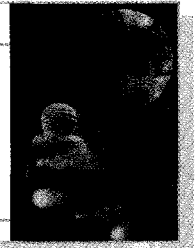


The effect of sperm morphology and testicular spermatozoa on embryo quality



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Objective. To determine the correlation between sperm morphology groups (strict criteria) and testicular spermatozoa, and day 2 and 3 embryo quality in intracytoplasmic sperm injection (ICSI) and *in vitro* fertilisation (IVF) cases.

Methods. A retrospective study was done of 2 402 IVF and ICSI-fertilised embryos classified as good-quality embryos (GOEs) or poor-quality embryos (POEs). Sperm morphology (strict criteria) was classified as teratozoospermia (P-pattern (< 5% normal), G-pattern (5 - 14% normal)), normozoospermia (N-pattern (> 14% normal)), and testicular spermatozoa (immature, only ICSI group).

Results. Sperm morphology (P, G, and N-patterns) and immature testicular sperm had no effect on day 2 or 3 embryo quality for ICSI ($p = 0.82$) and IVF-fertilised ($p = 0.64$) embryos. A significant increase in GOEs from day 2 to 3 in the P-pattern group (33 - 39%, $p = 0.002$) and testicular spermatozoa group (30 - 35%, $p = 0.014$) was found in ICSI cases.

Conclusion. Morphology of human spermatozoa according to Tygerberg's strict criteria and testicular spermatozoa had no predictive value for the outcome of day 2 and 3 embryo quality.

Factors known to negatively influence pregnancy rates are poor sperm morphology and poor embryo quality. Good-quality embryos (GOEs) have been associated significantly with increased pregnancy rates,¹ but reported results evaluating the relationship between sperm morphology and embryo quality are divergent. The questions that arise are whether poor sperm morphology (strict criteria) could be the cause of poor embryo quality, and if immature testicular spermatozoa used in intracytoplasmic sperm injection (ICSI) could also lead to an increase in poor-quality embryos (POEs).

Embryo selection is traditionally done using embryo morphology as a guide² and it has been proved that there is a significant increase in pregnancy rate after transfer of GOEs.^{1,3}

In the case of male infertility, continuous efforts have been made to establish a reproducible and reliable way to assess the fertilising ability of sperm samples. The introduction of strict criteria for sperm morphology assessment by the Tygerberg Hospital Reproductive Biology Unit⁴ was one such effort and is now accepted by the World Health Organization⁵ as the standard evaluation method. Several reports have evaluated the outcome after *in vitro* fertilisation (IVF) in relation to the

morphology of spermatozoa. The results are divergent. Some authors have proposed morphology of spermatozoa as an important, simple and cost-effective predictor of fertilisation, suitable for use in counselling of the couple for either IVF or ICSI.^{6,7} By means of a structured literature review of the IVF situation, Coetzee *et al.*⁷ studied the impact of sperm morphology on fertilisation and pregnancy rates. Results showed that in 92% of the articles evaluated there was a positive association between sperm morphology and IVF success.

However Host *et al.*⁸ have shown that neither the strict Tygerberg criteria nor the WHO criteria correlated with fertilisation rate, embryo development or pregnancies in couples with tubal factor or unexplained infertility undergoing IVF.

Cohen *et al.*⁹ and Parinaud *et al.*¹⁰ found that poor sperm morphology resulted in poor embryo quality in their systems. Embryo quality was influenced by semen quality and especially by sperm head abnormalities, suggesting an important role of the male gamete in the early stages of embryogenesis.¹⁰ In a review, Grow and Oehninger¹¹ also speculated that higher incidences of head abnormalities lead to embryos with a lower pregnancy potential.

Previous studies have indicated that the outcome of ICSI is not related to strict morphology of the sperm used for microinjection.¹² De Vos *et al.*¹³ concluded that individual sperm morphology assessed at the moment of ICSI correlated well with fertilisation outcome but did not affect embryo development.

Some studies also concluded that the fertilising ability and pregnancy results of sperm in ICSI is highest with ejaculated sperm and lowest with sperm extracted by testicular biopsy.¹⁴

The present study was therefore designed to evaluate sperm morphology (percentage of normal forms) among men participating in IVF or ICSI, and then to correlate these findings with embryo quality on day 2 and 3 post-insemination. We also wanted to establish if there was a possible correlation between immature testicular spermatozoa and embryo quality on day 2 and 3 post-injection for the ICSI group.

Materials and methods

A retrospective study was done of 965 IVF and 1 437 ICSI-fertilised embryos (days 2 and 3 post-insemination/injection) ($N = 2\ 402$) at the Reproductive Biology Unit at Tygerberg Hospital, South Africa. Only fertilised metaphase II oocytes (showing 2 pronuclei at ± 18 hours post-insemination/injection) developing into embryos were included in the study. The embryos were classified as GOEs or POEs according to specific criteria. Sperm morphology was assessed according to the Tygerberg strict criteria⁴ and classified into the following groups: (i) teratozoospermia (P-pattern, poor prognosis group (< 5% normal), G-pattern, good prognosis group (5 - 14% normal)); (ii) normozoospermia (N-pattern, normal group (> 14% normal); and (iii) testicular spermatozoa (immature, only ICSI group).

Patient selection

Included in the study were couples qualifying for ICSI, IVF and gamete intrafallopian tube transfer (GIFT) (January 1999 - January 2003), female patients below the age of 38 years, and male patients of all ages including those with teratozoospermia (P and G-patterns), normozoospermia (N-pattern) and azoospermia (immature testicular spermatozoa, ICSI only).

Stimulation protocol and follicle aspiration

The female partners underwent superovulation using a standardised regimen and follow-up procedure. Ovulation was induced by the administration of human chorionic gonadotrophin (HCG) as soon as the leading follicle reached a diameter of 18 mm.

Oocyte aspiration for ICSI and IVF was done 34 - 36 hours after HCG administration, under conscious sedation using transvaginal ultrasound guidance. Oocyte aspiration for GIFT was done laparoscopically and general anaesthesia was therefore administered.

Semen preparation

Motile spermatozoa were isolated by a standard wash and swim-up technique using HEPES-buffered Medicult (Harrilabs, South Africa) sperm preparation medium or by a mini-gradient (95%: 70%: 45%) centrifugation method.

The modified Papanicolaou method for staining the spermatozoa was performed⁵ and sperm cell morphology was assessed according to the strict Tygerberg criteria⁴ in which spermatozoa are scored as normal or abnormal.

Ejaculated, testicular biopsy, cryopreserved ejaculated, and cryopreserved testicular biopsy semen specimens were all included in this study.

ICSI

Retrieved oocytes were incubated (37°C, 6% CO₂ in air) with their cumulus in Sydney IVF fertilisation medium (Cook, Australia) for at least 3 hours until denuding. The cumulus mass was removed 3 - 5 hours post retrieval using 40 IU/ml hyaluronidase (Sigma, Cape Town, South Africa). Metaphase II oocytes were incubated in 50 μ l drops of Sydney IVF fertilisation medium, covered with Medicult paraffin oil, and injected with an immobilised, 'normal'-appearing sperm cell from an ejaculated sperm sample, or with a motile immature testicular sperm cell. Injected oocytes were incubated (37°C, 6% CO₂ in air) in Sydney IVF cleavage medium (Cook, Australia) under paraffin oil. Sequential culture media (fertilisation, cleavage, blastocyst) were used for the different cell stages.

IVF

Metaphase II oocytes were inseminated with 100 000 - 500 000 motile spermatozoa each in NUNC 4-well dishes and incubated in Sydney IVF fertilisation medium.

GIFT

Three or 4 metaphase II oocytes were transferred laparoscopically into the fallopian tube, with between 100 000 and 500 000 motile spermatozoa per oocyte. Supernumerary oocytes were inseminated and incubated exactly as those undergoing IVF. These supernumerary embryos were included in the IVF group for this study.

Embryo culture, grading and transfer

Fertilisation (presence of 2 pronuclei) was noted at 16 - 18 hours post insemination and transferred to fresh Sydney IVF cleavage medium drops. Evaluation at ± 26 hours identified 'early dividing' embryos (division to 2-cell stage). Embryos were evaluated for embryo quality (blastomere morphology, percentage fragmentation and cleavage to the 4- and 8-cell stage) at 48 hours (day 2) and 72 hours (day 3) respectively. Embryo transfer was either at the 4- or 8-cell stage into the fallopian tube or uterus.

Embryo grading (modified from Veeck³) was utilised to determine the embryo quality (morphology) on a scale of 1 - 5 (5 being best) and included blastomere number, blastomere regularity and size and blastomere fragmentation.

The embryos were divided into GOEs and POEs. GOEs were those at the 4- to 5-cell stage at 48 hours post-injection with a morphological grading of 4 or 5, or at the 6- to 8-cell stage, 72 hours post-injection with a morphological grading of 4 or 5. Embryos not adhering to this classification were classified as POEs.

Statistics

Patient details regarding sperm morphology and embryo development were entered into a specially designed Microsoft Access programme for retrospective statistical evaluation. Statistical analysis to establish relationships between sperm morphology and embryo quality was performed using chi-square tests to test the homogeneity of the day 2 and 3 embryo profiles across the morphological groups. Kappa statistics were used to calculate and compare across the morphological groups. All *p*-values reported were based on the assumption of independence between embryos. Values were considered significant when *p* < 0.05. The IVF and ICSI groups were analysed separately. According to the sperm morphology, 3 groups were identified and analysed: P-pattern (< 5% normal), G-pattern (5 - 14% normal), and N-pattern (> 14% normal). A fourth group, testicular spermatozoa (immature, only ICSI group), was also included and analysed.

Results

ICSI group (N = 1 437)

The results showed that 33.1% of embryos (476/1 437) became GOEs and 66.9% (961/1 437) POEs on day 2. There was no significant difference between the percentage of GOEs observed on day 2 across the 3 morphology groups for ICSI patients (35.0%, 30.2% and 31.6% respectively, *p* > 0.05) (Table I).

Two hundred and forty embryos were transferred on day 2 (16.7%, 240/1 437). Of the remaining embryos, 31.0% (445/1 437) were GOEs and 52.3% (752/1 437) were POEs on day 3. There was also no significant difference between the percentage of GOEs observed on day 3 across the 3 morphology groups for ICSI patients (31.1%, 28.8% and 31.8% respectively, *p* > 0.05) (Table II).

There was a significant change in the percentage of GOEs from day 2 to day 3 (33.4% to 39.2%, *p* = 0.002 in the P-pattern group; 29.5% to 35.2%, *p* = 0.014 in the testicular sperm group). The change was not significant in the G-pattern group (29.0% to 35.2%, *p* = 0.09) (Fig. 1).

Table I. Effect of sperm morphology on embryo quality in ICSI-fertilised embryos on day 2

	Day 2 quality		
	Good	Poor	Total
P-pattern			
Count	257	477	734
% within morphology	35.0	65.0	100
G-pattern			
Count	65	150	215
% within morphology	30.2	69.8	100.0
Testicular			
Count	154	334	488
% within morphology	31.6	68.4	100.0
Total			
Count	476	961	1 437
% within morphology	33.1	66.9	100.0

Table II. Effect of sperm morphology on embryo quality in ICSI-fertilised embryos on day 3

	Day 3 quality			
	Good	Poor	Transferred	Total
P-pattern				
Count	228	353	153	734
% within morphology	31.1	48.1	20.8	100.0
G-pattern				
Count	62	114	39	215
% within morphology	28.8	53.0	18.1	100.0
Testicular				
Count	155	285	48	488
% within morphology	31.8	58.4	9.8	100.0
Total				
Count	445	752	240	1 437
% within morphology	31.0	52.3	16.7	100.0

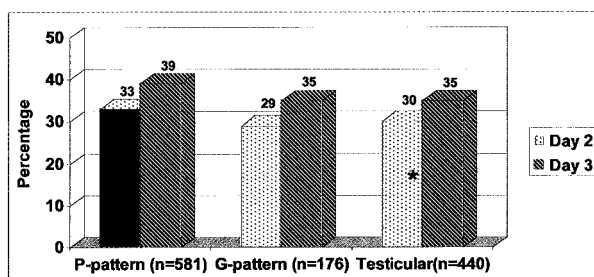


Fig. 1. Percentage of GOEs (ICSI-fertilised) from day 2 to day 3 in the different sperm morphology groups (p* < 0.05).**

IVF group (N = 965)

Results showed that 43.9% of embryos (424/965) became GOEs and 56.1% (541/965) POEs on day 2. There was no difference between the percentage of GOEs observed on day 2 across the 3 morphology groups for IVF patients (46.0%, 43.7% and 37.5% respectively, *p* > 0.05) (Table III).

One hundred and eighty-seven embryos were transferred on day 2 (19.4%; 187/965). Of the remaining embryos, 35.2% (340/965) were GOEs and 45.4% (438/965) were POEs on day 3. There was no difference between the percentage of GOEs observed on day 3 across the 3 morphology groups for IVF patients (42.0%, 33.4% and 34.4% respectively, $p > 0.05$) (Table IV).

There was no significant change from day 2 to day 3 in GOEs in the P-pattern group (42.1% to 49.1%, $p = 0.058$), the G-pattern group (42.5% to 42.2%, $p = 0.932$) and the N-pattern group (46.2% to 42.3%, $p = 1.00$) (Fig. 2).

From this study it is clear that sperm morphology (P, G, and N-patterns) and immature testicular sperm had no effect on day 2 or day 3 embryo quality for ICSI-fertilised and IVF-fertilised embryos.

Also interesting to note was that significantly more IVF embryos were graded as GOEs compared with ICSI embryos (day 2: 43.9% v. 33.1%, $p < 0.0001$; day 3: 43.0% v. 37.2%, $p = 0.0075$, respectively).

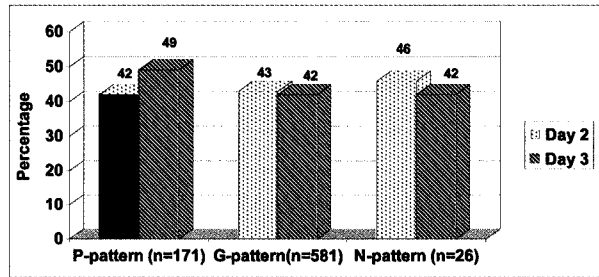


Fig. 2. Percentage of GOEs (IVF-fertilised) from day 2 to day 3 in the different sperm morphology groups.

Discussion

While sperm morphology assessment according to strict Tygerberg criteria has proved useful and predictive of fertilising capacity in conventional IVF, it seems to be less valuable when used for ICSI.¹² Many sperm cells classified as abnormal on the basis of morphological details might not interfere with fertilising capacity once they are introduced into the oocyte through ICSI. Previous studies have indicated that the outcome of ICSI is not related to strict morphology of the sperm used for microinjection.¹² However, De Vos *et al.*¹³ concluded that individual sperm morphology assessed at the moment of ICSI correlated well with fertilisation outcome, but did not affect embryo development.

Loutradis *et al.*¹⁵ and Salumets *et al.*¹⁶ concluded that it is the effect of the oocyte rather than the spermatozoon that influences embryo quality. Oocyte morphology correlates well with embryo quality and pregnancy rates after ICSI.¹⁵ In conventional IVF, oocyte maturity is assessed indirectly and the nuclear maturity and morphological appearance of the oocytes themselves cannot be examined. In ICSI, on the other hand, the morphological structure of the denuded oocytes can be assessed in a more detailed and precise manner.¹⁵ The results of the study by Salumets *et al.*¹⁶ provide compelling evidence that embryo morphology 2 days after insemination is predominantly determined by the properties of the oocyte, whereas the blastomere cleavage rate is simultaneously influenced by both the sperm cell and the oocyte.

The aim of this study was to evaluate the effect of sperm morphology (strict criteria) on embryo quality. The results clearly show that the sperm morphology status was not a factor in embryo quality in either the ICSI or IVF groups. Morphology (Tygerberg's strict criteria) therefore had no predictive value for the outcome of embryo quality as evaluated on either day 2 or day 3. These results are in accordance with those of other groups.^{8,13}

There is little information available on the mechanism whereby the spermatozoon could influence embryo development. It is generally assumed that only maternally produced transcripts and proteins govern the first 2 cell divisions in pre-implantation human embryo development as genes are not expressed until about the 4- to 8-cell stage. Therefore the spermatozoon could affect embryo development only after the activation of the

	Day 2 quality		
	Good	Poor	Total
P-pattern			
Count	92	108	200
% within morphology	46.0	54.0	100.0
G-pattern			
Count	320	413	733
% within morphology	43.7	56.3	100.0
N-pattern			
Count	12	20	32
% within morphology	37.5	62.5	100.0
Total			
Count	424	541	965
% within morphology	43.9	56.1	100.0

	Day 3 quality			
	Good	Poor	Transferred	Total
P-pattern				
Count	84	87	29	200
% within morphology	42.0	43.5	14.5	100.0
G-pattern				
Count	245	336	152	733
% within morphology	33.4	45.9	20.7	100.0
N-pattern				
Count	11	15	6	32
% within morphology	34.4	46.9	18.8	100.0
Total				
Count	340	448	187	965
% within morphology	35.2	45.4	19.4	100.0

embryonic genome. However, spermatozoa are known to carry some epigenetic factors regulating embryo development. The most important cellular contribution of the sperm cell to the zygote is the centrosome. During embryo development the sperm centrosome forms the poles of the mitotic spindle, thereby regulating the first and subsequent cell divisions.¹⁶ Significantly more information about the putative effect of spermatozoa on embryo quality has been obtained from the extended culture of human embryos to the blastocyst stage. It has been ascertained that the blastocyst formation rate as well as blastocyst morphology were significantly lower when sperm with impaired quality were used in fertilisation of oocytes in conventional IVF procedures.¹⁷ Future research should focus on understanding the exact mechanism whereby the oocyte and spermatozoa may influence early embryonic development.

The effect of sperm DNA packaging on early embryonic development is also of importance. Some studies^{18,19} have pointed out the association between impaired sperm morphology and increased DNA damage. Benchaib *et al.*²⁰ found that the proportion of spermatozoa with DNA fragmentation appears to be potentially useful as a predictor of ICSI outcome, whereas embryo quality based on morphological criteria appeared unaffected by DNA fragmentation. Based on the work of De Vos *et al.*¹³ it seems possible that the abnormal spermatozoa have a higher DNA fragmentation rate, leading to poorer fertilisation rates.

Additional data from this study showed a difference between IVF and ICSI when embryo quality progression from day 2 to 3 was calculated. In the ICSI group (Fig. 1) a higher percentage of embryos were of good quality on day 3 compared with day 2 (significant for P-pattern and testicular groups), while in the IVF group (Fig. 2), percentages were similar and not significant. The reason for this result is uncertain. It is possible that ICSI embryos are initially slower to develop during the first 2 days, but 'catch up' on day 3.

Our study also showed a significantly higher percentage of GOE in the IVF group compared with the ICSI group. This result can possibly be explained taking into account how the mechanism of fertilisation and embryo handling differs for the 2 methods. Most ICSI cycles are performed because of poor sperm characteristics (this is not the case in IVF). It is therefore possible to find a higher rate of sperm cells with elevated DNA fragmentation in ICSI than in IVF cycles. Moreover, it is possible that IVF leads to a natural selection of sperm, leading to more GOEs. The selected fertilising spermatozoon will be morphologically normal and highly motile, and is supposed to have an intact DNA. Several authors have shown that in a poor-quality sperm population (according to classic criteria), DNA damage is found at a high level.^{18,19} These spermatozoa would potentially have a reduced chance of fertilising the oocyte in the IVF procedure. The situation is different in ICSI, where the choice of the spermatozoon to be injected is made according to very rough criteria (motile, normal-looking spermatozoon, i.e. those lacking major defects such

as a broken neck, an elongated or amorphous head, or presence of a cytoplasmic droplet). The risk of injecting a spermatozoon with impaired DNA and resulting in POEs is therefore potentially high.²⁰ We can speculate that this may be a possible reason for a high percentage of GOEs in the IVF group compared with the ICSI group. ICSI oocytes are also exposed to potentially more stress than IVF oocytes during denuding and micro-injection. These factors can influence embryo quality if optimum pH and temperature conditions are not adhered to.

In the present study we only investigated the effect of sperm morphology on embryo quality and morphology. The results of this study stress the need for more research on understanding the exact mechanism whereby the oocyte and spermatozoa may influence early embryonic development. It is, however, clear that sperm morphology on the crude sample can predict fertilisation *in vitro*⁷ but not ICSI fertilisation rate and embryo quality where the individual sperm morphology characteristics play an important role in the fertilisation process.¹³ Our study also suggests that in the case of ICSI a better selection of GOEs is possible on day 3 than day 2.

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