Y-DELETIONS IN MEN WITH SEVERE OLIGOGENulia.
Eric J. Meuleman, Jan A. Kremer, Joep H. Tuerlings, Nijmegen, The Netherlands. (Presented by Dr. Meuleman)

The introduction of ICSI offered a successful treatment option for subfertile male with severe oligospermia, although the etiology of the disorder remains unclear in most cases. Recently, microdeletions in the AZF region of the Y chromosome have been detected in men with azospermia or severe oligospermia.

In this study we investigated the prevalence of microdeletions in the AZF region of the Y chromosome in our ICSI population (by PCR analysis) and looked for clinical differences between the men and without the disorder. Blood was drawn from 154 men, who were waiting for ICSI treatment: 24 azoospermic men, 98 oligospermic and 32 normospermic men. After previous fertilization failure, Chromosome analysis showed 4 Klinefelters in the azoospermic group and two Klinefelters in the oligospermic group. One translocation was observed in the oligospermic group. Microdeletions in the AZF region were present in 7 of the 88 oligospermic men (7%). None of these 7 men had abnormal findings on andrologic history and examination. No microdeletions were found in the azoospermic and normospermic group.

We conclude that microdeletions in the AZF region of the Y-chromosome are frequently found in men with severe oligospermia and with no other causal factors. We recommend DNA screening (and genetic counseling) in this population of subfertile men.

EVALUATION OF CANDIDATE AZOSPERMIA GENES CREM, hHR6B, PMS1, AND SMCY IN CASES OF NON OBSTRUCTED AZOSPERMIA.
John T. B. Houston, Alexander I. Agoulnik, Larry I. Lipshultz, Doreas J. Lash, Calgary, Alberta, Canada. (Presented by Dr. Houston).

INTRODUCTION AND OBJECTIVES: The increasing use of ICSI (Intracytoplasmic Sperm Injection) for cases of testicular failure underscores the importance of identifying genes involved in spermatogenesis. Evidence to date suggests that deletions within Y chromosome long arm interval 6 are associated with diffuse defects in spermatogenesis. A candidate gene distal to this region, DAZ (Deleted in Azoospermia), has been identified with deletions in only 11-18% of azoospermic patients. The possibility of deletions or aberrations in other genes controlling spermatogenesis exists. Recent studies involving gene knockout experiments of CREM (Cyclic AMP-responsive Element Modulator) and hHR6B (human homologue ubiquitin-conjugating yeast enzyme) in mice have demonstrated a variety of defects in spermatogenesis. Thus, other azoo or severely oligospermic. Mice mice were deleted for a region on the Y chromosome including SMCY (a Y specific chromosomal gene) exhibit failure of spermatogenesis. It has also been observed in yeast that deficiency of PMS1 (a DNA mismatch repair system) results in meliotic sterility. The role of these genes in human spermatogenesis is unknown. We report the screening of DNA from 50 azoospermic/oligoospermic males for candidate genes CREM, hHR6B, PMS1, and SMCY.

METHODS: DNA was obtained from 56 men presenting with idiopathic infertility. cDNA was to candidate genes CREM, hHR6B, PMS1, and SMCY were obtained and a molecular probe to each created. Using Southern Blot analysis, genomic DNA from each patient was then probed for each of the above candidate genes.

RESULTS: 34 men (61%) had positive results for at least one of the candidate genes. Of these, CREM, SMCY, and PMS1 were positive in 18 (32%), 9 (16%), and 8 (14%) respectively. There was no statistical difference in terms of sperm concentration, motility, or semen analysis.

CONCLUSIONS: Although large deletions were not observed, these data do not exclude the possibility that the above candidate genes may play a significant role in human male spermatogenesis. The possibility exists that microdeletions/mutations may contribute to gene impairment resulting in the observed phenotype.

THE EFFECTS OF PERCOLL ON THE OPTIMIZED SPERM PENETRATION ASSAY (SPA).
Steven Lewitan, Benjamin M. Tripp, Marin Gavrichka, Larry I. Lipshultz, Doreas J. Lash, Houston, TX. (Presented by Dr. Tripp).

INTRODUCTION: The SPA is an important sperm function test that reveals information about sperm capacitation, acrosome reaction, membrane fusion and chromatin decondensation. At our institution, the SPA has been highly correlated with successful outcome in IVF (Johnson et al, Fertil Steril 56:S28,1991). Percoll has been used extensively in sperm processing for IUI (intravaginal insemination), IVF and ICSI (intracytoplasmic sperm injection). The objective of this study was to determine the effect of Percoll processing on sperm penetrating ability.

METHODS: This is a retrospective study of 125 patients out of 1200 men who underwent the optimized SPA both before and after Percoll processing from January 1993 to July 1996. 97% of patients had more than 40 million motile spermatozoa on semen analysis. The SPA result is stored by the SCI (sperm capacitation index); the SCI >5 is normal, SCI < 5 is moderately abnormal, and SCI <1 is severely abnormal.

RESULTS: SPA RESULTS AFTER PERCOLL PROCESSING

<table>
<thead>
<tr>
<th>SPA Score</th>
<th>Patients (Before Percoll)</th>
<th>Normal</th>
<th>Improved Score After Percoll</th>
<th>Decreased Score After Percoll</th>
<th>Abnormal Score After Percoll</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>68 (59 (92%)</td>
<td>5 (10%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Moderately Abnormal</td>
<td>57 (40 (85%)</td>
<td>5 (8%)</td>
<td>39 (70%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severely Abnormal</td>
<td>14 (2 (14%)</td>
<td>6 (43%)</td>
<td>2 (14%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>139 (99 (89%)</td>
<td>10 (9%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The only group of patients that did not have their SCI significantly improved by Percoll processing was the group with a severely abnormal SCI (17% of the infertile males (191/1200) tested by the SPA). (Chi Squared Test, P = 0.05). CONCLUSIONS: Percoll processing was found to improve the SPA score in 89% of patients. Patients with mild to moderate penetration dysfunction as assessed by the SPA can potentially be treated with Percoll processing of their semen in conjunction with IUI, IVF and ICSI. However, patients with severe sperm penetration abnormalities were not significantly helped by Percoll processing, suggesting that this group can only be successfully treated with ICSI.

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EFFECT OF SODIUM NITROPRUSSIDE ON SPERM MOTILITY: A DOSE-RESPONSE CURVE.
Francis G. Martinis, Nestor Vizgi, Harris M Nagler, New York, NY. (Presented by Dr. Martinis).

Objective: Nitric Oxide (NO) has been shown to improve the maintenance of sperm motility in cryopreserved human sperm, as well as increase the yield of motile sperm using swim-up methods. However, an inhibition of sperm motility by NO at higher concentrations has also been reported. This suggests that NO may exert its effect on motility in a dose dependent manner. To investigate this hypothesis, we generated dose-response curves to evaluate the effects of NO on sperm motility over a wide range of concentrations using sodium nitroprusside as the NO donor.

Methods: Motile sperm obtained from two known fertile donors (two sperm samples each) and three infertile patients were separated on a Percoll gradient. Sodium nitroprusside was added in decreasing concentrations to the washed semen aliquots, to achieve final concentrations in the tube of 1x10^-6 to 1x10^-4 M. Sperm concentration in each tube was 20 million/ml. A control tube was maintained without sodium nitroprusside. Sperm motility was evaluated after two hours incubation at 37° C.

Results: Sperm motility in the control ranged from 71% to 84%. High concentrations of sodium nitroprusside (1x10^-4 M and 1x10^-3 M) were inhibitory to sperm motility resulting in final motility of 1% to 39%. At the concentration of 1x10^-2 M, sperm motility returned to baseline value (i.e. control motility). Between 1x10^-3 M and 1x10^-2 M sodium nitroprusside, there was a trend towards enhanced motility (range: 71% to 95%).

Conclusions: The dose-response curves for both donors and patients were identical, that is, inhibition at higher concentrations of sodium nitroprusside (1x10^-4 M and 1x10^-3 M) and no inhibition or mild enhancement of sperm motility at lower concentrations of sodium nitroprusside (1x10^-2 M and 1x10^-1 M).