

Thomas Bruckner
Institute of Medical Biometry and Informatics
University of Heidelberg
Heidelberg, Germany

Vanderleyen Biolchi
 Amal Alia, M.D.
 Thomas Strowitzki, M.D.
Department of Gynecological Endocrinology and
Reproductive Medicine
Women's University Hospital
University of Heidelberg
Heidelberg, Germany

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Need for standardization and confirmation of STS deletions on the Y chromosome

To the Editor:

As clinical investigators and scientists in the Y chromosome deletion field, we wish to express our concern about the study by Feng et al. (1) that purports to show a significantly increased risk of de novo Y chromosome deletions in children conceived by assisted reproductive technology (ART). We believe it has serious technical flaws and will serve to confuse and concern the readership.

First, in terms of patient selection, it is remarkable that the 37 ART fathers had the same semen quality as the control group with a mean sperm density of 91.5 million/mL. Such an inclusion criterion is at variance with worldwide ART practice and yet passes without comment.

Most critically, we question the performance of the Y deletion analysis. Overdetection (false positives) is a major issue in Y chromosome deletion studies, and for this reason there have been significant international efforts to provide a quality insurance program (2). Their brief method section suggests that the authors have not participated in any quality control program.

These authors have published previously (3) a staggering 33% incidence of Y chromosome deletions in idiopathic non-obstructive azoospermia. This is many fold higher than that reported by others using appropriately stringent methods for Yq deletion detection (4) and serves to raise questions about the molecular methods used to define Yq deletions and the overall validity of the data. Independent confirmation of their data should have been sought.

We also note their claim that two of the four deletions were limited to the AZFa region. The authors (and the referee) are

clearly not aware of the limits of the AZFa region because the STS marker sY82 is erroneously indicated as being “in the AZFa region.” Moreover, the four deletions found by the authors were all single STS deletions, which require further confirmation (at least with a few contiguous markers) given that dealing with STS plus/minus analysis there is a high probability of generating polymerase chain reaction (PCR) artifacts. In addition, given that the RNA recognition motif 1 is present several times along the Y chromosome, the likelihood of finding an isolated deletion for this specific marker is negligible. The European Academy of Andrology (EAA) Guidelines (2) for the valid detection of AZF deletions contain all the relevant methodological and clinical information, including the need for a reliable set of primers and the need for confirmation of results.

Finally, the authors state that “[t]he incidence of microdeletion was higher in children conceived via ICSI than in those conceived by IVF (3:18 vs. 1:19),” and yet they go on to say that this was not statistically significant. This reveals a lack of statistical understanding and underscores the limited power of this small study. Several of us were involved in a previous study that looked in detail into this issue in a properly selected group of 86 severely infertile men who were thus candidates for intracytoplasmic sperm injection (ICSI), and we found no evidence for de novo chromosome deletions in their 99 ICSI-conceived male offspring (5).

Robert Ian McLachlan, M.D., Ph.D.
Prince Henry's Institute of Medical Research
Clayton, Australia

R. John Aitken, Ph.D.
ARC Centre of Excellence in
Biotechnology and Development
University of Newcastle
Callaghan, Australia

David Cram, Ph.D.
Monash Immunology and Stem Cell Laboratories
Monash University
Clayton, Australia

Csilla Krausz, M.D., Ph.D.
Andrology Unit
Department of Clinical Physiopathology
Florence, Italy

Moira O'Bryan, Ph.D.
Monash Institute of Medical Research
Monash University
Clayton, Australia

March 25, 2008

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Reply of the Authors:

The letter to the editor by Dr. McLachlan et al. expressed their concern about our recent study (1), which showed a significantly increased risk of de novo Y chromosome deletions in children conceived by assisted reproductive technology (ART). We appreciate the concern from these doctors and others, and we would like to discuss our study with them.

The participants in our study were selected as follows. Couples requesting ART treatment for tubal infertility in our center in 2004 were informed about our research, and nearly 200 couples consented to take part. They were randomly divided into an in vitro fertilization (IVF) group and an intracytoplasmic sperm injection (ICSI) group. Thirty-seven male offspring conceived through ART constituted the study group. So semen quality is not a routine inclusion criterion for ICSI.

In our previously published study (2), Y-chromosome microdeletions were detected in 13.2% of patients with idiopathic azoospermia. Among those patients, there were microdeletions in 33.3% of the men with high serum levels of follicle-stimulating hormone (FSH). The generally accepted deletion frequency for idiopathic severe oligospermic and azoospermic men is 10% to 18%. We do not believe that the incidence we found was higher than that found in other studies.

As we have successfully performed Y chromosome microdeletion detection in infertile men and quite a few published papers have adopted similar methods, we used the same method to investigate microdeletions in offspring. The quality control program was as follows. A healthy man of proven fertility and a healthy woman were included as positive and negative controls, respectively, and were run in parallel with each reaction. Patients were considered negative only after three amplification failures. Because these details had been described in our previous report (2), we omitted them from the current report. The European Academy of Andrology (EAA) Guidelines for the valid detection of AZF deletions (3) have been recommended as being more stringent, so we will employ those methods in our future studies.

Because SY82 locates at the proximal region of the AZFa region and it is included in the AZFa region in some studies, we described it in the AZFa region. We performed poly-

merase chain reaction (PCR) using the primers reported previously; we found a section of RNA recognition motif 1 (YRRM1) was deleted, but we did not mean that all the copies of YRRM1 were deleted. No Y chromosome microdeletion was found in naturally conceived offspring and their fathers, indicating that the detected microdeletion in ART offspring should not be a false-positive result. Of course, if additional confirmation had been done, the results would be more convincing.

No statistically significant difference was found in the incidence of microdeletion between ICSI and IVF offspring. This may be due to the relatively small sample size. It is true that the presentation is not precise enough, but we were emphasizing the difference in incidence of microdeletion between ART and naturally conceived offspring, which was statistically significant. Cram et al. (4) found no de novo chromosome deletions in 99 ICSI-conceived male offspring (4), but it has been reported that ICSI may lead to vertical transmission, expansion, and de novo Y-chromosome microdeletion (5). Our study tries to show a potential risk that the incidence of microdeletion might increase in ART-conceived offspring. We will enlarge the sample size and adopt more specific methods in our subsequent studies to confirm our results.

Chun Feng, M.D.

Li-Quan Wang, M.D., Ph.D.

Min-Yue Dong, M.D., Ph.D.

Fan Jin, Ph.D.

He-Feng Huang, M.D.

*Department of Reproductive Endocrinology
Women's Hospital, School of Medicine,
Zhejiang University*

*Key Laboratory of Women's Reproductive Health
of Zhejiang Province*

Hangzhou, Zhejiang, People's Republic of China

April 17, 2008

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