

# Sexually communicable micro-organisms in human semen samples to be used for artificial insemination by donor

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**SUMMARY** Two hundred and thirty seven semen samples from 10 institutes for artificial insemination by donor (AID) in Belgium and the Netherlands were tested for the presence of *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, herpes simplex virus, and cytomegalovirus. The incidence of these micro-organisms in the semen samples was 0%, 6.3%, 4.6%, 35.9%, 0%, and 0.4% respectively, and 47% of all samples were infected with one or more of the micro-organisms. As the ejaculates from which the samples had been taken had already been, or would be, used for AID, the exclusion of microbiological contamination with sexually communicable micro-organisms before insemination is indicated.

## Introduction

Artificial insemination by donor (AID) is used thousands of times a year in the Netherlands and Belgium in cases of male infertility. In general, microbiological examination of the semen samples before insemination is incomplete, especially as fresh semen is often used. In view of the epidemic incidence of sexually communicable diseases it would seem to be important that semen samples intended for AID should be subjected to such tests as are necessary to prevent iatrogenic infections in women. For this reason a study was set up in Rotterdam, the Netherlands, to investigate the incidence of *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, herpes simplex virus (HSV), and cytomegalovirus (CMV) in frozen semen samples that had been or would be used for AID.

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## Materials and methods

### SEMEN SAMPLES

Two hundred and thirty seven semen samples frozen at  $-196^{\circ}\text{C}$ , from ten AID institutes in the Netherlands and Belgium were supplied in 0.25 ml or 0.5 ml straws. The semen had been given voluntarily by donors, who received a small sum for expenses. The samples were all diluted 2:1 or 1:1 with a cryoprotective liquid containing glycerol and sometimes also egg yolk or bovine serum albumin. Fifty samples contained ampicillin 380 mg/l and 36 contained kanamycin 5 mg/l. Forty samples came from an institution that used only fresh semen. The semen was diluted 1:1 with Eagle's modified minimum essential medium (EMEM) with 15% fetal calf serum, and was frozen to  $-196^{\circ}\text{C}$ . Volumes of 120-200  $\mu\text{l}$  were diluted with 2 ml of EMEM for culture for *N gonorrhoeae*, *C trachomatis*, HSV, and CMV. Volumes of 60-200  $\mu\text{l}$  were suspended in 2 ml of U-9 medium<sup>1</sup> for culture for *M hominis* and *U urealyticum*.

### CULTURE METHODS

#### Culture for *N gonorrhoeae*

Semen diluted with EMEM was inoculated with a loop on to a medium consisting of GC medium base (Difco), haemoglobin (Difco), and IsoVitalax (BBL), and was incubated for 48 hours.

### Culture for *C trachomatis*

A 200  $\mu$ l volume of semen diluted with EMEM was again diluted 1:1 with EMEM. A 200  $\mu$ l volume of each dilution was inoculated in duplicate wells on a HeLa 229 monolayer that had been rinsed with 30 mg/l diethylaminoethanol (DEAE) dextran and grown in a 96 well microtitre plate (Falcon 3070). After being centrifuged for one hour at 3000 x *g* the inoculum was replaced by 100  $\mu$ l EMEM with 4.5 mmol/l glucose, 5% fetal calf serum, 15 mg/l nystatin, 25 mg/l gentamicin, and 25 mg/l vancomycin. After being incubated for 48 hours at 37°C the monolayers were screened for the presence of inclusions after being stained with fluorescent monoclonal antibodies (Syva).

### Culture for HSV and CMV

A 200  $\mu$ l volume of the sample diluted with EMEM was put in a Leighton tube in which an HEL cell monolayer had been grown. Inoculation was carried out in duplicate. After being incubated for one hour at 37°C the inoculum was replaced by 1.5 ml of Dulbecco's MEM with 2% fetal calf serum. The medium was renewed every week. After three weeks the cells were transferred to new tubes. The cell culture was maintained for six weeks. If there was a cytopathogenic effect the monolayer was stained with virus specific fluorescent monoclonal antibodies (MA Bioproducts).

### Culture for *M hominis* and *U urealyticum*

The medium of Chanock *et al*<sup>1</sup> was used for culture for mycoplasmas. Yeast extract was prepared as described by Hers and Masurel,<sup>2</sup> and inactivated horse serum was applied. U-9 and A-7 media<sup>3,4</sup> were used for culture for ureaplasmas. Ureaplasmas were detected by the phenol red colouring in the U-9 medium and the typical colony form shown by microscopic examination of the A-7 plate. Mycoplasmas were recognised by the colony form on Chanock's medium, and further identified by the indirect immunofluorescence test of unfixed colonies.<sup>5</sup>

## Results

Neither gonococci nor HSV were cultivated from any of the 237 samples. The prevalence of *C trachomatis*, *M hominis*, *U urealyticum*, and CMV was 6.3%, 4.6%, 35.9%, and 0.4% respectively (table). Of the 237 samples, 112 (47.3%) were infected with one or more of the micro-organisms studied, 15 (6.3%) being infected with two or three micro-organisms. The microbiological screening of the semen samples carried out by the institutes and the upper limit of leukocytes accepted varied widely. Six institutes did not perform any microbiological test on their samples. None of the institutes cultured for HSV or CMV.

TABLE Culture results in 237 semen samples

Micro-organism	No (%) positive
<i>Chlamydia trachomatis</i>	15 (6.3)
<i>Mycoplasma hominis</i>	11 (4.6)
<i>Ureaplasma urealyticum</i>	85 (35.9)
Cytomegalovirus	1 (0.4)
None	142 (59.9)

All cultures for *Neisseria gonorrhoeae* and herpes simplex virus were negative.

Though two institutes examined semen for ureaplasmas and mycoplasmas, some samples were nevertheless found to be infected with these micro-organisms. Two of 50 samples containing ampicillin yielded chlamydiae. Eleven out of 36 specimens containing kanamycin yielded mycoplasmas or ureaplasmas. Two of the 10 institutes examined donors' urine for sediment.

## Discussion

Micro-organisms were found to be present in 47% of the 237 semen samples intended for AID. This percentage is probably an underestimate. The quantity of semen (120-200  $\mu$ l) that could be extracted from the straws varied fairly widely. Moreover, the samples had to be diluted to avoid a cytotoxic effect on the cell lines used. On the other hand, however, a decrease in the concentration of semen components that inhibit growth may enhance the recovery of chlamydiae.<sup>6</sup> The presence of kanamycin and ampicillin in 86 specimens tested would have inhibited the growth of gonococci, chlamydiae, mycoplasmas, and ureaplasmas.

The examinations carried out by the institutes are not sufficient to rule out the presence of these micro-organisms in the semen they use. The only way for the institutions to ensure that the samples they obtain are free of pathogens is for them to carry out the necessary examinations of donors and semen samples. The presence or absence of micro-organisms does not seem to be related to other properties of semen, such as motility, abnormal morphology, and leucospermia.<sup>7,8</sup> Neither do physical and simple laboratory examinations of donors provide conclusive evidence, as infection with sexually transmissible organisms is often symptomless.

In view of the large yearly number of inseminations carried out, it is notable that few reports of genital infections in the recipients of AID have been published. Gonococcal and ureaplasma infections transmitted through AID have, however, been described.<sup>9,10</sup> Moreover, gonococci, chlamydiae, and other bacteria, including  $\beta$ -haemolytic streptococci, have been reported to survive freezing (Jordan GW *et al*, unpublished observation).<sup>11-13</sup> The incidence of the

other bacteria was not investigated in this study, nor was the incidence of hepatitis B or human immunodeficiency virus (HIV). The occurrence of infections with HIV through AID has, been described, however,<sup>14</sup> and serological examination of donors for both of these viruses is therefore indicated.

It is not known whether the contaminated semen samples have caused infections in the recipients, nor is it clear whether the presence of the micro-organisms is of pathogenic importance. That would be assessed by investigating the number and serotype of the micro-organism in question and the condition of the recipients' defence mechanisms. The importance of *C trachomatis* as a pathogenic agent in urethritis, cervicitis, salpingitis, neonatal conjunctivitis, and pneumonitis, however, has been well documented.<sup>15</sup> *M hominis* is associated with salpingitis, pyelonephritis, and postpartum fever, and *U urealyticum* can be associated with chorioamnionitis and possibly low birth weight.<sup>16</sup> One of the causes of the "TORCHES" syndrome in neonates is congenital infection with CMV.

The results of this screening therefore point to the need to examine both the donor and the semen before insemination to avoid contamination with pathogenic micro-organisms. This implies that only frozen semen samples should be used for AID purposes.

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