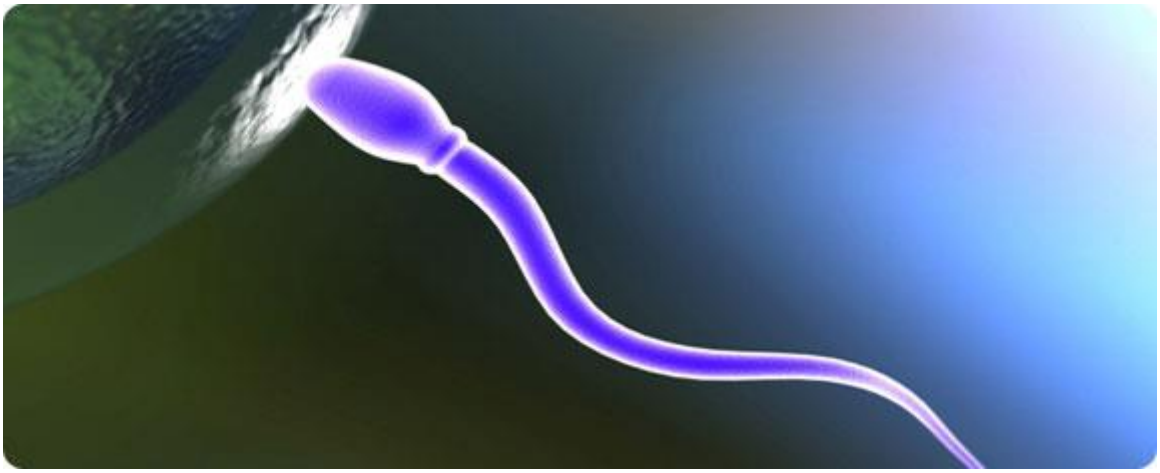
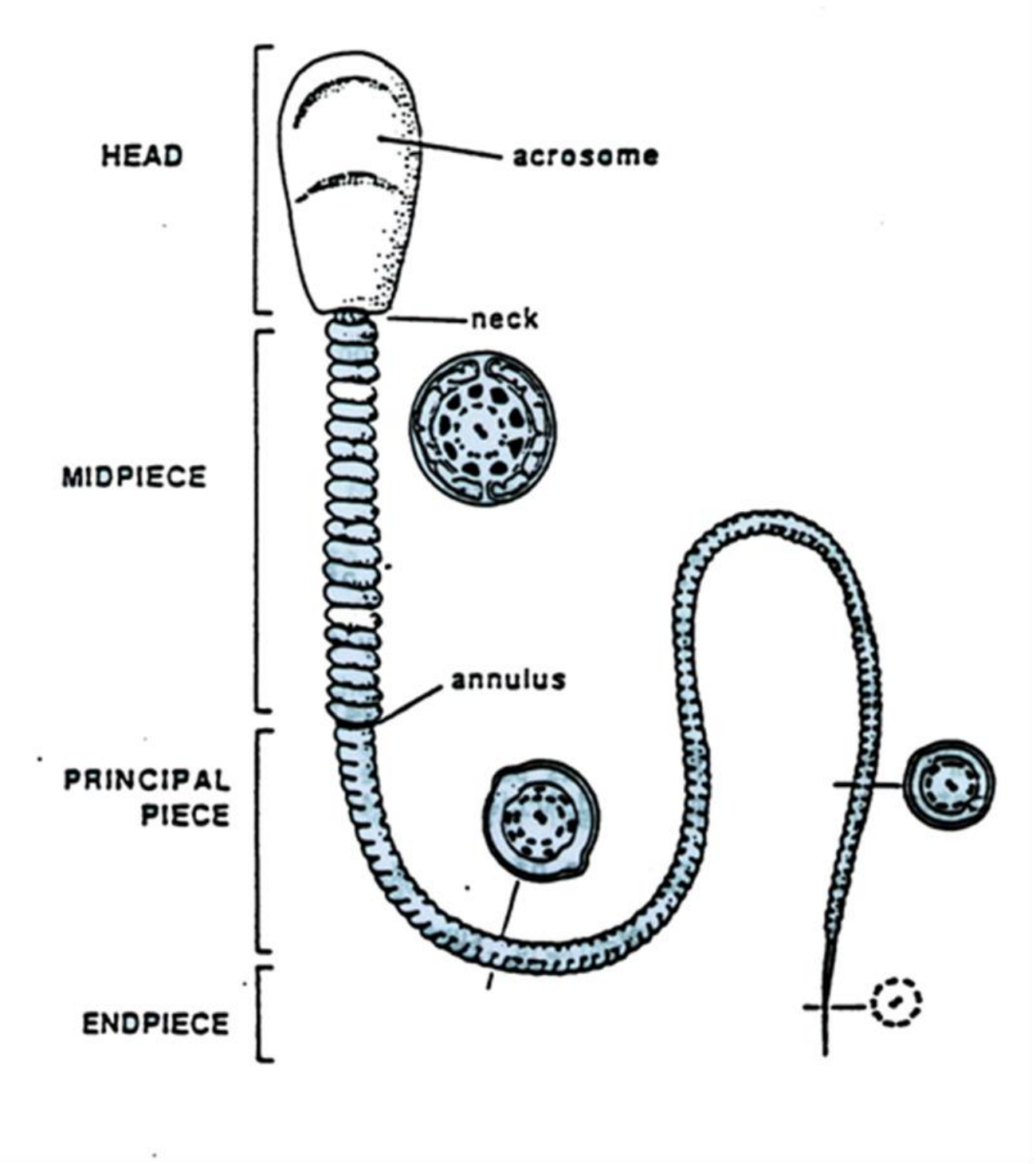
Sperm Morphology



Peter J Chenoweth **BVSc (Hons), PhD, DACT, MANZCVSc**

Sperm Morphology

Sperm Structure



Morphology Assessment

There are many ways to assess sperm morphology, with technical improvements occurring regularly. Methods vary according to complexity, cost effectiveness and the quest for finer detail.

Methods:

- **Bright-Field Microscopy**
- **Ordinary Phase Microscopy**
- **Differential Interference Contrast (DIC)**
- **Electron Microscopy**
- **CASA (Fourier) Analysis**

General Principles:

1. **Higher magnification (≥ 1000X) equals better visualization**
2. **Better optics (mostly objectives) also equals better visualization.**
3. **Sperm should only be placed into 1 category (e.g. normal, head, midpiece tail abnormality etc. based on assumed significance) regardless of the number of different defects present.**
4. **Sub-category thresholds are discouraged.**
5. **The threshold for normal sperm is 70%.**

Microscopic evaluation of sperm morphology should entail counting at least 100 sperm per sample, using 1000X microscopy wherever possible. This magnification can be achieved in the field with a bright-field microscope using an oil immersion objective and stained slides. However, it is preferable to use either ordinary phase or DIC microscopy with a semen sample which is "fixed" as a wet preparation. Appropriate fixatives include buffered formal saline (BFS) or PBS-gluteraldehyde.

Common stains used for depicting sperm include eosin-nigrosin (EN), William's stain and modified Giemsa, while Dif-Quik (DQ) and Papanicolaou ("Pap") stains are also useful. For a comprehensive review of stains and probes used for animal sperm, refer Lorton (2014). Note also that proprietary stains are available, such as SpermBlue, Cell-VU and Goldcyto-SB (using pre-stained slides). A comparison of different sperm stains is available at the site below;
https://scielo.conicyt.cl/scielo.php?script=sci_arttext&pid=S0717-95022012000400045

Currently, differential-interference phase contrast (DIC) microscopy of "fixed" (i.e. unstained) samples is the "gold standard" for certain types of abnormalities, particularly those involving the acrosome and for depicting subtle abnormalities of the sperm head or midpiece. The electron microscope (EM) is the ultimate method for depiction of individual sperm abnormalities, although complex, expensive and difficult to quantify.

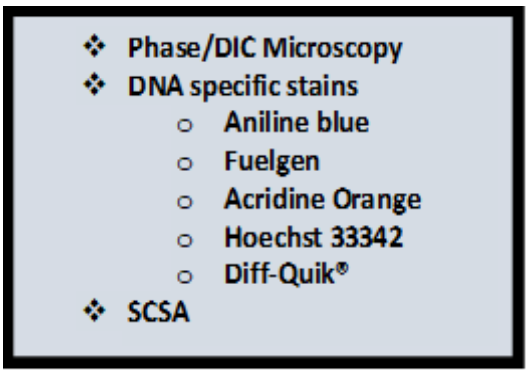
Applications for use with computer-assisted sperm analysis (CASA) systems are available to assess sperm morphology, with the advantage that they can process sperm at high speed, and thus provide easily quantifiable results.

Acrosome Assessment

A functional acrosome is necessary for fertilization under normal conditions. Different techniques exist to detect either the presence of the acrosome (percent intact acrosomes, or PIA), or aspects of its functionality. While brightfield preparations, such as eosin-nigrosin staining, can help to depict the acrosome, they are not ideal for more detailed visualization. Better results are obtained with specific stains (e.g..modified Giemsa, Wells-Awa, Casarret's), or use of phase-contrast microscopy (ordinary or DIC). Acrosome-specific fluorochromes (e.g. Lectins, FITC-PSA, CTC) may also be employed.

Disturbances in sperm DNA/chromatin structure and condensation are linked with infertility in different species. Although one can sometimes observe signs of incomplete condensation using phase or DIC microscopy alone, there are better methods for accurate quantification. However, biomarkers for such damage, such as the diadem/crater defect, can be relatively easily observed using 1000x microscopy.

DNA/Chromatin Assessment



A number of fluorochromes, alone or in combination, have been used either with fluorescent microscopy or with flow cytometry to depict different aspects of sperm DNA/chromatin status. The latter approach provides good quantifiable data via high throughput.

Some useful stains do not require fluorescent technology, thus providing advantages for non-institutional laboratories. These include Aniline Blue, Toluidine Blue and Diff-Quik®.

Acridine Orange (AO) is a fluorescent stain which distinguishes between native (double-stranded) DNA, which stains green, and single-strand DNA which stains orange-red. AO is the basis for the sperm chromatin structural assay (SCSA) test; a flow-cytometry based procedure which has been useful in diagnosing bull infertility.

Sperm Membrane Integrity

Sperm have several membrane systems within the outer cell membrane, or plasmalemma. All need to be competent for proper sperm function. The functional integrity of sperm membranes can be assessed in different ways. An indirect method is to use supra-vital stains (e.g. eosin-nigrosin) which rely on plasma membrane damage to reach and stain underlying structures. The hyperosmotic swelling test (HOS) relies on hypo-osmotically induced changes in membrane-intact sperm which include reflex bending of the flagellum within the distended cell-membrane. This characteristic response is easily observed with phase-contrast or DIC microscopy.

Causes of Abnormal Sperm

Sperm morphology assessment receives much attention because it is relatively simple to perform and it often links form with function. Disadvantages include our inability to detect all abnormalities using conventional technologies, and disagreements on interpretation.

The assumption that abnormal sperm appearance is associated with abnormal function is often proven correct. More problems occur with the reverse assumption, i.e. that normal sperm appearance indicates normal function. Here, it is useful to appreciate both the pre-requisites of fertile sperm and causes of dysfunction.

Classifying Abnormal Sperm

Classification systems employed for domestic animal sperm morphology have included;

1. **Site of Defect; Head, midpiece, tail etc**
2. **Primary/Secondary;** Site of presumed origin (Blom 1950).
3. **Major/Minor**: Presumed significance (Blom 1971) (see below)
4. **Systemic/Non-systematic.** (Chemes and Rawe, 2003) (see below)
5. **Compensable/Uncompensable** Mode/site of action re. infertility (Saacke 1990).

Table 1. Major/Minor Sperm Defect Classification (after Blom 1971)

"Major" Sperm Defects	"Minor" Sperm Defects	Other cells:
Underdeveloped (small, dark) heads Double forms Acrosome defects (eg Knob) Decapitated heads (active tails) Diadem defect Pear shaped (pyriform) defect Narrow at base Abnormal contour Midpiece defects (corkscrew, tail stump) Proximal droplet Strongly coiled/folded tails ("Dag" defect)	Narrow, narrow at base Giant/broad heads Undetached normal heads Detached acrosomal membranes Distal droplet Simple bent tail Terminally coiled tail	Epithelial cells Blood cells Leukocytes Round cells Bacteria

As the Site of Defect (SOD) system offers the advantages of simplicity and unambiguity, particularly as it emphasizes the 4 major attributes required by the spermatozoon to achieve and maintain fertilization, viz;

1. **Intact membranes**,
2. **A functional acrosome,**
3. **Purposeful movement,** &
4. **Competent DNA.**

Important Concepts for understanding and interpreting Sperm Morphology

1. **Specific (Genetic) sperm defects.**
2. **The Role of Oxidative Stress[1]**
3. **Compensable vs Uncompensable sperm defect categories.**

[1]*Oxidative stress adversely affects sperm in a number of ways including; 1. Disrupting disulfide bonds (as in sperm chromatin/DNA[2] and midpiece function), & 2. Causing lipid peroxidation (disrupting membranes and sperm motility).*

[2]*In turn, DNA damage is linked with poor semen quality, low fertilisation rates, impaired pre-implantation development, increased abortion and elevated disease levels (including cancer) in offspring. (Lewis and Aitken 2005).*

Sperm numbers and "quality" can be compromised by a variety of factors which include genetic, traumatic, nutritional, toxic or infectious considerations. Apart from genetic sperm defects and specific spermatoxic agents, both of which are relatively rare, the most common factor is spermatogenic stress. Here, the spermatogenic response is predictable, both qualitatively and temporally (as below).

Specific (Genetic) Sperm Defects

Some sperm defects in domestic animals are considered to be of genetic origin. Genetic sperm defects are consistent sperm defects which are genetically transmitted. However, some sperm defects may be caused either by genetic or environmental influences and some may be due to an interaction of environment and genetic predisposition while yet others are likely to be genetic in origin, although yet unproven. Categorization as a true heritable factor requires a burden of proof that has rigorous rules (Chenoweth, 2005), and thus the term 'genetic' should not be used lightly. This is particularly relevant in the context of the livestock purebred breeding industries, where genetics and marketability often overlap. The classification system of Systematic Sperm Defects (Chemes and Rawe 2003) is inclusive of these considerations, with a current list of recognized systematic (including genetic) sperm defects in domestic animals being shown in Table 2, below.

Table 2. SYSTEMATIC SPERM DEFECTS in ANIMALS

Defect	Sperm Location	Species	Genetic
Knobbed acrosome	Acrosome	Bull, Boar, Ram, Stallion	Yes & No
Ruffled and Incomplete Acrosome	Acrosome	Bull, Mouse	No
Miniacrosome	Acrosome		
"SME" defect	Acrosome/Head	Boar	Yes
Decapitated sperm head, Disintegrated, Decaudated, Headless, Acephalic, Microcephalic, Alteration of head-neck attachment	Head	Bull (Guernsey, Hereford, Swedish Red & White, Red Danish, Friesian, Jersey), Man, Dog	Yes & No
Round heads ("Globozoospermia")	Head	Man, Bull, Ram, Dog	?
Diadem/Crater, Pyriform	Head	Man, dog, bull, ram, boar, stallion	No (predisposition may occur)
Rolled head/Nuclear crest/Giant sperm/ Large heads	Head	Bull, Dog, Man	?
Macrocephalic, Multi nucleated, multiflagellate	Head, Principal piece	Human	?
Teratoid Teratospermia?	Head	Bull, Cat, Stallion, Dog	Yes & No
Pinhead/Megalo pinhead/ Micro-cephalic	Head	Bull, Man	?
Narrow head, Narrow at base		bull	?
Diploid, Polyploid	Head	Bull, mouse, man, rabbit	No
Abnormal DNA condensation	Head	Bull, Ram, Red Deer, Boar, Dog, Man	No
Pyriform head	Head	Bull, Alpaca	No
"Corkscrew"	Midpiece	Bull, boar, stallion	No
Spindle-shaped body, Microtubule mass	Midpiece	Man, rabbit	No
"Hairpin"	Midpiece	Man, Boar	No
Distal Midpiece Reflex (DMR)	Midpiece	All	No
Proximal cytoplasmic droplet	Midpiece	Boar, Bull, Stallion, Guinea Pig, Dog	No
Distal cytoplasmic droplet	Midpiece	Boar, Bull, Man	No
Immotile cilia syndrome, Primary cilary dyskinesis ("Kartagener's syndrome")	Midpiece	Man	Yes & No
Abaxial, Accessory/Offshoot	Midpiece	Bull, Ram, Man Boar, Stallion	Normal in some spp
Disrupted	Midpiece	Stallion, Buck, Bull	No

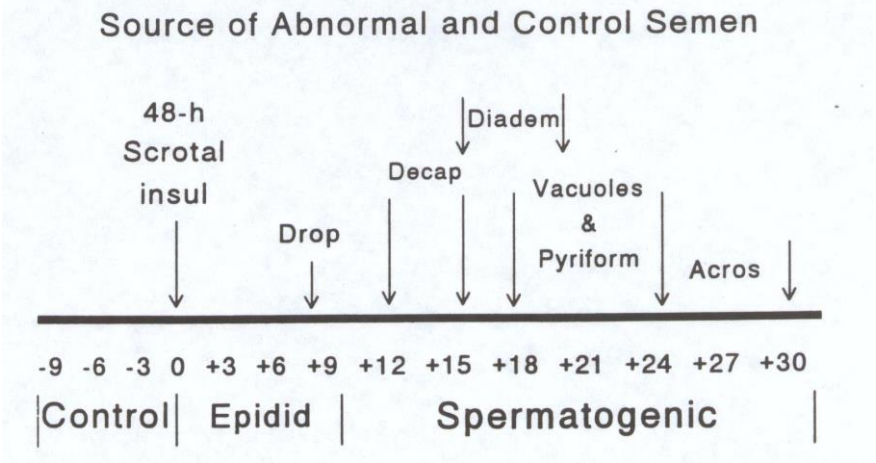
Pseudo-droplet	Midpiece	Bull, stallion, dog	Yes & No
Tail stump	Midpiece	Bull, Mouse, Rabbit, Dog, Boar, Stallion, Man	Yes
Short Tail	Midpiece	Boar, Bull	?
Centriolar-mitochondrial defect	Midpiece	Man	?
Dag defect, "Ard" defect, "Dag-like" defect	Midpiece, tail	Bull, Stallion, Buck, Boar.	Yes & No
Dysplasia of the fibrous sheath (DFS)	Midpiece	Man	Yes
Coiled tails	Tail	Buck, Man, Bull, Stallion, Dog	?
Multiple tails	Tail	Emu, Man	?
Hair pin sperm defect	Tail	Boar	No
Azoospermia	Absence of sperm in ejaculate	Dog, Stallion, Bull, Camel	?

Teratozoospermia = abnormal sperm; asthenozoospermia = immotile sperm; necrozoospermia = dead sperm

The Role of Oxidative Stress.

Although many culprits are implicated in spermatogenic stress, virtually all result in a very stereotypical response from the spermatogenic epithelium. This has been illustrated in a number of studies in which a standardized stressor (e.g. 48h scrotal insulation) is applied to the testicles. The result is a highly predictable temporal series of dominating abnormalities in the ejaculate, as below.

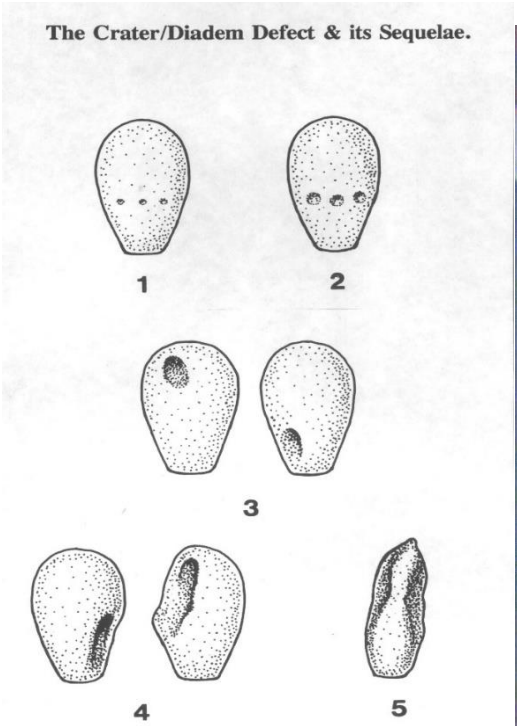
Temporal Appearance of Sperm Abnormalities following 48h Scrotal Insulation.

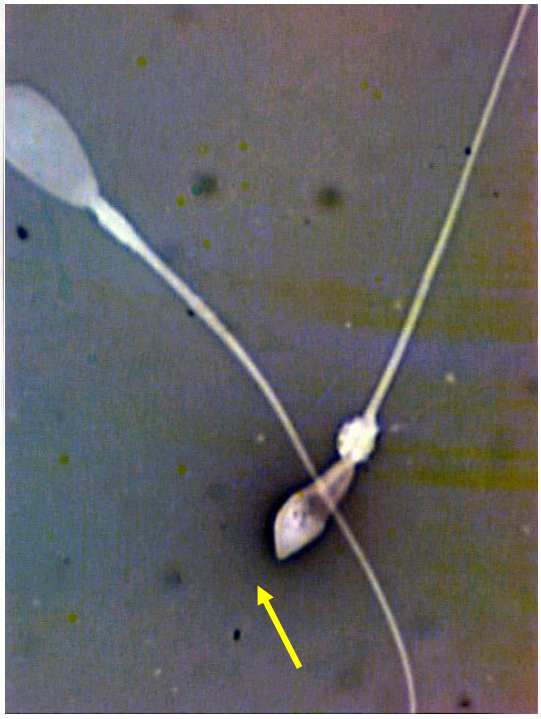


Various forms of head abnormalities occur during this highly predicable "cascade" of sperm defects, starting with the diadem defect (grade 1) and progressing to vacuoles/craters and pyriform defects. This series (the diadem/crater "cascade") is represented diagrammatically below (The Crater/Diadem Defect and its Sequelae).

These sperm head abnormalities are mainly due to problems in DNA/chromatin condensation and organization during spermiogenesis (or the sperm differentiation phase), when changes to chemical chromatin bonds are particularly vulnerable to oxidative damage. An early sign, or biomarker, of this process is the appearance of the diadem/crater defect in sperm heads (see below)

Oxidative damage can occur to other sperm structures apart from the head region. It is also associated with sperm midpiece and tail defects, particularly mitochondrial location and function. Reactive oxygen species (ROS) as well as ubiquitin are also present in residual sperm cytoplasm; i.e. proximal and distal droplets (Aitkin 2002), which act as their repositories.





Grade 5 Diadem/Crater Defect (EN 1000X)

Oxidative Stress and the Diadem/Crater Defect

The diadem/crater defect of sperm is part of a continuum that represents a stereotyped response to a wide variety of spermatogenic insults, as described above. It is observed in most species as part of a predictable, choreographed response to spermatogenic stress and thus represents a reliable early biomarker of such stress.

Although the diadem/crater defect represents a most useful biomarker of sperm oxidative damage, it has limitations. Those defects that we detect even with the most sophisticated microscopy are only part of the story; other damaged sperm remain undetected. In turn, a number of these can be detected with specialized staining procedures for sperm DNA/chromatin status, such as acridine orange, aniline blue or Diff-Quik®. However, even without such tools, we can still be reasonably predictive of sperm/semen potential fertility by applying the 30% threshold rule to the sperm population that we can observe with good microscopy, as below.

The oxidative damage concept of sperm abnormalities provides a relatively simple and integrative platform for sperm morphologists who have long endeavored to attribute significance as well as acceptable thresholds to individual sperm abnormalities. Maintenance of oxidative equilibrium also provides a rationale for the complex thermoregulatory mechanisms which are in place to maintain the temperature of the spermatogenic epithelium at several ^{0}C below body temperature. In addition, as few sperm problems caused by oxidative stress are expressed as a singular abnormality, either within the ejaculate or even within a single sperm, this places greater emphasis on the validity of recording the total proportion of damaged sperm in the ejaculate (i.e. the 30% rule, as below) and less emphasis on allowable levels of specific abnormalities (with the exception of genetic sperm defects).

The “70% rule”

From early on, there has been recognition of a “threshold” of observable abnormal sperm, above which fertility became compromised. This threshold has been quite consistent at the ~30% level for a number of species. In bulls, it was validated in a series of natural mating trials in Texas (Wiltbank and Parrish 1986) where bulls pre-selected for good sperm morphology (70% or greater normal sperm) achieved significantly more pregnancies than unselected bulls and more recently confirmed in the results of the Northern Bull Project (Fitzpatrick et al 2002). Other studies areas as varied as IVF and sperm DNA damage have come to similar conclusions. The threshold level of 70% normal sperm is now widely accepted for different species throughout the world.

Compensable/Uncompensable defects

Sperm abnormalities can cause infertility in a number of ways. One group of abnormalities may not be capable of successfully completing the journey to the fertilization site. These are generally characterized by abnormalities which adversely affect sperm movement, such as tail and midpiece abnormalities and misshaped heads. Barriers in the female tract act to constrain such abnormalities which have been termed ***compensable sperm*** factors (Saacke et al. 2000). Other abnormal sperm may succeed in fertilizing the ovum, but then result in reproductive wastage (either EED or abnormal development). Such problem sperm (***uncompensable sperm***) are less easy to characterize, but include more subtle forms of the diadem defect.

Identifying Defective Sperm.

The recognition & categorization of sperm abnormalities has benefited from recent insights, such as:

1. **Abnormal sperm head morphology** is related to **damaged DNA** (Erenpreiss 2006).
2. **Oxidative stress** is the major cause **of sperm DNA damage** (Aitken 2002: Lewis and Aitken 2005)
3. **Abnormal sperm DNA** causes **male-factor sub-fertility.**
4. Routine sperm assessment parameters are only partially successful in identifying such damage.

Rational Approach to Sperm Morphology Interpretation:

- Compromised sperm often have multiple "problems", some of which are not easily observed with routine techniques.
- Although many sperm abnormalities are recognized, *they mostly result from a small number of possible pathogenic pathways.*
- These pathogenic pathways are predictable, providing detectable ***patterns or clues***

Conclusions:

- Identify the "best" markers (or indicators) of the different causes of sperm damage (e.g. the diadem/crater defect and its sequelae).
- Use best diagnostic techniques and equipment available e.g. 1000X microscopy.
- Record total "abnormal sperm" (regardless of whether they have single or multiple defects)*
- In general, apply the 70% "rule" as the threshold for satisfactory levels of "normal" sperm.
  ** although it is advantageous to count specific defects for collation and monitoring purposes*

Notes on classifying certain sperm defects.

1. Bent or single bend tails

Traditionally, the rule of thumb has been to nominate bent or single bent tails as those being reflected 90^0 or more from the shaft. However, a more intuitive approach is to subjectively decide if the bend in question could be due to normal tail movement or to either a structural defect, or physical disruption.

2. Distal midpiece reflex

These are midpieces that have undergone a single 180^0 reflex. Usually the bend occurs in the more distal part of the midpiece, and often contains a distal cytoplasmic droplet,

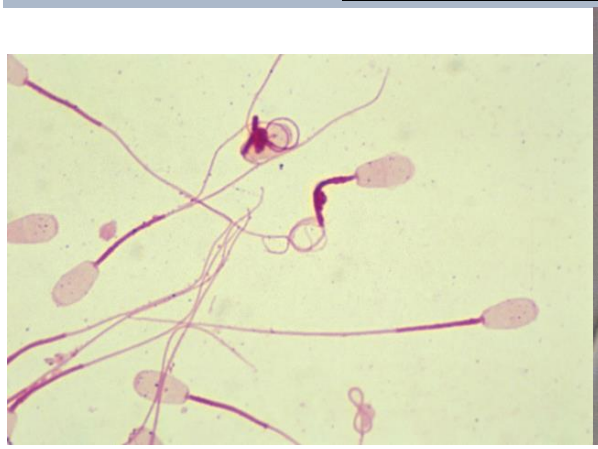
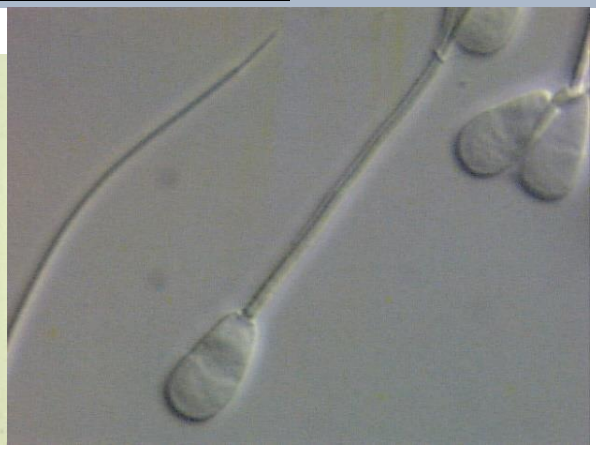
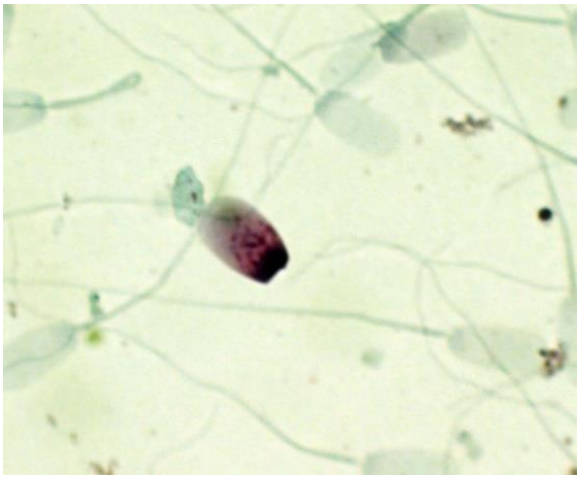
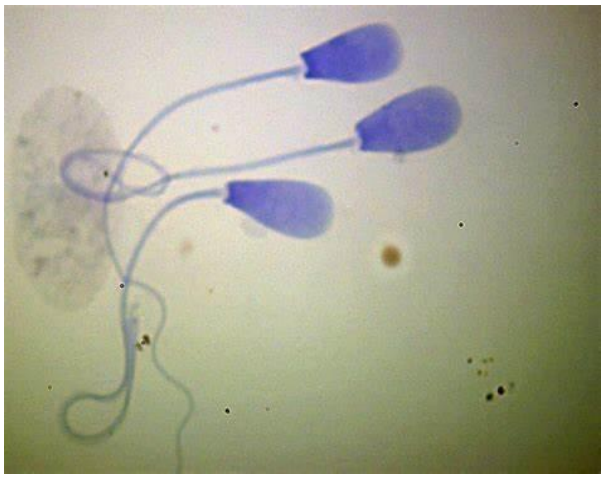
3. Tapered head, narrow at base.

These are difficult to categorize authoritatively for several reasons; a) they can be an observer error caused by variations in sperm positioning on the slide, especially with "wet" preparations", and b) they are generally not recognized as a systematic sperm defect.

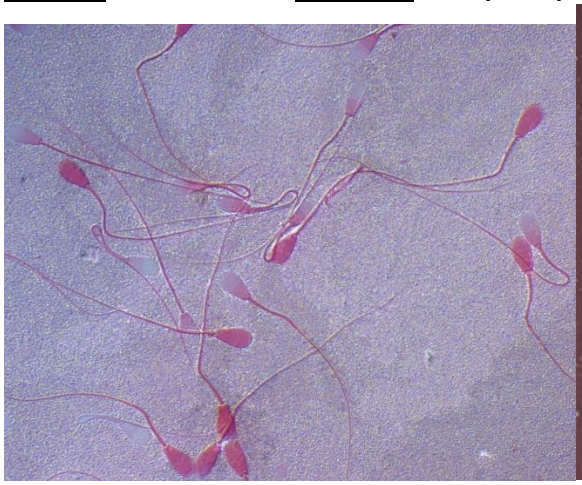
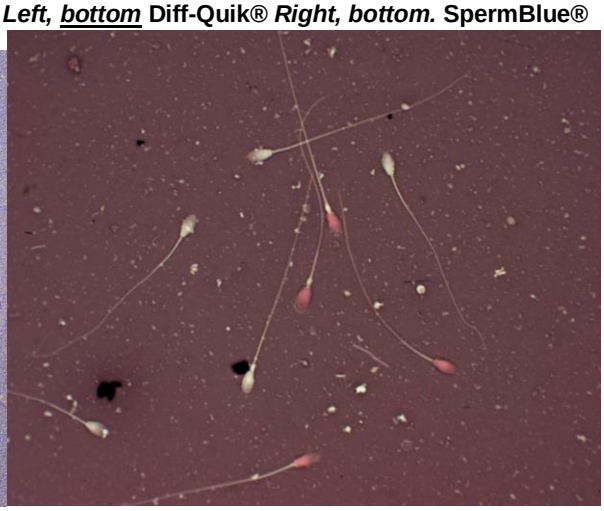
4. Abaxial midpiece.

Abaxial midpieces are observed when the insertion of the midpiece to the head is considered to be off-center. They are relatively common in some species (e.g. horse, pig). However, observer error also occurs due to variations which occur in sperm positioning on the slide. In some species, e.g. the bull, abaxial midpiece positioning is often associated with the presence of a second implantation site, suggesting that this defect is caused by aberrant centriole migration. If so, then it would be a defect which adversely affects sperm fertility, and should be regarded more seriously than some advise.

Semen Stains & Preparations:

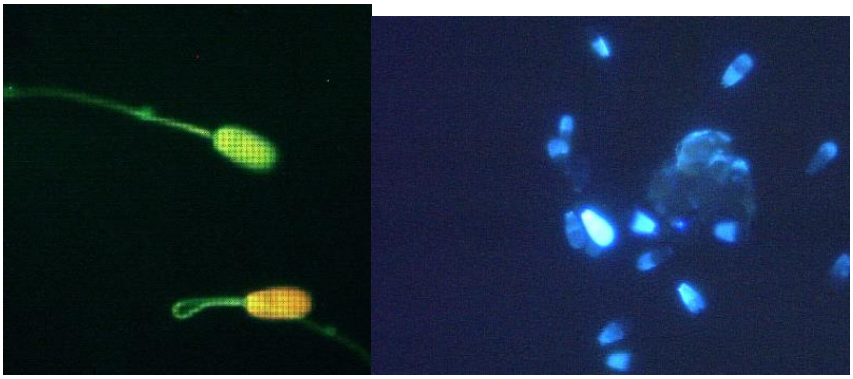
Left, top. **William's Stain,** ***Right, Top*****. DIC (1000x)** ***Left, bottom*** **Diff-Quik®** ***Right, bottom.*** **SpermBlue®**

Left. **Eosin stain (Bull, BF)**

Right. **EN stain, Stallion (BF)**

Notes:

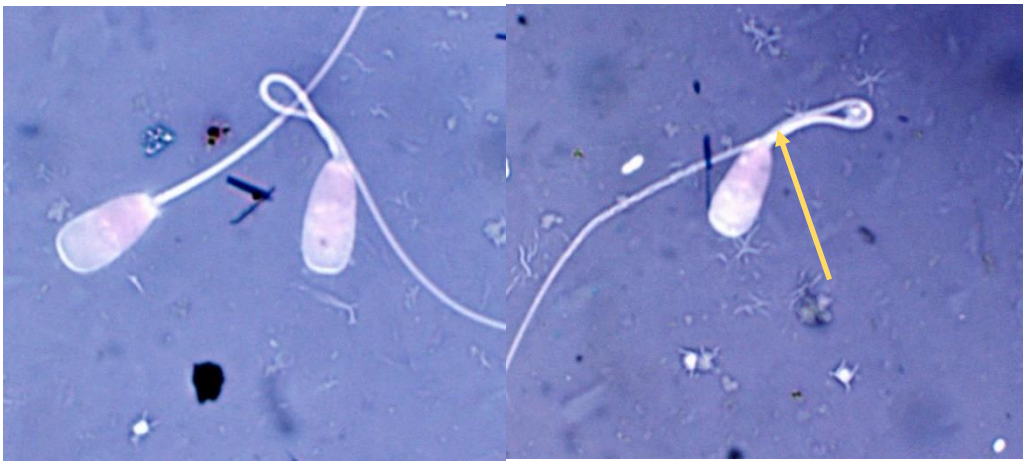


Fluorescent probes*. Left* Acridine orange, *Right* Hoechst 33342

Extenders: A number of extenders are used to prolong the useful life of sperm. To do this, they require different properties depending on whether the extended semen is relatively fresh, chilled or frozen. For frozen semen, a key ingredient is a cryoprotectant, often glycerol or ethylene glycol. Extender should always be added to semen, rather than vice versa, and both temperature and osmolarity can influence sperm viability as well as morphology. High opacity extenders, particularly those which contain much particulate matter (e.g. milk or egg yolk), may pose difficulties in the microscopic observation of both sperm motility and morphology.

Sperm obscured by milk extender; DIC 1000x

Temperature: For best results, add semen to extender or fixative at the same, physiologic temperature. Sperm that have suffered cold stress before or during extension/fixation often show distal midpiece reflexes (DMR) without a retained droplet. DMRs containing droplets are commonly observed as a result of male factors such as immaturity, response to stress or overuse (see below).



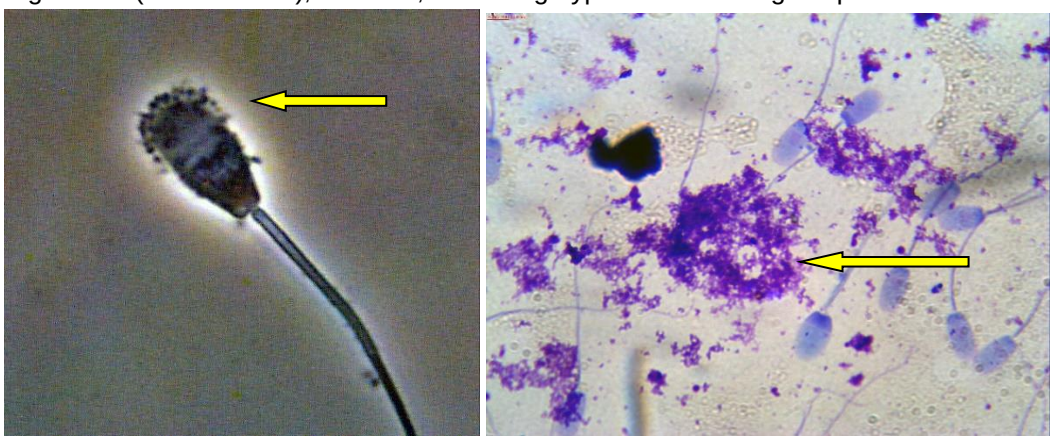
Left. **DMR without droplet, *Right.* Droplet retained within DMR (R). EN stain/BF**

Fixatives: Fixatives are used to kill and preserve sperm while retaining their normal structures. Osmolarity, physiological compatibility and temperature are important factors in achieving this goal. Suitable fixatives include 5% Formal Buffered Saline Methanol or PBS (phosphate-buffered saline) glutaraldehyde. A common approach is to mix raw semen with fixative in a 1.5ml re-sealable micro-centrifuge tube* at a ratio of 1:9 (i.e. 100µl extended semen in 900µl fixative).

**Ensure that all plastics employed are biologically safe for semen*

Slide Preparation: On a clean slide, write the animal's identification number/name. Mix the tube with fixed semen **very thoroughly**. Place a few drops of fixed semen on the slide and carefully place a coverslip on the semen. Leave the sperm to settle for at least 1 hour before reading morphology at 1000x magnification under oil immersion using DIC or Phase (1000X). Alternatively, read morphology using a stained preparation (as above) and bright field setting (BF) on the microscope @1000X.

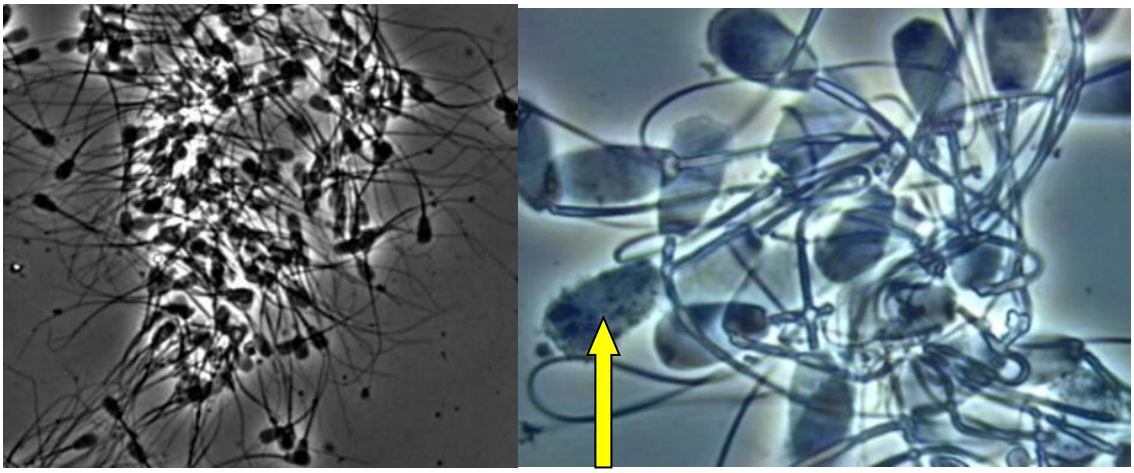
Bacteria: The presence of some bacteria in extended semen is normal, despite the common practice of adding antibiotics to extenders. However, significant numbers may be regarded as a flag to check any and all possibilities of contamination. Although such presence does not automatically disqualify semen from normal use or results, bacteria can adversely affect both the sperm environment and sperm structures themselves. Bacteria can be visualized under 1000x magnification as moving organisms (cocci or rods), colonies, branching hyphae or adhering to sperm membranes. (see below).



***Left*. Unstained bacteria adhering to sperm heads (1000x Phase); *Right.* Bacteria, Diff-Quik® (BF)**

Clumping: Clumping occurs for a number of reasons, and for some species is a normal event. However, in domestic species, it is often due to bacterial contamination. Clumping leads to problems in sperm morphology assessment as sperm within clumps are often difficult to fully visualize. Here, the morphologist should attempt to record only those sperm that can be clearly observed from acrosome to end of the tail. If the clumping is especially problematic (Figure 5), then it is suggested that another attempt at slide preparation be made from the same sample after thorough mixing.

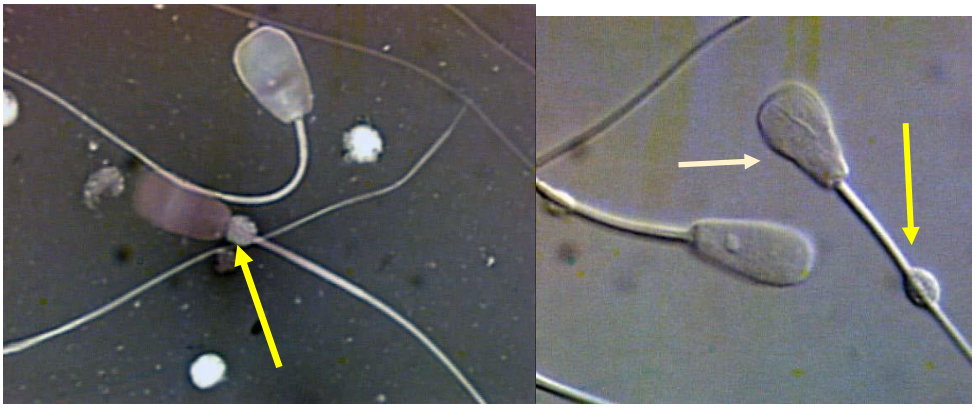
Notes:



***Left*. Severe clumping (Phase 400X) and *Right* (Phase 1000X) with bacteria (arrow).**

Morphological Defects:

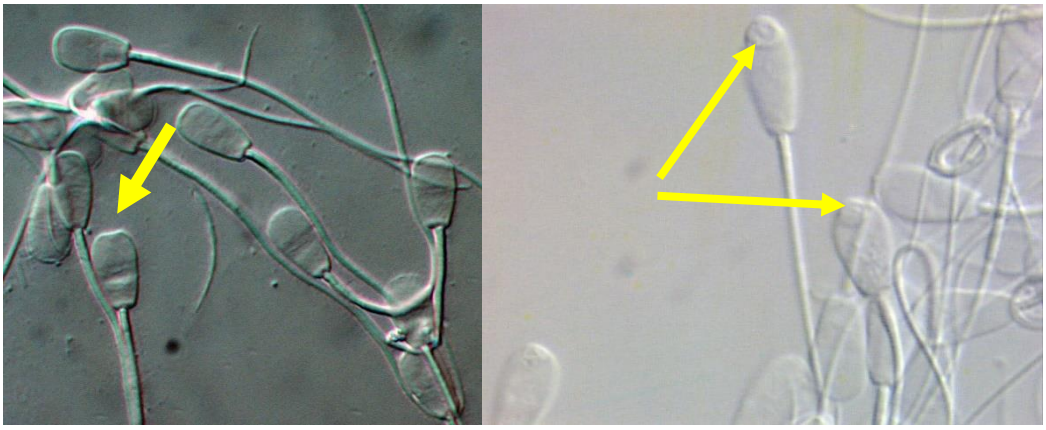
Cytoplasmic droplets: Droplets should normally detach from sperm before ejaculation. When present, droplets are regarded as a defect and are either classed as proximal (immediately under the head) or distal (at the distal end of the midpiece).



***Left.* Proximal cytoplasmic droplet (EN, 1000X) *Right.* distal droplet (DIC 1000X) plus pyriform sperm head.**

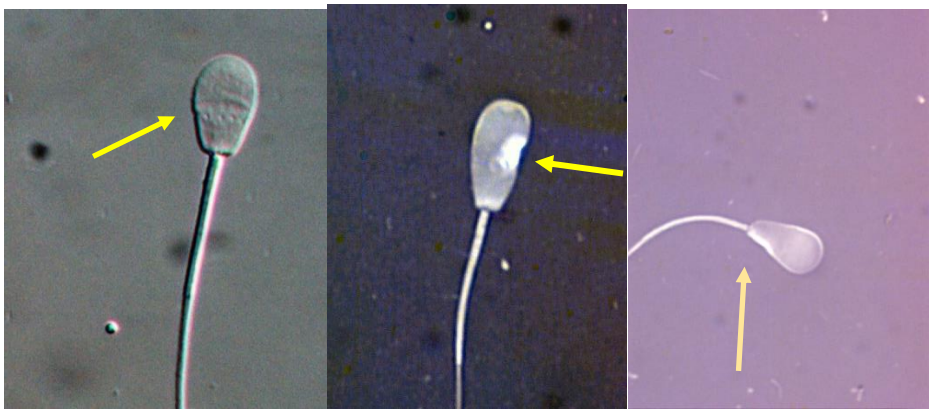
Acrosomes: An intact acrosome is essential for sperm binding to the zona pellucida membrane (ZP) and therefore fertilization. Defects include ruffled acrosomes, knobbed acrosomes and partially or completely absent acrosomes. Causes of acrosome defects include membrane deterioration (age, bacteria, osmotic problems), temperature and genetics. A count of percent intact acrosomes (PIAs) can be used as a proxy indicator of semen handling and processing.

Notes



Left. **Knobbed acrosome, notched form (most common) and** ***Right*****. bulbous form**

Heads: All of the DNA (chromatin) or genetic material of a sperm is located in the head in a tightly condensed form. Following different forms of stress, sperm heads usually show a progressive series of defects starting as subtle 'diadem formations' in the equatorial region of the sperm head, and then progressing to craters and deformed heads, including pyriform formations (see diagram above). This predictable progression of defects is caused by a variety of stressors including heat stress (including pyrexia), spermatoxins, glucocorticoids, and other causes.

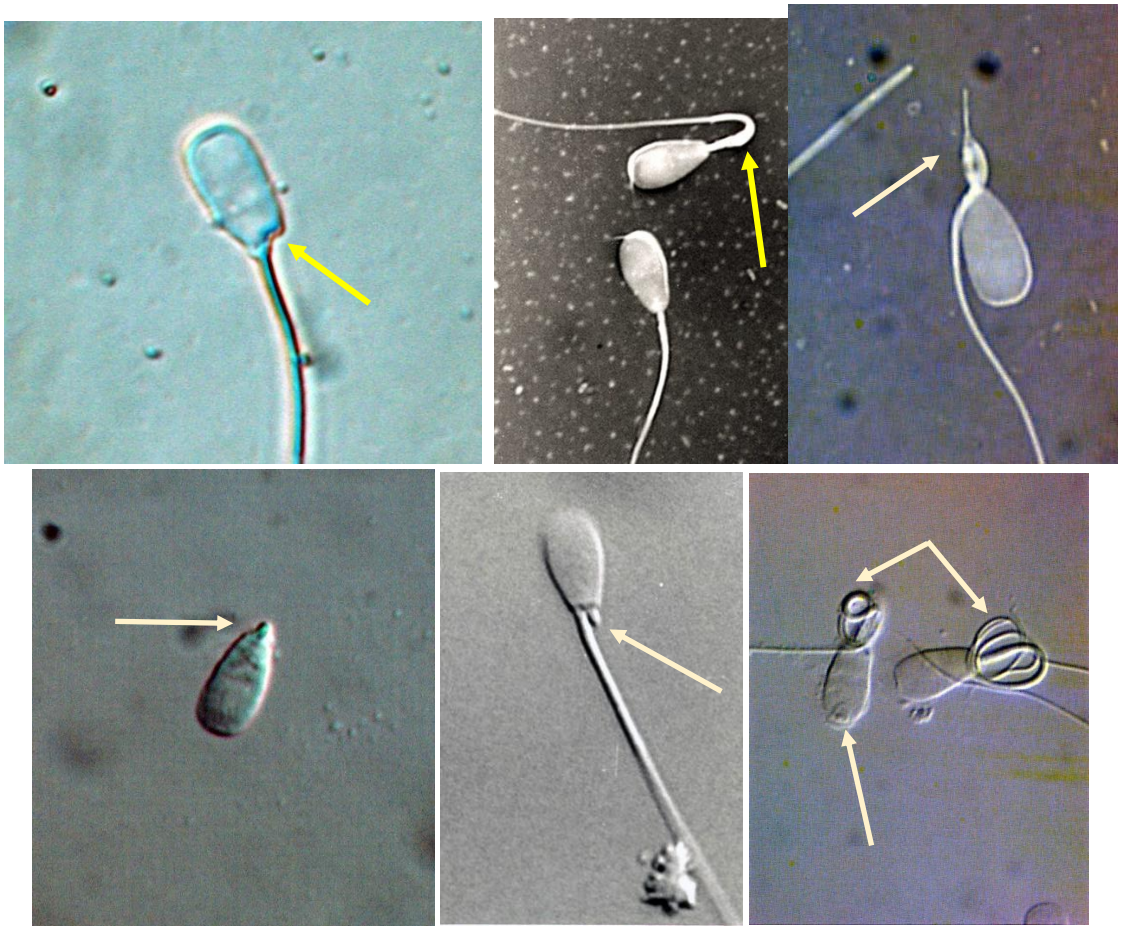


Left**. Crater/diadem Grade 1) in equatorial region,** ***Middle.*** **Grade3-4,** ***Right.*** **Pyriform head**

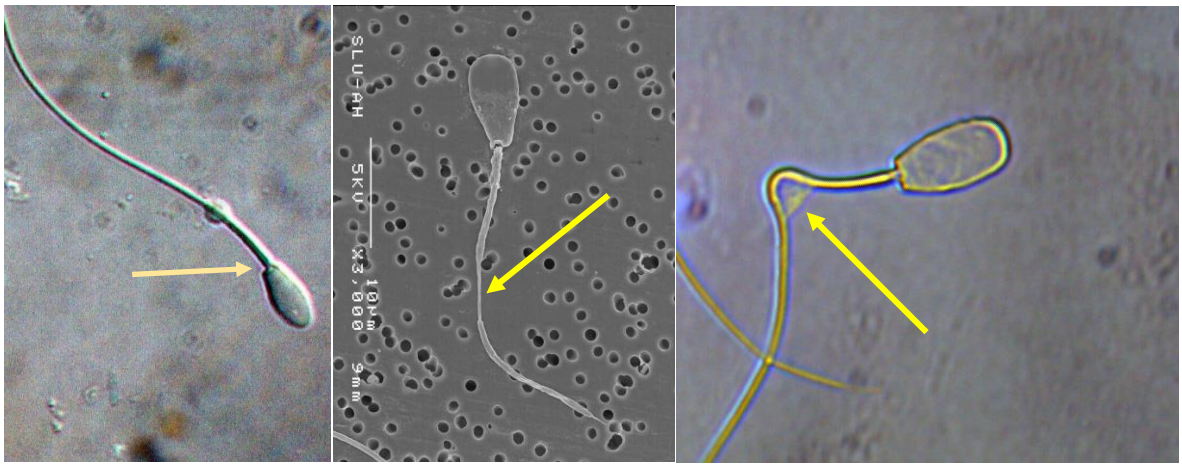
Other defects include small, round, detached and double heads (see list of systemic sperm defects above) as well as "rolled heads/nuclear crests/giant heads".; due to excessive chromosomal content.

Left. **Giant head (Boar, DIC,);** ***Middle.*** **Double head (Phase).** ***Right.*** **rolled head/ nuclear crest (Phase)**

Midpieces: Common midpiece defects include distal midpiece reflex (DMR; as above), abaxial attachment, accessory midpieces, tail stump defect as well as thickenings, kinks, notches, gaps etc

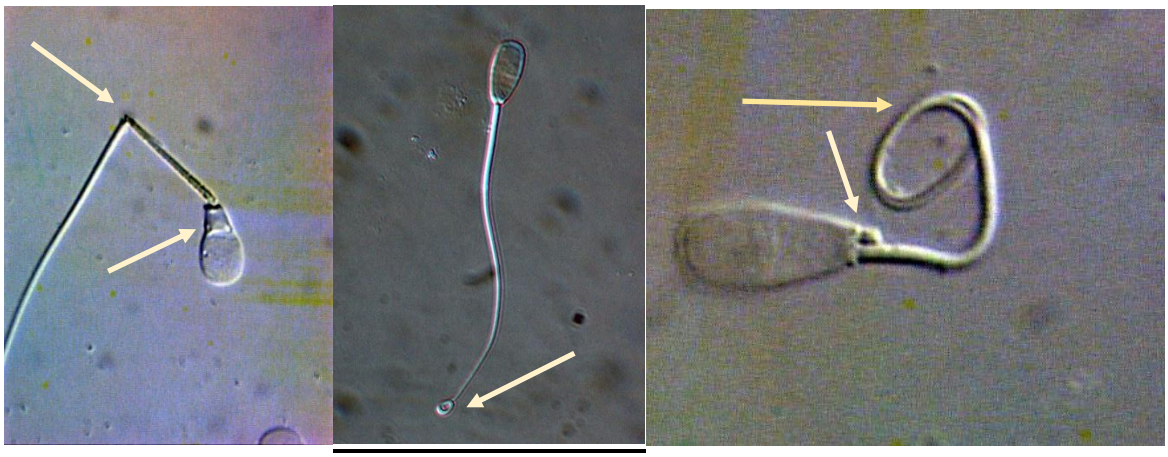


***Top Left*. Abaxial attachment (Bull. DIC 1000X. Note 2nd implantation socket, arrow). *Top Middle*. Thickened midpiece reflex (Phase 1000X) *Top Right.* Broken midpiece (DIC 1000X). *Lower Left.* Tail stump defect (DIC 1000X), *Lower Middle*. Accessory Midpiece (DIC 1000X), *Lower Right.* Coiled midpiece ("Dag defect") plus KA (DIC 1000X)**



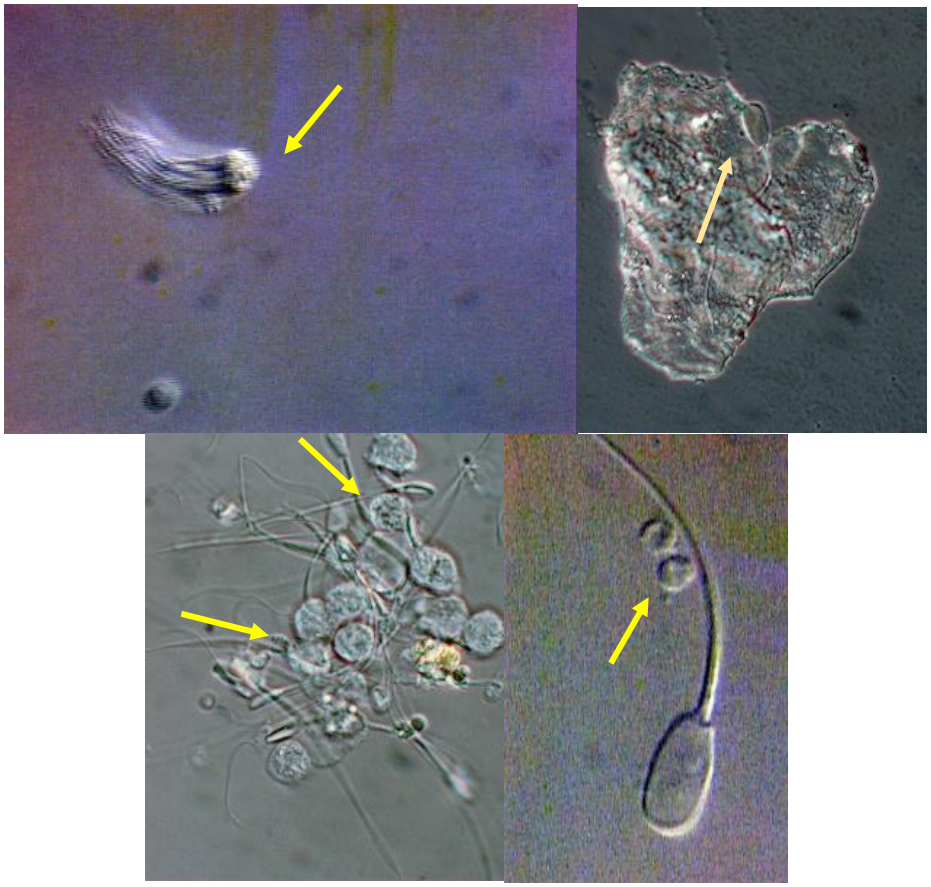
***Left.* Abaxial (normal) plus DD, Stallion (DIC 1000X), *Middle*. Mid-piece "gap" (Bull, SEM. *Courtesy Prof J Chacon, Costa Rica.*). *Right.* DMR due to hypo-osmolarity (note membrane) (DIC 1000X)**

Tails: Common defects include bent, folded, coiled and broken tails



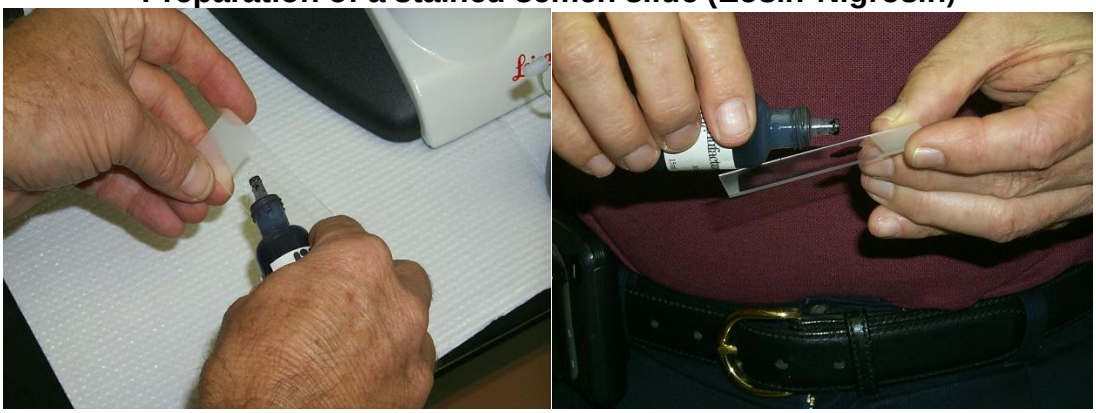
Left. **Broken tail plus pyriform head (DIC 1000X),** ***Middle*****. Terminal tail coil*** *Regarded as a cold-shock effect.* **(DIC 1000X),** ***Right*****. Coiled tail plus accessory Midpiece**.

Other cells: other cells observed in semen include round cells (which can include spermatogenic precursor cells and inflammatory cells), epithelial cells, red blood cells and "Medusa formations".

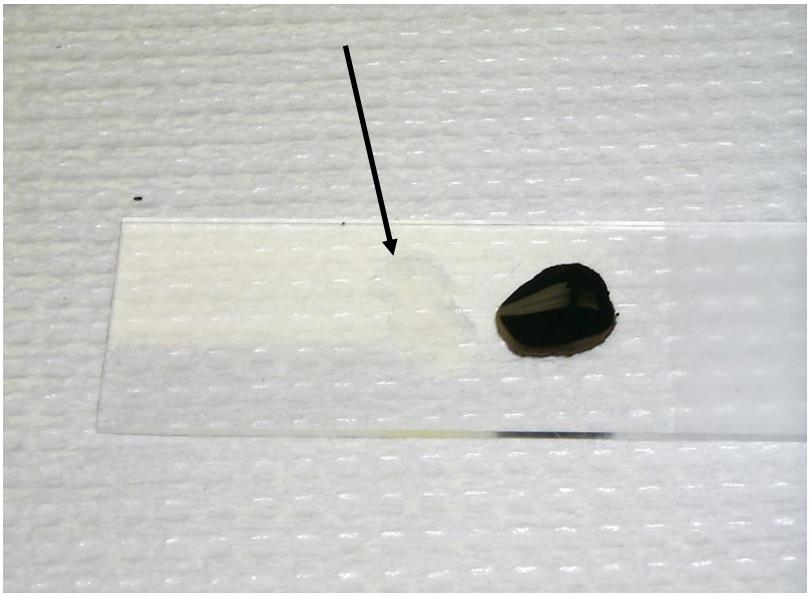


Top Left**. "Medusa formation (Bull DIC 1000X),** ***Top Right*****. Epithelial cell plus sperm (Bull DIC 1000X)**
Lower Left**. Round cells in poor semen sample (Ram Phase 1000X),** ***Lower Right*****. RBC (Bull DIC 1000X)**

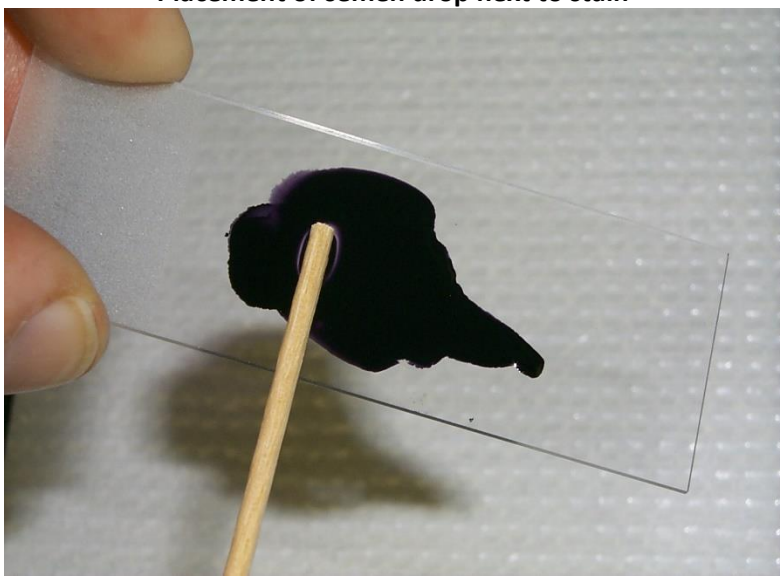
Preparation of a stained semen slide (Eosin-Nigrosin)



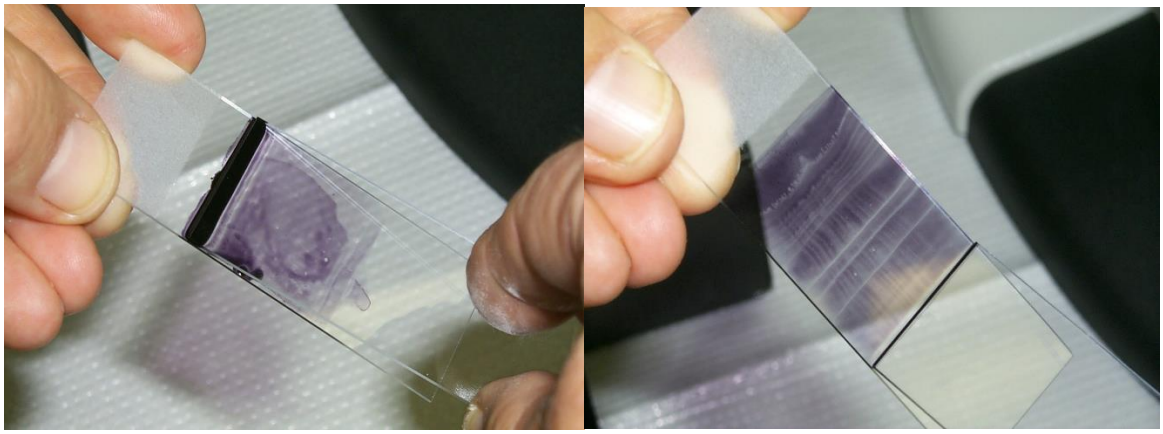
Left and Right. **Applying stain to slide.**



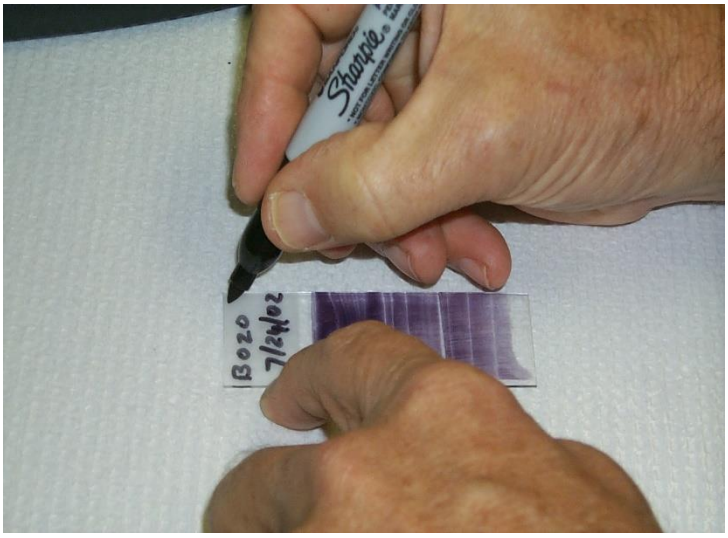
Placement of semen drop next to stain



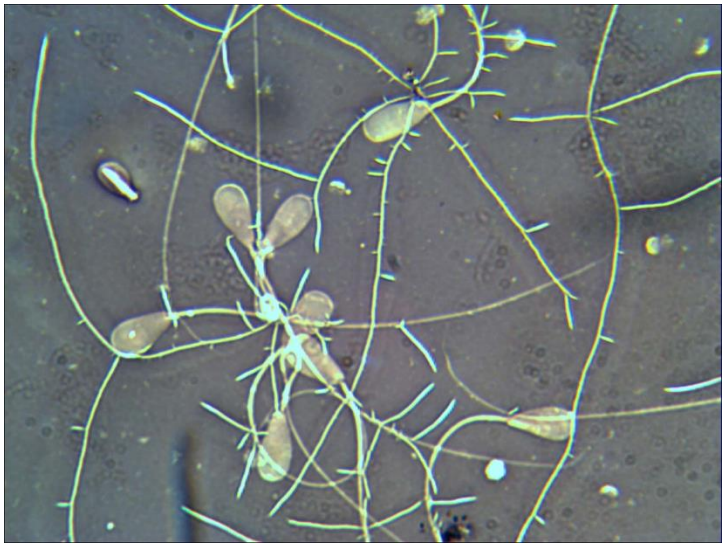
Gently mixing semen and stain with wooden applicator



Making smear by “feathering” mixture of semen and stain



Appropriately labelling the stained semen slide



Example of “cracking” of over-thick stain

Notes: