# Status of sperm morphology assessment: an evaluation of methodology and clinical value

Leonie van den Hoven, B.Sc.,[a] Jan C. M. Hendriks, Ph.D.,[b] Jozé G. M. Verbeet, B.Sc.,[a] Johan R. Westphal, Ph.D.,[a] and Alex M. M. Wetzels, Ph.D.[a]

[a] Fertility Laboratory, Department of Obstetrics and Gynaecology, and [b] Department for Health Evidence, Radboud University Medical Centre, Nijmegen, the Netherlands

**Objective:** To characterize methodological changes in sperm morphology assessment and to correlate sperm morphology with clinical outcome.
**Design:** In this observational study, sperm morphology profiles of patients were analyzed. The percentages of morphologically normal spermatozoa were evaluated with respect to changes in morphology assessment criteria; male aging; and prognostic value for outcomes after in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI).
**Setting:** Diagnostic and clinical laboratories.
**Patient(s):** A total of 8,846 men who visited the diagnostic laboratory; 133 samples from a sperm bank; and 3,676 IVF/ICSI couples.
**Intervention(s):** None.
**Main Outcome Measure(s):** The percentage of morphologically normal spermatozoa in semen samples. The regression of the individual morphologically normal cell profiles. The relation between the percentage of normal forms with pregnancy outcome after IVF/ICSI.
**Result(s):** The percentage of morphologically normal spermatozoa showed a decrease from roughly 30%–80% in 1984 to 0%–10% since 2004. With added evidence from sperm bank samples, this decrease was found to be attributable mainly to changes in morphology assessment criteria. Furthermore, an intraindividual aging effect of 0.51% per year was observed. A statistically significant relationship was found between decreases in percentage of normal forms and a lower probability of ongoing pregnancies after IVF, although the area under the curve was only 54%.
**Conclusion(s):** Methodological changes had a strong effect on the percentage of morphologically normal spermatozoa over the past few decades. In addition, male aging results in decreasing sperm morphology. The percentage of morphologically normal spermatozoa has no prognostic value for individual IVF/ICSI patients. (Fertil Steril® 2015;103:53–8. ©2015 by American Society for Reproductive Medicine.)
**Key Words:** Sperm morphology, male aging, prognostic value, IVF, ICSI

**Discuss:** You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/vandenhovenl-sperm-morphology-assessment/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Morphology determination of spermatozoa is a major component of the standard semen analysis that is advised by the World Health Organization (WHO) (1–5). Sperm morphology is usually evaluated on fixed and stained spermatozoa, according to predetermined criteria. These criteria have evolved over the past decades from the so-called "WHO criteria" (1, 2) into the "strict criteria" (3–5). The methodology of applying the strict criteria to sperm assessment was described by Menkveld et al. (6) and was based on studies of sperm in cervical mucus (7). A full background on the strict criteria was reviewed by Mortimer and Menkveld (8). Since the first publication regarding the clinical importance of the strict criteria in 1986 (9), sperm morphology has been studied extensively. In many studies, the fraction of normally shaped spermatozoa was found to be a strong indicator for male fertility classification (e.g., Guzick et al. [10]); successful (i.e., resulting in a live birth) in vitro fertilization (IVF) (reviewed by Coetzee et al. [11]); and/or successful intrauterine insemination (reviewed by Van Waart et al. [12]).

However, in the majority of clinics, sperm morphology is no longer an important factor in clinical decision making, for several reasons (13). First,

Received June 17, 2014; revised September 23, 2014; accepted September 25, 2014; published online October 24, 2014.
L.v.d.H. has nothing to disclose. J.C.M.H. has nothing to disclose. J.G.M.V. has nothing to disclose. J.R.W. has nothing to disclose. A.M.M.W. has nothing to disclose.
Reprint requests: Leonie van den Hoven, B.Sc., Department of Obstetrics and Gynaecology, Radboud University Medical Centre, 791 Fertility Laboratory, P.O. Box 91016500 HB, Nijmegen, the Netherlands (E-mail: Leonie.vandenHoven@Radboudumc.nl).

Fertility and Sterility® Vol. 103, No. 1, January 2015 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2014.09.036

the interpretation of the strict criteria by many laboratories has resulted over time in an increasingly lower percentage of what are considered morphologically normal spermatozoa (14). In our experience, this small range renders appropriate assignment of patients' fertility status virtually impossible.

Second, the introduction of intracytoplasmic sperm injection (ICSI) in 1992 (15) made assisted reproduction available to men (as part of a couple) who have a (very) limited number of spermatozoa in their ejaculate. In these semen samples, the determination of the percentage of morphologically normal spermatozoa is not relevant, as the low number of ejaculated spermatozoa is sufficient to assign the couple to ICSI treatment. Finally, the sperm morphology assay is poorly standardized, making comparison of the results from many laboratories to the WHO reference values impossible (13). As a consequence, in the Netherlands, the decision to assign an infertile couple to either an IVF or an ICSI treatment is currently based mainly on the total progressively motile sperm count (TPMSC) in ejaculate (16).

In this context, we decided to evaluate the performance and clinical significance of sperm morphology determination. First, the pattern of sperm morphology results was followed over time to observe the impact of methodological changes; second, the change in sperm quality within individual men (individual morphology profile) was determined. In addition, the relation between sperm morphology and pregnancy outcomes after IVF or ICSI was investigated to assess its clinical value.

## MATERIALS AND METHODS

### Study Population

The study population was comprised of all patients who visited our laboratory for semen analysis at least twice between January 1, 1986 and July 7, 2011. Multiple measurements of each patient are essential to evaluate the stability of the sperm morphology assessment procedure and determine the relationship of the outcome to changes in fecundity. As sperm morphology is not routinely evaluated in surgically retrieved semen, only data from ejaculated semen samples meant for fertility diagnosis were included in this study.

Furthermore, we used cryopreserved semen samples from patients who refrained from further storage of their semen. These samples, which were cryopreserved between May 17, 1981 and November 22, 2007, were thawed, and sperm morphology was reassessed. The percentage of normal spermatozoa was compared with that in the same samples before cryopreservation.

Finally, to correlate sperm morphology with IVF and ICSI outcome, data regarding positive pregnancy tests, ongoing pregnancies, and abortions after IVF and ICSI treatments were collected. For this part of the study, we used data from the period when morphology assessment methods were more or less stable. Relevant parts of this research were approved by the local ethics committee.

### Semen Analysis

In all cases, semen analysis was performed as described previously (17). Briefly, volume was determined by aspirating the ejaculate with a scaled pipette; sperm concentration was determined by counting in a Makler chamber; and the fraction of progressively motile spermatozoa was determined in a 20-$\mu$m–deep wet preparation. For morphology assessment, a small drop of semen was mixed with an equal amount of aniline blue/eosin solution (consisting of 2 g eosin yellow and 25 g aniline blue [VWR] in 100 ml of phosphate-buffered saline [Gibco-Invitrogen] and 1 ml of ethanol). The mix was spread on a microscopic slide and flame fixed. A total of 200 spermatozoa per slide were evaluated according to the current WHO criteria (1–5) at $\times$1,000 magnification.

This staining method leads to clearly visible spermatozoa with a white nucleus and a transparent acrosome against a blue background. Dead sperm are colored red as a result of staining with eosin. Compared to Papanicolaou and Diff-Quik staining, the sperm size in this method is, respectively, 4.5% bigger and 9.1% smaller (A.M.M. Wetzels, unpublished data). In general, the results of this method in external quality assessments were in line with the reference method ($<2 \times$ SD; reference method: Papanicolaou staining).

### Cryopreserved Semen Samples

Between April 24, 1981 and November 22, 2007, several changes were made in the semen cryopreservation protocol, with regard to cryopreservation medium, the straws used for storage, and the actual freezing procedure. For this study, these changes were presumed to have no effect on sperm morphology. The thawing procedure was the same for all samples. After thawing at room temperature, the semen were washed twice with 5 ml human tubal fluid medium (Lonza) supplemented with 10% human albumin solution (Sanquin) at 600 g for 5 minutes. The final pellet was used to make a semen smear for morphology evaluation.

### IVF and ICSI

In vitro fertilization and ICSI procedures were performed as described elsewhere (18). Patients received an indication for an ICSI treatment when the TPMSC (TPMSC = volume $\times$ concentration $\times$ the fraction of progressively motile sperm) was below $1 \times 10^6$, or in cases of a previous IVF attempt with $<$10% of the oocytes fertilized. Pregnancy tests were performed on day 15 after embryo transfer, using a commercially available urinary kit. Five to 8 weeks later, ongoing pregnancies were routinely confirmed by ultrasound. A positive pregnancy test without an adequate gestational sac was considered to be an abortion.

### Statistical Methods

Initially, differences in the percentages of morphologically normal spermatozoa from January 1, 1986 and July 7, 2011 were studied, using descriptive statistics. To study the individual morphology profiles (i.e., the percentage of morphologically normal semen cells from an individual at consecutive points of measurement), all men were included with at least 3 morphology outcomes in a 7-year period, specifically, from January 1, 2004 to December 31, 2010.