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Y-DELETIONS IN MEN WITH SEVERE OLIGOSPERMIA.
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 deletion. Blood was drawn from 154 men, who were waiting for ICSI treatment: 24 azoospermie men, 98 oligospermie and 32 normospermie men. After previous fertilization failure, Chromosome analysis showed 4 Klinefelters in the azospermic group and two Klinefelters in the oligospermic group. One translocation was observed in the azospermic group. Microdeletions in the AZFc region were present in 7 of the 98 oligospermic men (7%). None of these 7 men had abnormal findings on andrologic history and examination. No microdeletions were found in the azospermic and normospermic group.

We conclude that microdeletions in the AZFc 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.

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EVALUATION OF CANDIDATE AZOOSPERMIA GENES CREM, hHR6B, PMS1, AND SMCY IN CASES OF NON OBSTRUCTED AZOOSPERMIA John T. B. Houston, Alexander I. Agoulnik, Larry I. Lipszultz, Dolores J. Lamb, Collin E. Bishop, Houston, TX (Presented by Dr. Houston).

INTRODUCTION AND OBJECTIVES: The increasing use of ICSI (Intracytoplasmic Sperm Injection) for cases of bilateral 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 azospermic patients. The possibility of deletions or aberrations in other genes controlling spermatogenesis exists. Recent studies involving gene knockouts 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, including either azoospermic or severely oligospermic. Mutant mice 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 meiotic sterility. The role of these genes in human spermatogenesis is unknown. We report the screening of DNA from 50 azoospermic/oligospermic males for candidate genes CREM, hHR6B, PMS1, and SMCY.

METHODS: DNA was obtained from 56 men presenting with idiopathic infertility. cDNA to a 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: CREM, SMCY, PMS1 and hHR6B genes were identified in all 50 patients. Under stringent conditions, Southern Blot analysis did not detect any large deletions resulting in differential banding patterns in any of the 34 azoospermic or 16 oligospermic patients compared to normal controls.

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.

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THE EFFECTS OF PERCOLL ON THE OPTIMIZED SPERM PENETRATION ASSAY (SPA)
Steven Lewiston, Benjamin M. Tripp, Mariana Gvakharia, Larry I. Lipszultz, Dolores J. Lamb, 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 56:328,1991). Percoll has been used extensively in sperm processing for IUI (intrarutine 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 spermatozoon on semen analysis. The SPA result is stored by the SCI (sperm capacitation index); the SCI >5 is normal, SCI <5 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>Improved Score (After Percoll)</th>
<th>Decreased Score (After Percoll)</th>
<th>Abnormal Score (Before Percoll)</th>
<th>Combined 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 (83%))</td>
<td>5 (8%)</td>
<td>39 (70%)</td>
<td>9 (16%)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Severely Abnormal</td>
<td>2 (14%)</td>
<td>2 (14%)</td>
<td>2 (14%)</td>
<td>2 (14%)</td>
<td>2 (14%)</td>
</tr>
<tr>
<td>Total</td>
<td>135 (99 (89%))</td>
<td>10 (9%)</td>
<td></td>
<td>39 (70%)</td>
<td>2 (3%)</td>
</tr>
</tbody>
</table>

The only group of patients that did not have their SPA significantly improved by Percoll processing was the group with a severely abnormal SPA (77% of the infertile males [199/1200] 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 penetrance 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 penetrating 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, Nastini Vizji, 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 specimens 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^{-5} 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^{-3} M, sperm motility returned to baseline value (i.e. control motility). Between 1x10^{-3} M and 1x10^{-4} 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^{-5} M and 1x10^{-4} M).