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EDITORIALS



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The Hemizona Assay (HZA): Finding Sperm that Have the "Right Stuff"^{1,2}

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THE STORY

Once upon a time there were two male-factor patients who presented for infertility evaluation. These men were believed to have severe infertility conditions based on a detailed workup by conventional semen evaluation criteria. The first man was Mr. Andro L. Ogy. After 4 years without impregnating his wife, he was counseled that a new laboratory test was available and that it may provide crucial evidence prognostic of his response to infertility treatment. Subsequently, Mr. Ogy's sperm were tested in the hemizona assay (HZA). Despite his apparently diminished seminal quality, he achieved a HZA index of 71, consistent with a reduced, but reasonable potential for fertilization in vitro. Because his wife had tubal endometriosis, it was agreed they would proceed with in vitro fertilization and embryo transfer (IVF/ET) therapy.

The second man, Mr. Epi D. Idymis, had undergone microsurgery for vasectomy reversal 2 years earlier and, on similar advice, contributed a specimen for HZA evaluation. The score of 13 left his medical advisors troubled about this couple's treatment opportunities. Since a few "good" sperm were apparently present, as indicated by HZA, this encouraged his physicians to consider gamete intrafallopian transfer (GIFT) treatment, because Mrs. Idymis was believed to be reproductively unimpaired. It was decided to treat him with prednisolone to alleviate high sperm antibodies. Mr. Idymis' sperm would be retested in the HZA, after 3 months of immune suppression.

THE HEMIZONA ASSAY (HZA)

The advent of IVF ushered in the first direct laboratory assessment of a man's fertilization potential. While the appropriate uses of GIFT or intrauterine insemination (IUI) in conjunction with controlled ovarian hyperstimulation have proven to be significant additions to the repertoire of infertility specialists, IVF/ET uniquely provides actual observations about the number of eggs fertilized and the number of early-cleaving embryos available at transfer for pregnancy.

As a diagnostic tool per se, IVF/ET is unsuitable,

at \$5000 and up for each cycle of treatment. Here the problems include the high emotional burden. We need quantifiable objective assessments of sperm quality having a high reliability, a low cost of operation, ease of application, a wide availability, and rapid results. In brief, we wish for a male-factor "litmus test."

The idea to develop the hemizona assay as a measure of sperm quality derived from endocrine studies of 20 years ago when we used split (hemi-) rat anterior pituitaries in culture to bioassay hypothalamic peptide fractions. One-half served as control tissue; the matching half was exposed to experimental hormone preparations. Similarly, by hemisectioning the human egg (animal eggs do not substitute because of species-specific sperm binding), we have obtained mathematically reproducible measurements of sperm binding from a single oocyte (1). In contrast, several whole oocytes would be required to provide equivalent statistical validity, because of the inherent variability of sperm-binding capabilities among eggs (2). Thus, the limited supply of precious human oocytes having useful zonae is extended by HZA; only one human oocyte is needed per test to achieve useful data. An important ethical safeguard is provided, in that inadvertent fertilization of a whole egg cannot occur in the HZA.

Perhaps the fundamental utility of the HZA stems from its being a functional bioassay of sperm performance in relation to the oocyte, where the requisite first step which the sperm and egg must accomplish together is tight binding. Whereas tight binding to the zona pellicuda does not guarantee fertilization, subsequent penetration leading to fertilization and development cannot otherwise be achieved naturally. Among severe male-factor cases, failure of sperm attachment to the egg(s), despite the addition of motile sperm to apparently "good" oocytes, is not uncommonly encountered in IVF/ET therapy. We sought to determine whether the HZA could provide a useful discrimination between men capable of achieving fertilization in vitro versus those who are unlikely to be successful. Of course, such data would be invaluable in counseling couples *prior* to their entry into various infertility treatments.

After determining the optimal kinetics of tight sperm binding, our next aim was to increase the available supply of human eggs by an extended intercontinental network. This was done by storing

the oocytes in a salt solution for shipment to Norfolk (3). After desalting, such eggs worked well in the HZA, as was hoped for based on earlier reports using whole oocytes stored in salt solution (4).

Our clinical results to date are gratifying, albeit limited to a few dozen patients. Thus far, we have found the HZA index to be accurate in predicting IVF outcome to the point of embryo transfer (5). In time, we will know whether HZA aids in the prediction of pregnancy rates and outcome. Obviously, a much larger and statistically valid data set must be collected prospectively before we can expound on the utilities of HZA for predicting the fertilizing potential of a given man. Equally important will be defining the limits of HZA, that is, situations where reliability is highest versus conditions where HZA is not useful. For example, the HZA index is computed by making a simple equation: after 4 hr of incubation, the number of sperm bound with the test specimen \div the number of sperm bound with the known fertile specimen $\times 100 =$ the HZA index. Already, we know that if the egg used has an inferior zona pellicuda that binds fewer than 30 sperm from the known fertile specimen, its power of discrimination is compromised. Likewise, the use of a marginal control specimen or a control man whose sperm quality fluctuates often could be confounding. But again, there remains much to refine in order to maximize application of the HZA.

As stated above, expectations that the HZA is a litmus test for severe male-factor cases are premature at best, perhaps unlikely in reality. However, we are confident that the HZA offers a genuine technical advance that does assist us in both diagnosis and prognosis. Importantly, given the increased attention to treatment intervention on behalf of male-factor cases, the HZA may be a useful tool by which to monitor the progress of therapeutic courses or to select a "better" sperm for microsurgical injection than would a random choice.

On the flip side of this scientific coin, contraceptive effectiveness may be estimated by employing the HZA, again as a functional bioassay. Examples include residual or intermittent oligospermia in men receiving a gonadotropin-releasing hormone (GnRH) antagonist plus testosterone or vaccines employing specific antigens of the sperm or zona pellicuda. Indeed, ethics may dictate the need for such data before undertaking trials of clinical efficacy, where contraceptive failure could lead to unwanted pregnancy.

THE OUTCOME

By the way, Mrs. Ogy's three metaphase eggs did fertilize *in vitro*, with two embryos transferred. As for Mr. Idymis, his HZA index improved to 64 and encouraged the couple to repeat GIFT.

While in reality there is much research remaining to be done before we will know the utility of HZA on behalf of couples like the Ogys and the Idymises, our preliminary findings suggest that the HZA will be an important new tool in the armamentarium for assessment and treatment of male-factor infertility (6).

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Short-Term Use of Gonadotropin-Releasing Hormone Agonist

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When we first read the study by Katayama *et al.* we had just initiated a similar study of our own and were extremely impressed and hopeful after reading their results. The concept of using the gonadotropin-releasing hormone (GnRH) agonist response at the onset of stimulation was very attractive as a potential treatment for our low responders. Along with the authors of that study, we hypothesized that the initial elevation in gonadotropins may serve to sensitize the follicles with an early boost in estradiol (E₂) and follicle-stimulating hormone (FSH) that may synergize to increase the number of FSH receptors and thereby improve follicular recruitment.

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We saw this as being particularly useful in our premenopausal patients, who may be defined by virtue of their endocrinologic and follicular response to gonadotropins and their poor pregnancy rates. In our laboratory these patients typically have relatively elevated baseline (day 3) serum FSH levels, >20 IU/liter. We theorized that the high luteinizing hormone (LH) response one gets with the GnRH agonist may combine with the elevated FSH levels to create a superhyperstimulation that may overcome the apparent diminished ovarian sensitivity in these patients.

In the report by Katayama *et al.*, some of the patients were likewise selected for previously demonstrated poor follicular development and/or poor E₂ response. In addition, three patients had demonstrated an LH surge during previous cycles. The age range of their patients was 24 to 36 years, which is relatively young compared to our patients.

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