The semen analysis and sperm preparation

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human reproduction update

World Health Organization reference values for human semen characteristics

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SEMEN ANALYSIS

Evaluation of male fertility

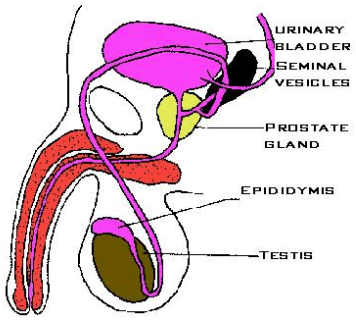
Testis function and male genital tract
Accessory sex glands (prostate and seminal vesicles)

Under given conditions of collection

A complete medical history and physical examination

It is impossible to characterize a man's semen quality from evaluation of a single semen sample

SEMEN PRODUCTION



SEMEN ANALYSIS (WHO)

The results of laboratory measurements of semen quality will depend on :

- ❑whether a complete sample is collected
- ❑the activity of the accessory sex glands
- ❑the time since the last sexual activity
- ❑the penultimate abstinence period
- ❑the size of the testis

PREPARATION

Private room near the laboratory

A minimum of 2 days and a maximum of 7 days of sexual abstinence

A complete sample

Should be reported :

- ❑Man's name, birth date and personal code number
- ❑The period of abstinence
- ❑The date and time of collection
- ❑The completeness of the sample
- ❑Any difficulties in producing the sample
- ❑The interval between collection and start of the semen analysis

PREPARATION

- ❑The sample should be obtained by masturbation and ejaculated into a clean container made of glass or plastic (non toxic)
- ❑The specimen container is placed on the bench or in an incubator (37°C) while the semen liquefies
- ❑For ART or microbiological analysis, specimen containers and pipettes must be sterile
- ❑Analyze ASAP
- ❑Collection of semen at home : NO
- ❑Collection of semen by non-toxic condom during sexual intercourse
- ❑Coitus interrupts : NO
- ❑Safe handling of specimen : infectious agents (HIV…)

Evaluation

Initial macroscopic examination

- ❑Liquefaction (30 minutes)
- ❑Semen viscosity
- ❑Appearance of the ejaculate
- ❑Semen volume
- ❑Semen pH

Table 1: Macroscopic Parameters of the Semen Analysis and Clinical Significance of Some of the Abnormal Values

Parameters	Normal values	Abnormalities	Clinical significance
pH	7.6	Acidic, <6.5/7	Low volume and non-coagulation: congenital absence of the vas Ejaculatory duct obstruction Partial retrograde ejaculation
Coagulation/liquefaction	Coagulates and liquefies within 20 minutes at room temperature	No coagulation	Congenital absence of the seminal vesicles
		Prolonged liquefaction	Poor prostatic secretions
Color	Homogenous, pearl-white	Yellowish Reddish-brown	Jaundice, carotenemia, drugs Hematospermia secondary to urethral bleeding or inflammation of the seminal vesicles, but other genito-urinary tumors will need work-up to be excluded
Viscosity	Within threading	Watery No threading	Of importance when associated with low motility
Volume	2-6ml	Oligospermia Azoospermia	Retrograde ejaculation Incomplete collection Partial retrograde ejaculation Short duration of sexual abstinence Anejaculation, gland overproduction Prolonged sexual abstinence

Evaluation

Initial microscopic examination

- ❑Thorough mixing and representative sampling of semen
- ❑Making a wet preparation
- ❑Aggregation of spermatozoa
- ❑Agglutination of spermatozoa
- ❑Cellular elements others than spermatozoa

Evaluation

- ❑Sperm motility
- ❑Sperm vitality
- ❑Sperm numbers
- ❑Counting of cells other than spermatozoa
- ❑Sperm morphology
- ❑Leukocytes
- ❑Immature germ cells
- ❑Antibody coating of spermatozoa

Table 1. Cut-off values for semen variables as published in consecutive WHO manuals [6–9] and as proposed in the fifth World Health Organization (WHO) manual [1].

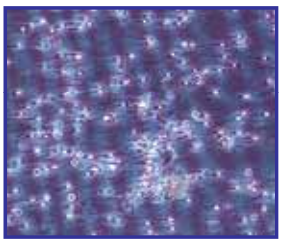
Semen variable	1980	1987	1992	1999	2010
Volume (mL)	–	≥ 2.0	≥ 2.0	≥ 2.0	1.5
Concentration (10^6 mL^{-1})	20–200	≥ 20	≥ 20	≥ 20	15
Total sperm number (10^6/ejaculate)	–	≥ 40	≥ 40	≥ 40	39
Motility (% motile)	≥ 60	≥ 50 (a + b)[a]	≥ 50 (a + b)	≥ 50 (a + b)	40 (a + b + c)
Forward progression (for 1980 only)	≥ 2[a]	≥ 25 (a)	≥ 25 (a)	≥ 25 (a)	32 (a + b)
Morphology (% normal)	80.5[a]	≥ 50	≥ 30[b]	(14)[c]	4
Viability/vitality (% live)	–	≥ 50	≥ 75	≥ 75	58
White blood cells (10^6 mL^{-1})	< 4.7	< 1.0	< 1.0	< 1.0	< 1.0

[a]Lower reference limit. Obtained from the lower fifth centile value. [b]Grade a + rapid progressive motility (> 25 μm s^{-1}); Grade b + slow/ sluggish progressive motility (5–25 μm s^{-1}); Grade c + non-progressive motility; Grade d + immotility; Normal = ≥ 50% motility (grades a + b) or ≥ 25% progressive motility (grade a) within 60 min of ejaculation. [c]Forward progression on a scale of 0–3, in which 0 = no forward progression. [d]Mean of a fertile population (range = 48%–98%); modified Bryan Leishman staining. [e]Arbitrary value. [f]No actual value given. Multicentre studies in progress refer to > 14% for in vitro fertilization (IVF).

Sperm motility 1H and 4H

ASTHENOZOOSPERMIA

At least 200 spermatozoa in a total of at least 5 fields in each replicate



WHO (IV edition)
a>25µm/s: progressive (rapid)
b 5-25µm/s: progressive (slow)
c<5µm/s: non progressive
d: immotile
Motility a + b ≥ 50% ; a ≥ 25%

WHO (V edition)
progressive PR a + b
non-progressive NP
Immotile IM
Motility a + b + c ≥ 40% ; PR a+ b ≥ 32 %

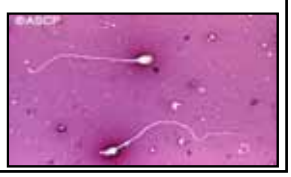
Vitality

NECROSPERMIA

Eosin – Nigrosin

Evaluate 200 spermatozoa in each replicate

Spermatozoa
❑ With red or dark pink heads are considered dead (membrane damaged)
❑With white heads or light pink heads are considered alive (membrane intact)



WHO (IV edition) ≥ 75% live
WHO (V edition) ≥ 58% live

Vitality

Hypo-osmotic swelling (HOS) test (if ICSI)

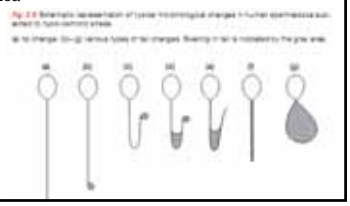
Evaluate 200 spermatozoa in each replicate

❑Swollen spermatozoa are identified by changes in the shape of the cell

❑Live cells are distinguished by evidence of swelling of the sperm tail : all forms of swollen tails = live spermatozoa

WHO (IV edition) ≥ 75% live

WHO (V edition) ≥ 58% live



Sperm numbers

Evaluate 200 spermatozoa in each replicate

OLIGOZOOSPERMIA

WHO (IV edition)
≥ 20M/ml ; **≥** 40M/ejaculate

WHO (V edition)
≥ 15M/ml ; **≥** 39M/ejaculate

Sperm numbers

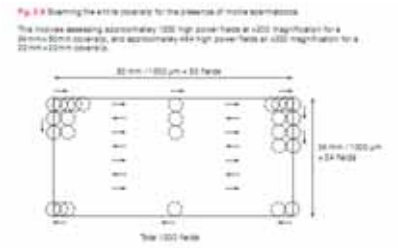
Low sperm numbers : examination of the sediment of a centrifugated sample (two slides)

❑cryptozoospermia : the presence of spermatozoa in either replicate

❑suspected azoospermia : the absence of spermatozoa from both replicates

Sperm numbers

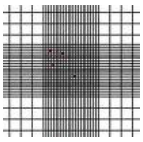
Low sperm numbers : examination of non-centrifugated samples to detect motile spermatozoa (when an accurate assessment of low sperm numbers is not required)



Sperm numbers

Low sperm numbers : examination of non-centrifugated samples to detect motile spermatozoa (when an accurate assessment of low sperm numbers is required)

Assessing low sperm numbers in the entire improved Neubauer chamber



Counting of cells other than spermatozoa

Calculation of the concentration of round cells in 10^6 per ml in semen (C)

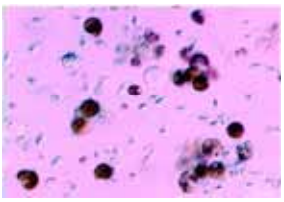
N = the number of round cells counted in the same number of fields as 400 spermatozoa
S = the concentration of spermatozoa in 10^6 per ml
C = S x (N/400)

If the estimated of round cell concentration exceeds 10^6 per ml, their nature should be assessed :

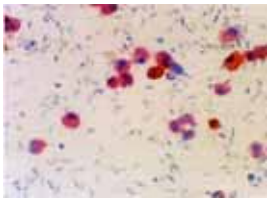
- peroxidase activity or leukocyte markers
- Identify immature germ cells

WHO (IV edition) Leukocytes <1M/ml

WHO (V edition) Leukocytes <1M/ml



Peroxidase



CD45

LEUKOCYTOSPERMIA

Cells other than spermatozoa (1)

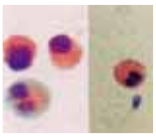
Spermatogony

Spermatocyte I

Spermatocytes II

Cells other than spermatozoa (2)

Round spermatid

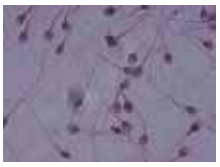


Elongated spermatid

Sperm morphology

Shorr-stained spermatozoa

Papanicolaou –stained spermatozoa



Sperm morphology

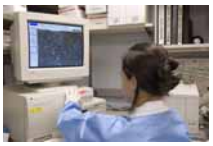
Semen smearing method for sperm morphology

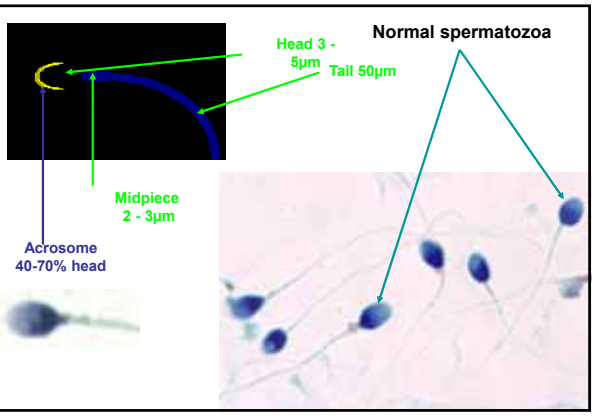
Computer – aided sperm morphometric assessment

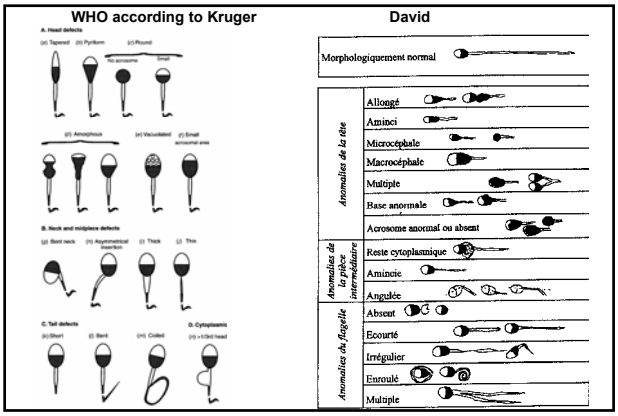
TERATOZOOSPERMIA

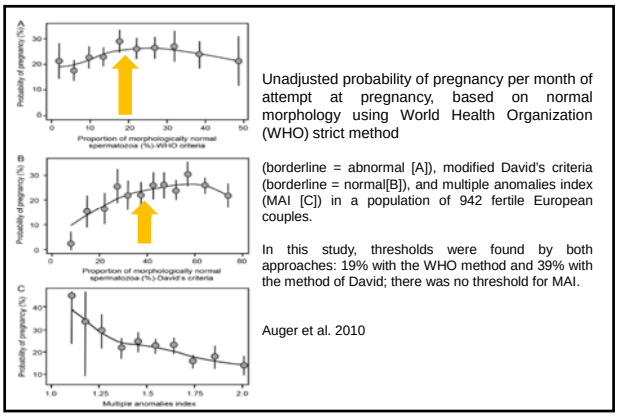
WHO (IV edition) ≥ 14% (Kruger)

WHO (V edition) ≥ 4% (Kruger)







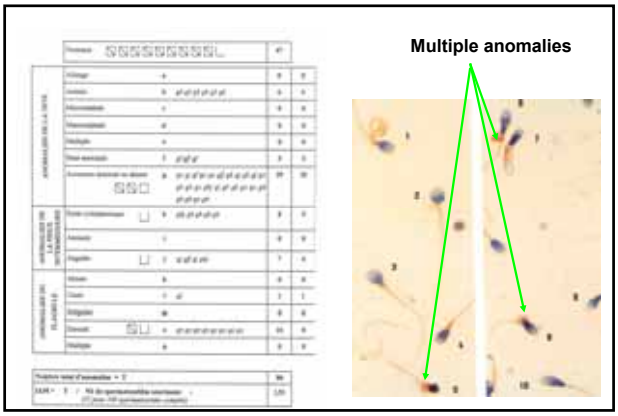


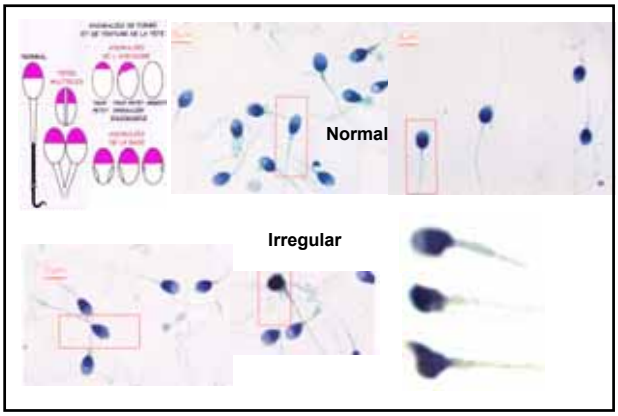
Unadjusted probability of pregnancy per month of attempt at pregnancy, based on normal morphology using World Health Organization (WHO) strict method

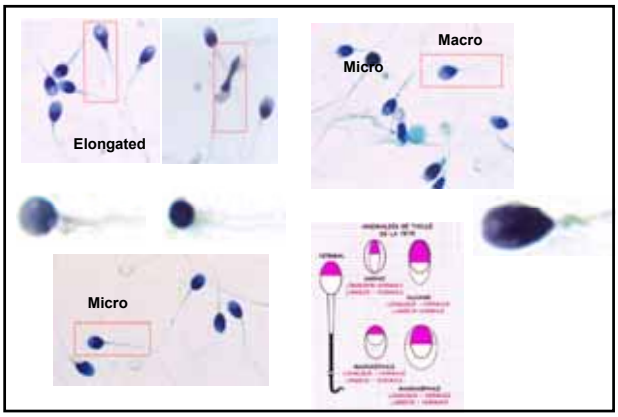
(borderline = abnormal [A]), modified David's criteria (borderline = normal[B]), and multiple anomalies index (MAI [C]) in a population of 942 fertile European couples.

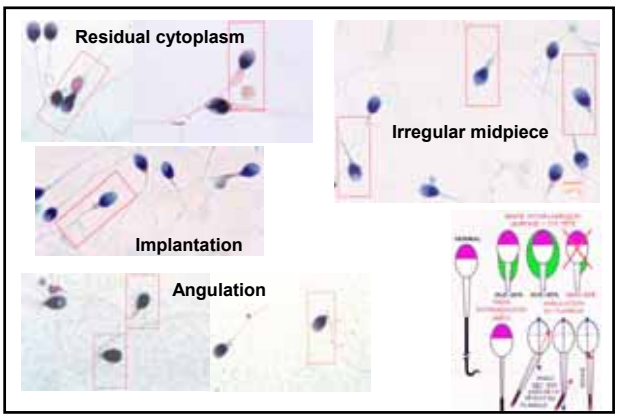
In this study, thresholds were found by both approaches: 19% with the WHO method and 39% with the method of David; there was no threshold for MAI.

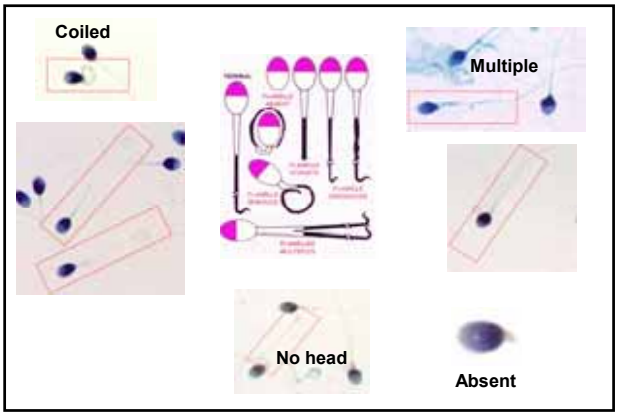
Auger et al. 2010




Normal
Irregular


Elongated
Macro
Micro
Micro


Residual cytoplasm
Irregular midpiece
Implantation
Angulation



MORPHOLOGY AND FERTILITY

- Each spermatozoa is evaluated as normal or abnormal
- The % of normal sperm cells in the ejaculate is evaluated and related to fertility according to a threshold

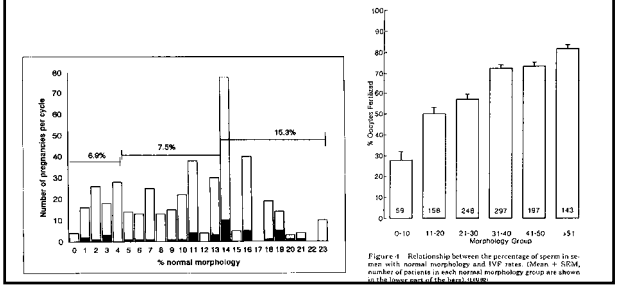


Figure 4 Relationship between the percentage of sperm in semen with normal morphology and IVF rates. (Mean + SEM, number of patients in each normal morphology group are shown in the lower part of the bars) (1998)

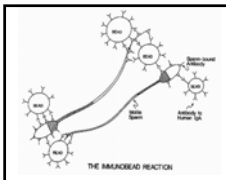
MORPHOLOGY AND FERTILITY

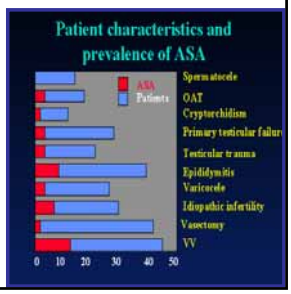
Table 2. Comparison of fifth World Health Organization (WHO) manual for normal morphology values with recently published literature values.

Author	Population	Cut-off value				
		Fertile	Subfertile	Infertile	TZI	AI
Van Zyl et al. [23, 29, 31]	*In vivo* pregnancies	20	10	5		
Kruger et al. [32, 33]	IVF fertilization rates					
Initial published intervals		≥ 15	14–4	≤ 3		
Intervals as used in practice		≥ 15	14–4	≤ 4		
Eggert-Kruse et al. [34]	*In vivo* pregnancies	14	7	4		
Oudshoff et al. [35]						
Tenth percentile	Fertile population	11				
ROC curve analysis	Fertile vs. subfertile	5				
Zinaman et al. [36]	Healthy couples	8				
Gonsalves et al. [26]	Fertile vs. subfertile	12	9			
Menkveld et al. [37]						
ROC curve analysis	Fertile vs. subfertile	4			1.44	0
Adjusted	Fertile vs. subfertile	5			2.09	1
Tenth (upper) percentile	Fertile population	2			1.82	0
Guzick et al. [38]	Fertile vs. subfertile	> 12	9–12	< 9		
Haugen et al. [40]						
Tenth percentile	Fertile population	4				
Fifth percentile		3			1.72	
Fifth WHO manual [1]						
Fifth percentile	Recent fathers	4				

Abbreviations: IVF, in vitro fertilization; ROC, receiver operating characteristic; TZI, zona-pellucida index; AI, acrosome index. [a] See text for more detailed description of populations investigated. [b] Based on the positive and negative predictive values (see Guzick et al. [38]).

Anti-sperm antibodies (ASA)





10% infertile (3-20%) / 2% fertile men

When the blood – testis barrier is violated

- ❑ Surgical, infectious (50%), testicular trauma
- ❑ Vasectomy
- ❑Torsion
- ❑ Unexplained infertility
- ❑ Agglutination, mobility, vitality, MAR test +,
- ❑ « shaking phenomenon » abnormal PCT

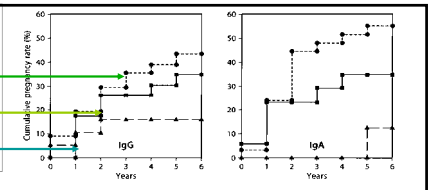
% ASA
Spontaneous pregnancy

10-49%

50-90%

>90%

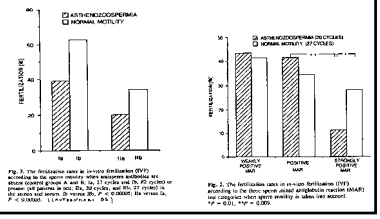
Abshagen 1998



ASA and fertility

FIV %Fertilization <<<

ICSI :++++



SPERM PREPARATION

- ❑Simple washing
- ❑Direct swim-up
- ❑Discontinuous density gradients

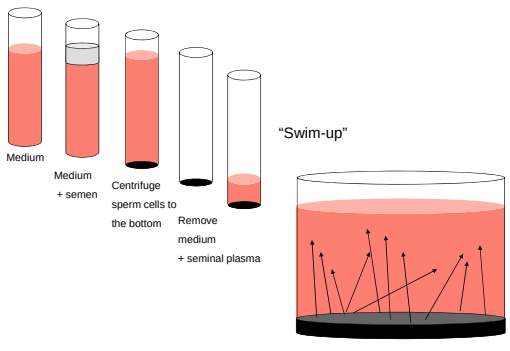
Simple washing

- This simple washing procedure provides the highest yield of spermatozoa
- Adequate only if semen samples are of good quality.
- It is often used for preparing spermatozoa for intrauterine insemination (donor).

Direct swim-up

- Spermatozoa may be selected by their ability to swim out of seminal plasma and into culture medium = the “swim-up” technique.
- The semen should preferably not be diluted and centrifuged prior to swim-up, because this can result in peroxidative damage to the sperm membranes
- The direct swim-up technique can be performed either by layering culture medium over the liquefied semen or by layering liquefied semen under the culture medium.
- Motile spermatozoa then swim into the culture medium.
- Gives a lower yield of spermatozoa than washing, but selects them for their motility and is useful where the percentage of motile spermatozoa in semen is low, e.g. for IVF and ICSI.

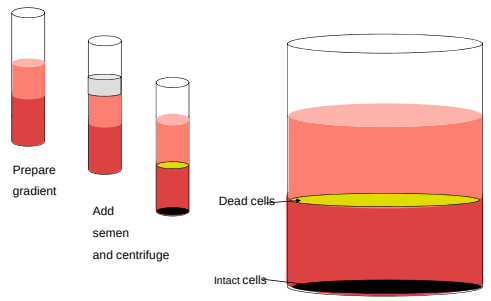
Sperm Preparation: Swim-up



Discontinuous density gradients

- The best selection of good quality spermatozoa, giving good separation from other cell types and debris.
- Used to recover and prepare spermatozoa for use in IVF and ICSI.
- Centrifugation of seminal plasma over density gradients consisting of colloidal silica coated with silane which separates cells by their density. Motile spermatozoa swim actively through the gradient material to form a soft pellet at the bottom of the tube.
- A simple two-step discontinuous density gradient preparation method is most widely applied, typically with a 40% (v/v) density top layer and an 80% (v/v) density lower layer.
- Sperm preparation using density gradient centrifugation usually results in a fraction of highly motile spermatozoa, free from debris, contaminating leukocytes, non-germ cells and degenerating germ cells.

Sperm Preparation: Gradient centrifugation



Semen preparation techniques for intrauterine insemination (Review)

Boomsma CM, Heineman MJ, Cohlen BJ, Farquhar C

Authors' conclusions

There is insufficient evidence to recommend any specific preparation technique. Large high quality randomised controlled trials comparing the effectiveness of a gradient and/ or a swim-up and/ or wash and centrifugation technique on clinical outcome are lacking. Further randomised trials are warranted.

CONCLUSION (1)

One ejaculate: No conclusion !
≥ 2 or 3 samples – 3 months

VARIATIONS !!!!!

If necessary, complete with :

- Microbiological analysis
- Computer-aided sperm analysis : motility, concentration, morphology
- Biochemical assays for accessory sex organ function : citric acid, zinc, fructose, acid phosphatase, neutral α-glucosidase in seminal plasma
- Electron microscopy

CONCLUSION (2)

Research :

- Reactive oxygen species (ROS)
- Sperm–mucus interaction or a post-coital test (PCT)
- The sperm penetration assay (SPA)
- The sperm capacitation index (SCI)
- Sperm chromatin : TUNEL, COMET, SCSA
- Birefringence
- MSOME
- Emerging technologies : microarray, metabolomics, atomic force microscopy….

CONCLUSION (3)

If azoospermia, complete with :

- A complete medical history
- Physical examination
- Measurement of selected hormones
- Genetic testis
- Testicular biopsy….