Spermatoanalysis

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Reproduction

- the basic property of living organisms
- the ability to form the basis of a system, which is the same as the founding system
- allows to preserve the species and time continuity of life, to develop, increase the number of individuals, ensure the survival of the genetic lineages
- sexual and asexual, or their alternation (metagenesis)
- the level of reproduction is an indicator of the well-being of an organism in a given environment
- an indicator of the balance of conditions in the external and internal environment of the organism

Male fertility

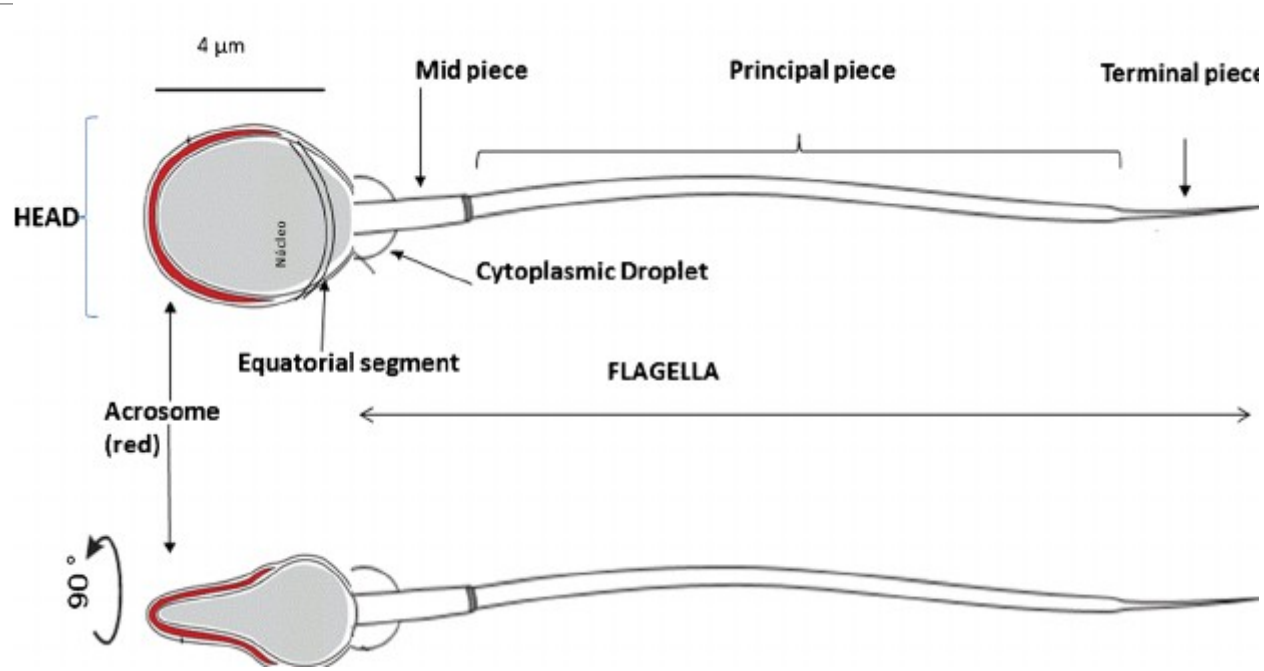
Fertility:

- basic biological property
- a prerequisite for the time existence of the species
- complex feature based on the ability to have healthy offsprings in the optimal number (in a given time period)
- determined by a number of genes (polygenic inheritance)
- heritability, (h^2) of fertility indicators is low, fertility of animals is decided mainly by environmental conditions (eg. breeding conditions)

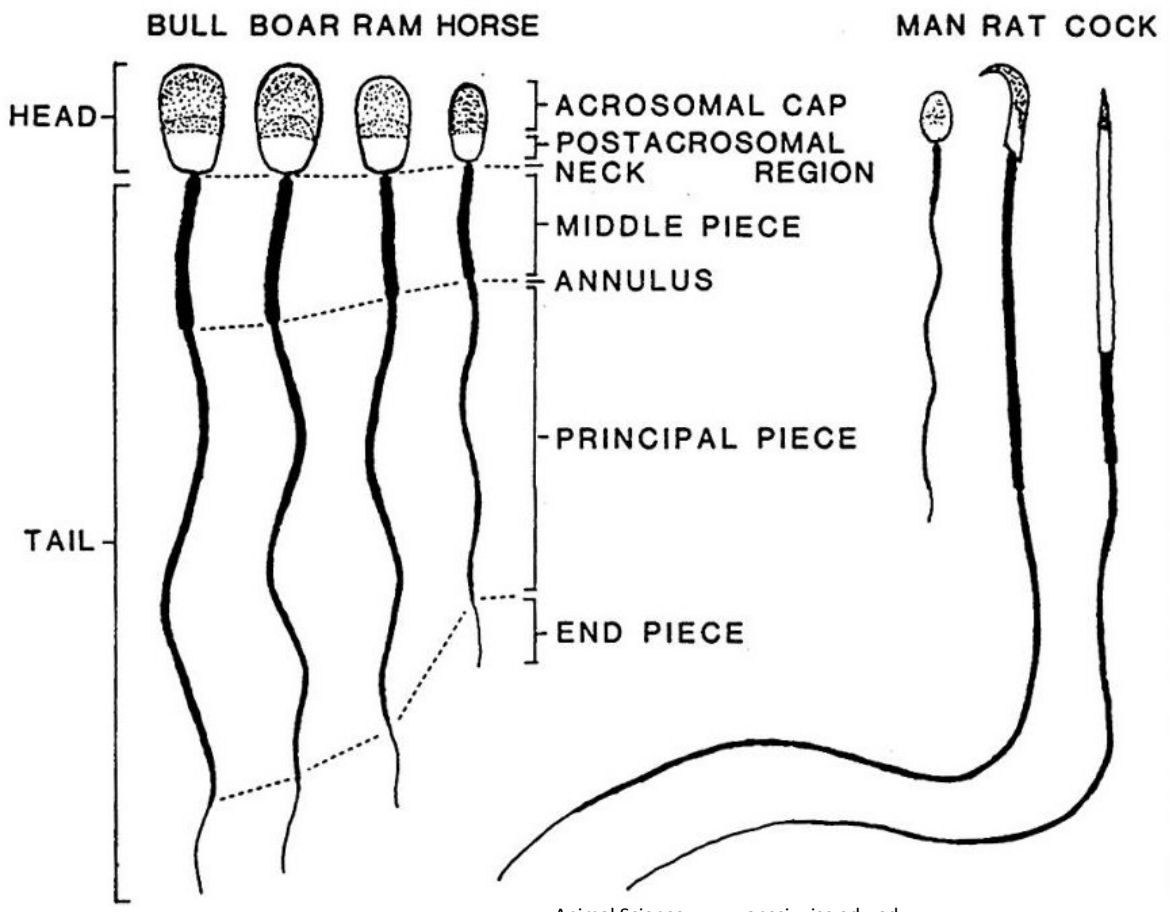
- **SPG** (spermiogram) = basic examination of a semen sample, evaluation of male fertility: https://www.youtube.com/watch?v=T9_XkaXCXqc

Spermatozoa

- head, neck, tail
- the head: flattened, the nucleus with condensed chromatin; cca 2/3 of the nucleus covered by the acrosome (enzymes important tor fertilization).
- the neck: short (cca 1 µm)
- the tail = middle piece + principal piece + end piece;
- the middle piece: axonema (arrangement of microtubules), a sheath of mitochondria.
- the principal piece: axonema



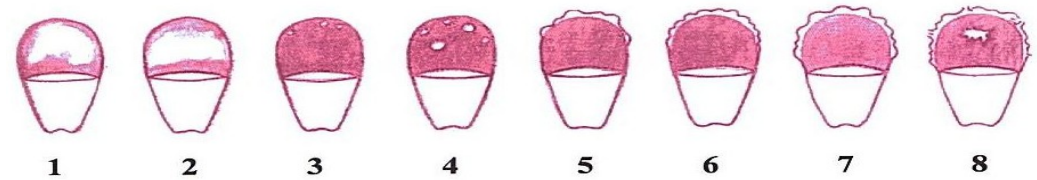
Spermatozoa in different animal species



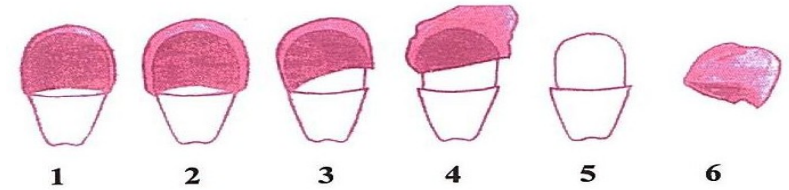
Animal Science, www.ansci.wisc.edu.edu

	Sus (µm)	*Homo (µm)*
Heald		
length	8,7	4,0-5,0
width	4,6	2,5-3,5
Flagellum		
middle piece	10	5,0-6,5
main piece	30	38,5-40
Total length	48,2	47,5-51,5

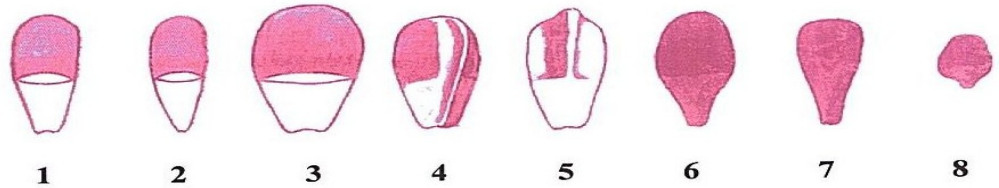
Morfology abnormalities



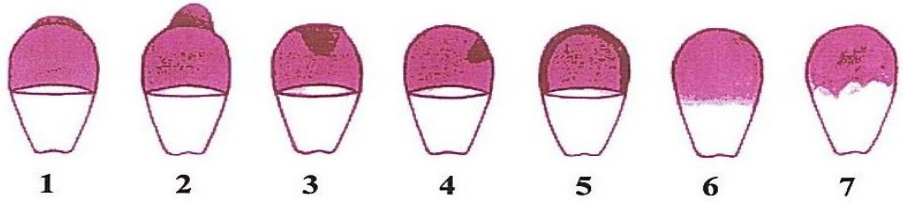
1 – 2	změny homogenity akrozomové hmoty
3 – 4	vakuoly v akrozomové hmotě
5 – 6	bulózní edém lehkého stupně
7 – 8	bulózní edém těžkého stupně



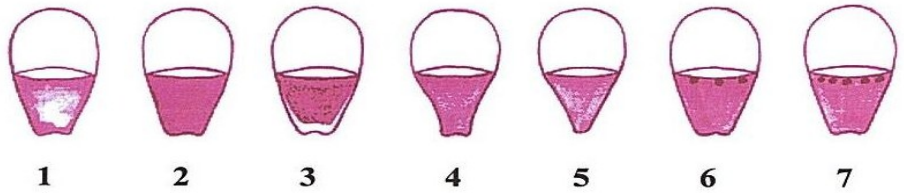
1 – 2	zbobtnání akrozomu
3 – 4	uvolnění akrozomu
5	hlavička bez akrozomu
6	volný akrozom



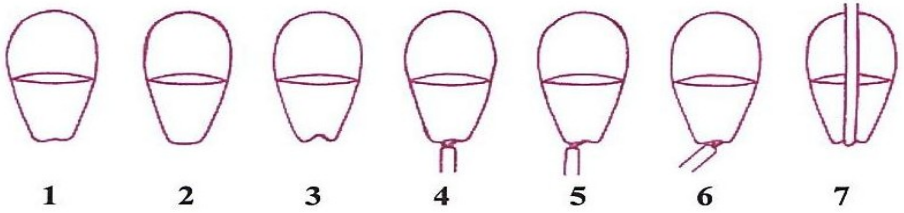
1 – 2	zúžená hlavička
3	velká – gigantická hlavička
4 – 5	hřebenovitá hlavička – krista
6 – 7	teratoidní formy
8	abortivní forma



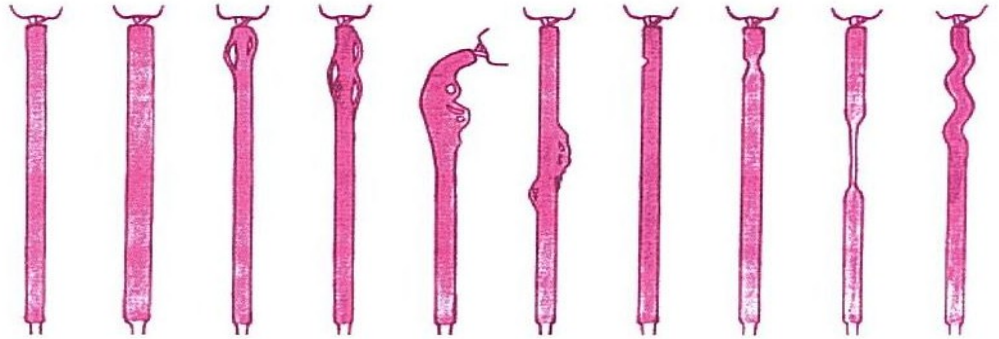
1 – 4	perzistující akroblast
5	homogenní kondenzace akrozomální hmoty
6 – 7	ztráta struktury ekvatoriálního segmentu



1	nehomogenní struktura kalíšku jádra
2	kondenzovaná hmota kalíšku jádra
3	uvolnění kalíšku jádra
4 – 5	zúžená hlavička v oblasti pars posterior (hruškovité hlavičky)
6 – 7	diadémový defekt

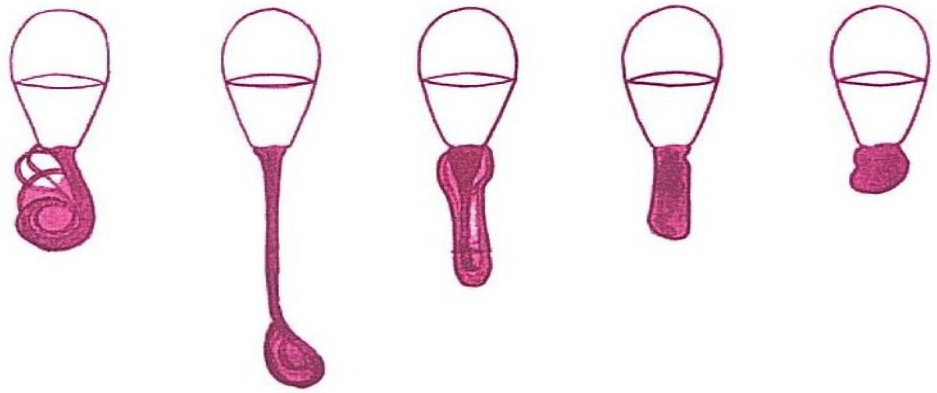


1	normální báze hlavičky spermie
2	příliš rovná báze
3	příliš vyklenutá báze
4	normální uložení bičíku
5	abaxiální uložení
6	paraaxiální uložení
7	retroaxiální uložení



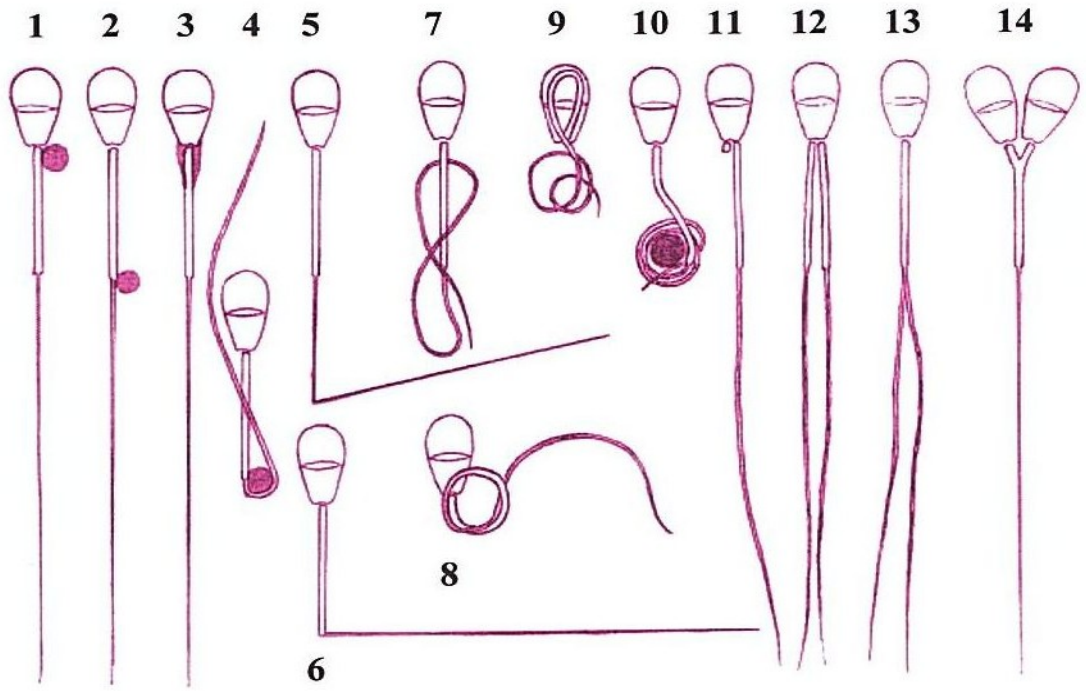
1 2 3 4 5 6 7 8 9 10

1	normální spojovací část
2	zesílená spojovací část
3 – 4	rozvolnění mitochondriální spirály
5 – 6	pseudokapénka
7 – 9	absence mitochondriální spirály
10	vývrtkovitý tvar spojovací části



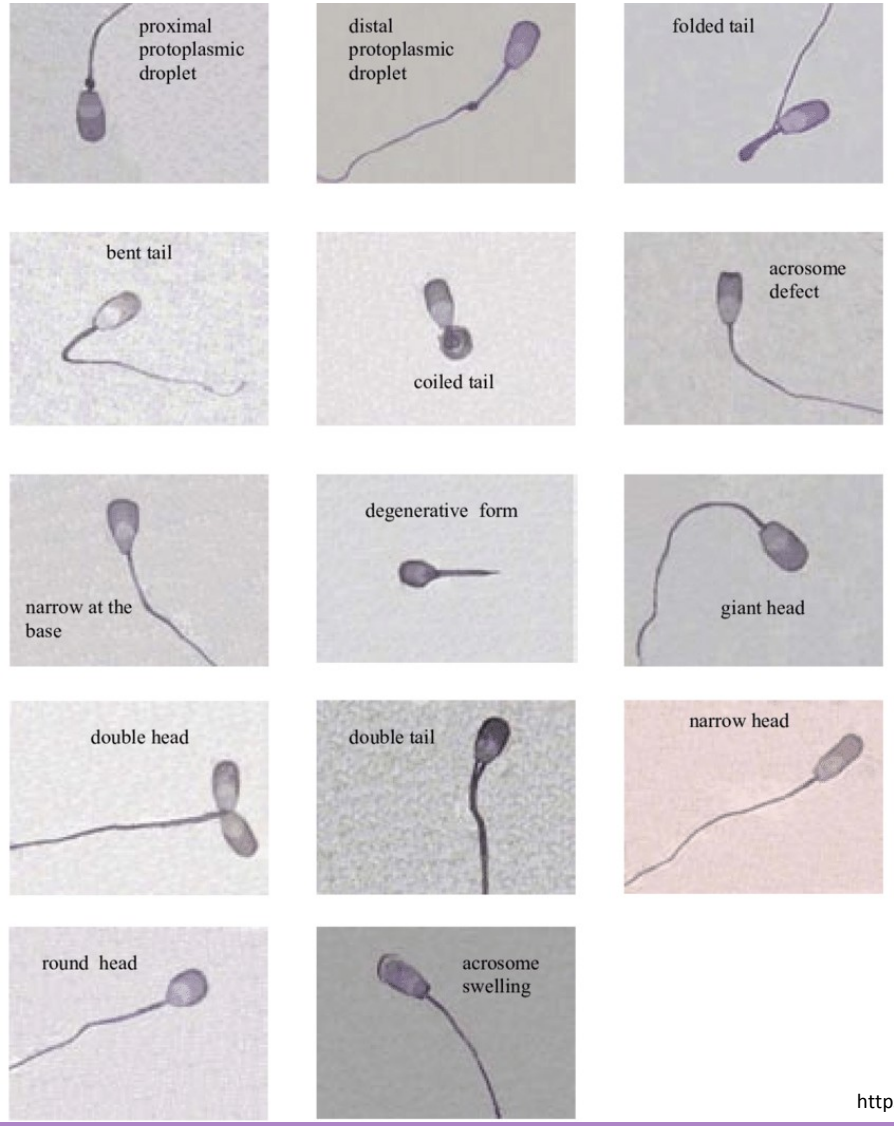
1 2 3 4 5

1	stočený bičík ve vaku povrchové membrány
2 - 4	hypogenese bičíku spermie
5	agenese bičíku spermie



I	1	proximální protoplazmatická kapénka
	2	distální protoplazmatická kapénka
	3	retardovaný protoplazmatický relikt na krčku spermie
	4	jednoduché ohnutí bičíku
	5 – 6	zlomení bičíku
	7 – 10	různé stupně svinutí bičíku
	11	zdvojení bičíku s jednostrannou agenesí
	12	totální zdvojení bičíku
	13	zdvojení hlavní části bičíku
	14	zdvojení iniciální partie spojovací části bičíku při zdvojení hlaviček

Boar (domestic pig) – morfology abnormalities



https://www.researchgate.net/figure/Morphologically-abnormal-spermatozoa-monitored_fig1_242709808

Basic values of native semen in men

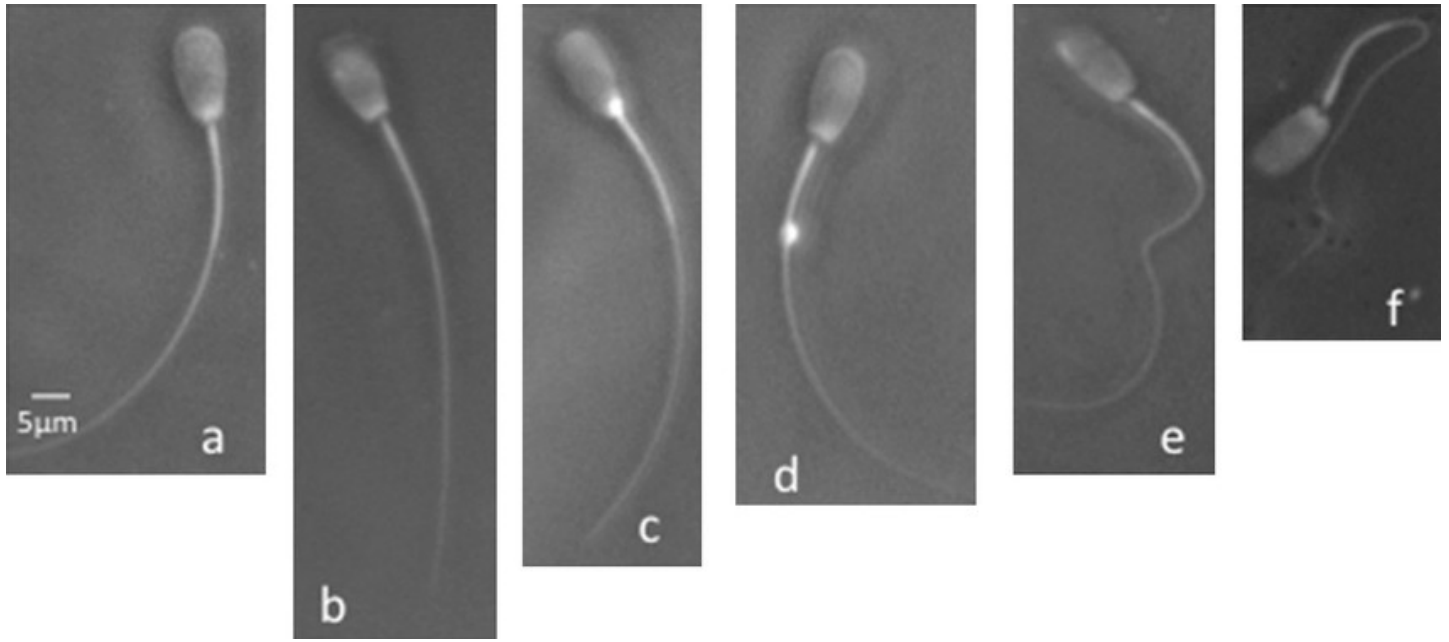
WHO laboratory manual for the examination and processing of human semen 2010:

Note: reference values in 2009 for men: 20 mil./ml, motility 50 % (25 % progressive motility), morphology 30 %; **reference values in 1960:** 80 mil./ml

total sperm count (mil./ejaculate)	39 mil.
liquefaction	do 60 minut
pH of liquefied sample	7,2-7,8
sperm concentration	15 mil./ml
vitality (eosin staining)	58 %
total motility	40 % (progressive + non progressive)
progressive motility (linear)	32 %
normal morphology (head, neck and tail morphology)	4 %
presence of white blood cells, prokaryotic cells	

Basic values of native semen in boars

pH	7,2
sample volume	> 100 ml
sperm concentration	> 150 mil./ml
total motility	> 70 %
abnormal sperm morphology inc. cytoplasmatic droplets	< 25 %



a) normál morfology; b) abnormal acrosome; c) proximal droplet; d) distal droplet; e+f) bent flagellum

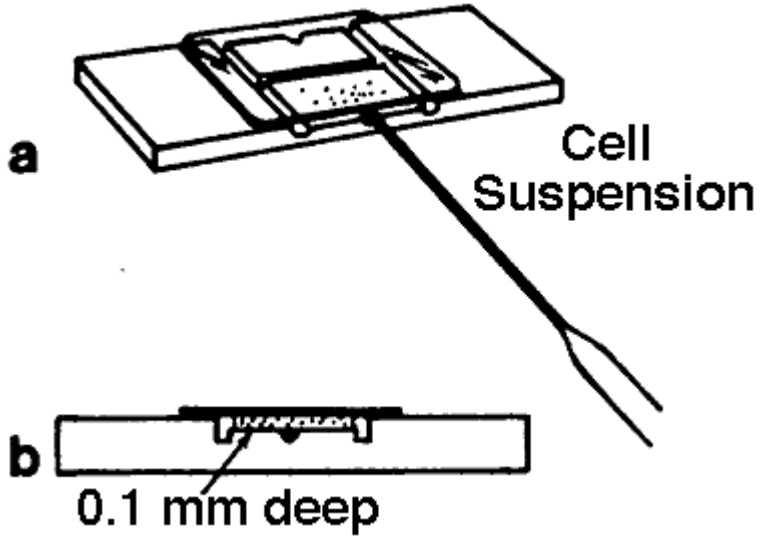
https://www.researchgate.net/figure/Boar-spermatozoa-a-Normal-morphology-b-reacted-acrosome-c-proximal-droplet_fig2_275366299

Basic findings and abnormalities:

normozoospermia	see reference values
oligozoospermia	low sperm count
asthenozoospermia	reduced sperm motility
teratozoospermia	pathological shapes
cryptozoospermia	less than 1 mil./ml
azoospermia	semen contains no sperm cells
nekrozoospermia	dead sperm cells
OAT syndrome	oligo-astheno-teratospermia

Sample evaluation using Bürker chamber (BCh):

- Bürker chamber = a counting-**chamber** device originally designed and usually used for counting blood cells.

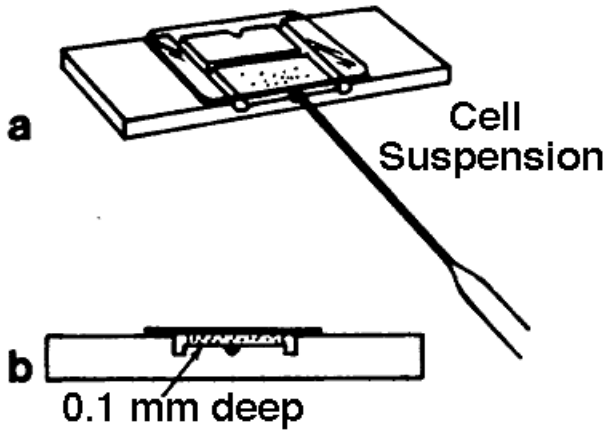


https://cz.vwr.com/store/product/2991822/komurky-pocitaci-burker, http://www.ansci.wisc.edu/jjp1/equine/lab_supplement/hemacytometer/Hemocytometer%20use.html

Sample evaluation using Bürker chamber

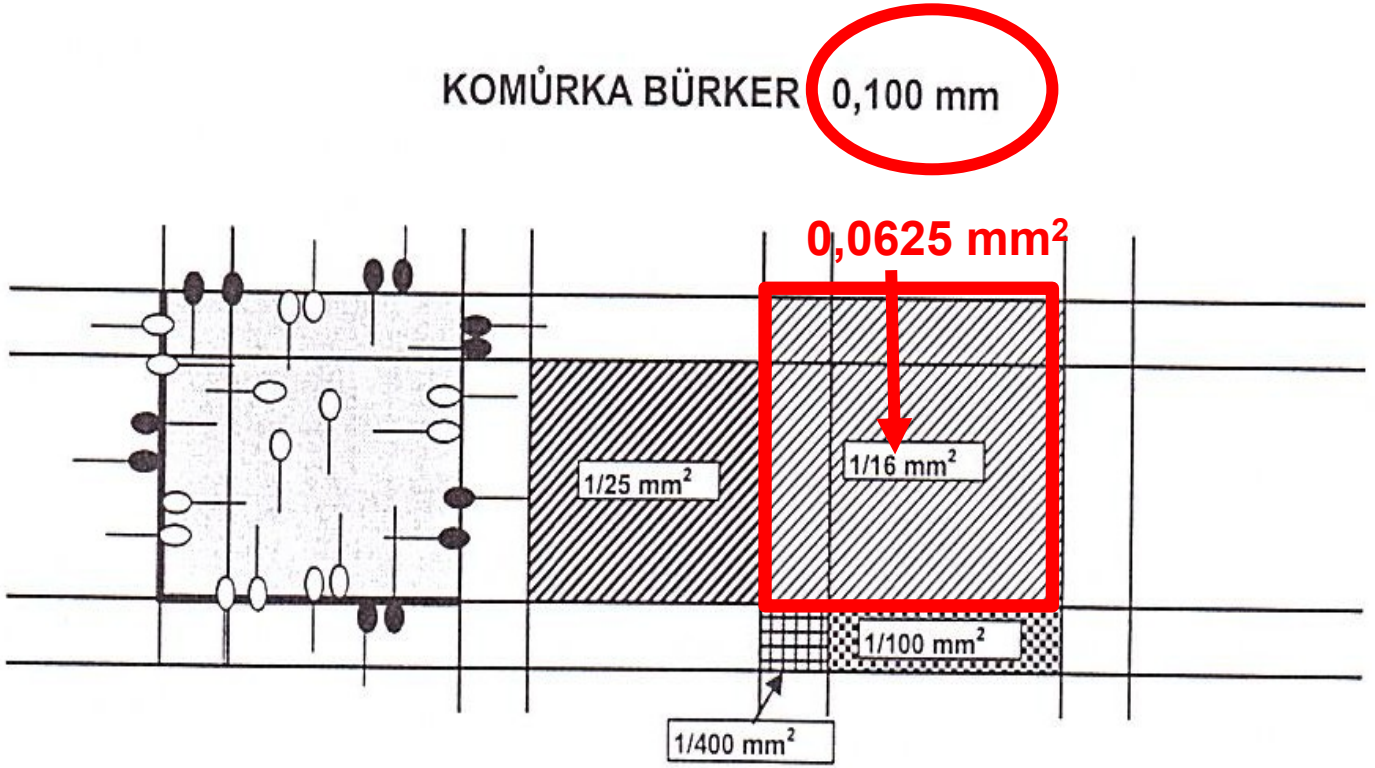
a) evaluate the sample under a microscope as you received it. Is a dilution of the sample necessary (compare your sample with this video: https://www.youtube.com/watch?v=T9_XkaXCXqc)? Find a suitable dilution if necessary.

b) sample dilution: mix 50 µl of the sample with **.....** µl of buffered physiological saline solution in a test tube. Mix the sample well and apply under the cover glass of the Bch. Wait 3 minutes. Then evaluate the sample under the light microscope using the magnification from 200 x to 400 x. **Evaluate the sample in 10 squares of Bch, unless otherwise indicated**. Count the cells inside the square and on 2 sides (see graphic materials). Calculate the concentration of the sample →



http://www.ansci.wisc.edu/jjp1/equine/lab_supplement/hemacytometer/Hemocytometer%20use.html

Sample evaluation using Bürker chamber



- we count sperms with heads completely inside a specified sector/syuare, or when they touch two adjacent sides (marked in bold) of the relevant square (ie only those white marked sperms, we do not count black marked sperm)
- chamber depth: 0,100 mm

http://atraktivnibiologie.upol.cz/docs/img/databaze/genetika/slides/Burkerova%20po%c4%8d%c3%adtac%c3%ad%20kom%c5%afrka-po%c4%8d%c3%adtac%c3%ad%20s%c3%ad%c5%a5%20(Fellnerov%c3%a1%20I.).jpg

Sample evaluation using Bürker chamber

b) concentration assessment (c): count motile and immotile cells separately (= motile cells in 10 squares + immotile cells in 10 squares) , then calculate *c*:
c = (total number of counted cells x dilution) / (number of squares x square area x chamber depth)

c) motility assessment:
i) total motility in %:

total motility in % = c of motile sperm cells / total c

ii) % of cell with progressive motility: evaluate min. 100 cells (optimally 200 cells), calculate % of cells with progressive movement (https://www.youtube.com/watch?v=T9_XkaXCXqc, Progressive motility = sperm swimming in a mostly straight line or in large circles = generally healthy, functioning sperm).

d) morphology evaluation: evaluate min. 100 cells (optimally 200 cells), calculate % of cells with wi normal morpsholgy, calculate abnormalities of head, neck and tail separately

e) record the presence of bacteria, white blood cells and ather abnormalities

Informační zdroje

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- URL 1: http://humrep.oxfordjournals.org/content/16/12/2710/F1.expansion
- URL 2: https://www.researchgate.net/figure/Sperm-morphology-Schematics-of-human-sperm-The-different-cell-regions-are-indicated_fig1_260527307
- Světová zdravotnická organizace: WHO laboratory manual for the examination and processing of human semen, 2010.
- další literatura u autorky