

THE IMPORTANCE OF SEMEN PARAMETERS AND THE USE OF IVF-ET TO IDENTIFY MALE FACTOR

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ABSTRACT

Male factor is considered to be an etiologic parameter in approximately 40% of infertility cases. This is based on semen analysis and whether a given individual is above or below arbitrary numbers considered to be the dividing line of fertile vs. subfertile. Various therapies have been employed based on these numbers and claims for pregnancy success have been made. The normal values have been reduced approximately to 20% of what they were originally considered yet there is evidence that even these lower figures cannot really distinguish fertile from subfertile males. A reasonable percentage of "subnormal" men are quite fertile and vice versa, many "normal" males are subfertile. Only the hypo-osmotic swelling test (HOS) when $<50\%$ has been highly predictive of an infertile male but a normal level does not indicate that he is fertile. The hamster oocyte sperm penetration assay has too many false positives/negatives to be useful though a new modification with short/long capacitation, and different salt concentrations shows promise. The hemi-zona assay (HZA), though of scientific interest, could never be of commercial importance because of lack of enough human oocytes. A new concept to distinguish the fertile male is provided and that is IVF-ET but with the couple in question sharing the wife's oocytes with a woman in ovarian failure (costs are shared) so that a comparison of fertilization can be made. Failure for either donor or recipient to fertilize would require one more IVF cycle but now with the use of known fertile donor sperm for a portion of the oocytes. Performing these latter studies has allowed confirmation of the general lack of the standard semen analysis, except when semen quality is extremely poor, to predict male subfertility. Though IVF-ET is expensive, by

sharing oocytes not only is the cost reduced by 50% but the patient still has an increased chance of conceiving because of the test. Many expensive non-IVF cycles may be eliminated because of earlier use of this test. Some may provide simply IVF using all oocytes for fertilization the first time, using shared oocytes the next cycle only with poor fertilization. It is realized that some males showing adequate fertilization with IVF-ET may still not be able to fertilize *in vivo* but is unlikely that a male who cannot fertilize by IVF can do so *in vivo*.

INTRODUCTION

The diagnosis/treatment of male infertility has received much attention. There are several methods for treatment modalities including the time honored varicocelectomy, administration of clomiphene citrate, tamoxifen, hCG, hMG, testosterone replacement, and/or antibiotic therapy. Surgical treatment of the varicocele has been claimed to be successful to treat male infertility during the past 35 years (Tulloch, 1955). The success rates of correcting infertility, even in the earlier days of surgery was 50% (Dubin, 1976; Charny, 1962). At that time, semen analysis of $80-100 \times 10^6/\text{ml}$ was considered normal. Many of these so-called "successes" may have been in men undergoing needless surgery despite having normal semen parameters by our present standards.

Certain studies demonstrated slightly higher success despite lowering the limits for an abnormal semen analysis (Baker, 1985; Pryor, 1987). Thus the actual benefits of the surgical procedure has been questioned (Vermeulen, 1984). A well randomized/carefully controlled study has never been reported. In fact, one partially controlled study demonstrated only a limited improvement in sperm motility, from 34% to 39%, following varicocelectomy, but a similar improvement was also noted in the control group. In one study where clomiphene citrate was administered after therapy, 75% of the 28 males showed a persistent increase in sperm count per ml by at least $10 \times 10^6/\text{ml}$, with 57% demonstrating increases above $20 \times 10^6/\text{ml}$ (Check, 1980). Furthermore 68% demonstrated at least an additional increase in sperm motility by 25%, and 36% achieved a pregnancy in an average of 8 cycles (Check, 1980). This study, like those following surgery, was not well controlled. The following questions should be answered:

- Does clomiphene therapy work as well as the varicocele operation?
- Or are neither of the two therapies any better than expectant therapy?

I. DETECTING SUBNORMAL MALES

A major problem in determining whether the therapies claiming to "treat" male infertility are truly effective is identifying the subfertile male. We evaluated 135 infertile couples in whom a female factor was identified and thought to be corrected. Pregnancy rates were then evaluated during the following 6 months. When both the hypo-osmotic swelling test (Jeyendran et al, 1984) and the semen analysis were normal, 89% of the couples conceived in comparison to 83% with normal HOS scores but subnormal semen analysis (below $20 \times 10^6/\text{ml}$ with $< 50\%$ motility). When various parameters of the semen (employing computer assisted semen analysis) were evaluated, there was no specific parameter that is capable of distinguishing the subfertile male (Check, 1990).

II. MOTILE DENSITY IN DETECTING MALE SUBFERTILITY

Another study was performed to evaluate the motile density (MD) to predict subfertile males (Check, 1991). Once again a group of infertile couples were selected for evaluation with the requirement that a female factor be identified and considered corrected. The men were divided into 5 groups based on the motile density of two averaged specimens. The results in group 1 showed pregnancies in 7 of the 32 (22%) couples with motile densities $< 2.5 \times 10^6/\text{ml}$ and in the groups as follows:

- group 2 (≥ 2.5 to $< 5 \times 10^6/\text{ml}$) 9 of 13 (69%);
- group 3 (≥ 5 to $< 10 \times 10^6/\text{ml}$) 25 of 31 (81%);
- group 4 (≥ 10 to $< 15 \times 10^6/\text{ml}$) 27 of 34 (74%);
- group 5 ($\geq 15 \times 10^6/\text{ml}$) 139 of 171 (81%).

No statistical differences were found comparing group 2 to groups 3, 4, 5, but statistical differences were found comparing group 1 (with the lowest motile density) to the other four groups. It would appear that the $10 \times 10^6/\text{ml}$ level established by the World Health Organization is set too high to effectively distinguish fertile from subfertile males. Strictly adhering to the WHO level could lead to unnecessary treatment of the male.

III. SPERM MORPHOLOGY IN DETECTING MALE SUBFERTILITY

Several researchers believe that sperm morphology may be the best parameter of the semen analysis to identify the subfertile male (Rogers, 1983). WHO establishes 50% as normal. In infertile couples with all female factors corrected, the 6 month pregnancy rates were compared in couples where the males had $\geq 10 \times 10^6/\text{ml}$ and $\geq 50\%$ normal morphology versus those with motile densities

$\geq 10 \times 10^6$ /ml but $< 50\%$ motility; there was no difference in rates of pregnancy with 95 (87%) in former vs 18 of 22 (82%) in the latter (Check, 1989).

IV. STRICT CRITERIA FOR MORPHOLOGY-IS THIS MORE HELPFUL?

Another method suggested for evaluating sperm morphology is the use of much stricter morphology criteria (Kruger et al, 1988). This evaluation has been identified as one of the most accurate methods of predicting whether a given sperm specimen will fertilize oocytes during IVF (Oehninger, 1988).

Check (1990) performed 3 separate *in vivo* studies to evaluate this test. Kruger noted that the IVF pregnancy rate was the same whether the semen had normal values ($> 14\%$) or below normal (subnormal) values as low as 4% normal sperm morphology was maintained. However, below this level the pregnancy rate was poor. Since it is estimated that only 400 sperm reach the oocyte *in vivo*, but a least 50×10^3 are added to each oocyte *in vitro*, we reasoned that a higher level than 4% might discriminate fertile from subfertile males. The first study in men with motile density $\geq 10 \times 10^6$ resulted in 6 month pregnancy rates according to the "strict criteria" as follows:

- 9 of 24 (38%) for $> 14\%$;
- 4 of 8 (50%) for 5 to 14%;
- 0 of 1 for $< 5\%$.

Once again sperm motile density (MD) failed to predict fertilization. The pregnancy rates were higher when the MD was $< 10 \times 10^6$ /ml and distributed as follows:

- 1 of 2 (50%) with normal morphology of $> 14\%$;
- 4 of 6 (67%) for scores of 5-14%.

The second study involved a retrospective study of infertile couples (Check, 1990). With normal morphology of $> 14\%$ ($n=76$), 38 of 41 (93%) couples conceived when MD was $\geq 10 \times 10^6$ /ml and 8 of 35 (23%) conceived when MD $< 10 \times 10^6$ /ml. Although the data might suggest the importance of the MD in fertility prediction, it must be noted that when morphology scores of $\leq 4\%$ were considered, 10 of 18 (56%) conceived with MD $< 10 \times 10^6$ /ml versus 4 of 10 (40%) with MD $\geq 10 \times 10^6$ /ml. The 93% pregnancy rate in the wives of men with normal MD but strict criteria scores $> 14\%$ compared to only 40% with normal morphology of $\leq 4\%$ seem to support Kruger's original conclusion (Kruger, 1988); However, the fact that 56% conceived in the poor MD and poor morphology group compared with 40% with the poor MD but good morphology fails to confirm Kruger's original conclusions.

Table 1. Predictability of Male Fertility Testing

Test	Subnormal predicting infertile	Normal predicting fertile
Motile Density (MD) ^a	Poor	Poor
Sperm morphology-strict criteria ^b	Poor	Poor
Shared oocytes with IVF-ET ^c	Good	Good?
Hypo-osmotic swelling test (HOS)	Good	Poor
Hemizona Assay ^d (HZA)	Poor	Good?
Hamster Ova Penetration ^e (HOP)	Poor	Poor

^a poor predictor unless MD $< 2.5\% \times 10^6/\text{ml}$

^b may be predictive for IVF, but no reliable *in vivo* - even when $< 4\%$. Reason for dichotomy unclear.

^c may still fail to detect subtle male infertility factor *in vivo* due to large number of sperm used for insemination of oocytes.

^d theoretically this assay should be useful to distinguish fertile from subfertile male, but clinical studies are lacking, and it is difficult to perform for a large number of patients.

^e poor predictor of subfertile vs. fertile male; but future modification may improve sensitivity/specificity (Muller, 1990)

The prospective study was performed in which an infertile couple with a poor morphology evaluation in the male partner was matched with the closest temporally infertile couple where the male's morphology was normal. These parameters failed to confirm the morphology score as a good predictor of male subfertility, since 41% of couples conceived where the score was $\leq 4\%$ and yet only 24% pregnant when $> 14\%$. There was no increase in the abortion rate in either study in couples with low rates, as based on Kruger scores.

V. USE OF SHARED OOCYTES TO DETECT INFERTILE MEN

The fact that our donor oocyte program uses the donor patients who are undergoing IVF and willing to give away 50% of their oocytes, provides a unique opportunity to compare the fertilization potential of sperm from two different men against the same pool of oocytes. We evaluated 19 of 63 donor-recipient cycles in which there was $< 30\%$ fertilization of oocytes by the donors (Check, 1991). When compared to the matching recipients, 10 of 19 (53%) demonstrated fertilization $> 30\%$ fertilization. The respective mean (\pm SD) MD for the donors

with poor fertilization was 42 ± 39 compared to 34 ± 23 for the recipients. The MD for 8 donors with "zero" fertilization was 28 ± 26 and the MD for the 10 recipients with good fertilization amounted to only 37 ± 29 ($p=0.58$ unpaired t-test). In 4 of 8 cycles the recipient had good fertilization and the donor "zero", yet in 3 of these cycles the MD's for the donors were 31, 86 and 34 compared to 10, 14, and 10.1×10^6 respectively in the recipients. These data thus demonstrate that a) apparently normal sperm may fail to fertilize even under ideal circumstances eg. IVF, and b) this phenomenon is not so rare.

VI. USE OF A DONOR SPERM PROBE TO DETECT MALE INFERTILITY

The fact that so-called "normal sperm" may be subfertile was demonstrated in a totally different *in vivo* study (Check, 1990). Once again, taking couples with at least 18 months of infertility and a female factor identified and deemed corrected, therapeutic donor insemination (TDI) was offered to all patients failing to attain conception within 8 months. It was hoped to determine that if MD was not a particularly reliable index of conception during the first 8 months, perhaps it could distinguish fertile from subfertile couples during the second 8 months. The theoretical results, utilizing the "TDI probe", would be a high 6 months pregnancy rate in couples where oligozoospermia existed and low pregnancy rates in those with normal MD's (probably an occult female factor). The fact that 13 of 15 (87%) couples with $MD < 10 \times 10^6/ml$ achieved pregnancies in 8 cycles is not surprising. However, the 53 pregnancies in 73 couples (73%) where the MD was $\geq 10 \times 10^6/ml$ were unexpected.

VII. HYPO-OSMOTIC SWELLING TEST TO DETECT MALE SUBFERTILITY

If evaluating standard semen parameters fails to distinguish fertile from subfertile males, perhaps tests which evaluate sperm function may be of value. A functionally intact sperm membrane is needed for capacitation, acrosome reaction, and fusion with the vitelline membrane of the oocyte. Since a functionally intact membrane is needed to actively transport water from higher to lower concentration, a test was designed where sperm would be placed in a hypo-osmolar solution and then subsequent percentage of tail swelling would be noted (Jeyendran, 1984). When this hypo-osmotic swelling (HOS) test was used there was a higher correlation between the HOS test and IVF than between the HOS test and other semen parameters (Van der Ven, 1986). Data from other studies were not as supportive regarding its usefulness (Chan, 1985; VanKooij, 1986).

We have recently performed two clinical studies involving the HOS test. The first study involved 40 infertile couples; 23 of 25 (92%) couples with normal semen analyses and normal HOS scores ($\geq 60\%$) conceived in 6 months, 3 of 3 couples with normal semen parameters in the male but grey-zone HOS (50-90%) conceived. Only one male had normal semen parameters and a subnormal ($<50\%$) HOS score and that couple failed to conceive (Check, 1988). In a separate more extensive study of infertile couples with all female factors corrected, 83 of 93 men (89%) with normal semen parameters and normal or grey-zone HOS achieved pregnancies in 6 months compared to 0 of 7 (zero %) of men with normal semen parameters and subnormal HOS test (Check, 1989). It would appear that a poor HOS test may indicate a subfertile male but it is unlikely that all men with normal HOS scores are fertile.

VIII. HUMAN SPERM/HAMSTER ZONA-FREE OOCYTE PENETRATION TEST

The human sperm zona-free hamster oocyte penetration test was designed to evaluate the competence of acrosome reacted spermatozoa to fuse with the vitelline membrane of the oocyte (Yanagimachi, 1975). There has been a wide range of diverse opinions about the clinical efficacy of this procedure. Certain studies suggested good predictability of male fertilizing potential both *in vivo* (Rogers, 1985; Corsson, 1988; Karp, 1981; Rogers 1979) and *in vitro* (Rogers, 1986; Tyler 1981). Other studies, however, failed to demonstrate any such diagnostic potential (Wickings, 1983; Check, 1986; Wolf, 1983). A summary of the discrepancies has been previously reported (Check, 1986).

IX. NEW MODIFICATIONS OF SPERM PENETRATION ASSAY

Several reports praised the "Enhanced Sperm Penetration" and "Zona Binding" assays as well as "Follicular Fluid Enhanced SPA" to identify new categories of male infertility. In these patients, semen analysis does not preclude the presence of more subtle sperm dysfunctions. Adjustments in the preincubation time prior to the SPA have been done to identify capacitation defects (Muller, 1990). Of all patients tested, 22% failed at the longer time of capacitation when it came to the hamster oocyte penetration. This group was further subdivided into those who failed either incubation period with the SPA. Various agents were added during a short (1-2 h) capacitation including high salt, follicular fluid, and or calcium ionophore A23187, 10 and 20 μM . A 55% rate of patients responded to one these agents (Muller, 1990). These enhanced tests may identify a very small sub-population of idiopathically infertile men, but it is not known whether these results remain consistent with repeated semen samples over time. The fact

that the SPA is used as an endpoint for capacitation/acrosome reaction in itself is suspected. The SPA is not species specific and is also devoid of the zona-pellucida, the very glyconjugate that may play the definitive role in sperm/egg interaction. Each ejaculate may consist of a highly asynchronous population of cells with many differences among the groups. By performing manipulations leading up to the SPA and additions to the samples, this only serves to magnify this diversity in population. Even if these tests can identify a sub-population of sperm defects, there is no known treatment for the "defects".

CONCLUSION

Perhaps the best way to assess sperm function is to attempt IVF. Even this may not completely exclude a male factor problem in that only 400 sperm may reach the oocyte *in vivo*, whereas a minimum of 50×10^3 sperm are incubated with each oocyte *in vitro*. Some sperm may have fairly normal fertility potential initially, but quickly lose this function in time, making IVF difficult. Failure to fertilize despite normal appearing oocytes might indicate an occult male factor problem but could also suggest a subtle oocyte defect.

Thus, with all of the new/highly sophisticated methodology now available for semen analysis, the diagnoses of male factor problems has become more difficult than ever (Table 1). Thus early reports claiming the success of male factor therapies should be re-evaluated. We are most encouraged at the present time of identifying male factor by comparing the fertilization results of the partner of a woman providing donor oocytes for fertilization by the partner of a recipient for half of the oocytes, who is in ovarian failure. This obviates the need to find non-used oocytes for the hemi-zona assay and evaluates the sperm-zona interaction in man (in contrast to even the new "sophisticated" hamster test). At the same time, the cost of IVF is reduced by 50% and the cost to the patient may result in one or two pregnancies.

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