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# The clinical usefulness of the hamster ova penetration test in unexplained infertility

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## INTRODUCTION

Frequently a physician is faced with the problem of unexplained infertility. One potential explanation might be a subtle abnormality with the sperm not discernible by standard semen analysis. The hamster ova penetration test or sperm penetration assay (SPA) is a test that has been purported to determine the ability of spermatozoa to penetrate inside an ovum<sup>1</sup>. The assumption is that there is a fairly good correlation between the zona-free hamster egg and the human ova. Thus, this test may prove useful in evaluating a cause of unexplained infertility.

Though there is no known therapy as yet for a poor SPA, finding an abnormal one might allow the physician to offer artificial insemination by donor (AID) or try some experimental approaches, e.g. medical therapy of the male with clomiphene citrate<sup>2-4</sup>. The incidence of normal versus abnormal SPA tests in 100 men with unexplained infertility was thus evaluated and also studied was their choice of treatment and subsequent results.

## MATERIALS AND METHODS

100 patients were selected who had at least 8 months of all fertility factors being normal or if no infertility factors were found. The couple had to have at least 18 months of infertility. A minimum concentration of  $20 \times 10^6$ /ml spermatozoa was required with at least 60% motility, grade 3 or 4 quality, 60% normal morphology, 2 ml volume. Ovulation was considered normal as determined by two endometrial biopsies taken in the late luteal phase dating appropriately<sup>5</sup>, and each patient was required to demonstrate a mature follicle of 18-24 mm<sup>6,7</sup>, a serum estradiol level of at least 200 pg/ml and also demonstrate release of the ovum from the follicle<sup>8</sup>. Furthermore, a postcoital test taken at the time of a mature follicle needed to show at least five sperm per

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high powered field with progressive forward motion (PFM). All women in the study had laparoscopies and needed to demonstrate bilateral tubal patency with no more than Stage I endometriosis and at most mild adhesions not appearing to hinder fertility.

The hamster ova penetration tests were performed by The Fertility Testing Laboratory, Philadelphia, Pennsylvania. A level under 15% penetration was considered abnormal. The test was patterned after the description by Yanagimachi *et al.*<sup>1</sup>. In all cases the donor control had to be normal or the test was considered invalid.

Each patient, whether the SPA was normal or subnormal, was given the option of (1) continuing the same treatment regime, (2) treating the husband with clomiphene citrate (25 mg daily for 25 days each month), (3) treating the husband with clomiphene citrate and doxycycline (200 mg 1st 10 days of each of wife's cycle), or (4) treatment with AID.

### RESULTS

The incidence of abnormal SPA results and the efficacy of various treatments are seen in Table 1. The incidence of normal SPA tests and the results of no further therapy of the male or AID are seen in Table 2.

30 of the 56 patients with abnormal SPA results who were subjected to

**Table 1** Hamster test results prior to treatment and after treatment

	<i>Total number</i>	<i>No therapy to male</i>	<i>AID</i>	<i>Clomi- phene</i>	<i>Clomiphene and doxycycline</i>
No. of abnormal hamster results prior to treatment	56	5	13	17	21
No. of normal hamster results after treatment	14	0	not performed	9	5
No. of pregnancies within 8 months	30	0	8	10	12
No. of pregnancies with hamster test improved to normal	5	0	Not applicable	3	2

**Table 2** Pregnancy results in men with normal hamster tests

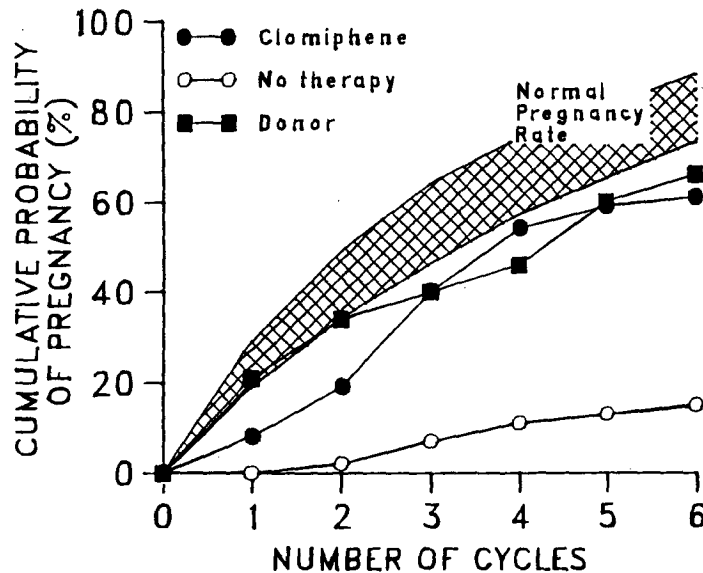
	<i>Total number</i>	<i>No therapy to male</i>	<i>AID</i>
No. of normal hamster results	44	41	3
No. of pregnancies within 6 months	9	7	2

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therapy (53%) achieved a pregnancy within 8 months as compared to only 9 of 44 (20%) patients with normal SPA results with no therapy ( $\chi^2 = 11.5$  and  $p < 0.001$ ). This difference cannot be explained on the basis of more frequent choosing of AID since this would account for only six extra pregnancies. 58% (22/38) of patients with poor SPA treated with clomiphene citrate achieved a pregnancy as compared to 61% (8/13) who went to AID (not statistically significant). Adding doxycycline to the clomiphene did not seem to improve the pregnancy results.

There was a statistically significant difference in the pregnancies achieved with clomiphene citrate (22 of 38) in patients with poor SPA results prior to treatment versus untreated patients with poor SPA results (0 of 5) and untreated patients with normal SPA results (7 of 41) with  $p < 0.025$  in the first instance and  $p < 0.005$  in the latter. Though 14 of the 38 SPA men with poor SPA results did show improvement to 15% or greater penetration into the hamster ova following clomiphene therapy, only five of the pregnancies occurred in men with improved SPA while 17 occurred in men where abnormal penetration persisted. Doxycycline did not seem to improve the SPA results (9 of 17 improved with clomiphene alone while 5 of 21 improved with clomiphene and doxycycline).

A life table analysis of all clomiphene-treated men, versus couples receiving AID, versus untreated men is seen in Table 3 and is illustrated in Figure 1.



**Figure 1** Cumulative probability of pregnancy as determined by life table analysis in couples with unexplained infertility treated by AID versus clomiphene citrate therapy of the male versus no additional therapy<sup>14</sup>

## DISCUSSION

Wang *et al.* published a study suggesting that clomiphene citrate does not improve the zona-free hamster penetration in men with idiopathic oligospermia<sup>9</sup>. Though 14 of 38 men did improve from subnormal penetration to normal penetration in our study we did not provide any untreated controls

**Table 3** Cumulative probability of pregnancy as determined by life table analysis in couples with unexplained infertility treated by AID versus clomiphene citrate therapy of the male versus no additional therapy

No. of cycles	No. of patients	Pregnancies achieved	Lost to follow-up	Patients months of treatment	Pregnancy rate per month	Cumulative probability of pregnancy
<i>Clomiphene</i>						
1	38	3	0	36.50	0.08	0.08
2	35	4	0	33.00	0.12	0.19
3	31	7	0	27.50	0.25	0.40
4	24	5	0	21.50	0.23	0.54
5	19	2	0	18.00	0.11	0.59
6	17	1	0	16.50	0.06	0.61
		<u>22</u>				
<i>No therapy</i>						
1	46	0	0	46.00	0.00	0.00
2	46	1	0	45.50	0.02	0.02
3	45	2	0	44.00	0.05	0.07
4	43	2	0	42.00	0.05	0.11
5	41	1	0	40.50	0.02	0.13
6	40	1	0	39.50	0.03	0.15
		<u>7</u>				
<i>Donor</i>						
1	16	3	0	14.50	0.21	0.21
2	13	2	0	12.00	0.17	0.34
3	11	1	0	10.50	0.10	0.40
4	10	1	0	9.50	0.11	0.46
5	9	2	0	8.00	0.25	0.60
6	7	1	0	6.50	0.15	0.66
		<u>10</u>				

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where the SPA assay was repeated. Thus our data cannot refute Wang's statement since it is possible that time alone may result in improvement in some men's SPA results, the same as some sperm counts may improve spontaneously in time. The improvement, however, should not have been due to problems with internal consistency of the SPA since potential variables were consistently controlled<sup>10</sup>.

Since only 5 of 14 (35%) men with improved to normal zona-free hamster penetration test achieved a pregnancy as compared to 17 of 24 (70%) with men who did not improve their SPA, we conclude that the apparent improved fertility in men with normal spermograms but poor SPA results is not related to improving the zona-free hamster ova penetration but through some other unknown mechanism. This statement is further supported by the fact that so far six men with abnormal SPA results without therapy who failed to achieve a pregnancy during the 8 months of the study have been placed on clomiphene citrate and three of six so far have conceived in the 8 months.

One very important aspect of this study was that the women were meticulously evaluated including very sophisticated investigation of slight aberrations in ovulation, so that we feel that the improved fertility reflects the exclusive improvement of some factor increasing the sperm's ability to fertilise the ova. Some studies, by not controlling so well other factors in the women, may not show the benefit of clomiphene in improving male infertility since a female factor may be missed.

When one sees a subnormal spermogram an SPA test is not needed since the male infertility has been established. All men in the study had to have at least a low normal spermogram to be included. When given the choice of clomiphene citrate it was explained to the men that this would be experimental in that there was no data as yet to suggest that the drug would improve the SPA. In fact, it was explained that clomiphene citrate for male infertility has not been approved by the Food and Drug Administration. They were advised that there was some data suggesting an improvement in oligospermia and asthenospermia following clomiphene therapy<sup>2-4</sup> but that there have also been reports that it is not effective<sup>11</sup>.

The average age of the men with poor SPA tests treated with clomiphene was 34.3 years and that of the women was 32.2 years, and they averaged 4.2 years of infertility. The average age of the untreated men with normal SPA results was 33.5 years and their wives 31.8 years and they averaged 3.6 years of infertility. We feel that the groups turned out fairly close as far as age and duration of infertility and if there was any slight bias it was against the group of men with poor SPA results. Antisperm immunoglobulin was not measured in this study. Though there is evidence that this can inhibit sperm penetration, once again if any bias occurs it would disfavour the men with poor SPA results since no immunotherapy was given<sup>12</sup>. Since no hamster ova penetration was performed unless there was at least 8 months of all female factors correct, the distribution of associated female problems should not influence the results.

There has been some evidence that doxycycline can improve the SPA<sup>13</sup>. We mentioned this study to all men with poor SPA assays, explained the side-effects and gave them the option of clomiphene or clomiphene and doxycycline. 21 of 38 men chose to add the doxycycline but this did not

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either improve the SPA results or improve the percentage of conceptions as compared to the group receiving clomiphene alone.

Thus, our results indicate that the human sperm/hamster ova penetration test may not prove that useful in deciding on treating the male in unexplained infertility. Rather the data suggest that either empiric therapy of the male with clomiphene or switching to AID might achieve pregnancies regardless of the SPA results. We hope to further support this statement when more data are obtained from clomiphene-treated men with normal SPA results since so far we have only been able to collect data from six men. We caution, however, that despite its use for over 10 years in male infertility, there still are conflicting reports about the efficacy of clomiphene citrate in oligospermia and asthenospermia so that corroboration of this data is needed. We emphasise, that to be meaningful the women must be meticulously evaluated so that an occult female factor does not negatively influence the results.

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### REFERENCES

1. Yanagimachi, R., Yanagimachi, H. and Rogers, B. J. (1976). The use of zona-free animal ova as a test-system for the assessment of the fertilizing capacity of human spermatozoa. *Biol. Reprod.*, **15**, 471-6
2. Paulson, D. and Wacksman, J. (1976). Clomiphene citrate in the management of male infertility. *J. Urol.*, **115**, 73-6
3. Check, J. H. and Rakoff, A. E. (1977). Improved fertility in oligospermic males treated with clomiphene citrate. *Fertil. Steril.*, **28**, 746-8
4. Check, J. H. (1980). Improved semen quality in subfertile males with varicocele-associated oligospermia following treatment with clomiphene citrate. *Fertil. Steril.*, **33**, 423-6
5. Noyes, R. W., Hertig, A. and Rock, J. (1950). *Fertil. Steril.*, **1**, 3-25
6. Hackeloer, B. (1978). Ultrasonic demonstration of follicular development. *Lancet*, **1**, 941
7. Check, J. H., Goldberg, B. B., Kurtz, A., Adelson, H. G. and Rankin, A. (1984). Pelvic sonography to help determine the appropriate therapy for luteal phase defects. *Int. J. Fertil.*, **29**, 156-8
8. Check, J. H., Chase, J. S., Adelson, H. G. and Dietterich, C. (1986). New approaches to the diagnosis and therapy of the luteinized unruptured follicle syndrome. *Int. J. Fertil.*, **30**, 29-32
9. Wang, C., Chan, S. Y., Tang, L. C. and Yeung, K. K. (1985). *Fertil. Steril.*, **44**, 102-5
10. Rogers, B. J., Perrault, S. D., Bentwood, B. J., McCarville, C., Hale, R. W. and Soderdahl, D. (1983). Variability in the human hamster *in vitro* assay for fertility evaluation. *Fertil. Steril.*, **39**, 204-11
11. Charney, C. W. (1979). Clomiphene therapy in male infertility: a negative report. *Fertil. Steril.*, **32**, 551-5
12. Haas, G. G., Ausmanus, M., Culp, L., Tureck, R. W. and Blasco, L. (1985). The effect of immunoglobulin occurring on human sperm *in vivo* on the human sperm/hamster ova penetration assay. *Am. J. Reprod. Immunol. Microbiol.*, **7**, 109-12
13. Berger, R. E., Smith, W. D., Critchlow, C. W., Stenchever, M. A., Moore, D. E., Spadoni, L. R. and Holmes, K. K. (1983). Improvement in the sperm penetration (hamster ova) assay (SPA) results after doxycycline treatment of infertile men. *J. Androl.*, **4**, 126-30

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14. Goldfarb, A. F., Schlaff, S. and Mansi, M. L. (1982). A life-table analysis of pregnancy yield in fixed low-dose menotropin therapy of patients in whom clomiphene citrate failed to induce ovulation. *Fertil. Steril.*, **37**, 639-44