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RESEARCH HIGHLIGHT

New genetic markers for male fertility

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Studies in the last years definitively demonstrate the high prevalence of genetic causes of spermatogenic impairment. A large proportion of infertile males does not receive a clear diagnosis and are reported as idiopathic or unexplained, also because standard semen analysis cannot clearly distinguish fertile from infertile populations. Researches aimed at identifying new potential genetic markers of sperm fertilizing ability and male fertility are therefore necessary. Two recent papers addressed these questions and provided interesting results on new possible genetic factors involved in male fertility and as prognostic markers of fertilizing potential of human spermatozoa.

Male infertility represents one of the clearest examples of complex phenotype with substantial genetic basis.[1] Numerous male mouse models, mutation screening and association studies performed in the last few years definitively demonstrate the high prevalence of genetic causes of spermatogenic impairment. Genetic causes account for 10%–15% of severe male infertility, including chromosomal aberrations and single-gene mutations. However, a large proportion of infertile males does not receive a clear diagnosis and are reported as idiopathic or unexplained, reflecting our poor understanding of the basic mechanisms regulating spermatogenesis and sperm function. Indeed, it has been suggested that up to 50% of infertility in humans can be attributed to genetic abnormalities.[1] On the other hand, standard semen analysis, although being able to give information on the probability of conception, cannot clearly distinguish fertile from infertile populations. This is particularly evident in cases of infertility or repeated assisted reproduction failure with normal routine semen parameters. In these cases, abnormal sperm function or unknown molecular defects can be hypothesized, making researches aimed at identifying new potential genetic markers of fertilizing ability and male fertility necessary.

Two recent papers[2,3] addressed these questions and provided interesting results on new possible genetic factors involved in male fertility and as prognostic markers of fertilizing potential of human spermatozoa.

The first study[2] utilized a two-stage strategy to identify candidate genes for male fertility: it first performed a genome wide association study of two fertility traits (family size and birth rate) in 269 Hutterite men, and then significant single nucleotide polymorphisms (SNPs) were analysed in a validation study of 123 ethnically diverse men from Chicago who underwent semen analysis. The Hutterites are a founder population of European descent that represents an ideal population in which to study the genetics of normal human fertility because they proscribe contraception and uniformly desire large families, and in which variation in non-genetic factors that affect reproductive practices is minimized between individuals. As a consequence, the Hutterites are among the most fertile human populations[4,5] with only ~2% childless couples, median sibship size >10 persons and mean interbirth interval <2 years in the 1960s.[4] The study included Hutterite men with at least one child and considered two quantitative measures of fertility: family size (number of births) and birth rate (calculated for couples with two or more children as the number of births per year of marriage). Genotyping of 248 210 autosomal SNPs yielded 61 SNPs located at 28 independent loci with a significant threshold of $\leqslant 10^{-4}$ for family size, 25 SNPs defining 15 loci for birth rate and 4 SNPs in two regions for both phenotypes. The most significant SNPs at each locus (41 SNPs) were then selected and analysed in 123 men (72 Hispanic, 32 African American, 11 Middle Eastern, 5 European, and 3 Asian) for validation and assessment for the functional or clinical effects on semen parameters. This analysis showed that alleles or genotypes for nine of the 41 SNPs that were associated with reduced natural fertility in Hutterites were also associated with reduced measures of sperm count and/or motility in this cohort of men, representing therefore new potential loci for human male fertility. Among them, outstanding candidates for male fertility genes include *USP8*, a gene encoding an essential deubiquitinating enzyme that has a role in acrosome assembly, *UBD* and *EPSTI1* that have a role in innate immunity, and *LRRC32* that encodes a latent transforming growth factor-β receptor of regulatory T cells.

This study[2] therefore identified new autosomal regions and genes with potential roles in male fertility and candidates as infertility genes. The major value of this study resides in the two-step approach and the identification of an ideal population in which the genetics of fertility were studied and two quantitative measures for fertility were performed. Although there are some limitations in this study, including the relative small sample sizes (269 Hutterites and 123 men who provided semen samples) and the identification of SNPs that are near a gene or intronic in a gene but not in exons, future studies will clarify whether more severe mutations in these candidate genes might result in spermatogenic impairment and male infertility and whether they can explain cases of idiopathic male infertility.

The second study[3] aimed at identifying gene markers able to provide information on the fertilizing potential of human spermatozoa in cases in which standard semen analysis does not detect any abnormality. The basic concept was that, in a situation analogous to the male partner in couple with unexplained infertility, some sperm donors for intrauterine insemination (IUI) have low pregnancy rates even with different female recipients. The hypothesis to test was

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that the analysis of the expression of some genes in the sperm could identify genetic markers of the fertilizing potential. To this aim, the authors studied 68 normozoospermic donors (43 for training phase I and 25 for validating phase II) who have been used for a total of 1631 IUI cycles in 545 women during the period 1994–2006, and who differed only for the different pregnancy rate (PR) obtained after IUI: low (0%–14.7% PR), medium (15.7%–23.0% PR) and high (23.0%–45.0% PR). After exclusion of possible confounding factors, 43 randomly chosen donors (frozen–thawed semen samples) were used for expression analysis by quantitative real-time PCR of 95 genes. Only 39 genes obtained good amplification, and 21 of these were further analysed in the 25 validating donors. Main results of the study included the identification of five genes (*RPL23A*, *RPS27A*, *RPS3*, *RPS8* and *TOMM7*) whose expression was significantly reduced in donors with low PR compared to those with high PR. The transcription level of the same genes was also found to have a significant positive correlation with PR of the insemination cycles in which the donor sample was used, and the expression levels of three additional genes (*RPL10A*, *RPS6* and *RBM9*) correlated with miscarriage rate. A multivariate logistic regression analysis showed that a model including four genes (*EIF5A*, *RPL13*, *RPL23A* and *RPS27A*) has good sensitivity (82%) and specificity (90%) for predicting donors with low PR, far better than that obtained from the combination of traditional semen variables (23% sensitivity and 96% specificity).

This study[3] has the merit to contribute to the search for valuable genetic markers which are potentially useful as tools for predicting pregnancy. Although this expression model, if confirmed, could complement classical semen analysis in order to identify sperm donors with a less favourable IUI reproductive outcome, the translation of this information to the general physiology of reproduction or to men with idiopathic infertility is not immediate. Limitations of this study include the low number of subjects studied, the use of frozen–thawed semen samples for expression analysis, the retrospective nature of the analysis and the absence of comparison with new second-step parameters to study sperm quality, such as DNA fragmentation or high-magnification microscopy. Furthermore, the expression level of the identified genes in the sperm is actually very low and the differences in the expression levels between groups, albeit significant, are limited. Furthermore, there are large inter-individual variations that render application of this method to the single case quite difficult. The function of the identified genes is not known and the significance of residual mRNA in the sperm is still speculative.[6] Nevertheless, this report gives the basis for additional studies dealing with the identification of genetic markers of natural fertility, effective spermatogenesis,[7] idiopathic infertility cases with normal standard semen parameters and oligo-astheno-teratozoospermia, and reproductive potential during assisted reproduction technologies, including *in vitro* fertilisation and intracytoplasmic sperm injection cases with repeated implantation failure or pregnancy loss.

Future researches in other populations and in selected cases of infertile males will clarify whether the candidate genes identified in these studies are indeed key regulators of spermatogenesis and whether mutations in their coding regions are responsible for cases of male infertility so far defined as idiopathic. Similarly, deeper investigations will reveal the usefulness of genetic markers identified in these studies in predicting natural pregnancy and reproductive potential during assisted reproduction technologies.

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