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ORIGINAL ARTICLE

Male Fertility

The hamster egg penetration test may decrease intracytoplasmic sperm injection utilization while maintaining high conventional fertilization rates

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This was a cohort study of *in vitro* fertilization (IVF) subjects at the University of Utah, Salt Lake City (UT, USA) utilizing partner sperm. Cycles where both the hamster egg penetration test (HEPT) and semen analysis were performed within 2 years prior to IVF cycles were stratified into four groups based on a normal or an abnormal HEPT and morphology. The mean conventional and intracytoplasmic sperm injection (ICSI) fertilization rates were calculated in each group. We performed a univariate analysis on the primary outcome comparing clinically interesting subjects. We performed a cost-effectiveness analysis of a policy of HEPT *versus* universal ICSI in couples with an abnormal morphology. Among patients with a normal HEPT, there was no difference in the mean conventional fertilization rates between those with a normal and an abnormal morphology. There was no difference in the mean conventional fertilization rates between subjects with a normal morphology without a hamster test and those with a normal HEPT without a morphology assessment. In 1000 simulated cycles with an abnormal morphology, a policy of HEPT was cost saving compared to universal ICSI, yet produced similar fertilization rates. The HEPT is similar to the World Health Organization edition 5 (WHO-5) morphology in predicting successful conventional fertilization while allowing decreased utilization of ICSI. A policy of HEPT for males with abnormal morphology saves cost in selecting couples for a fertilization method.

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INTRODUCTION

The routine semen analysis remains the cornerstone of the male fertility evaluation. However, male infertility continues to be a significant clinical challenge because some men with normal semen parameters can be infertile, and there is a need for the development of functional sperm assessment tools. The hamster egg penetration test (HEPT) was first developed in the 1970s.[1] In 1976, Yanagimachi and colleagues observed that upon removal of the zona pellucida of hamster ova, the eggs allowed penetration of sperm by other species.[2] This test known as the hamster egg penetration test measures the ability of sperm to undergo capacitation, the acrosome reaction, fusion with the egg membrane, and decondensation within the cytoplasm of the oocyte resulting in the formation of the male pronucleus[1] (**Figure 1**). Several studies have demonstrated that this test is a useful predictor of fertilization in conventional *in vitro* fertilization (IVF). Freeman *et al.*[3] demonstrated that a threshold of 20% of hamster oocytes penetrated had a 98% positive predictive value and a 2% false positive rate in predicting the chances of fertilization of fewer than 50% of oocytes in an IVF cycle with conventional insemination. Similarly, Soffer *et al.*[4] found a positive correlation between percentage of hamster oocytes penetrated and IVF fertilization rate, and there was no fertilization with IVF in 74% of cases with HEPT <20%. However, Ausmanas *et al.*[5] found false positive rates as high as 25% among men whose partners achieved pregnancy in an IVF cycle, and that fertilization and pregnancy with IVF could occur even with 0% hamster oocyte penetration. Assay variability is also a concern, with 14% of men showing significantly different values in two consecutive assays.[3,6,7] A systematic review and meta-analysis found that the HEPT was not adequate for predicting IVF success.[8] While the HEPT provides useful information, it is not commonly utilized today in most IVF clinics.

ICSI is indicated primarily for the treatment of male factor infertility and is also widely used during IVF in men with borderline semen parameters.[9] However, fertile men can have wide variation from one semen analysis to the next and we do not understand if ICSI is necessary for men with borderline semen parameters. Furthermore, ICSI has been associated with a slight increase in imprinting disorders over conventional IVF.[10] It also adds an additional cost to IVF therapy; 1250 US dollars to one IVF cycle at Utah Center for Reproductive Medicine, Salt Lake City (UT, USA). According to the National Assisted Reproductive Technology Surveillance System, 69% of all assisted reproductive technology

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(ART) cycles that were reported from all states within the USA in 2015 utilized ICSI. Morphology is a major component of the semen analysis that is advised by the World Health Organization (WHO).[11] Studies demonstrated that using the 5% threshold for morphology positively predicted IVF success with conventional insemination.[12] The WHO has had several revisions for its strict criteria for the lower limits of normal semen parameters. The most recent one is edition 5 (WHO-5) which uses strict criteria of ≥4% normal forms[11] and many clinicians have adopted this and use it as a cutoff for assigning a method of fertilization.

At the Utah Center for Reproductive Medicine, we utilize the HEPT to determine if ICSI is indicated in the absence of a severe male factor infertility and especially in cases of isolated teratozoospermia. We use a criterion of penetration of 80% of 15 oocytes with greater than two penetrations per egg to assign the individual to conventional microdroplet insemination. If the hamster test of the male sample does not meet the criteria described, the couple would be assigned to ICSI fertilization. Our clinic utilized ICSI in 46% of our cycles in 2015, more than 20% lower than the national average.[13]

Therefore, the objective of our study is to determine if the HEPT compares to WHO-5 morphology in predicting successful conventional fertilization. We hypothesized that in cycles where the male partner's HEPT was normal and morphology was <4%, fertilization rates with conventional insemination would be similar to cycles in which the male partner's morphology was ≥4%. In addition, we aim to determine if it is a cost-effective approach for our patient population.

MATERIALS AND METHODS

Approval was granted by the Institutional Review Board at University of Utah, Salt Lake City (UT, USA) for this retrospective cohort study. The proposal for the study underwent an expedited review by the institutional review board, who determined that the risk of the study to subjects was minimal and approved a waiver of consent and authorization. We included IVF cycles between May 2013 and November 2017. We excluded donor sperm cycles, cycles cancelled prior to egg retrieval, and those with missing data on fertilization method used. For both conventional and ICSI fertilization, the fertilization rate was calculated as the number of two pronuclear zygotes divided by the total number of meiosis II oocytes. Oocyte maturity is not assessed in cycles with conventional insemination until the day following oocyte retrieval. Statistical analysis was performed using STATA statistical software (release 15, StataCorp LLC, College Station, TX, USA). In the large group containing all cycles in which fertilization was attempted, we identified cycles with a failed fertilization regardless of method of fertilization and utilized a Poisson regression to attempt to identify any variables predictive of failed fertilization. Any variable with $P < 0.20$ would be entered into a multivariable regression to identify the most significant variable predictive of failed fertilization.

The mean conventional and ICSI fertilization rates were calculated for each group. We utilized one-way ANOVA to test globally any differences in conventional and ICSI fertilization rates among all possible nine groups (**Figure 2**) and Mann–Whitney U test to compare the differences in the mean fertilization rates between clinically interesting groups. $P < 0.05$ was considered statistically significant. Cycles in which the male partner had both a WHO-5 semen analysis and an HEPT result in the 2 years preceding oocyte retrieval were stratified into four groups: Group 1, HEPT <80% and morphology <4%; Group 2, HEPT ≥80% and morphology <4%; Group 3, HEPT <80% and morphology ≥4%; and Group 4, HEPT ≥80% and morphology ≥4%.

To compare the economic impact of using HEPT in our institution, a decision tree analytic model was constructed, utilizing all cycles in which both an HEPT and WHO-5 morphology had been performed. In cycles with abnormal morphology on semen analysis, a policy of HEPT, with conventional fertilization if the HEPT was normal, was compared to a policy of universal ICSI without HEPT. Couples first undergoing HEPT would accrue the cost of HEPT (460 US dollars) but avoid the cost of ICSI (1250 US dollars) if HEPT was normal. If HEPT was abnormal, couples would accrue the cost of both HEPT and ICSI. The mean fertilization rates under various clinical circumstances were incorporated into the model, including the proportions of cycles with a normal HEPT despite abnormal morphology in our cohort. Cost inputs for the model were derived from our local estimates. Probabilistic sensitivity analysis (PSA) was performed to evaluate parametric uncertainty in the model. Beta distributions for each probability in the model were defined using 95% confidence intervals (95% CIs) and means. These distributions were then incorporated into PSA using second-order Monte Carlo simulations, sampling each variable across its distribution. The PSA was reported as the percentage of simulations, of 1000, in which each strategy was cost saving.[14]

RESULTS

Fertilization rates

Out of 1564 cycles, 302 were excluded due to cycle cancellation, use of donor sperm, or missing data on fertilization outcome (**Figure 2**).

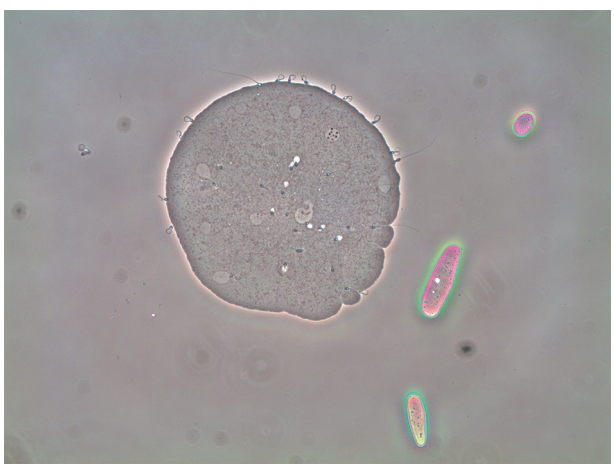

Figure 1: The hamster egg penetration test.

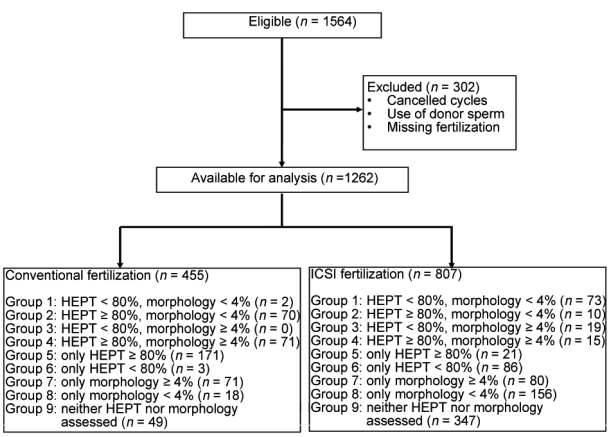

Figure 2: Flowchart of study participants. HEPT: hamster egg penetration test.

Details of the sperm test results and fertilization method are shown in **Table 1**. Of the 1262 cycles initially included, there were 11 cycles with failed fertilization (0.9%). Female age, male age, HEPT, and morphology were not predictive of failed fertilization and therefore no variable could be entered into a multivariable Poisson regression. Of the 11 failed fertilization cycles, 8 (72.7%) occurred as a result of an absence of mature oocytes following oocyte retrieval and 2 (18.2%) were planned for ICSI, but no sperm was present in the ejaculate on the day of oocyte retrieval.

There were 260 cycles where both HEPT and semen analysis were performed within 2 years of the IVF cycle. We present demographic data and mean fertilization rates by group in **Table 2** and **3**, respectively.

WHO-5 morphology versus HEPT in predicting successful conventional fertilization

There were no differences in the mean conventional and ICSI fertilization among all nine possible groups (**Supplementary Table 1**) included in **Figure 2** (P = 0.8184 and 0.1232, respectively). Among cycles with a normal HEPT, there was no clinically significant difference in the mean conventional fertilization rates between those with normal and abnormal morphology (95.8% [95% CI: 93.6%–98.0%] *vs* 91.4% [95% CI: 87.1%–95.8%]; P = 0.4173). Between the 71 cycles with a normal morphology and no HEPT, and the 171 cycles with a normal HEPT and no morphology, there was no difference in the mean conventional fertilization rates between these groups (92.7% [95% CI: 88.4%–97.0%] *vs* 92.5% [95% CI: 89.7%–95.2%]; P = 0.7447). There were 3 (1.8%) cycles with failed conventional fertilization despite a normal HEPT but no morphology, and 2 (2.8%) cycles with failed conventional fertilization despite a normal morphology but no HEPT preventing any clinically meaningful comparison. There were only two subjects with abnormal HEPT and morphology who underwent conventional insemination; therefore, no meaningful comparisons could be made. One cycle with abnormal morphology but a normal HEPT had fertilization failure with conventional fertilization (1.4%).

Table 1: Sperm test results within 2 years of *in vitro* fertilization cycle and method of fertilization

Variables	*HEPT ≥80%*	*HEPT <80%*	*No HEPT*
WHO-5 morphology ≥4%	71 conventional 15 ICSI Group 4	0 conventional 19 ICSI Group 3	71 conventional 80 ICSI
WHO-5 morphology <4%	70 conventional 10 ICSI Group 2	2 conventional 73 ICSI Group 1	18 conventional 156 ICSI
No WHO-5 morphology	171 conventional 21 ICSI	3 conventional 86 ICSI	49 conventional 347 ICSI

WHO-5: World Health Organization edition 5; HEPT: hamster egg penetration test; ICSI: intracytoplasmic sperm injection

Table 2: Demographic information of subjects who had both semen analyses and hamster egg penetration test performed within 2 years of *in vitro* fertilization cycle

Variables	*Group 1*	*Group 2*	*Group 3*	*Group 4*
Subjects (*n*)	75	80	19	86
Male age (year), mean±s.d.	33±7	35±6	34±5	37±7
Female age (year), mean±s.d.	31±6	33±5	33±5	34±5
Oocytes retrieved (*n*), mean±s.d.	15±8	16±8	14±6	13±7
Mature oocytes (*n*), mean±s.d.	11±6	12±5	9±4	10±6

s.d.: standard deviation. Group 1: HEPT < 80%, morphology < 4%; Group 2: HEPT ≥ 80%, morphology < 4%; Group 3: HEPT < 80%, morphology ≥ 4%; and Group 4: HEPT ≥ 80%, morphology ≥ 4%.

Cost-effectiveness calculations for policy of HEPT versus ICSI in men with abnormal morphology

Forty-five percent (70/155) of cycles with abnormal morphology were able to avoid ICSI due to a normal HEPT and have a mean conventional fertilization rate of 91.4%. There were 156 cycles where ICSI was utilized due to an abnormal morphology without a HEPT and these subjects demonstrated a mean fertilization rate of 90.5%. These values were incorporated in our cost-effectiveness model (**Table 4**).

We demonstrated that for couples with abnormal morphology, a policy of HEPT with conventional fertilization in those with normal results is cost saving compared to ICSI without HEPT with an average cost saving of 168.30 US dollars per patient (95% CI: 343.04 US dollars cost savings to 8.20 US dollars additional cost compared to routine ICSI). The base case estimate for per-patient cost was 1081.62 US dollars for HEPT *vs* 1250.00 US dollars for ICSI. HEPT with conventional fertilization if normal led to similar fertilization rates under this policy compared to a policy of universal ICSI without HEPT (fertilization rate 89.7% *vs* 90.5%). In a probabilistic sensitivity analysis, HEPT remained cost-saving compared to universal ICSI in 96.6% of 1000 simulations using second-order Monte Carlo simulation.

DISCUSSION

The absence of a functional sperm assessment tool continues to be a limitation to the treatment of men with borderline semen parameters. Strict sperm morphology is often used to identify couples at risk for poor

Table 3: Fertilization rates by groups where both hamster egg penetration test and World Health Organization edition 5 were performed

Variables	*Group 1*	*Group 2*	*Group 3*	*Group 4*
ICSI fertilization rate (%), mean (95% CI)	88.0 (84.1–91.8)	96.2 (93.0–99.5)	88.9 (82.0 –95.9)	81.9 (72.2–91.7)
Conventional fertilization rate (%), mean (95% CI)	81.3 (44.4–118.1)	91.4 (87.1–95.8)	NA	95.8 (93.6–98.0)
Failed fertilization, *n* (%)	0 (0)	1 (1.3)	0 (0)	0 (0)

95% CI: 95% confidence interval; ICSI: intracytoplasmic sperm injection; NA: not applicable. The definition of Groups 1–4 is the same as that in the **Table 2**.

Table 4: Model input parameters for the cost-effectiveness analysis

Model input	*Base-case estimate, mean*	*95% CI*
Cost of HEPT (US dollar)	460	NA
Cost of ICSI (US dollar)	1250	NA
Mean ICSI fertilization if no HEPT (%)	90.5	88.5–92.4
Mean ICSI fertilization if failed HEPT (%)	88.0	84.1–91.8
Mean conventional fertilization if normal HEPT (%)	91.4	87.1–95.8
Proportion of HEPT pass with abnormal morphology (%)	50.3	38.0–68.0*

*Assumed 95% CI for model. PSA: probabilistic sensitivity analysis; HEPT: hamster egg penetration test; ICSI: intracytoplasmic sperm injection; NA: not applicable; CI: confidence interval

fertilization or fertilization failure during IVF, and ICSI is commonly employed in this setting. This practice has come under scrutiny as a meta-analysis did not demonstrate a significant association between isolated teratozoospermia and a decreased probability of pregnancy with IVF with conventional insemination.[15] While ICSI is generally considered safe and useful for the treatment of male factor infertility, there is insufficient evidence for its utility in men with borderline semen parameters, and in fact, some red flags have been raised. One study utilizing Society for Assisted Reproductive Technology (SART) data between 2004 and 2008 demonstrated that ICSI resulted in lower clinical pregnancy rates than conventional insemination in couples with male factor infertility, but not lower rates of live birth.[16] In addition, animal studies have shown that fertilization with ICSI does not follow the normal chromatin decondensation and histone replacement kinetics as natural fertilization, hence raising concerns that epigenetic reprogramming may be abnormal.[17,18] These epigenetic changes can present as imprinting disorders or may not present until later in life such as increased risk of diabetes and heart disease in offspring produced.[19] In the mouse embryo, there is differential expression of approximately 1000 genes between blastocysts created with ICSI and those conceived naturally, and the implications of these changes are unknown.[20] Not only is ICSI more expensive for patients and third-party payers,[21,22] it requires more medical resources and laboratory time than conventional insemination.[23] Interestingly, the use of ICSI in the United States has increased substantially since 1995 despite the fact that the proportion of patients receiving treatment for male factor infertility has remained stable.[24] This is likely as a result of multiple factors including other indications for the use of ICSI, for example, the uptake of preimplantation genetic testing. However, ICSI for routine use in the absence of a male factor has not been demonstrated to be justified and may even hurt pregnancy rates in an IVF cycle.[25]

The results of our study suggest that using the hamster egg penetration test in couples, we can select couples with an abnormal morphology for conventional insemination and still obtain fertilization success in a cost-effective way. It should be noted that the HEPT is a complex test to perform, requiring ovarian stimulation of hamsters over a 4-day period, with timed sacrifice of the animals and collection of cumulus oocyte complexes from the hamster oviducts under microscopy. The skills required are consistent with those of embryologists performing ICSI and conventional fertilization; thus, technical skill should not be a barrier to test performance in modern embryology laboratories. However, as with any new laboratory assay with a technical component, hands-on training is required to become proficient. For example, removal of the zona is a qualitative step in the assay that can affect the sensitivity of the assay. Correctly mounting the oocytes for observation is critical to interpretation of sperm penetration.

The fertilization failure rate was only 0.9% in our cohort, which is much lower than national averages (typically around 1.0%–3.0%). Also of note is that 72.7% of fertilization failure in our cohort was as a result of a female factor with no mature eggs available for fertilization, while 18.2% were planned ICSI cycles where no sperm was available in the ejaculate at the time of oocyte retrieval. We recognize that the hamster egg penetration test was not originally designed to predict fertilization potential. However, given the large cycle cost borne by couples in states without a mandate to cover fertility treatment, this method can serve as a cost-effective approach to select for ICSI in our patient population without jeopardizing fertilization success. Furthermore, the concerns raised about ICSI in animal studies give reason for caution before routinely adopting ICSI without a clear benefit.

Several limitations of our study should be discussed. The first limitation is the small sample size as it was not unusual to have couples in whom greater than a year passed between their semen analyses and initiating IVF. To ensure that the method of fertilization for the IVF cycle was clinically determined based on the most recent semen analyses, we chose to include only the most recent evaluation performed within 2 years prior to the IVF cycle. Live birth was not assessed in this study, and thus, it is unclear whether results of the HEPT are associated with chances of live birth in IVF. Furthermore, the results of our cost-effectiveness analysis may not be generalizable to institutions in which HEPT and ICSI are priced differently or bundled into a single or universal charge. The expected results may also not be valid for institutions with a different population of IVF patients, as our patients are younger on average than the mean age of women undergoing IVF in the United States and our failed fertilization rate was only 0.9% and mostly as a result of a female factor.

We conclude that the hamster egg penetration test appears similar to WHO-5 morphology in predicting successful conventional fertilization while allowing decreased utilization of ICSI. It also appears to be a reasonable additional test to determine which couples with an abnormal morphology might have a successful conventional fertilization in our population. In addition, a policy of hamster egg penetration test for males with WHO-5 morphology <4% saves cost in selecting couples for conventional fertilization in our patient population without jeopardizing fertilization success. Further studies in other populations are required to evaluate the potential application of the hamster test as a supplemental test to the semen analysis in selecting couples for a fertilization method.

AUTHOR CONTRIBUTIONS

YI contributed to the conception and design of the study, acquisition of data, analysis and interpretation of data, and writing the manuscript. BE contributed to the conception and design of the study, performed the cost-effectiveness analysis and interpretation, and critically reviewed the manuscript. DTC and BRE contributed to the conception and design of the study and acquisition of data and critically reviewed the manuscript. EJ contributed to the conception and design of the study, acquisition of data, interpretation of data, and critical revision of the article. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing interests.

Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

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Supplementary Table 1: Mean conventional fertilization and intracytoplasmic sperm injection fertilization by group with 95% confidence interval

Groups	Conventional fertilization rate (95% CI)	ICSI fertilization rate (95% CI)
1	81.3 (44.4 - 118.1)	88.0 (84.1 - 91.8)
2	91.4 (87.1 - 95.8)	96.2 (93.0 - 99.5)
3	NA	88.9 (82.0 - 95.9)
4	95.8 (93.6 - 98.0)	81.9 (72.2 - 91.7)
5	92.5 (89.8 - 95.2)	84.1 (75.1 - 93.2)
6	95.8 (87.6 - 104.0)	90.1 (86.1 - 94.1)
7	92.7 (88.5 - 97.0)	91.8 (89.1 - 94.6)
8	92.8 (81.9 - 103.8)	90.5 (88.5 - 92.4)
9	92.7 (88.1 - 97.3)	88.8 (87.1 - 90.6)

ICSI: intracytoplasmic sperm injection; NA: not applicable; CI: confidence interval