source of contamination, semen and the genital tracts of 45 artificial insemination bulls were cultured for these organisms. The results indicate that mycoplasmas colonize the prepuce and the distal part of the urethra. Only rarely were they found in the ampullae or seminal vesicles. In 92% of the bulls with contaminated semen the same *Mycoplasma* species or *Ureaplasma diversum* was isolated from the prepuce and urethral orifice as was found in the semen. This suggests that the prepuce and distal urethra is the source of contamination. Colonization of the genital tracts with *Mycoplasmas* or *U. diversum* was not associated with histological changes.

Key words: Mycoplasma, ureaplasma, genital tract, semen, artificial insemination, microbial colonization.

RÉSUMÉ

La distribution des mycoplasmes et des uréaplasmes dans les voies génitales de taureaux sains de centres d'insémination artificielle

Le sperme de taureau est souvent contaminé par des mycoplasmes. Afin de déterminer la source de cette contamination, les auteurs ont procédé à la recherche de ces microorganismes, dans le sperme et les voies génitales de 45 taureaux de centres d'insémination artificielle. Les résultats de leurs cultures révélérent que les mycoplasmes se développent dans le prépuce et la partie distale de l'urètre, mais rarement dans les ampoules ou les vésicules séminales. Chez 92% des taureaux dont le sperme était contaminé, les mêmes espèces de mycoplasmes et *Ureaplasma diversum* se retrouvaient dans le prépuce, l'orifice urétral et le sperme. Cette constatation amena les auteurs à penser que le prépuce et la partie distale de l'urètre représentent la source de contamination du sperme. La présence des microorganismes précités dans les voies génitales ne s'accompagnait pas de lésions histologiques.

Mots clés: *Mycoplasma* spp., *Ureaplasma diversum,* voies génitales, sperme, insémination artificielle, développement microbien.

INTRODUCTION

Organisms of the genera *Mycoplasma, Acholeplasma* and *Ureaplasma* have been isolated from the distal part of both the male and female bovine genital tract (1,2,3,4). *Mycoplasma bovis, M. bovigenitalium* and *U. diversum* appear to be the most important pathogens of the genital tract, involved in such diseases as lowered sperm motility (*M. bovigenitalium* (5)), seminal vesiculitis, epididymitis (*M. bovis* and *M. bovigenitalium* (6,7)) endometritis (*M. bovis* (8)) granular vulvovaginitis, infertility and abortion (*U. diversum* (9)). Semen may be an important vehicle for transmission because it is often contaminated with mycoplasmas (10,11,12). It has been suggested that the preputial flora is the source of the contaminating mycoplasmas (13); others have found bulls shedding mycoplasmas from testis, epididymis, ductus deferens, prostate and bulbourethral glands (10). We undertook the present study to establish which sites of the male genital tract were colonized with mycoplasmas and therefore might be a source for semen contamination. With this knowledge it may be possible to control contamination by sanitary measures before and during semen collection.

MATERIALS AND METHODS

A group of 45 bulls from artificial insemination centres was studied. Semen samples were collected from all bulls at the artificial insemination unit by means of an artificial vagina. The semen containers were sealed and placed in a styrofoam container with commercial freezer packs to maintain refrigeration. The samples were brought to the laboratory within two hours of collection. Once the fresh semen samples reached the laboratory they were cultured immediately or placed in a -70°C freezer until culture. Processed semen stored in liquid nitrogen was obtained also for culture.

The bulls were shipped for slaughter within 48 hours after semen collection. One of us (NAF) attended at the kill floor and obtained the entire intact genital tract from the slaughtered animal during the evisceration process. The genital tracts of the bulls were taken directly to the laboratory within one hour for culture and collection of histopathological specimens. No more than two bulls were slaughtered at one time period. This afforded an opportunity to collect the genital tracts under aseptic conditions and to permit early culture of the tracts.

Mycoplasma and Ureaplasma Isolation

The following media were used:

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1) Hayflick's medium with 20% horse serum (14), 2) Hayflick's medium with 20% swine serum and 3) *Ureaplasma* medium prepared according to Waelchli-Suter *et al* (15). All solid media contained 1% Noble Agar No. 1.

Fresh and processed semen were streaked onto plates of each medium and 0.1 mL was added to 2 mL of broth of each of the three media. Processed semen was also cultured after being washed twice in PPLO broth (Difco, Detroit, Michigan). The washing procedure involved centrifugation at 500 *g* for 5 min followed by resuspension of the sediment in a volume of PPLO broth corresponding to the original volume of semen. The washing was undertaken in order to remove the antibiotics of processed semen.

In order to culture the genital tracts, cuts were made under aseptic conditions into the testis, epididymis, ampullae and seminal vesicles. The urethra at the flexure and 2 cm from the orifice was accessed through cross sections of the penis. Cotton tipped wooden applicator sticks (swabs) were rubbed onto the mucosal surfaces or the cut surfaces before being streaked onto the agar plates and subsequently placed in broth. Swab samples from testes were combined, as were samples from both epididymides, both ampullae and both seminal vesicles. The prepuce was cultured by rubbing a swab on the preputial mucosa as well as the surface of the penis. Tissue adjacent to the culture sites was placed in 10% buffered formaldehyde and processed for histopathological evaluation after haematoxylin-eosin staining.

Blind subcultures onto homologous plates were made from the broth tubes on days 3 and 6 after seeding. All plates except *Ureaplasma* plates were incubated in atmosphere with 7% CO_2 and 90% relative humidity. All broth cultures were capped and incubated under normal conditions. The *Ureaplasma* plates were incubated anaerobically using the Gas Pak system (BBL, Canlab, Toronto).

Ureaplasma plates were examined for growth after two days, all other plates were examined after four days and reincubated for another four days if negative. *Ureaplasmas* were recognized on the basis of their colony morphology, urease activity and growth requirement. Mycoplasma colonies were cloned and identified by biochemical and serological testing using growth inhibition and fluorescent antibody techniques.

TABLE I
RECOVERY OF MYCOPLASMAS AND UREAPLASMAS FROM THE SEMEN AND GENITAL TRACTS OF NORMAL BREEDING BULLS

Bull	Fresh Semen	Unwashed Processed Semen	Washed Processed Semen	Prepuce	Urethral Orifice	Urethra	Seminal Vesicles	Ampullae	Epididymides	Testicles
1	—	—	—	U	—	—	—	NS	—	—
2	—	—	B	U	—	U	—	NS	—	—
3	BU	—	—	BU	U	—	—	NS	—	—
4	U	—	—	BU	BU	U	—	NS	—	—
5	BU	BU	BU	A	BU	—	—	NS	—	—
6	U	NS	NS	BU	—	BU	—	NS	—	—
7	—	—	—	B	BU	—	—	NS	B	—
8	BU	—	—	BU	BU	—	—	—	—	—
9	B	—	—	AU	—	—	—	—	—	—
10	—	—	—	BU	BU	—	—	—	—	—
11	U	—	—	BU	U	U	—	—	—	—
12	U	—	—	AU	AU	—	—	—	—	—
13	U	—	—	U	U	—	—	—	—	—
14	U	—	—	BU	BU	—	—	—	—	—
15	—	—	—	BU	BU	—	—	—	—	—
16	U	—	—	U	U	—	—	—	—	—
17	BU	U	U	BU	BU	—	B	—	—	—
18	—	—	—	BU	—	—	B	—	—	—
19	B	—	—	BU	BU	B	—	B	—	—
20	U	—	—	U	B	—	—	—	—	—
21	BU	—	B	B	BU	U	—	—	—	—
22	U	—	—	U	U	—	—	—	—	—
23	BU	—	—	BU	BU	—	—	B	—	—
24	—	—	—	BU	BU	B	—	B	—	—
25	U	—	—	BU	BU	—	—	—	—	—
26	U	—	—	BU	U	—	—	—	—	—
27	—	—	—	B	—	—	—	—	—	—
28	AU	—	A	AU	—	—	—	—	—	—
29	—	—	M	—	A	A	—	—	—	—
30	—	—	—	—	—	—	—	—	—	—
31	BU	—	—	BU	B	—	—	—	—	—
32	—	—	—	BU	BU	B	B	—	—	—
33	—	—	—	U	U	—	B	—	—	—
34	—	—	B	A	—	—	A	—	—	—
35	—	U	—	B	—	—	—	—	—	—
36	—	—	—	BU	BU	—	—	—	—	—
37	—	—	—	B	B	—	—	—	—	—
38	M	—	—	B	B	—	—	—	—	—
39	—	—	—	—	U	—	—	—	—	—
40	—	—	—	U	U	—	—	—	—	—
41	—	—	—	BU	BU	—	B	—	—	—
42	BU	—	—	BU	BU	—	—	—	—	—
43	—	—	—	—	—	—	—	—	—	—
44	BU	—	M	BU	—	—	—	—	—	—
45	—	—	B	B	B	—	—	—	—	B

Note:
A: *Mycoplasma arginini* isolated
B: *Mycoplasma bovigenitalium* isolated
M: Unidentified organism of genus *Mycoplasma* isolated
U: *Ureaplasma* isolated
—: Negative for mycoplasmas or ureaplasmas
NS: Not sampled

RESULTS AND DISCUSSION

The results of the recovery of *Mycoplasmas* and *Ureaplasmas* from semen and the genital tract of mature breeding bulls are shown in Table I. It

appears that these organisms colonize the prepuce and the distal part of the urethra. Only rarely were they isolated from the ampullae and seminal vesicles.

The epididymis was positive in one case and the testicles were positive in another. The most commonly isolated species of mycoplasmas were *M. bovigenitalium* and *U. diversum.* No *Acholeplasmas* were found, which is in contrast to other reports (2,3). Perhaps we would have identified a wider variety of species if we had tested the growth plates directly with the fluorescent antibody technique before purifying the cultures (16).

There was no correlation between histological appearance of the tissue and the cultural results. The mucosa of the prepuce and surface of the penis had occasional areas of focal epithelial and subepithelial lymphoplasmacytic and macrophage infiltration. This was interpreted as a normal host response to a rich mucosal flora of microorganisms. A few of the bulls had mild plasma cell and macrophage infiltration in the interstitium of the seminal vesicles, but none of these animals was positive for mycoplasmas. No changes were found in the epididymis or testis of the two bulls with mycoplasmas in these tissues. Twenty-four of the 45 bulls had mycoplasmas in the fresh semen samples. In 22 of these (92%) the same mycoplasma species or *U. diversum* was subsequently isolated from either the prepuce, the urethral orifice or both of these sites. This suggests that the prepuce and the distal urethra is the souce of the contamination. This conclusion agrees with the opinion of Taylor-Robinson *et al* (13). Only in one of the five bulls with mycoplasmas in the seminal vesicles was the fresh semen positive for mycoplasmas. This suggests that colonization of this gland under normal conditions is not an important source of contamination. Only three of 44 samples of processed semen were positive before washing, whereas nine were positive after inhibitors had been eliminated ($\chi^2 = 3.4$; $p < 0.10$). Although the mycoplasmas or ureaplasmas colonizing the genital tracts of artificial insemination bulls do not appear to cause inflammation in the bulls their potential pathogenicity in the female genital tract can not be excluded. It would appear that in an attempt to control mycoplasma contamination of semen during ejaculation both the prepuce and the distal urethra would have to be cleansed and disinfected before collection of semen in the artificial vagina. Future studies may determine if this is practical.

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