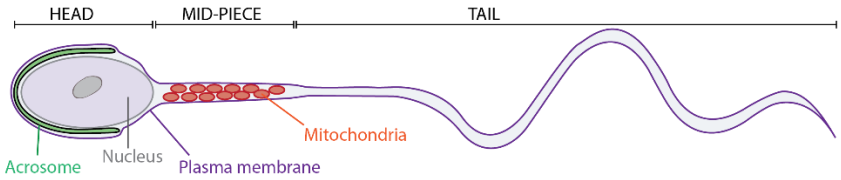
Part B: Heads or tails? Identify the cause of male factor infertility

Infertility can be caused by problems with the sperm. This is called male factor infertility. Semen is analysed in the laboratory to identify if the sperm cells are normal, healthy, motile and plentiful.

Analyse semen and sperm samples

REFER TO THE SPERM MORPHOLOGY CHART AT YOUR LAB BENCH

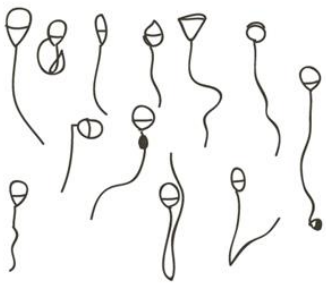


Fertilisation – a numbers game

Fertility depends on having a high enough concentration of normal, live, motile sperm cells that are capable of swimming to the eggs for fertilisation to occur. Cells with an abnormal shape may not swim properly or may not be able to penetrate the Zona pellucida, a gel-like layer surrounding the oocyte (egg).

Fertility also depends on a healthy developing ovum at the correct stage of meiosis (see the Meiosis and Oocyte Development activity - GTAC resource).

Abnormal sperm cells fail to reach or fertilise an egg



A healthy oocyte, ready for fertilisation, has 1 polar body and is surrounded by Zona pellucida and cumulus cells.

In the Sperm Lab - Practical activity you learned how to prepare a wet mount for viewing live motile sperm and how to prepare a smear to look closely at cell morphology. A full laboratory semen analysis includes tests for the number of sperm cells in semen using a cell counting chamber, measuring progressive motility (the ability to swim normally in a straight line), counting the number of live healthy cells in a sample (cell viability), and determining whether the cells have the normal size and shape (morphology).

Student Worksheet 2

Worksheet 2 provides you with practice analysing a full set of semen data for two patients undergoing tests for male factor infertility.

Patient Information

Aaron A. and partner - 2 years trying for a pregnancy

- 29yo, computer technician, keen cyclist, healthy diet, non smoker, social drinker
- Partner has been tested: normal cycles, hormone levels and ovulation
- Clinical history: no serious illnesses; first sperm tests ever done

Barry B. and partner - 5 years trying for a pregnancy

- 38yo, artist, former smoker, social drinker, reasonable diet
- Partner has been tested: normal cycles, hormone levels and ovulation
- Clinical history: chlamydia infections; 5 years ago-low sperm count detected

Semen analysis – the clinic collects two semen samples (sample 1, sample 2) per patient 4 weeks apart to know whether any changes are long term issues. They are analysed for sperm number, motility (ability to swim), viability (alive or dead) and morphology (shape and structure). The values for the two samples are given in the data tables below.

Sperm Concentration

The semen was diluted 1/20 and the sperm cells were counted in a specialised counting grid.

VIEW AN EXAMPLE OF SPERM IN A CELL COUNTING CHAMBER, LOW POWER MICROSCOPY - IMAGE 1

Step 1 Calculate the average number of sperm cells in the two samples tested
Calculation: (sample 1 + sample 2) ÷ 2.

Step 2 Then calculate the number of sperm cells per millilitre of semen (cells/mL)
Calculation: Cells/mL = cells per grid x dilution factor x 10^4

patient	Sperm per grid (two tests)	Average cells per grid	Sperm/mL
A	sample 1: 79 cells sample 2: 73 cells		
B	sample 1: 21 cells sample 2: 15 cells		

Sperm Viability

The eosin/nigrosin stain is used to show live (viable) and unhealthy or dead (non-viable) cells. Under the light microscope, viable sperm cells appear white; dead sperm cells stain pink.

VIEW AN EXAMPLE OF THE NIGROSIN-EOSIN VIABILITY STAIN - IMAGE 2A, 2B

Two samples were tested. 100 cells from each sample were counted under the microscope.

Step 1 Calculate the average number of live cells in the two samples
Calculation: (sample 1 + sample 2) ÷ 2

Step 2 Calculate the percentage of live (viable) sperm cells in the samples.
Calculation: % viability = (live cells/total cells) x 100

patient	Viable Live cells (white)	Non-viable Dead cells (pink)	Total cells (live + dead)	% viability
A	sample 1: 72 cells sample 2: 66 cells average:	sample 1: 28 cells sample 2: 34 cells average:		
B	sample 1: 20 cells sample 2: 18 cells average:	sample 1: 80 cells sample 2: 82 cells average:		

Sperm Motility

Two semen samples were tested.
100 cells in each sample were scored for motility.
Progressive motility means swimming forwards continuously.
Non-progressive motility means the cells can move but may just wriggle on the spot, swim in circles, move in random directions or similar motion.

Calculation: % motility = (motile cells ÷ total cells) x 100

patient	progressive motility	non-progressive motility	non-motile	total cell count	% motility
A	sample 1: 24 sample 2: 16 average:	sample 1: 11 sample 2: 9 average:	sample 1: 81 sample 2: 59 average:		
B	sample 1: 4 sample 2: 6 average:	sample 1: 11 sample 2: 9 average:	sample 1: 87 sample 2: 83 average:		

Sperm Morphology

More than 100 sperm cells from 2 samples were observed for each patient. The averages for the 2 samples are provided below.
Calculate the percentage of normal cells in each patient's sample.

VIEW AN EXAMPLE OF HUMAN SPERM MORPHOLOGY: STAINED, VIEWED AT 1000X - IMAGE 3A,B AND COMPARE IT TO THE SPERM MORPHOLOGY CHART

Calculation: % normal morphology = (number normal cells ÷ total cells) x 100

patient	Normal head & tail	Tail defects: Hairpin tail & Bent tail	Defects - head/neck/ midpiece (e.g. small, flat, cytoplasmic blebs)	Other tail defects (e.g. short, duplicate, coiled)	**Total cell count**	**% Normal**
A	60	52	18	20		
B	5	24	36	80		

Summary table

patient	**Sperm morphology* (% normal)**	**Progressive motility* (% estimate)**	**Sperm count * (cells/mL)**	**Sperm viability* (% viable)**
A				
B				

* average for 2 samples over 4 weeks

Analysis

Compare the patients' results to the WHO normal range values. Refer to Clinical Recommendations sheet for suggested reproductive technology treatment approach.

Table 1: World Health Organisation standards table

SEMEN ANALYSIS PARAMETER	NORMAL VALUES
Volume	1.5 ml or more
pH	> or equal to 7.2
Sperm concentration	15,000,000/ml or more
Total motility	40% or more
Progressive motility	32% or more
Morphology	4% or more normal forms
Vitality/viability	58% or more live
White blood cells	Less than 1,000,000/ml

Based on your analysis, conclude whether the semen sample is healthy and normal. Comment on whether you think there is a good chance of fertilisation occurring (assuming the eggs and ovulation in the female partner are normal).

REFER TO THE CLINICAL RECOMMENDATIONS SHEET FOR THE SUGGESTED REPRODUCTIVE TECHNOLOGY TREATMENT FOR THIS PERSON

Treatment Recommendations

patient	Recommended assisted reproductive technology approach	Give the reasons for your suggestion
Aaron A.		
Barry B.		