Bacteriologic Study of Donor Semen

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Abstract : *Semen samples from 63 donors were bacteriologically studied to determine the bacterial isolates, their influence on sperm quality and the recipients. Two of the 63 samples had no bacterial growth, 61 cultures yielded one or more aerobic organisms, and 42 yielded anaerobic organisms. The 4 common aerobes were S. epidermidis (50.8%), Streptococcus group D enterococci (30.2%), S. viridans (25.4%), and Diphtheroides (25.4%). These organisms had no influence on semen parameters. The anaerobic organisms, which included Peptostreptococcus (60.3%), nonsporing gram-positive rods (17.5%), Bacteroides (14.3%), were related to sperm concentration and motility. All recipients had no complications after donor insemination. Four of the 21 recipients became pregnant, and the semen inseminated to these women contained from 1 to 6 types of organisms. The routine aerobic and anaerobic bacterial culture of selected donor semen seem not be necessary. (Thai J Obstet Gynaecol 1991;3:89-94.)*

Key words: bacteriologic study, donor semen

Donor insemination is widely practiced for treatment of male infertility. It has been shown previously that semen used for artificial insemination may be contaminated with a variety of microorganisms, some of which are potentially pathogens[1,2]. Despite this phenomenon the symptomatic rate of pelvic infection following insemination was very low[3]. Furthermore, semen quality seem not related to aerobic or anaerobic bacterial cultures of semen[4].

The purpose of the present study was to determine the bacterial isolates in donor semens and the influence of such isolates on sperm quality.

Materials and Methods

Sixty-three semen samples were obtained from healthy adult males of 21 to 36 years of age and were randomly selected from those attending the artificial insemination donor program of Infertility Unit, Department of Obstetrics and Gynaecology, Siriraj Hospital, from March 1988 to June 1989. Before collecting the specimens, all donors were instructed to wash their hands and the glans penis with soap and water thoroughly, or to clean the glans penis with a sterile cloth. The semen was collected by masturbation - ejaculation

into a sterile wide-mouthed glass jar and was divided into three portions, 0.5-0.8 ml for bacterial culture, 0.5 ml for analysis and the rest for insemination. Culture for aerobic and anaerobic organisms was carried out, within 1 hour after collection of specimen, by conventional method. Semen analysis was performed by the standard technique described by WHO[5].

The ovulatory day of the cycle was determined by BBT and cervical mucus. The donor semen, 0.3 ml, was inseminated into the cervical canal while the rest was deposited in the posterior fornix.

Statistical analysis of the results was performed employing the Student's unpaired t-test or ANOVA as appropriate.

Results

Twenty one women with normal infertile work-up, whose husbands were azoospermic, were included in the study. Their ages ranged from 21 to 36 with the infertility period between $1\frac{1}{2}$ to 10 years. Of the 63 cultures, no organism was found in only 2 (3.2%). Among the remaining 61 samples, 1 to 4 organisms were isolated. Only one specimen harboured 7 different organisms (Fig. A).

The aerobic organisms isolated and the sperm characteristics of the ejaculates are shown in Table 1. To determine whether semen quality was affected by certain aerobic organisms, it was found that there was no association between a rich culture of aerobic organisms and sperm concentration and motility ($p > 0.05$).

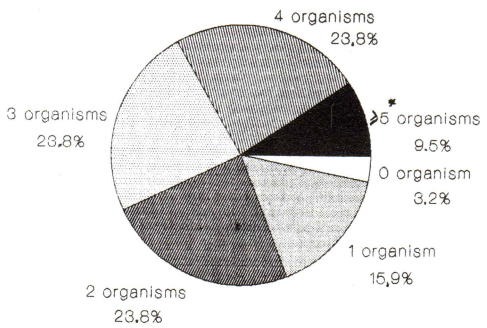

Fig. A Number of organisms isolated from 63 donor semens.
* maximum = 7.

Table 2 shows the anaerobic organisms isolated and their correlation to sperm concentration and motility. When comparing 21 ejaculates without anaerobic organsims with 42 ejaculates with anaerobic organisms, it was found that the sperm concentration and motility was significantly better among those with than those without growth of organisms ($p<0.05$).

Four women (19.0%) became pregnant. The bacterial growths in the semens that inseminated these women are shown in Table 3. All semens harboured 1 to 6 varieties of organisms.

None of the women in this study had either fever, abnormal vaginal discharge, or lower abdominal pain after insemination.

Discussion

Despite several species of organism, as many as 7, were identified from the same ejaculates. These organisms could account for the con-

Table 1 Aerobic bacteria and semen characteristics

Aerobic bacteria	No. of samples	Sperm count (x 10^6 / ml) ($\bar{x} \pm SD$)	Sperm motility (%) ($\bar{x} \pm SD$)
S. epidermidis	32	71.4 ± 56.3	74.6 ± 13.4
S. group D enterococci	19	67.6 ± 56.0	76.2 ± 13.6
S. viridans	16	77.6 ± 65.6	68.4 ± 17.6
Diphtheroides	16	65.4 ± 35.9	71.1 ± 12.1
Nonfermentative gram - negative rods	7	73.8 ± 66.0	79.8 ± 9.5
B. subtitis	5	55.0 ± 44.3	78.0 ± 8.8
Hemolytic streptococcus non group A,B,D.	4	47.0 ± 30.9	73.5 ± 11.1
P. mirabilis	4	64.7 ± 35.6	76.0 ± 4.1
H. parainfluenza	2	50.5 ± 0.7	66.5 ± 33.2
K. pneumoniae	2	41.0 ± 1.4	73.5 ± 13.4
P. morgagni	1	63	65
E. coli	1	48	72
S. aureus	1	52	80
S. saprophyticus	1	65	89
A. hydrophila	1	44	88
Corynebacterium species	1	63	85
Micrococcus species	1	169	84
No growth	3	56.3 ± 44.7	65.0 ± 11.1

Table 2 Anaerobic bacteria and semen characteristics

Anaerobic bacteria	No. of samples	Sperm count (x 10^6 / ml) ($\bar{x} \pm SD$)	Sperm motility (%) ($\bar{x} \pm SD$)
Peptostreptococcus	38	63.4 ± 39.0	71.4 ± 14.4
Nonsporing gram - positive rods	11	63.1 ± 54.3	68.8 ± 11.6
Bacteroides	9	56.8 ± 20.8	69.4 ± 18.7
Fusobacterium	2	26.5 ± 19.1	70.5 ± 13.4
V. parvula	1	111	75
No growth	21	91.9 ± 64.3	80.2 ± 9.7

Table 3 Bacterial growths in semen inseminated to 4 pregnant recipients

Pregnancy	Aerobic bacteria	Anaerobic bacteria
1	S. epidermidis S. viridans Diphtheroides	Peptostreptococcus Bacteroides Non-sporing gram positive rods
2	S. epidermidis S. viridans S. group D enterococci	Peptostreptococcus
3	S. epidermidis	
4	S. epidermidis S. viridans Non-fermentative gram - negative rods	Peptostreptococcus

tamination of bacterial flora of the skin or distal urethra. However, these findings are similar to those previously reported[1,2,4,6-9].

With regard to the relationship between the presence of organisms in the semen and the sperm quality, it seems that aerobic bacteria does not play an important role in reducing sperm concentration and sperm motility, but it seems to be reduced among ejaculates who harbour anaerobic bacteria. These figures are slightly different from other studies which reported no association between sperm characteristics and the presence of aerobes or anaerobes isolated from the semen[4,10,11]. Several authors reported that U. urealyticum was found in significant numbers among the semen of poor quality[4,12,13]. On the other hand, there is no relationship between abnormal semen parameters and the presence of U. urealyticum as reported by others[14,15]. This organism was not of interest in this series.

Despite potentially pathogenic organisms, such as hemolytic streptococci, Klebsiella, E.coli and S. aureus were identified in this study but none of the patients developed clinical infection after insemination. It is believed that cervical mucus may play a role of the effective mechanical and immunologic barrier thereby preventing the ascending infection to the upper female genital tract. However, it has been demonstrated *in vitro* that bacteria can be carried by sperm through a cervical mucus column[16]. Stone et al[17] reported that after artificial insemination, microorganisms were discovered from the peritoneal fluid.

The influence of the presence of bacteria in the semen on the fertilizing capacity of sperm is not known. Since all pregnancies from artificial

insemination of the semen harboured 1 to 6 different organisms, further study should be carried out to disclose this event.

As a result of this study, although a conclusion cannot be made, it is felt that routine culture of aerobic and anaerobic bacteria of selected donor semen is not necessary. However, one should practice restricted precautions in order to prevent a transfer of a sexually transmitted disease or pathogenic bacteria by means of ejaculate used for donor insemination.

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References

1. Garcia A, Sierra MF, Friberg J. Survival of bacteria after freezing of human semen in liquid nitrogen. Fertil Steril 1981;35: 549-51.
2. Leiva JL, Peterson EM, Wetkowski ME, de la Maza LM, Stone S. Microorganisms in semen used for artificial insemination. Obstet Gynecol 1985;65:669-72.
3. Stone SC. Complications and pitfalls of artificial insemination. Clin Obstet Gynecol 1980;23:667-82.
4. Naessens A, Foulon W, Debrucker P, Devroey P, Lauwers S. Recovery of microorganisms in semen and relationship to semen evaluation. Fertil Steril 1986;45: 101-5.
5. WHO laboratory manual for the examination of human semen and semen cervical mucus interaction. Cambridge : Cambridge University Press, 1987:3-13, 27.
6. Megory E, Zuckerman H, Shoham Z, Lunenfeld B. Infections and male fertility. Obstet Gynecol Surv 1987;42:283-90.
7. Busolo F, Zanchetta R, Lanzone E, Cusinato R. Microbial flora in semen of asymptomatic infertile men. Andrologia 1984;16:269-75.
8. Moberg PJ, Eneroth P, Ljvng A, Nord CE. Bacterial flora in semen before and after doxycycline treatment of infertile couples. Int J Androl 1980;3:46-58.
9. Bowie WR, Pollack HM, Forsyth PS, et al. Bacteriology of the urethra in normal men and men with non-gonococcal urethritis. J Clin Microbiol 1977;6:482-8.
10. Makler A, Urbach Y, Lefler E, Merzbach D. Factors affecting sperm motility. VI sperm viability under the influence of bacterial growth in human ejaculates. Fertil Steril 1981;35:666-70.
11. Comhaire F, Verschraegen G, Vermeulen L. Diagnosis of accessory gland infection and its possible role in male infertility. Int J Androl 1980;3:32-8.
12. Fowlkes DM, Mac Leod J, O'Leary WM. T-mycoplasmas and human infertility: Correlation of infection with alterations in seminal parameters. Fertil Steril 1975;26: 1212-8.
13. Upadhyaya M, Hibbard BM, Walker SM. The effect of Ureaplasma urealyticum on semen characteristics. Fertil Steril 1984; 41:304-8.
14. Lewis RW, Harrison RM, Domingue GJ. Culture of seminal fluid in a fertility clinic. Fertil Steril 1981;35:194-8.
15. Cintron RD, Wortham JWE, Acosta A. The association of semen factors with the recovery of Ureaplasma urealyticum. Fertil Steril 1981;36:648-52.
16. Toth A, O'Leary WM, Ledger W. Evidence for microbial transfer by spermatozoa. Obstet Gynecol. 1982;59:556-9.
17. Stone SC, de la Maza LM, Peterson EM.

Recovery of microorganisms from the pelvic cavity after intracervical or intrauterine artificial insemination. Fertil Steril 1986;46:61-5.