Unravelling the genetics of spermatogenic failure

Liesbeth Visser and Sjoerd Repping

Department of Obstetrics and Gynecology, Centre for Reproductive Medicine, Academic Medical Centre, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

Correspondence should be addressed to S Repping; Email: s.repping@amc.uva.nl

Abstract

Subfertility, defined as the inability to conceive within 1 year of unprotected intercourse, affects 10–15% of couples. In up to 55% of couples, the male partner is diagnosed with spermatogenic failure, i.e. one or more semen parameters fall below the WHO criteria for normozoospermia. In these cases, assisted reproductive technology is usually used to achieve pregnancy. Both genetic and environmental factors are thought to underlie spermatogenic failure. Despite years of research, only few genetic factors have clearly been shown to cause spermatogenic failure, and the identification of additional genetic causes or risk factors has proven to be extremely difficult. In this review, we will present an overview of established genetic causes of spermatogenic failure, describe pitfalls in searching for novel genetic factors and discuss research opportunities for the future.

Reproduction (2010) **139** 303–307

Male infertility: introduction to the problem

Subfertility is of major clinical, social and economical concern. In the western world, one in eight couples does not conceive spontaneously within 1 year of unprotected intercourse (de Kretser 1997). In up to 55% of couples seeking medical attention, the male partner is diagnosed with spermatogenic failure, defined as one or more semen parameters falling below the WHO cut-off for normozoospermia (De Kretser & Baker 1999). The prognostic value of this diagnosis is limited, as female parameters also contribute to the couple's (sub)fertility. As semen parameters therefore cannot fully discriminate between fertile and subfertile men, reduced semen parameters are generally interpreted as a reduced chance of spontaneous pregnancy (Bonde *et al.* 1998, Guzick *et al.* 2001, Aitken 2006).

Less than 20 years ago, couples of which the man had azoospermia or severe oligozoospermia were doomed to remain childless. It was the introduction of ICSI that allowed these men to obtain offspring (Palermo *et al.* 1992). Subsequently, this technique was used successfully in men with ejaculatory azoospermia by injecting spermatozoa that were surgically retrieved from the epididymis (through microsurgical sperm aspiration) or testis (by testicular sperm extraction). At the same time, the development of ICSI spurred research into the genetic causes of low semen quality, following questions on the health status of children conceived using ICSI and the risk of transmitting spermatogenic failure to male ICSI offspring (Silber & Repping 2002). Follow-up data of ICSI children at 5 years of age indicate that, compared

with naturally conceived children, children conceived through ICSI have an increased risk of congenital malformations, especially urogenital malformations in ICSI boys (Bonduelle *et al.* 2005).

Besides uncovering genetic causes of spermatogenic failure in light of diagnostic testing and counselling, the ultimate goal of studying spermatogenic failure is to develop a treatment for spermatogenic failure. Currently, the 'treatment' of spermatogenic failure consists of ovarian hyperstimulation of the unaffected female partner, which is both costly and burdensome and with potential unknown long-term side effects. Strikingly, as of today, there are no means to treat spermatogenic failure directly, except for treatment with a dopamine agonist in case of hyperprolactinaemia and treatment with gonadotrophins in case of hypogonadotrophic hypogonadism.

Known causes of spermatogenic failure

Spermatogenesis is governed by the parallel and serial actions of thousands of genes (Sha *et al.* 2002, Schultz *et al.* 2003, Schlecht *et al.* 2004, Ellis *et al.* 2007, Zamudio *et al.* 2008). In theory, alterations in any of these genes or their expression may cause spermatogenic failure. In reality, only a handful of genetic alterations have clearly been shown to cause spermatogenic failure (Nuti & Krausz 2008).

First, numerical and structural chromosomal abnormalities interfere with normal meiosis and can therefore cause spermatogenic failure (Tuerlings *et al.* 1998).

Klinefelter's syndrome (47, XXY) is the most commonly found numerical abnormality. Structural abnormalities that are often encountered include reciprocal translocations, Robertsonian translocations and pericentric inversions (Tuerlings *et al.* 1998, Dohle *et al.* 2002).

Second, five recurrent Y-chromosome deletions, the so-called AZFa, P5/proximal P1 (AZFb), P5/distal P1, b2/b4 (AZFc) and gr/gr deletions, are associated with spermatogenic failure (Reijo *et al.* 1995, Vogt *et al.* 1996, Repping *et al.* 2002, 2003, Visser *et al.* 2009). These deletions all remove multiple genes that are potentially involved in normal human spermatogenesis. It is currently unknown which of these individual genes or any combination thereof play an essential role, and in which dosage (Noordam & Repping 2006).

Third, a number of monogenic disorders, listed in the Online Mendelian Inheritance in Man database, are associated with spermatogenic failure. Among these are Kallmann syndrome and Noonan syndrome. In monogenic disorders, spermatogenic failure results from hypogonadotropic hypogonadism, cryptorchidism, delayed puberty or male pseudohermaphroditism and ambiguous genitalia (Hardelin & Dode 2008). In cystic fibrosis (CF), spermatogenesis is unaffected, but congenital bilateral aplasia of the vas deferens (CBAVD) causes ejaculatory azoospermia. Besides being part of the disorder CF, CBAVD may also present without the other clinical symptoms common to CF, depending on the type of mutation in the CF transmembrane receptor gene. Although monogenic disorders are rare, they should not be overlooked in the workup of the male partner of the subfertile couple (Meschede & Horst 1997).

Apart from these established causes, there is a large body of literature either claiming or refuting the association of certain genetic variants with spermatogenic failure (Matzuk & Lamb 2008). Although the evidence in some of these studies is stronger than in others, the vast majority are single studies and for most of them no subsequent studies by other groups have confirmed the initial findings. Moreover, methodological and biological issues often confound these studies as outlined below.

Methodological issues in the study of the genetics of spermatogenic failure

First, many previous studies have included men based on their (reported) fertility, instead of their semen quality. Case-control association studies frequently included 'subfertile' men as cases and/or 'fertile', 'proven fathers' as controls without knowledge of their semen parameters. Since normozoospermic subfertile cases and oligozoospermic fertile controls are likely to have been included in these studies, no conclusions can be drawn with regard to the association between the genetic variant studied and spermatogenesis. It cannot be stated

too often that subfertile is not the same as oligozoospermic, just as fertile is not synonymous with normozoospermic.

Second, due to the substantial interindividual variability of sperm counts, men may erroneously have been classified as normo- or oligozoospermic when only a single semen sample was used for classification (Tielemans *et al.* 1997, Alvarez *et al.* 2003).

Third, the case-control design in itself may not be the most suited design to study the effect of a putative genetic risk factor on spermatogenesis. Spermatogenesis is a quantitative, continuously distributed trait that shows large intra- and interindividual variation (Table 1). Therefore, a design that dichotomizes sperm counts is unable to detect a leftward shift of the sperm distribution curve, i.e. an overall reduction in sperm count, due to a genetic risk factor (Fig. 1).

Fourth, in many previous case-control studies, cases and controls were unmatched. Drawing cases and controls from different populations, for example using cases drawn from a clinical subfertile population and population samples as controls, may introduce selection bias. Another potential source of selection bias is the different ethnic background of included subjects. How to deal with these 'racial' differences is frequently debated in clinical, epidemiological and molecular research (Ioannidis *et al.* 2004). Some researchers argue that classification of patients according to ethnicity should be abandoned, because there would not be a correlation between common complex diseases and race, and genetic variation would be continuous and discordant with race (Cooper *et al.* 2003). However, there are known racial and ethnic differences in the cause, expression and prevalence of various diseases. Even with similar allele frequencies, i.e. similar genetic variation, the effect of a genetic risk factor may differ between ethnic groups (Burchard *et al.* 2003). In studies on spermatogenesis, geographic and ethnic differences in sperm quality have been reported between men in the Nordic-Baltic area (Jorgensen *et al.* 2002, Tsarev *et al.* 2005). In addition, a possibly differential effect of *POLG* genotype on spermatogenesis was described in men from Dutch Caucasian or African descent (Westerveld *et al.* 2008a).

Table 1 Semen parameters in a cohort of 1014 consecutively included men, part of a subfertile couple, compared to WHO criteria.

Semen parameter	WHO cut-off	Cohort ^a
Volume (ml)	2	3.3 ± 1.5
Concentration (10 ⁶ /ml)	20	52 (16–89)
Motility (% progressive)	25	32 ± 16
Morphology (% normal) ^b	30	37 (25–46)
Total count (10 ⁶)	40	149 (44–281)
Total motile count (10 ⁶)	10	49 (7–110)

^aData are presented as mean ± s.d. or median (25th–75th percentile).

^bAccording to WHO (1992).

Figure 1 Distribution of total sperm counts in an unselected cohort of men (straight line). The sperm count distribution curve is shifted to the left, i.e. towards spermatogenic failure, in men carrying risk factors for spermatogenic failure (dashed lines). The red line indicates the WHO cut off value for normozoospermia.

Biological issues in the study of the genetics of spermatogenic failure

Besides the methodological issues described above, there are also some biological principles that should be taken into account when studying the genetics of spermatogenic failure.

The first issue concerns the biological consequences of the spermatogenic failure phenotype, notably the limited reproductive success of this phenotype. If a genetic locus is strongly linked to spermatogenic failure, the chances are that this locus will not, or scarcely, be spread to the next generation. This greatly hampers the application of linkage analysis, which is preferably carried out on large extended pedigrees. Although familial clustering of subfertility has been reported and segregation analysis has pointed to a heritable background, linkage analysis is unlikely to provide novel genetic loci, simply because spermatogenic failure and large pedigrees are likely to be mutually exclusive (Lilford *et al.* 1994, Gianotten *et al.* 2004).

Another biological issue relates to the characteristics of human spermatogenesis as compared with other mammals, notably rodents. Even though spermatogenesis in mice and men involves similar, highly evolutionary conserved processes, there are also many different processes involved that are likely to be governed by different sets of genes. This may limit the usefulness of another method to search for genetic factors underlying spermatogenic failure, namely the mutation screening of candidate genes derived from mouse knockout models. To date, there are over 400 mouse models with a reproductive phenotype (Matzuk & Lamb 2008). Other studies providing candidate genes are those analysing expression profiles of rodent gametes throughout

gametogenesis (Schultz *et al.* 2003, Schlecht *et al.* 2004, Chalmel *et al.* 2007). Although the generation of a multitude of mouse knockout models is of great value in fundamental research on mammalian fertility and spermatogenesis, the equivalents of artificially induced homozygous knockouts in mouse are indeed not commonly found in humans. In humans, it is usually heterozygous single base pair mutations or small deletions that are detected (Westerveld *et al.* 2005, 2006). Interestingly, in mouse knockout studies, the heterozygous deletion most often does not have a phenotype (O'Bryan & de Kretser 2006). The natural occurrence of heterozygous gene mutations rather than the artificially induced homozygous gene deletions in humans reflects the fact that thousands of genes are involved in spermatogenesis and hence each single mutant is likely to occur spontaneously at extremely low frequency. Another difference between mice and men is that, in contrast to inbred mouse models, non-inbred human populations harbour large variation in environmental and genetic factors that may modulate the effects of genetic variants.

Finally, an enduring complicating factor of studies in the field is the lack of functional assays to assess the biological effect of genetic variants found in men with spermatogenic failure. Such assays are unavailable, either because the function of the encoded protein is unknown, or because there is no suitable cell system to test its function. To date, no human spermatogonial stem cell (SSC) line is available to let alone an *in vitro* system for SSC differentiation. Although such systems are becoming available in rat and mouse, these might not be ideal to study human spermatogenesis as mentioned earlier (van Pelt *et al.* 2002, Kanatsu-Shinohara *et al.* 2003).

Investigating the genetic basis for low semen quality: the way forward

Evidently, all issues discussed above, both methodological as well as biological, should be taken into account when studying the genetic causes of spermatogenic failure. Below we will indicate how to deal with some of these issues.

When using a case-control design, it is a prerequisite that both cases and controls are defined and selected on the basis of their respective semen parameters to avoid bias/confounding that goes with the use of subfertile cases and fertile controls.

As it both avoids the introduction of a possible selection bias and does not require dichotomization of quantitative data, a cohort design is the most powerful to study genetic risk factors. By assembling a large cohort of men with varying sperm counts and comparing the sperm counts of men with and without a genetic variant, the effect of a genetic variant on the distribution of sperm

counts can be observed. Using this design, we were able to clearly refute an association between the CAG repeat in the androgen receptor and the CAG repeat in the polymerase γ -gene and spermatogenic failure (Westerveld *et al.* 2008a, 2008b).

Another way to improve the chances of identifying genes associated with spermatogenic failure is by selecting groups of men with a very homogeneous phenotype, for example isolated asthenozoospermia, isolated teratozoospermia, meiotic arrest or Sertoli cell-only syndrome. It may well be that the phenotypes that are usually studied (azoospermia and oligozoospermia) are too heterogeneous, and actually include men with different underlying (testicular) phenotypes. The advantage of this approach is illustrated by the fact that causal mutations in the *SYCP3* and *SPATA16* genes were found in groups of men with a very homogeneous phenotype, although for *SYCP3* subsequent studies failed to find mutations in this gene (Miyamoto *et al.* 2003, Stouffs *et al.* 2005, Dam *et al.* 2007, Martinez *et al.* 2007).

Apart from the different methodological problems in previous studies, one technical issue applies to all of them. Nearly, all studies studied the effect of a single gene on spermatogenesis. As stated earlier, with thousands of genes involved in spermatogenesis and thus theoretically just as many in spermatogenic failure, chances of identifying individuals carrying one particular causal variant are extremely low. With the advent of high throughput screening methods, thousands of genes can now be studied at the same time. This should open up new opportunities in studying the genetic causes of spermatogenic failure (Aston & Carrell 2009). Besides investigating base pair mutations, attention should also be given to the so-called copy number variants (CNV), DNA segments of 1 kb or larger which are present at variable copy numbers in comparison with a reference genome, including deletions, duplications and insertions (Iafate *et al.* 2004, Sebat *et al.* 2004, Feuk *et al.* 2006). CNVs make up a very large source of structural variation in the human genome and are thought to even outweigh the variation that is contained by single nucleotide polymorphisms (SNPs; Redon *et al.* 2006). Also, whole-genome expression arrays can be used to identify differentially transcribed genes in patients and controls. However, expression array data have to be interpreted with caution since differential expression can simply mirror the presence or absence of specific cell types in the testis rather than provide clues on which genes are involved.

The future of genetic studies in spermatogenic failure

Considering the few genetic factors that have so far been found to cause spermatogenic failure and the exhaustive lists of candidate genes, much work remains to be done. As outlined above, new insights should become available by 1) assembling large cohorts of men with

2) homogenous spermatogenesis-based phenotypes for 3) whole-genome analysis, at the level of SNPs, CNVs and eventually whole-genome sequencing. In addition, much progress can be made by establishing a human SSC culture and *in vitro* differentiation system to study the effects of genetic variants on human spermatogenesis. Hopefully, the findings from these types of studies will eventually lead to treatments for male subfertility.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the work/research reported.

Funding

This work did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Acknowledgements

We thank Prof. Fulco van der Veen for his comments on the manuscript.

References

- Aitken RJ 2006 Sperm function tests and fertility. *International Journal of Andrology* **29** 69–75 (discussion 105–108).
- Alvarez C, Castilla JA, Martinez L, Ramirez JP, Vergara F & Gaforio JJ 2003 Biological variation of seminal parameters in healthy subjects. *Human Reproduction* **18** 2082–2088.
- Aston KI & Carrell DT 2009 Genome-wide study of single nucleotide polymorphisms associated with azoospermia and severe oligozoospermia. *Journal of Andrology* **30** 711–725.
- Bonde JP, Ernst E, Jensen TK, Hjollund NH, Kolstad H, Henriksen TB, Scheike T, Giwercman A, Olsen J & Skakkebaek NE 1998 Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. *Lancet* **352** 1172–1177.
- Bonduelle M, Wennerholm UB, Loft A, Tarlatzis BC, Peters C, Henriët S, Mau C, Victorin-Cederquist A, Van Steirteghem A, Balaska A *et al.* 2005 A multi-centre cohort study of the physical health of 5-year-old children conceived after intracytoplasmic sperm injection, *in vitro* fertilization and natural conception. *Human Reproduction* **20** 413–419.
- Burchard EG, Ziv E, Coyle N, Gomez SL, Tang H, Karter AJ, Mountain JL, Perez-Stable EJ, Sheppard D & Risch N 2003 The importance of race and ethnic background in biomedical research and clinical practice. *New England Journal of Medicine* **348** 1170–1175.
- Chalmel F, Rolland AD, Niederhauser-Wiederkehr C, Chung SS, Demougin P, Gattiker A, Moore J, Patard JJ, Wolgemuth DJ, Jegou B *et al.* 2007 The conserved transcriptome in human and rodent male gametogenesis. *PNAS* **104** 8346–8351.
- Cooper RS, Kaufman JS & Ward R 2003 Race and genomics. *New England Journal of Medicine* **348** 1166–1170.
- Dam AH, Kosciński I, Kremer JA, Moutou C, Jaeger AS, Oudakker AR, Tournaye H, Charlet N, Lagier-Tourenne C, van Bokhoven H *et al.* 2007 Homozygous mutation in *SPATA16* is associated with male infertility in human globozoospermia. *American Journal of Human Genetics* **81** 813–820.
- Dohle GR, Halley DJ, Van Hemel JO, van den Ouwel AM, Pieters MH, Weber RF & Govaerts LC 2002 Genetic risk factors in infertile men with severe oligozoospermia and azoospermia. *Human Reproduction* **17** 13–16.
- Ellis PJ, Furlong RA, Conner SJ, Kirkman-Brown J, Afnan M, Barratt C, Griffin DK & Affara NA 2007 Coordinated transcriptional regulation patterns associated with infertility phenotypes in men. *Journal of Medical Genetics* **44** 498–508.

- Feuk L, Carson AR & Scherer SW 2006 Structural variation in the human genome. *Nature Reviews. Genetics* **7** 85–97.
- Gianotten J, Lombardi MP, Zwiderman AH, Lilford RJ & van der Veen F 2004 Idiopathic impaired spermatogenesis: genetic epidemiology is unlikely to provide a short-cut to better understanding. *Human Reproduction Update* **10** 533–539.
- Guzick DS, Overstreet JW, Factor-Litvak P, Brazil CK, Nakajima ST, Coutifaris C, Carson SA, Cisneros P, Steinkampf MP, Hill JA *et al.* 2001 Sperm morphology, motility, and concentration in fertile and infertile men. *New England Journal of Medicine* **345** 1388–1393.
- Hardelin JP & Dode C 2008 The complex genetics of Kallmann syndrome: KAL1, FGFR1, FGF8, PROKR2, PROK2, *et al.* *Sexual Development* **2** 181–193.
- Iafrate AJ, Feuk L, Rivera MN, Listewnik ML, Donahoe PK, Qi Y, Scherer SW & Lee C 2004 Detection of large-scale variation in the human genome. *Nature Genetics* **36** 949–951.
- Ioannidis JP, Ntzani EE & Trikalinos TA 2004 'Racial' differences in genetic effects for complex diseases. *Nature Genetics* **36** 1312–1318.
- Jorgensen N, Carlsen E, Nermoen I, Punab M, Suominen J, Andersen AG, Andersson AM, Haugen TB, Horte A, Jensen TK *et al.* 2002 East–West gradient in semen quality in the Nordic–Baltic area: a study of men from the general population in Denmark, Norway, Estonia and Finland. *Human Reproduction* **17** 2199–2208.
- Kanatsu-Shinohara M, Ogonuki N, Inoue K, Miki H, Ogura A, Toyokuni S & Shinohara T 2003 Long-term proliferation in culture and germline transmission of mouse male germline stem cells. *Biology of Reproduction* **69** 612–616.
- de Kretser DM 1997 Male infertility. *Lancet* **349** 787–790.
- De Kretser DM & Baker HW 1999 Infertility in men: recent advances and continuing controversies. *Journal of Clinical Endocrinology and Metabolism* **84** 3443–3450.
- Lilford R, Jones AM, Bishop DT, Thornton J & Mueller R 1994 Case–control study of whether subfertility in men is familial. *BMJ* **309** 570–573.
- Martinez J, Bonache S, Carvajal A, Bassas L & Larriba S 2007 Mutations of SYCP3 are rare in infertile Spanish men with meiotic arrest. *Fertility and Sterility* **88** 988–989.
- Matzuk MM & Lamb DJ 2008 The biology of infertility: research advances and clinical challenges. *Nature Medicine* **14** 1197–1213.
- Meschede D & Horst J 1997 The molecular genetics of male infertility. *Molecular Human Reproduction* **3** 419–430.
- Miyamoto T, Hasuike S, Yogev L, Maduro MR, Ishikawa M, Westphal H & Lamb DJ 2003 Azoospermia in patients heterozygous for a mutation in SYCP3. *Lancet* **362** 1714–1719.
- Noordam MJ & Repping S 2006 The human Y chromosome: a masculine chromosome. *Current Opinion in Genetics & Development* **16** 225–232.
- Nuti F & Krausz C 2008 Gene polymorphisms/mutations relevant to abnormal spermatogenesis. *Reproductive Biomedicine Online* **16** 504–513.
- O'Bryan MK & de Kretser D 2006 Mouse models for genes involved in impaired spermatogenesis. *International Journal of Andrology* **29** 76–89 (discussion 105–108).
- Palermo G, Joris H, Devroey P & Van Steirteghem AC 1992 Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet* **340** 17–18.
- van Pelt AM, Roepers-Gajadien HL, Gademan IS, Creemers LB, de Rooij DG & van Dissel-Emiliani FM 2002 Establishment of cell lines with rat spermatogonial stem cell characteristics. *Endocrinology* **143** 1845–1850.
- Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD, Fiegler H, Shaper MH, Carson AR, Chen W *et al.* 2006 Global variation in copy number in the human genome. *Nature* **444** 444–454.
- Reijo R, Lee TY, Salo P, Alagappan R, Brown LG, Rosenberg M, Rozen S, Jaffe T, Straus D, Hovatta O *et al.* 1995 Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. *Nature Genetics* **10** 383–393.
- Repping S, Skaletsky H, Lange J, Silber S, Van Der Veen F, Oates RD, Page DC & Rozen S 2002 Recombination between palindromes P5 and P1 on the human Y chromosome causes massive deletions and spermatogenic failure. *American Journal of Human Genetics* **71** 906–922.
- Repping S, Skaletsky H, Brown L, van Daalen SK, Korver CM, Pyntikova T, Kuroda-Kawaguchi T, de Vries JW, Oates RD, Silber S *et al.* 2003 Polymorphism for a 1.6-Mb deletion of the human Y chromosome persists through balance between recurrent mutation and haploid selection. *Nature Genetics* **35** 247–251.
- Schlecht U, Demougin P, Koch R, Hermida L, Wiederkehr C, Descombes P, Pineau C, Jegou B & Primig M 2004 Expression profiling of mammalian male meiosis and gametogenesis identifies novel candidate genes for roles in the regulation of fertility. *Molecular Biology of the Cell* **15** 1031–1043.
- Schultz N, Hamra FK & Garbers DL 2003 A multitude of genes expressed solely in meiotic or postmeiotic spermatogenic cells offers a myriad of contraceptive targets. *PNAS* **100** 12201–12206.
- Sebat J, Lakshmi B, Troge J, Alexander J, Young J, Lundin P, Maner S, Massa H, Walker M, Chi M *et al.* 2004 Large-scale copy number polymorphism in the human genome. *Science* **305** 525–528.
- Sha J, Zhou Z, Li J, Yin L, Yang H, Hu G, Luo M, Chan HC & Zhou K 2002 Identification of testis development and spermatogenesis-related genes in human and mouse testes using cDNA arrays. *Molecular Human Reproduction* **8** 511–517.
- Silber SJ & Repping S 2002 Transmission of male infertility to future generations: lessons from the Y chromosome. *Human Reproduction Update* **8** 217–229.
- Stouffs K, Lissens W, Tournaye H, Van Steirteghem A & Liebaers I 2005 SYCP3 mutations are uncommon in patients with azoospermia. *Fertility and Sterility* **84** 1019–1020.
- Tielemans E, Heederik D, Burdorf A, Loomis D & Habbema DF 1997 Intraindividual variability and redundancy of semen parameters. *Epidemiology* **8** 99–103.
- Tsarev I, Gagonin V, Giwercman A & Erenpreiss J 2005 Sperm concentration in Latvian military conscripts as compared with other countries in the Nordic–Baltic area. *International Journal of Andrology* **28** 208–214.
- Tuerlings JH, de France HF, Hamers A, Hordijk R, Van Hemel JO, Hansson K, Hoovers JM, Madan K, Van der Blij-Philipsen M, Gerssen-Schoorl KB *et al.* 1998 Chromosome studies in 1792 males prior to intracytoplasmic sperm injection: the Dutch experience. *European Journal of Human Genetics* **6** 194–200.
- Visser L, Westerveld GH, Korver CM, van Daalen SK, Hovingh SE, Rozen S, van der Veen F & Repping S 2009 Y chromosome gr/gr deletions are a risk factor for low semen quality. *Human Reproduction* **24** 2667–2673.
- Vogt PH, Edelmann A, Kirsch S, Henegariu O, Hirschmann P, Kiesewetter F, Kohn FM, Schill WB, Farah S, Ramos C *et al.* 1996 Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Human Molecular Genetics* **5** 933–943.
- Westerveld GH, Repping S, Lombardi MP & van der Veen F 2005 Mutations in the chromosome pairing gene FKBP6 are not a common cause of non-obstructive azoospermia. *Molecular Human Reproduction* **11** 673–675.
- Westerveld GH, Korver CM, van Pelt AM, Leschot NJ, van der Veen F, Repping S & Lombardi MP 2006 Mutations in the testis-specific NALP14 gene in men suffering from spermatogenic failure. *Human Reproduction* **21** 3178–3184.
- Westerveld GH, Kaaij-Visser L, Tanck M, van der Veen F & Repping S 2008a CAG repeat length variation in the polymerase gamma (POLG) gene: effect on semen quality. *Molecular Human Reproduction* **14** 245–249.
- Westerveld H, Visser L, Tanck M, van der Veen F & Repping S 2008b CAG repeat length variation in the androgen receptor gene is not associated with spermatogenic failure. *Fertility and Sterility* **89** 253–259.
- Zamudio NM, Chong S & O'Bryan MK 2008 Epigenetic regulation in male germ cells. *Reproduction* **136** 131–146.

Received 4 June 2009

First decision 13 July 2009

Revised manuscript received 25 August 2009

Accepted 22 September 2009