In search for the general population’s semen profile: the study of sperm parameters in partners of women with chronic anovulation

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Abstract

Background: Human fertility is linked to sperm quality and therefore the establishment of reference values for normality is mandatory.

Aims: The first aim was to establish a reference profile of men in the general population by examining the semen of partners of women with chronic anovulation. The second aim was to determine the prevalence of sperm abnormalities in this patient group.

Methods: Sperm samples of 304 partners of patients with chronic anovulation were analysed prospectively. Semen samples were examined according to WHO guidelines, for sperm morphology Tygerberg strict criteria were used. We compared the results of this study with the cut-off values for normality we obtained in a previous study performed in our centre.

Results: The mean value was 3.1 ml for volume, 64.7 mill / ml for concentration, 51.9% for progressive motility (grade a + b motility) and 7.4% for sperm morphology. Single parameter and double parameter abnormalities were observed in 42.7% and 8.2% of cases respectively. A normal sperm sample for all three parameters was noted in 46% of cases. Oligo-Astheno-Teratozoospermia was present in 3.0% of cases while azoospermia was found in two patients (0.7%).

Conclusion: We believe that the study of sperm parameters in partners of patients with chronic anovulation can be used to study the prevalence of sperm abnormalities in the general population. Our data show that semen abnormalities are not uncommon in partners of women with chronic anovulation, highlighting the importance of a semen examination in every infertility work-up, even in case of obvious female pathology.

Key words: Chronic anovulation, general population, human, semen analysis, sperm morphology.

Introduction

Although the problem of managing male subfertility changed dramatically since the introduction of assisted fertilization (IVF, ICSI), routine semen examination still remains the cornerstone in male subfertility diagnosis, although the usefulness of sperm evaluation in predicting human fertility has often been questioned (Polansky and Lamb, 1988; Bonde et al., 1998; Chia et al., 1998; Jequier, 2005, 2006; Lewis, 2007). The most important reason for the limited usefulness of the semen analysis is that it is not performed optimally in many laboratories (Pacey, 2006). Other factors influencing the value of semen analyses are the inter ejaculatory and geographical differences in semen quality. In the WHO manual (1987, 1999) a graph is shown of 60 consecutive sperm samples of one man. In one sample the concentration peaked to more than 170 mill/ml, in seven cases it fell below 20 mill/ml, the WHO cut-off level. This finding was confirmed in other studies (Mallidis et al., 1991; Alvarez et al., 2003). It is also important to realize that a regional variation in semen quality exists. Many studies have shown evidence of intra- and intercontinental variations (Fisch et al., 1996; Auger and Jouannet, 1997; Jorgensen et al., 2001; Nallella et al., 2006), making it even more difficult to develop male fertility predictors.
Since many decades more sophisticated tests such as sperm function tests, assessment of nuclear and mitochondrial DNA and oxidative stress tests were introduced to increase the accuracy of semen testing, but some of the promising initial data have already been questioned (Lewis 2007; Collins et al., 2008).

From a clinician’s point of view a routine semen examination remains the most important parameter in deciding which treatment will be used. The conventional parameters given most importance are the concentration, progressive motility (grade a + b motility) and sperm morphology. Other popular additional tests are the vitality test or hypo-osmotic swelling test (HOST) and the presence of anti-sperm antibodies (Lewis, 2007).

If semen analyses are used in clinical practice to assess the male fertility potential, the methods for semen examination should be standardized and threshold values for fertility/subfertility should be calculated for the most important semen parameters. Although the WHO (1999) suggests that each laboratory should determine its own reference range for each variable, until today only six studies have been attempted to define threshold values for normality by comparing a fertile with an infertile population in their specific population (Table 1, Ombelet et al., 1997a; Gunalp et al., 2001; Menkveld et al., 2001; Guzick et al., 2001; Haugen et al., 2006; Ho et al., 2007). In most studies the predictive power of sperm parameters was calculated using the ROC (receiver-operating characteristic) curve. Guzick et al. (2001) used the calcification and regression tree (CART) for analysis. By using ROC analysis a cut-off point for normality can be calculated with a relatively high sensitivity and specificity, but with low positive predictive value. According to such threshold values too many men will be classified as subfertile probably leading to unnecessary and costly infertility treatment (van der Merwe et al., 2005). Others have used the 10th percentile of the fertile population as the cut-off value which seems to be more advisable for clinical use since these values have a much higher positive predictive value, with the negative predictive value not much lower compared to the higher cut-off values based on ROC curves. A summary of the different cut-off values published in the literature is shown in Table 1.

Also important but difficult to obtain is the study of the sperm profile in a general population. To select a population not biased by important interfering factors is not easy because the population we are searching for has to be a mirror of the general population during the reproductive period. Using donors of a semen donor insemination program as the reference population for normality is certainly not the best option since this population is positively biased for fertility. Army recruits are biased by age. Partners of tubal factor patients can be biased by a positive history of infection (tubal factor due to pelvic infection) or a good fertility history (women with tubal sterilisation). A good reference group to study semen profiles in a general population are partners of women who have been diagnosed to have chronic anovulation. Patients with chronic anovulation are not infrequently seen in an infertility clinic and it is very easy to convince the partners to deliver a semen sample.

On the other hand, when the diagnosis of chronic anovulation is made during an infertility work-up, it is not uncommon that ovulation induction with timed coitus are offered as a first line treatment without examining a sperm sample. To investigate the prevalence of sperm abnormalities in partners of patients with chronic anovulation was a secondary goal of this prospective study.

Table 1. — Threshold levels for semen parameters in a fertile versus subfertile population using ROC (= receiver-operating characteristic) or CART = calcification and regression tree) or based on the assumed incidences of subfertile male in the respective populations (P10 = percentile 10 of the fertile population).

| First author | Country | Year of Publication | Morphology (% normal) | Progressive motility (%) | Concentration (mill/ml) |
|--------------|---------|---------------------|-----------------------|-------------------------|-----------------------|
| Ombelet (P10)| Belgium | 1997                | 5%                    | 28%                     | 14.3                  |
| Ombelet (ROC)|         |                     | 10%                   | 45%                     | 34                    |
| Gunalp (P10)| Turkey  | 2001                | 5%                    | 30%                     | 9.0                   |
| Gunalp (ROC)|         |                     | 10%                   | 52%                     | 34                    |
| Menkveld (P10)| South Africa | 2001 | 3%                   | 20%                     | 20                    |
| Menkveld (ROC)|         |                     | 4%                    | 45%                     | 20                    |
| Guzick (CART)| USA     | 2001                | 9%                    | 32%                     | 13.5                  |
| Haugen (P10)| Norway  | 2006                | 4%                    | 43%                     | 16.9                  |
| Ho (P10)    | Singapore| 2007                | 3%                    | 28%                     | –                     |
| Ho (ROC)    |         |                     | 7%                    | 50%                     | –                     |
Materials and Methods

All sperm samples of 304 consecutive partners of patients with chronic anovulation were analysed prospectively during a 10-years period from 01-01-1999 until 31-12-2008 at the Genk Institute for Fertility Technology. Chronic anovulation was defined as fewer than 3 menstrual periods per year. Patients were classified according to the WHO classification for anovulation (Figure 1). Patients delivered a fresh semen sample after two days of abstinence. None of the men reported fever or other illnesses during the 8 weeks prior to and during the study. Semen samples were processed within 1 hour of production and liquefaction. Semen analysis was performed according to World Health Organization (WHO, 1992, 1999) guidelines by the same laboratory technician. In our laboratory standardisation of sperm analysis is ensured by participation in internal (monthly) and external quality assessment schemes (organised by the Belgian Scientific Institute of Department of Health). The following fresh WHO semen parameters were studied: volume, concentration, morphology grade a motility, progressive motility (grade a + b), the hypo-osmotic swelling test (HOST) and antisperm antibodies IgG and IgA. Motility was analysed on a wet semen preparation on a glass slide at 37°C, using a fixed volume (14.5 µl for neat semen and 18.5 µl for prepared or washed semen). The grading system described by the WHO (1992, 1999) was implemented: Grade a motile spermatozoa are rapid progressive spermatozoa with a speed of 25 µm/seg; grade b spermatozoa are slow progressive with a speed of 5-24 µm/seg; grade c spermatozoa are non-progressive sperm cells and grade d spermatozoa are immotile spermatozoa. Sperm morphology was scored using strict criteria (Kruger et al., 1986, 1988) on Papanicolau modified stained sperm smears. Sperm concentration was determined using the haemocytometer method on two separate sperm preparations of the semen sample, one for each side of the Neubauer counting chamber.

A hypo-osmotic swelling test was performed in 275 semen samples (Jeyendran et al., 1984). In our search for seminal antisperm antibodies, we performed the modified mixed antiglobin reaction (MAR) for immunoglobulin G and A (SpermMar, Fertipro, Belgium) (Jager et al., 1978; Andreou et al., 1995).

Using the MedCalc programme, summary statistics of different sperm parameters were calculated. The results of this study were compared with the results obtained in a previous study (Ombelet et al., 1997a), using the threshold levels for normality based on the 10th percentile of the fertile population.

The cut-off values used were 14.3 mill/mll for concentration, 28% for progressive motility, 5% for sperm morphology and 48% for HOST. The results were also compared with the WHO (1992) cut-off values. Subsequently the percentage of abnormal results could be examined for all investigated semen parameters in our study population. The study was approved by our local ethical committee.

Results

Three hundred and four different sperm samples of partners with chronic anovulation could be evaluated. The women were classified as WHO class I (hypogonadotrophic hypogonadal anovulation) in 6.9% of cases. The majority of women (83.2%) were WHO class II anovulatory patients, most of them with polycystic ovary syndrome (PCOS). In 9.3% of the studied patients a premature ovarian failure or ovarian resistance could be observed (Table 2, Fig. 1).

Two cases of azoospermia could be detected (0.7%). The mean and median values for the most conventional sperm parameters were the following: 3.1 and 2.8 ml for volume, 64.7 and 57.0 mill/ml for sperm count, 16.2 and 16.0% for grade a motility, 51.9 and 54.0% for progressive motility and 7.4 and 7.0% for sperm morphology (Fig. 2). As mentioned before, we compared the results of this study with the threshold values for normality observed in a previous study using cut-off values for normality based on the 10th percentile of a fertile population (Table 1, Ombelet et al., 1997a). An abnormal result for concentration (Oligozoospermia) and motility (Asthenozoospermia) was shown in respectively 10.2% and 27.3% of the samples. In 29.9% a low sperm morphology score could be found (Teratozoospermia). If we compared our results with the threshold levels described by the WHO (1999), oligozoospermia and asthenozoospermia were found in respectively 13.8 and 55.9% of cases. For sperm morphology all samples (100%) should be classified as teratozoospermic if the WHO cut-off values are used. Table 3 & 4 show the distribution of the different sperm abnormalities. A normal sperm sample for all investigated parameters was noted in 46% of our patients. Figure 3 shows the percentages of the combination of abnormalities. Single parameter abnormalities and double parameter abnormalities were observed in 42.7% and 8.2% of cases respectively. Oligo-astheno-teratozoospermia (OAT) was present in 3.0%.

A subnormal HOST was found in 8.3% and 8.5% of cases using the Genk and WHO cut-off values respectively. A positive MAR for IgG and/or IgA (> 50%) was observed in 6 cases (2.2%).
Discussion

There is general agreement that standardization of semen analysis is useful and essential, but this is where the consensus ends. According to previous reported questionnaires (Helmerhorst et al., 1995; Ombelet et al., 1997b), there is a wide divergence in opinion as to what can be considered as normal semen. Sperm analysis results show a wide range of values for any given sample, most probably not only related to different methodology, but also as a result of persistent errors in laboratory evaluation of sperm samples (Matson, 1995; Ombelet et al., 1997d; Cooper et al., 2002; Pacey, 2006; Lewis, 2007).

An important breakthrough in the history of semen analysis was the publication of the WHO guidelines for semen analysis (WHO, 1980), three times revised during the following twenty years (WHO, 1987, 1992, 1999). The WHO criteria published in 1992 and 1999 were obviously authority based and not based on evidence. Meanwhile, more stringent criteria for sperm morphology assessment known as the Tygerberg strict criteria were introduced by Kruger and Menkveld (Kruger et al., 1986, 1988; Menkveld et al., 1990). When using strict Tygerberg criteria for sperm morphology, many reports showed an excellent association of sperm morphology and fertilization, not only in vitro (Kruger et al., 1986; 1988; Oehninger et al., 1988; Ombelet et al., 1994, 1995) but also in vivo (Ombelet et al., 1997a; 1997c, Van Waart et al., 2001; Lee et al., 2002; Slama et al., 2002). On the other hand, several quality control studies also demonstrated that considerable variation existed with visual evaluation of sperm morphology, not only between but also within different observers (Cooper et al., 1992; Ombelet et al., 1997b; 1997d; Keel et al., 2002; Eustache and Auger, 2003). Identical conflicting results considering the quality of semen examinations was described for all semen parameters in so far that accreditation should be mandatory for all andrology laboratories in order to improve the standards of accuracy and to justify the value of a semen analysis in any clinical situation (Jequier 2005, 2006).

Concerning the study for ‘normal’ ranges for fertile and infertile sperm, Bonde et al. (1998) studied the association between semen quality and the probability of conception in a single menstrual cycle in

| WHO Class | Description |
|-----------|-------------|
| WHO Class I: Hypogonadotropic hypogonadal anovulation |
| Women with low or low-normal serum follicle-stimulating hormone (FSH) concentrations and low serum estradiol concentrations due to decreased hypothalamic secretion of gonadotropin-releasing hormone (GnRH) or pituitary unresponsiveness to GnRH. |
| WHO Class II: Normogonadotropic normoestrogenic anovulation |
| Normal amounts of gonadotropins and estrogens are possible FSH secretion during the follicular phase of the cycle is normal. This group includes women with polycystic ovary syndrome (PCOS). |
| WHO Class III: Hypergonadotropic hypoestrogenic anovulation |
| The primary causes are premature ovarian failure (absence of ovarian follicles due to early menopause) and ovarian resistance (follicular form). |
| Hyperprolactinemic anovulation |
| These women are anovulatory because of hyperprolactinemia. They may have regular anovulatory cycles, but most have oligomenorrhea or amenorrhea. Their serum gonadotropin concentrations are usually normal. |

**Table 2.** — General data of the studied population of 304 couples (WHO Classification of anovulation).

| WHO Class | Number (%) | Mean | Median | Range |
|-----------|------------|------|--------|-------|
| Age Women | 29.7       | 29.0 | 19-45  |
| Age Men   | 32.2       | 31.0 | 20-63  |
| WHO Class I | 21/304 (6.9%) |
| WHO Class II | 253/304 (83.2%) |
| WHO Class III | 30/304 (9.8%) |

Fig. 1. — World Health Organization classification of anovulation
|                          | Range       | Arithmic mean (95% CI)       | Median (95% CI)     | Percentile 5-10-90 |
|--------------------------|-------------|-----------------------------|---------------------|-------------------|
| **Volume (ml)**          | 0.2 - 8.4   | 3.1 (3.0-3.3)               | 2.8 (2.6-3.1)       | 1.0 - 1.4 - 5.2   |
| **Concentration (mill/ml)** | 0.0 - 257.0 | 64.7 (59.8-69.5)            | 57.0 (51.0-65.2)    | 8.0 - 12.8 - 120.0 |
| **Morphology (%)**       | 0.0 - 23.0  | 7.4 (6.9-7.9)               | 7.0 (6.0-8.0)       | 1.0 - 1.5 - 13.5  |
| **Motility grade A (%)** | 0.0 - 49.0  | 16.2 (15.0-17.4)            | 16.0 (14.0-17.0)    | 2.0 - 3.0 - 30.0  |
| **Progressive motility (grade a + b) (%)** | 0.0 – 82.0 | 51.9 (50.4-53.4)            | 54.0 (53.0-56.0)    | 26.7 - 36.9 - 66.0 |
| **HOST (%)**             | 21.0 - 90.0 | 65.1 (63.6-66.6)            | 65.0 (64.0-67.0)    | 42.0 - 50.0 - 80.0 |

**Fig. 2.** — Semen profile results of 304 partners of infertile women with chronic anovulation (CI = Confidence Interval, HOST = hypo-osmotic swelling test).
According to Nallella et al. (2006) sperm motility and concentration provide more accurate information than morphology (WHO and Tygerberg strict criteria) during infertility evaluation. They concluded that redefining the reference values for concentration and morphology may significantly increase the importance of a routine semen analysis. Other studies in search for normal semen ranges for fertile and infertile men used many different population groups such as “presumed fertile” (Fish et al., 1996; Saidi et al., 1999), pregnant wives (Chia et al., 1998; Swan, 2006) or spontaneous pregnancy (Iwamoto et al., 2006; Gao et al., 2008).

Until now, only six studies succeeded to find threshold values and to discriminate between fertile and infertile men by comparing a fertile and infertile population (Table 1, Ombelet et al., 1997a; Gunalp et al., 2001; Guzik et al., 2001; Menkveld et al., 2001; Haugen et al., 2006; Ho et al., 2007).

Table 3. — Characteristics of sperm abnormalities in semen samples of 304 partners of women with chronic anovulation (HOST = hypo-osmotic swelling test, O = Oligozoospermia, A = Asthenozoospermia, T = Teratozoospermia). Thresholds levels for normality are based on the Percentile 10 of a fertile population in the same centre (Ombelet et al., 1997).

| Number | % |
|--------|---|
| No sperm defects - Normozoospermia | 140 | 46.0% |
| Single parameter defect | 128 | 42.7% |
| Oligozoospermia (< 14.3 mill/ml) | 7 | 2.3% |
| Asthenozoospermia (< 28% total motility) | 60 | 19.7% |
| Teratozoospermia (< 5% normal forms) | 61 | 20.0% |
| Double parameter defect | 25 | 8.2% |
| Oligo-Asthenozoospermia | 4 | 1.3% |
| Oligo-Teratozoospermia | 11 | 3.6% |
| Astheno-Teratozoospermia | 10 | 3.3% |
| Triple parameter defect (OAT) | 9 | 2.9% |
| Azoospermia | 2 | 0.7% |
| HOST (< 48%) | 23 / 275 | 8.3% |
| Ig G +/- IgA sperm antibodies > 50% | 6 / 267 | 2.2% |

Table 4. — Individual sperm characteristics in our population. Results were considered abnormal if they were below the thresholds values for normality based on (1) the WHO classification (1999) or (2) the 10th Percentile of a fertile population in the Genk Institute for Fertility Technology (Ombelet et al., 1997a).

| Sperm parameter | Number of men | % of abnormal results |
|-----------------|---------------|-----------------------|
| Volume          |               |                       |
| < 1.5 ml (WHO)  | 31/304        | 10.2%                 |
| < 1.3 ml (Genk) | 20/304        | 6.6%                  |
| Concentration   |               |                       |
| < 20 mill/ml (WHO) | 42/304    | 13.8%                 |
| < 14.8 mill/ml (Genk) | 33/304 | 10.8%                 |
| Progressive Motility |           |                       |
| < 50% (WHO) | 170/304 | 55.9%                 |
| < 28% (Genk) | 89/304 | 29.2%                 |
| Normal Morphology |           |                       |
| < 30% (WHO) | 292/292 | 100.0%                |
| < 5% (Genk) | 91/292 | 31.1%                 |
Although it seems that threshold values for sperm concentration, motility, and morphology can be used to classify men as subfertile, of indeterminate fertility, or fertile, none of the measures, however, are diagnostic of infertility.

To establish semen profiles for men in the general population, Lemcke et al. (1997) analysed the semen parameters of 187 men attending the Institute of Reproductive Medicine between 1977 and 1993 as volunteers for clinical studies. More than half of the ejaculates of these healthy men showed at least one abnormal parameter, so that only 46% of the volunteers could be classified as being ‘normozoospermic’ according to WHO guidelines. They concluded that the limits of normality for semen parameters may require redefinition.

According to a structured review of the literature published on semen parameters and in vivo fertility potential and to establish fertility/subfertility thresholds for sperm morphology using Tygerberg strict criteria, sperm concentration, and sperm motility, van der Merwe et al. (2005) suggested that thresholds of < 5% normal sperm morphology, a concentration < 15 mill / ml and a progressive motility of < 30% should be used to identify the subfertile male.

In our study, sperm parameters were analysed in a reference population of 304 partners of patients with chronic anovulation. We found that individual sperm parameters were below the cut-off value for normality in more than 40% of samples. Sperm morphology turned out to be the most common sperm abnormality in our population with 20% of cases below 5%. According to our results, teratozoospermia and asthenozoospermia are very common in our population. We found a normal sperm sample (concentration > 14.3 mill/ml, progressive motility > 28% and sperm morphology > 5%) in 46% of our patients. Surprisingly, the same figure (46% normozoospermia) was also described in two other studies (Lemcke et al., 1997; Siebert et al., 2007). Oligo-astheno-teratozoospermia was found in 3.0% of cases. Our results highlight the importance of studying the fertility potential of both partners and it seems that a lot of woman conceive with ‘so-called’ subfertile men. On the other hand, in the diagnostic evaluation of couples with women diagnosed as subfertile due to chronic anovulation, the examination of a sperm sample is often neglected although our study proves that an abnormal semen analysis is not infrequently found in this population group.

The clinical significance of antisperm antibodies (ASA) in male subfertility remains unclear. Most studies demonstrate a clear association between sperm surface antibodies and the fertility potential of the male (Adeghe et al., 1988; Hammitt et al., 1988; Acosta et al., 1994; Lombardo et al., 2001). Although Barratt et al. (1992) demonstrated a lack of correlation between low (< 10%) and moderate (< 50%) ASA positive binding cases and the probability of conception or the time to conception, it is generally accepted that male subfertility may be caused by the presence of sperm surface antibodies, at least if a level of more than 50% binding is reached. In our study, severe ASA positive binding (> 50% for IgG and/or IgA) was observed in only six cases (2.2%).

Another interesting semen parameter is the hypoosmotic swelling test (HOST). This parameter has been proposed as a useful assay for the evaluation of the functional competence of the human sperm membranes. However, the usefulness of the hypoosmotic swelling test for evaluation of human sperm quality remains questionable due to conflicting reports (Uchida et al., 1992; van den Saffele et al., 1992; Datta et al., 1996; Tartagni et al., 2002). According to Check et al. (1995), the achievement of pregnancies in vivo is rare in couples where the male partner has defective sperm membranes as shown by...
HOST scores of < 50%. The same author claims that a subnormal hypo-osmotic swelling test does not result in fertilization failure, but implantation failure (Check, 2006). In our study, 8.3% of men had a subnormal HOST-score. It is obvious that more prospective studies in well-defined cohorts of men in various populations are required to evaluate the potential effect of different external (environmental) factors on male reproductive health. Subsequently, it is really important from an epidemiological point of view that the fertility status of different populations are examined and compared. Prevention of male subfertility remains an important goal, but this goal can only be achieved if more information is available on the present fertility potential in different populations.

This paper describes the results of the first study examining the sperm profile in partners of women with chronic anovulation. Because this patients group is unbiased by selection for male fertility prognostic factors, it can be used as a reference group to study sperm quality in the general population. This might prove an important tool in male infertility research. This novel strategy may open the way to better epidemiological studies on sperm quality in the future.

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References

Acosta A, van der Merwe J, Doncel G, Kruger T, Sayilgan A, Franken D, Kolm P. Fertilization efficiency of morphologically abnormal spermatozoa in assisted reproduction is further impaired by antispem antibodies on the male partner’s sperm. Fertil Steril. 1994;62:826-33.

Adeghe J. Male subfertility due to sperm antibodies: a clinical overview. Obstet Gynecol Surv. 1992;48:1-8.

Alvarez C, Castilla JA, Martinez L, Ramírez JP, Vergara F, Gaforio JJ. Biological variation of seminal parameters in healthy subjects. Hum Reprod. 2003;18:2082-8.

Andreu E, Mahmoud A, Vermeulen L, Schoonjans F, Comhaire F. Comparison of different methods for the investigation of antispem antibodies on spermatozoa, in seminal plasma and in serum. Hum Reprod. 1995;10:125-31.

Auger J, Jouannet P. Evidence for regional differences of semen quality among fertile French men. Fédération Francaise des Centres d’Étude et de Conservation des Õufs et du Sperme humains. Hum Reprod. 2003;17:240-5.

Barratt C, Dunphy B, McLeod I, Cooke I. The poor prognostic value of low to moderate levels of sperm surface-bound antibodies. Hum Reprod. 1992;7:95-8.

Bonde JP, Ernst E, Jensen TK, Hjolmund NH, Kolstad H, Henriksen TB, Scheike T, Wiemer J, Olsen J, Skakkebaek NE. Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. Lancet. 1998;352(9135):1172-7.

Check J, Stumpo L, Lurie D, Benfer K, Callan C. A comparative prospective study using matched samples to determine the influence of subnormal hypo-osmotic test scores of spermatozoa on subsequent fertilization and pregnancy rates following in-vitro fertilization. Hum Reprod. 1995;10:1197-1200.

Check JH. The infertile male-diagnosis. Clin Exp Obstet Gynecol. 2006:33:133-9.

Chia SE, Tay SK, Lim ST. What constitutes a normal seminal analysis? Semen parameters of 243 fertile men. Hum Reprod. 1998;13:3394-8.

Collins JA, Barnhart KT, Schlegel PN. Do sperm DNA integrity tests predict pregnancy with in vitro fertilization? Fertil Steril. 2008;89:823-31.

Cooper T, Neuwinger J, Bahrs S, Nieschlag E. Internal quality control of semen analysis. Fertil Steril. 1992;58:172-8.

Cooper T, Bjorndahl L, Vreeburg J, Nieschlag E. Semen analysis and external quality control schemes for semen analysis need global standardization. Int J Androl. 2002;25:306-11.

Datta S, Giri A, Datta A. Role of hypo-osmotic sperm swelling test in assisted reproduction. J Indian Med Assoc. 1996;94:440-2.

Eustache F, Auger J. Inter-individual variability in the morphological assessment of human sperm: effect of the level of experience and the use of standard methods. Hum Reprod. 2003;18:1018-22.

Fisch H, Ikuguchi EF, Goluboff ET. Worldwide variations in sperm counts. Urol. 1996;48:909-11.

Gao J, Gao ES, Walker M, Yang Q, Wu JQ, Zhu QX, Wen SW. Reference values of semen parameters for healthy Chinese men. Urol Int. 2008;81:256-62.

Gunapal S, Onculoglu C, Gurgan T, Kruger T, Lombard C. A study of semen parameters with emphasis on sperm morphology in a fertile population: an attempt to develop clinical thresholds. Hum Reprod. 2001;16:110-4.

Guizick D, Overstreet J, Factor-Litvak P, Brazil C, Nakajima S, Coutifaris C, Carson S, Cisneros P, Steinkampf M, Hill J et al. National Cooperative Reproductive Medicine Network. Sperm morphology, motility, and concentration in fertile and infertile men. N Engl J Med. 2001;345:1388-93.

Hammitt D, Muench M, Williamson R. Antibody binding to greater than 50% of the tail tip does not impair male fertility. Fertil Steril. 1988;49:1.

Haugen TB, Egeland T, Magnus O. Sperm parameters in Norwegian fertile men. J Androl. 2006;27:66-71.

Helmerhorst F, Oei S, Bloemenkamp K, Keirse M. Consistency and variation in fertility investigations in Europe. Hum Reprod. 1995;10:2027-30.

Ho LM, Lim AS, Lim TH, Hum SC, Yu SL, Kruger TF. Correlation between semen parameters and the Hamster Egg Penetration Test (HEPT) among fertile and subfertile men in Singapore. J Androl. 2007;28:158-63.

Iwamoto T, Nozawa S, Yoshikie M, Hoshino T, Baba K, Matsushita T, Tanaka SN, Naka M, Skakkebaek NE, Jørgensen N. Semen quality of 324 fertile Japanese men. Hum Reprod. 2006;21:760-5.

Jager S, Kremer J, van Slochteren-Draaisma T. A simple method of screening for antisperm antibodies in the human male: detection of spermatozoal surface IgG with the direct mixed antoglobulin reaction carried out on untreated fresh human semen. Int J Fertil. 1978:23:12-21.

Jequier AM. Is quality assurance in semen analysis still really necessary? A clinician’s viewpoint. Hum Reprod. 2005;20:2039-42.

Jequier AM. The importance of diagnosis in the clinical management of infertility in the male. Reprod Biomed Online. 2006;13:311-5.

Jeyendran R, Van der Ven H, Perez-Pelaez M, Crabo B, Zaneveld, L. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. J Reprod Fert. 1984;70:219-28.

Jørgensen N, Andersen AG, Eustache F, Irvine DS, Suominen J, Petersen JH, Andersen AN, Auger J, Cawood EH, Horte
A. Jensen TK, Jouannet P, Keiding N, Vierula M, Toppari J, Skakkebaek NE. Regional differences in semen quality in Europe. Hum Reprod. 2001;16:1012-9.

Keel B, Stembridge T, Pineda G, Serafy N. Lack of standardization in performance of the semen analysis among laboratories in the United States. Fertil Steril. 2002;78:603-8.

Kruger T, Menkveld R, Standar F, Lombard C, Van der Merwe J, Van Zyl J, Smith K. Sperm morphologic features as a prognostic factor in in vitro fertilization. Fertil Steril. 1986;46:1118-23.

Kruger T, Acosta A, Simmons K, Swanson R, Matta J, Oehninger S. Predictive value of abnormal sperm morphology in in vitro fertilization. Fertil Steril. 1988;49:112-7.

Lee R, Hou J, Ho H, Hwu Y, Lin M, Tsai Y, Su, J. Sperm morphology analysis using strict criteria as a prognostic factor in intrauterine insemination. Int J Androl. 2002;25:277-80.

Lemcke B, Behre H, Nieschlag E. Frequently subnormal semen profiles of normal volunteers recruited over 17 years. Int J Androl. 1997;20:144-52.

Lewis SE. Is sperm evaluation useful in predicting human fertility? Reproduction. 2007;134:31-40.

Lombardo F, Gandini L, Dondero F, Lenzi A. Antisperm immunity in normal and assisted reproduction. Hum Reprod Update. 2001;7:450-6.

Mallidis C, Howard EJ, Baker HW. Variation of semen quality in normal men. Int J Androl. 1991;14:99-107.

Matson P. External quality assessment for semen analysis and sperm antibody detection: results of a pilot scheme. Hum Reprod. 1995;10:620-5.

Menkveld R, Standar F, Kotze T, Kruger T, Van Zyl J. The evaluation of morphological characteristics of human spermatozoa according to stricter criteria. Hum Reprod. 1990;5:586-92.

Menkveld R, Wong W, Lombard C, Wetzels A, Thomas C, Merkus H, Steegers-Theunissen R. Semen parameters, including WHO and strict criteria morphology, in a fertile and subfertile population: an effort towards standardization of in-vivo thresholds. Hum Reprod. 2001;16:1165-71.

Nallella KP, Sharma RK, Aziz N, Agarwal A. Significance of sperm characteristics in the evaluation of male infertility. Fertil Steril. 2006;85:629-34.

Oehninger S, Acosta T, Kruger T, Vecek L, Flood J, Jones, H.W. Failure of fertilization in in vitro fertilization: The “occult” male factor. J in vitro Fertil Embryo Transfer. 1988;5:181-7.

Ombelet W, Fourie F, LeR, Van de Puthe H, Bosmans E, Cox A, Janssen M, Kruger T. Teratozoospermia and in-vitro fertilization: a randomized prospective study. Hum Reprod. 1994;9:1479-84.

Ombelet W, Menkveld R, Kruger T, Steeno O. Sperm morphology assessment: historical review in relation to fertility. Hum Reprod Update. 1995;1:543-57.

Ombelet W, Bosmans E, Janssen M, Cox A, Vlasselaer J, Gyselaers W, Van de Puthe H, Gielen J, Pollet H, Maes M. et al. Semen parameters in a fertile versus subfertile population: a need for change in the interpretation of semen testing. Hum Reprod. 1997a;12:987-93.

Ombelet W, Pollet H, Bosmans E, Vereecken A. Results of a questionnaire on sperm morphology assessment. Hum Reprod. 1997b;12:1015-20.

Ombelet W, Van de Puthe H, Van de Putte G, Cox A, Janssen M, Jacobs P, Bosmans E, Steeno O, Kruger T. Intrauterine insemination after ovarian stimulation with clomiphene citrate: predictive potential of inseminating motile count and sperm morphology. Hum Reprod. 1997c;12:1458-63.

Ombelet W, Wouters E, Boels L, Cox A, Janssen M, Spierens C, Vereecken A, Bosmans E, Steeno O. Sperm morphology assessment: diagnostic potential and comparative analysis of strict versus WHO criteria in a fertile versus subfertile population. Int J Androl. 1997d;20:367-72.

Pacey AA. Is quality assurance in semen analysis still really necessary? A view from the andrology laboratory. Hum Reprod. 2006;21:1105-9.

Polansky FF, Lamb EJ. Do the results of semen analysis predict future fertility? A survival analysis study. Fertil Steril. 1988;49:1059-65.

Saidi JA, Chang DT, Goluboff ET, Bagiella E, Olsen G, Fisch H. Declining sperm counts in the United States? A critical review. J Urol. 1999;161:460-2.

Siebert TI, van der Merwe FH, Kruger TF, Ombelet W. How to define male subfertility and what is the prevalence in the general population. In Oehninger SC and Kruger TF (eds) Male Infertility. Diagnosis and treatment. Informa UK Ltd, London, UK, 259-76.

Slama R, Eustache F, Ducot B, Jensen T, Jorgensen N, Horte A, Irvine S, Suominen J, Andersen, A, Auger J et al. Time to pregnancy and semen parameters: a cross-sectional study among fertile couples from four European cities. Hum Reprod. 2002;17:503-15.

Swan S, Brazil C, Drobnis E, Liu F, Kruse R, Hatch M, Redmon J, Wang C, Overstreet J. Study For Future Families Research Group. Geographic differences in semen quality of fertile U.S. males. Environ Health Perspect. 2003;111:414-20.

Tartagni M, Schonauer M, Cicinelli E, Selman H, De Ziegler D, Petruzzelli F, D’Addario V. Usefulness of the hypo-osmotic swelling test in predicting pregnancy rate and outcome in couples undergoing intrauterine insemination. J Androl. 2002;23:498-502.

Uchida A, Takahashi K, Kitao M. Usefulness of the hypo-osmotic swelling test for evaluation of human sperm fertilization. Hum Reprod. 1992;7:1264-7.

van den Saffele J, Vermeulen L, Schoonjans F, Comhaire F. Evaluation of the hypo-osmotic swelling test in relation with advanced methods of semen analysis. Andrologia. 1992;24:213-7.

van der Merwe FH, Kruger TF, Oehninger SC, Lombard CJ. The use of semen parameters to identify the subfertile male in the general population. Gynecol Obstet Invest. 2005;59:86-91.

Van Wart J, Kruger T, Lombard C, Ombelet W. Predictive value of normal sperm morphology in intrauterine insemination (IUI): a structured literature review. Hum Reprod. 2001;7:495-500.

World Health Organization. (1980) WHO laboratory manual for the examination of human semen and semen-cervical mucus interaction.1st edn., Press Concern, Singapore.

World Health Organization (1987) WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction, 2nd edn. Cambridge University Press, Cambridge, UK.

World Health Organization (1992) WHO laboratory manual for the examination of human semen and semen-cervical mucus interaction. 3rd edn; Cambridge University Press, Cambridge, UK.

World Health Organization WHO (1999) Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction. 4th edition, Cambridge University Press, Cambridge, UK.