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Correlation Between Semen Parameters and the Hamster Egg Penetration Test (HEPT) Among Fertile and Subfertile Men in Singapore

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Abstract

The objective of this retrospective study was to distinguish between fertile and subfertile men based on their semen parameters and hamster egg penetration test (HEPT) outcome. This study involved 110 subfertile men recruited from an infertility clinic and 48 fertile men attending an antenatal clinic in Singapore. The men were required to donate a semen specimen for semen analysis and HEPT assay. The results indicated that the subfertile group had significantly lower normal sperm morphology according to the Tygerberg strict criteria, and lower progressive motility ($P < .05$). Semen volume, density, HEPT decondensation rate, and sperm penetration index were not significantly different between the 2 groups. Receiver operating characteristic curve analysis indicated that sperm morphology had the highest predictive power of 65.7% with a threshold value of 7%, and progressive motility had a predictive power of 61.8% with a threshold value of 50%. Using the tenth percentile of the fertile population as the cutoff, lower adjusted thresholds of 3% for sperm morphology and 28% for progressive motility were obtained, giving higher positive predictive values of 81.8% and 84.4%, respectively. This study shows that these new cutoff values can be used to screen the general population to identify subfertile men. In contrast, the HEPT proved to be an insensitive and unreliable assay in identifying subfertile males. To our knowledge the comparison of HEPT and semen parameters between subfertile and fertile men has not been previously reported in an Asian population.

Key words: Semen analysis, sperm, sperm function assay, sperm morphology, normal motile count

The hamster egg penetration test (HEPT) is a complex sperm function assay that is based on the capacity of the human spermatozoa to fuse with zona pellucida-free golden hamster oocytes, leading to subsequent decondensation of the sperm nuclei. This screening test is thought to reduce wastage of precious oocytes and costly in vitro fertilization (IVF) cycles.

Several studies have reported good correlation between HEPT scores and IVF rates (Wolf et al, 1983; Ausmanas et al, 1985; Shibahara et al, 1998). On the other hand, there are reports that suggest that the HEPT assay has a poor predictive value of IVF fertilization and pregnancy rates (O'Shea et al, 1993; Rashid et al, 1998). Therefore, previous reports regarding the ability of the HEPT to predict natural human fertility have come to opposing findings. Part of this discrepancy may be due to the many different experimental protocols used in individual laboratories in inducing the acrosome reaction. For example, some protocols involve a short capacitation time with artificial induction using calcium ionophore (Saito et al, 1984; Aitken et al, 1987) or calcium ionophore with or without pentoxifylline (Ford et al, 2001). Other protocols use TEST-yolk buffer (Romano et al, 1998) or exposure to progesterone during the spermatozoa/oocyte coincubation period (Francavilla et al, 2002).

At the Department of Obstetrics and Gynaecology of the Singapore General Hospital, the zona-free HEPT was used as a screening test to simulate an IVF cycle using the patient's husband's spermatozoa. The HEPT protocol with the overnight capacitation system which has been previously described (Wolf et al, 1996) was used in the present study because it is similar to the actual capacitation protocol used in our actual IVF cycles. The calcium ionophore challenge or the test-yolk buffer systems were not employed, as these are not used in real IVF cycles. A poor HEPT result suggested that intracytoplasmic sperm injection (ICSI) was a better option than IVF. However, the threshold value for the assay is difficult to determine, as is apparent from the reported false positive and negative results. In addition, the actual threshold value based on our local population had never truly been established and had been based largely on empirical data. Therefore it was essential to study the HEPT outcome of both the fertile and subfertile populations of local men in order to establish the cutoff predictive value.

In addition, while the laboratory had started using the Tygerberg strict criteria (SC) morphology scoring since early 1999, its predictive value for fertilization capability of spermatozoa has not been fully assessed. The objectives of our study were therefore to compare the semen parameters, including morphology, progressive motility and density, and the zona-free HEPT outcome between fertile and subfertile males, and to determine the parameters which provide good predictive value.

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Study Population

This study involved 158 participants comprising 110 subfertile and 48 fertile men. Male partners of pregnant women attending the Antenatal Clinic at the O&G Centre, Department of Obstetrics & Gynaecology, Singapore General Hospital were approached to participate in the study. The fertile group consisted of men who had successfully impregnated their spouses by natural conception at the time of recruitment (Chia et al, 1998), either within 1 year of attempted conception or having more than 1

child with the youngest child less than 1 year old. In addition, these men had not undergone any surgical procedures related to male infertility, and the couples had never attended any assisted reproductive program.

The subfertile group was recruited from those undergoing IVF or IVF with ICSI procedures at the hospital's IVF unit, the Centre for Assisted Reproduction (CARE), Department of Obstetrics & Gynaecology, Singapore General Hospital. The subfertile group consisted of couples who were unable to conceive despite having unprotected coitus over a period of 1 year. Men who were azoospermic and men whose wives were more than 40 years old were excluded from the study. Prior Institution Review Board approval was given to this study by the hospital's Ethics Committee. All participants had read, understood, and signed a consent form to allow their semen to be used solely for the purpose of this study.

Preparation of Zona-Free Hamster Oocytes

Sexually mature female Golden Syrian hamsters (*Mesocricetus auratus*) were superovulated with 40 IU pregnant mare serum gonadotrophin (Folligon; Intervet, Angers, France) on the morning of day 1 of their estrus cycle. Ovulation was induced with 40 IU human chorionic gonadotropin (hCG) (Chorulon; Intervet) on the evening of day 3. The hamsters were sacrificed 16 to 18 hours post-hCG, their oviducts excised, and the cumulus masses released from the oviducts. The cumulus cells from all the hamsters were mixed and dispersed to free the oocytes by a brief exposure to 0.1% hyaluronidase (Sigma Chemical Co, St Louis, Mo). The zona pellucida was digested using 0.1% trypsin (Sigma). The zona-free metaphase II-oocytes were then quickly but thoroughly rinsed in Biggers, Whitten, and Whittingham medium (BWW; Biggers et al, 1971), supplemented with 8.0 mg/mL human serum albumin (HSA, Fraction V; Sigma) and with an osmolarity of 410 mOsmol/kg.

Seminal Analysis and Sperm Preparation

On day 3 of the stimulation of the hamsters, fresh semen samples from both fertile and subfertile men were collected by masturbation into sterile containers after 2 to 5 days of abstinence and sent to the same laboratory for the semen analysis and HEPT assay. The semen samples were left to liquefy at room temperature for 30 minutes. Prior to HEPT assay, sperm density and progressive motility (rapid, slow, or sluggish) were determined according to the World Health Organization (1999) criteria, using phase-contrast microscopy at 400x magnification. Sperm morphology was performed on Papanicolaou-stained semen smears according to the Tygerberg SC (Kruger et al, 1986; 1988). The total normal motile count (semen volume x % SC morphology x % progressive motility x sperm density) was also calculated and included in the evaluation.

Following initial assessments, the semen samples were washed once in BWW medium. For swim-up, sperm pellets were overlaid with 1.25 mL of BWW and incubated at 37 °C in an atmosphere of 5% CO_2 for 1 hour. After the incubation period, 1.0 mL of the topmost sperm suspension containing highly motile spermatozoa was aspirated and kept at room temperature to capacitate for 16 to 18 hours.

Insemination and Scoring of HEPT Decondensation and Sperm Penetration Index

In the following morning, the concentration of motile spermatozoa was adjusted to 5 million cells/mL and resuspended in 2 50-µL droplets in a 35-mm petri dish. Thirty zona pellucida-free hamster oocytes were distributed for insemination, with 15 oocytes per sperm suspension droplet. Coincubation was carried out for the next 3 hours at 37 °C in an atmosphere of 5% CO_2. The oocytes were then washed by gentle pipetting to remove loosely bound sperm and placed onto the middle of a specially prepared microscope slide with 4 paraffin wax supports. A 22 x 22-mm glass coverslip was

mounted over the wax supports, and then a gentle pressure was applied to compress the oocytes.

A successfully decondensed sperm head appeared as a large clear vacuole with a closely associated tail within an oocyte when observed under phase contrast microscopy (400x magnification). The percentage of HEPT decondensation was calculated as the number of oocytes with at least 1 decondensed sperm head, divided by 30 oocytes multiplied by 100. The sperm penetration index (SPI) was calculated as the mean number of decondensed sperm heads per oocyte penetrated. Cryopreserved donor sperm was used as controls. The means for HEPT decondensation of the controls for intraassay were regularly more than 88%, and the coefficient of variation (CV) was less than 10%. The inter-assay means for HEPT decondensation were more than 86%, with CV of less than 17%.

Statistical Analysis

All statistical analyses were performed with the SPSS package version 10.1 (SPSS Inc, Chicago, Ill). Basic descriptive statistics including means, standard deviation, medians, and ranges were calculated for the fertile and subfertile groups separately. Semen parameters and HEPT values of the 2 groups were compared for statistically significant differences at *P* less than .05 (2-tailed) using the nonparametric Mann-Whitney U test.

The predictive value of the individual semen variables and HEPT outcome to differentiate between the fertile or subfertile status was analyzed using the receiver operating characteristics curve (ROC) analysis. The predictive power for fertility status is the area under the curve (AUC) and is expressed as a percentage. The ROC curves were plotted to compare the diagnostic performance of the semen parameters and HEPT outcome for the prediction of the fertility status of the males.

This study has defined sensitivity or true positive rate as the proportion of men with poor semen parameters (the subfertile group). Specificity or true negative rate is defined as the proportion of men with normal parameters (the fertile group) who are classified as fertile. The threshold values were calculated for optimal sensitivity and specificity. The positive predictive value (+PV) is the proportion or theoretical accuracy of identification of subfertile men with poor semen parameter with respect to the general population. The negative predictive value (−PV) is the proportion or theoretical accuracy of identification of fertile men with normal semen parameter with respect to the general population.

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The descriptive statistics for the semen parameters and HEPT outcomes are presented in Table 1. The mean progressive motility was significantly lower in the subfertile group compared to the fertile group (40.6% vs 47.0%, *P* = .041). The SC morphology and total normal motile count were also significantly lower in the subfertile group (5.6% versus 7.9%, *P* = .003; and 5.8 x 10^6 vs 8.2 x 10^6, *P* = .006, respectively). Semen volume, sperm density, and HEPT decondensation (%) were lower in the subfertile group but were not significantly different from the fertile group (*P* > .05). HEPT SPI was also not significantly different between the 2 groups of men (*P* > .05).

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Table 1. *Semen parameters and HEPT outcome (subfertile men, N=110; fertile men, N=48)**

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When the ROC curve analysis was used to predict fertility status, the SC morphology was found to be the best parameter, with a predictive power of 65.7%, *P* = .002 (95% CI: 56.6% to 74.7%) (Table 2). This was followed by progressive motility 61.8%, *P* = .02 (95% CI: 52.6% to 71.1%). On the other hand, HEPT decondensation (%) and HEPT SPI were not predictive, as the AUCs were only 54.0% and 51.1% (*P* > .05), respectively.

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Table 2. *Predictive test characteristics of semen parameters that significantly predict fertility status**

Based on the results of this study, the optimal cutoff values that identify men as subfertile are 7% for SC morphology, with a sensitivity of 67.3% and a specificity of 60.4%, and 50% for progressive motility, with a sensitivity of 66.4% and a specificity of 50.0% (Table 2).

Although sperm morphology and progressive motility showed good predictive power, the clinical value of semen analysis increased when the parameters were used in combination, that is, the total normal motile count (AUC of 64.1%, *P* = .006, 95% CI: 55.0% to 73.2%). The optimal threshold value for total normal motile count was 4 x 10^6, with a sensitivity of 59.1% and a specificity of 52.1%.

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Of all the parameters studied, the present study showed that the semen parameters that are of diagnostic value in identifying the subfertile male population are sperm morphology according to the Tygerberg SC and progressive sperm motility. In contrast, the high complexity HEPT assay and its SPI are found to be poor predictors, as are semen volume and sperm density.

SC sperm morphology at a threshold of 7% had the best sensitivity and specificity when calculated as its AUC using the ROC curve. This finding is in agreement with the few papers published on the comparison between fertile and subfertile men, in which the calculated threshold for sperm morphology ranged from 4% to 10% (Ombelet et al, 1997; Günlap et al, 2001; Guzick et al, 2001; Menkveld et al, 2001). The predictive power of sperm morphology according to these 4 articles ranges from 66% to 78.2%, similar to our value of 65.7%. The tenth percentile of the fertile population was then used to derive a lower cutoff value of 3% sperm morphology. This resulted in a higher positive predictive value of 81.8%, the theoretical accuracy in identifying a man with subfertility with respect to the general population (Table 3). Using this lower threshold of 3% instead of 7% results in a more accurate classification of the subfertile population by excluding some fertile men who may have normal morphology at the upper limits of the abnormal range. Our finding was comparable with the earlier reports of 2% and 5% (Ombelet et al, 1997; Menkveld et al, 2001).

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Table 3. *Adjusted thresholds based on the tenth percentile of the fertile population**

Apart from sperm morphology, progressive motility was also found to be a fairly good predictor of fertility at the threshold of 50%, with an AUC of 61.8%. In the 4 articles that were mentioned earlier, motility also had a high predictive power, with AUC values ranging from 59% to 79.1% and calculated threshold values between 32% and 52%. When the threshold was set lower, to 28%, using the tenth percentile of the fertile population, the positive predictive value (or theoretical accuracy in identifying a man with subfertility) of 84.4% was obtained and was comparable to the adjusted lower thresholds in 3 of the articles, which were between 20% and 30% for motility.

It was found that the clinical value of semen analysis could actually be further enhanced when semen volume, sperm morphology, density, and progressive motility parameters were combined (Table 2). The total normal motile count gave an AUC value of 64.1% at the threshold of 4.0 x 10^6, compared to 65.7% and 61.8% for sperm morphology and progressive motility, respectively. This threshold of 4.0 x 10^6 was comparable to 3.24 x 10^6, as reported earlier (Ombelet et al, 1997). Using the tenth percentile of the fertile population, a lower threshold value of 0.35 x 10^6 was derived for the total normal motile count, with a positive predictive value of 86.2% (Table 3), as compared to 0.52 x 10^6 in the earlier report.

No other semen parameters were found to differ significantly between the subfertile and the fertile groups. In particular, neither the HEPT decondensation rate (69.8% vs 79.2%) nor the SPI (3.5 vs 3.1) were significantly different. This was a somewhat unexpected finding, as this assay had been offered to patients at our hospital for several years as a screening test for patients before their actual IVF cycle. This routine test was offered because of the good correlation found between the assay and in vitro outcome (Wolf et al, 1983; Ausmanas et al, 1985; Coetzee et al, 1989; Ibrahim et al, 1989; Johnson et al, 1991; Freeman et al, 2001), despite several later opposing findings (Lui and Baker et al, 1992; O'Shea et al, 1993; Rashid et al, 1998). These contrasting findings could have been in due part to the different protocols used in the assay and to the various sperm capacitation times used. Another reason could be that this screening test may not be representative of an actual IVF scenario because the sperm-zona pellucida interaction is totally precluded in the HEPT assay, leading to potentially artificially inflated HEPT decondensation rates and SPI scores in the subfertile group. This suggestion is supported by a study by Lui and Baker (2000) which found that defective sperm-zona pellucida binding and penetration are the major factors of zero or low fertilization rates in IVF, since the zona pellucida selectively acts as a barrier to morphologically abnormal spermatozoa.

In conclusion, only sperm morphology according to the Tygerberg SC, progressive sperm motility, and total normal motile count were found to be the most useful parameters in discriminating the subfertile group from the fertile group of males. Using the tenth percentile values of the fertile population, the adjusted lower thresholds were 3% for sperm morphology, 28% for progressive motility, and 0.35 x 10^6 for total normal motile count, with positive predictive values or theoretical accuracy in identifying a man with subfertility being 81.8%, 84.4%, and 86.2%, respectively. These findings are in concept with those reported from the United States (Guzick et al, 2001), Turkey (Günlap et al, 2001), and Europe (Ombelet et al, 1997; Menkveld et al, 2001). This study sought to determine the reliability of the HEPT assay in comparison with semen analysis. In

contrast, the HEPT was found to be an insensitive and unreliable assay in identifying subfertile men. To our knowledge, the comparison of HEPT and semen parameters between subfertile and fertile men has not been previously reported in an Asian population.

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Footnotes

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