Epigenetic Sperm Quality Test (SpermQT™)

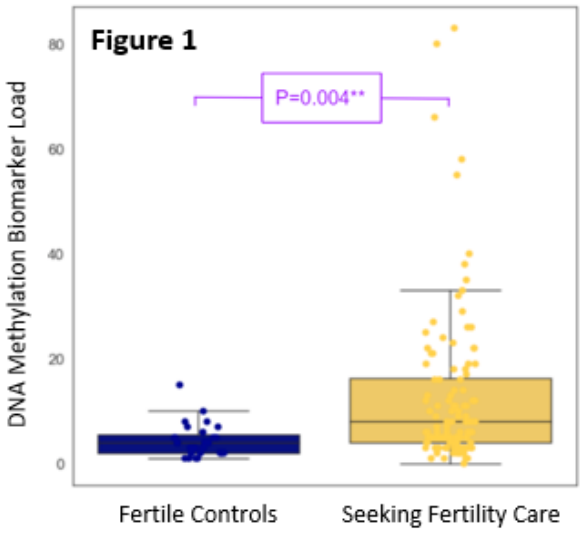
SpermQT™ is a novel DNA Methylation analysis developed by **Path Fertility** that significantly improves the measurement of sperm quality. By detecting poor sperm quality, SpermQT can direct patients to IVF treatment and help avoid unnecessary procedures and loss of precious time.

Highlights:

- Path Fertility's DNA methylation sperm quality test (SpermQT) is a novel assessment of male fertility
- Presence of the SpermQT biomarker is associated with poor fertility outcomes
- IVF shows potential for overcoming decreased sperm quality detected by SpermQT
- SpermQT identifies a male infertility factor that is missed by standard semen analysis

PROBLEM

Better tools are needed for diagnosing male factor infertility. The mainstay of male factor infertility diagnosis is the standard semen analysis (sperm concentration, motility, and morphology). Numerous studies have evaluated the prognostic value of the parameters evaluated by the standard semen analysis and have shown the predictive value of the semen analysis for fertility is modest at best, with the exception of severely diminished sperm count or motility[1]. Current semen analysis only has a 14.8% sensitivity in diagnosing male factor infertility[2]. In cases where there is an absence of female infertility factors and standard semen analysis parameters fall within normal ranges, undiagnosed male factor infertility may be the missing piece.



SOLUTION

Sperm epigenetics as a measure for male factor infertility. Studies have reported abnormal sperm DNA methylation patterns are associated with male factor infertility.[3] DNA methylation is a common epigenetic modification found on DNA where a CH_3 (Methyl) molecule binds to the cytosine base of DNA. This modification changes the expression of genes and is the focus of SpermQT™. **Path Fertility** has identified specific DNA methylation patterns on genes associated with important biological functions such as spermatogenesis (the production and development of sperm) and embryo development. SpermQT™ analyzes the DNA methylation pattern at these genes and assesses if a male patient is at high risk for poor sperm quality.

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[1] Schlegel PN, et al. Diagnosis and Treatment of Infertility in Men: AUA/ASRM Guideline Part I. J Urol. 2021 Jan;205(1):36-43. Barratt CL, et al. Diagnostic tools in male infertility-the question of sperm dysfunction. Asian journal of andrology. 2011;13(1):53-8. Bonde JP, et al. Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. Lancet. 1998;352(9135)

[2] Guzick DS, et al. Sperm morphology, motility, and concentration in fertile and infertile men. NEJM. 2001;345(19):1388-93 (Table 4)

[3] Benchaib M, et al. Quantitation by image analysis of global DNA methylation in human spermatozoa and its prognostic value in in vitro fertilization: a preliminary study. Fertil Steril. 2003;80(4):947-53. Houshdaran S., et al. Widespread epigenetic abnormalities suggest a broad DNA methylation erasure defect in abnormal human sperm. PloS one. 2007;2(12)

EVIDENCE

The initial discovery of a DNA methylation biomarker. DNA methylation analysis of sperm was conducted on 112 men seeking fertility care and 54 men known to be fertile ("fertile controls"). Couples with moderate-to-severe female factor infertility were excluded (including advanced maternal age, severe endometriosis, or polycystic ovarian syndrome). DNA methylation analysis of 10,000 gene promoters showed a statistically significant difference in methylation levels between men seeking fertility care and men known to be fertile (**Figure 1**). Investigation of the sites that were differentially methylated indicated a role in sperm development, sperm maturation, and embryogenesis. Interestingly, 70% of men with this novel DNA methylation biomarker displayed normal concentration and motility semen parameters.

Secondary study with 1,336 semen samples and live birth outcomes. **Path Fertility** analyzed sperm DNA methylation data from 1,336 men who were seeking fertility care in the NIH FAZST Study.[4] A blinded analysis of these samples confirmed the existence of the DNA methylation biomarker described previously and showed a negative correlation with live birth outcomes. The DNA methylation biomarker was present in 10% of all semen samples analyzed, and 77% of those samples displayed normal semen concentration and motility parameters.

Figure 2

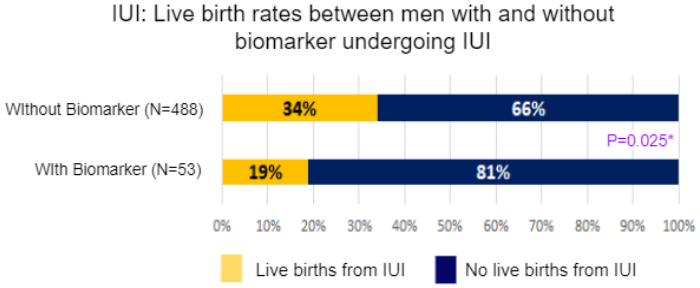
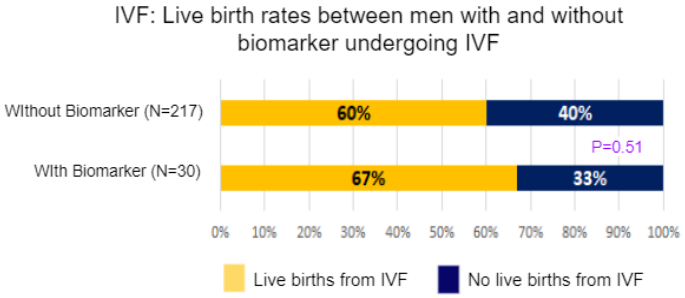


Figure 3



Couples treated with intrauterine insemination (IUI) had a statistically significant lower live birth rate when the DNA methylation biomarker was present in the male partner (**Figure 2**). However, in couples treated with IVF, there was no significant difference in live birth rates (**Figure 3**). This suggests lower sperm quality, as indicated by the DNA methylation biomarker, can be overcome with the use of IVF. <u>If the male partner has the DNA methylation biomarker, there is an 84% positive predictive value of accurately diagnosing the need for IVF.</u>

Taking advantage of this large data set, **Path Fertility** established the DNA methylation biomarker cut offs and derived an analysis algorithm - all analyses moving forward are completed using a fixed algorithm based on the 1,336 semen samples within this confirmatory study.

Tertiary study of the SpermQT biomarker. Applying the fixed analysis pipeline to an independent dataset, **Path Fertility** analyzed the sperm DNA methylation of 74 men seeking infertility care and 60 fertile controls. 38% of men seeking infertility care presented with the DNA methylation biomarker, compared to only 6.5% of the fertile controls. The difference is statistically significant (p=3.8 E-0.6**) and established a negative predictive value of 94.5% for needing IVF.

Further validation of the SpermQT biomarker: In collaboration with Dr. Larry Lipshultz and his urology clinic at Baylor College of Medicine, **Path Fertility** is collecting semen samples from all consenting incoming patients with fertility concerns. To date 78 patient samples have been collected and associated treatment and outcome data are being collected. Of the current patients, 22% present with the DNA Methylation biomarker, of which 73% have normal semen parameters. In analysis of fertility outcomes from non-IVF procedures, <u>0%</u> of the men with the DNA methylation biomarker have had a successful pregnancy while 20% of the men <u>without</u> the biomarker have since had a pregnancy.

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[4] Schisterman EF, et al. A Randomized Trial to Evaluate the Effects of Folic Acid and Zinc Supplementation on Male Fertility and Live birth: Design and Baseline Characteristics. Am J Epidemiol. 2020 Jan 3;189(1):8-26.