Sperm morphological defects in dogs: causes and consequences
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Abstract
Evaluating fertility (or ‘fertility potential’) of a stud dog is an important part of breeding management. True indices of canine fertility are high pregnancy rates (≥ 75%) and large litters. However, both are retrospective measures and are strongly influenced by factors independent of the stud dog, including fertility of the bitch and breeding management. In clinical practice, predicting likely fertility of a male is usually required prior to breeding. Within the context of designating dogs to have ‘satisfactory breeding potential,’ it is generally accepted that thorough physical and reproductive examinations and conventional semen evaluation generally provide a useful alternative to actual fertility data. Although poor semen quality is a good indicator of subfertility, conversely, good semen quality is not a guarantee of acceptable fertility. Therefore, considerable effort is being invested in identifying tests and markers to determine functional sperm capacity that can more accurately predict a male’s fertility. This is no easy task, given that any one test is likely to measure only one (or perhaps a few) of the many attributes that a sperm must possess for successful fertilization of an oocyte. Notwithstanding that important limitation, the objective is to describe morphological sperm defects likely to affect fertility potential of a male dog.

Introduction
Since the discovery of sperm by Van Leeuwenhoek in 1677, sperm morphological assessment has been employed in semen evaluation and it is widely accepted that specific structural defects are associated with male infertility. Although new methods have been introduced for semen examination, light microscopy examination of stained semen smears are still used for routine morphological evaluation. This approach provides a general assessment of the sperm head, mid-piece and tail, whereas advanced microscopy methods yield more detailed insights regarding the inner and outer fine structures of sperm. Regardless of animal species, evaluation of sperm morphology and its clinical usefulness has always been somewhat contentious, as a subjective evaluation of sperm morphology can lack precision, repeatability and accuracy. Therefore, well-defined criteria for normal sperm morphology, and those with morphological defects, are a prerequisite for clinical evaluation of male fertility potential.

Sperm structures
Although sperm of various animal species have great variation in ultrastructure, their principal components are head, mid-piece, and tail, and their goal is to deliver an intact haploid genome to an oocyte at fertilization for a successful pregnancy. Sperm length varies among animal species, ranging from 50 μm in boars to 90 μm in bulls (Table 1).

The plasma membrane surrounding sperm has regional surface domains, consisting of specific glycoproteins and lipids. These surface domains are important for specific functions, e.g. capacitation and fertilization. A disintegrin and metalloproteinase (ADAM) family of transmembrane peptidase protein that is essential for fertilization is localized on the sperm plasma membrane. The acrosome is a cap-like organelle covering the anterior two-thirds of the sperm head (acrosome morphology differs widely among animal species). The acrosome, which is derived from the Golgi apparatus of the spermatid, contains proteolytic enzymes such as acrosin, hyaluronidase and many other hydrolases and esterases. These enzymes promote lysis of the zona pellucida, facilitating penetration of the corona radiata of the oocyte. During an acrosome reaction, these enzymes are released when the outer acrosomal membrane coagulates with the plasma membrane. Mature dog sperm (from cauda epididymis or and fresh ejaculates) have a functional membrane-bound progesterone receptor; induction of this receptor (by either progesterone or a calcium ionophore) initiates an acrosome reaction. However, acrosomal damage renders a sperm incapable of binding to the zona pellucida and penetrating the corona radiate.
A mature sperm nucleus is highly condensed and very resistant to chemical and physical insults. The sperm nucleus is usually dorsoventrally flattened, although the shape of the outline is very species-specific (varies from oval to falciform). Condensed nucleus and lamella-like arrangements containing protamines are apparent with transmission electron microscopy. A small region in the neck of sperm has uncondensed chromatin where transcription, translation and protein synthesis are possible; this explains the existence of sperm RNAs and their possible epigenetic and developmental functions. Incomplete condensation of nuclear chromatin and the presence of nuclear vacuoles are two morphological abnormalities caused by protamine deficiency. The concave implantation fossa serves as the attachment of the head to the mid-piece. Cytoplasm and the cytoskeleton only exist between the plasma membrane and acrosomal membrane, as well as between the acrosome and nucleus.

The neck links the sperm head and the flagellum, and contains segmented columns and a dense fibrous structure "capitulum". There are nine columns and nine outer dense fibers of the flagellum at the junction of neck and middle piece, and mitochondria with small projections between the longitudinal columns into the connecting piece. The neck serves an articular piece, whereas mitochondria supply energy.

The tail consists of mid piece, principal piece, and end piece (Figure 1). The mitochondrial sheath surrounds the axonemal complex and the nine outer dense fibers. The outer dense fibers participate in internal fertilization. The axonemal complex consists of a central pair of two single microtubules, surrounded by uniformly arranged nine double microtubules. The mitochondrial helices surround the contractile elements for high flexibility. The number of gyres and total length of mitochondria vary widely among animals. For example, there are 10-12 gyres in humans and bulls, 15-17 gyres in dogs, 90 gyres in mice and 350 gyres in rats. The principal piece is the longest segment of the tail and is enclosed by fibrous sheaths containing two longitudinal columns and circumferentially oriented connecting ribs halfway around the tail. This sheath abruptly ends in the tail, where the principal piece merges into the end piece. The fibrous sheath not only provides proteins for signaling pathways, but is also involved in regulation of sperm maturation, motility, capacitation, hyper-activation, and the acrosome reaction.

Staining methods

In clinical practice, morphology of individual sperm is determined by examining an eosin-nigrosin stained semen smear under oil immersion (×1000 or ×1200 magnification). The recommended technique for the preparation of an eosin-nigrosin stained semen smear:

- Place a 4- or 5-mm drop of warm stain near the end of a warm microscopic slide.
- Place a drop of semen near the stain and mix the two on the slide using a Pasteur pipette. The size of the drop of semen varies with the density of the semen sample. For very concentrated semen use a 2-mm diameter (small) drop and for dilute semen use a 6-mm diameter (big) drop.
- To make a smear, a second slide held at 30° to 40° angle is pushed against the drop of stained semen and then pulled slowly across the slide.
- The smear should result in sperm being evenly distributed on the slide to facilitate evaluation of individual cells. If smear is too concentrated, sperm will overlap, making evaluation of individual cells difficult.

Advanced staining methods for determination of sperm structural integrity

Individual fluorochromes for specific structure, or combinations of fluorochromes for concurrently evaluating more than one sperm compartment, have been established. Various probes are utilized to determine structural and functional integrity of sperm organelles such as viability, DNA fragmentation, mitochondrial function, and acrosome integrity. Morphology classification

Sperm abnormalities are often classified as primary and secondary (based on their origin) or as major and minor (effect on fertility). Regardless, it is generally recommended that morphological
abnormalities should be designated by their descriptive name. Various sperm morphological defects, including their origin and classification, are shown (Table 3).

Primary abnormalities are due to abnormal spermatogenesis, whereas secondary abnormalities are caused during transit through the epididymal duct system, during semen handling, or as a consequence of pathological conditions. A greater incidence of major defects is associated with impaired fertility probably as a result of abnormal conditions of the testis or epididymis, or from genetic defects. Minor defects are considered less important for male fertility, unless present in a large percentage.

Normal potential males should have > 70% morphologically normal sperm, with < 10% and < 20% primary and secondary abnormalities, respectively. Regardless, some males with 50 to 70% normal sperm had higher than acceptable fertility, whereas some males with > 70% morphologically normal sperm fail to do so. Furthermore, combining two characteristics, namely total numbers of morphologically normal and progressively motile sperm per ejaculate was more accurate for predicting fertility than a single criterion. In that regard, artificial insemination with 200 x 10⁶ morphologically normal, progressively motile sperm increased pregnancy rates in bitches.

Sperm abnormalities may also be classified as compensable and uncompensable defects. Males requiring more sperm to reach their optimal fertilization rate were considered to have compensable sperm deficiencies, whereas males having lowered fertility independent of sperm dosage were considered to have uncompensable sperm defects. Increasing total sperm number in an insemination dose may compensate for abnormal sperm that are not transported to the oviduct or are incapable of penetrating the zona pellucida. However, abnormal sperm not filtered by the uterus and are capable of oocyte penetration, resulting in a zona reaction that cannot be compensated for by increasing the sperm number in a breeding dose.

Morphological defects: causes and consequences

Detached heads, knobbed acrosomes, detached acrosomes, proximal and distal cytoplasmic droplets, bent midpieces, bent tails, tightly coiled tails over the midpiece and proximally coiled tails are common sperm abnormalities in dogs. Specific abnormal morphology associated with infertility in the dog include abnormalities of mid-piece attachment or ultra-structure, microcephalic sperm, and proximal retained cytoplasmic droplets.

Any insults to male reproductive organs, caused by trauma, inflammation, infection, or neoplasia, and/or indirectly by chemical, behavioral, thermal metabolic, immune-mediated or hormonal insults will decrease sperm production and increase morphological defects.

Defects of the sperm head

Sperm with intact and structurally normal plasma and acrosome membranes are generally capable of undergoing capacitation and an acrosome reaction. In contrast, abnormal acrosomes are often associated with abnormal spermiogenesis, sub-fertility and infertility in several species, including stallions, bulls, boars and rams. Acrosomal defects have several underlying causes, including prolonged sexual rest, and the fixation process for morphological evaluation. In addition, genetic causes have also been suggested.

Detached acrosomes, partially or completely lost acrosomes are frequently detected as acrosomal abnormalities. Asymmetric thickening of the acrosome cap, small acrosomes, and droplets attached to the acrosome membrane are also described. The knobbed sperm defect, a protrusion of the acrosome, has been identified in man, stallion, bull, boar, ram and dog; this defect is often associated with other morphological deviations and results in sub-fertility and infertility. Sperm with acrosomal defects are incapable of binding and penetrating the zona pellucida and they may undergo premature capacitation and a spontaneous acrosome reaction.

In various species, nuclear and acrosomal vacuoles, fluid-filled membranous cavities similar to cytoplasmic droplets, membrane bound vesicles with clear fluid in the acrosomal and mid-piece region, swollen acrosomes and mitochondria at the mid-piece, are also observed.
Abnormal size and integrity of the nucleus and irregular chromatin condensation primarily constitute sperm head defects. Light microscopy identifies giant and dwarf heads, deformed heads and double heads, whereas electron microscopy recognizes the ultra-structure of sperm such as rolled heads and nuclear crests. Giant heads are often diploid or even tri- or tetraploid. Round-head-syndrome has also been observed; this was associated with defective acrosome biogenesis and tail defects. Sperm head morphological defects including, heads with small and large vacuoles, are attributed to defects in chromatin condensation or incomplete condensation. Nuclear vacuoles have also been reported in pathologies such as inflammation of accessory sex glands, varicocele, hyperthermia, testicular tumors, and inflammatory bowel disease. Incomplete condensation is a sign of immaturity and it is associated with low chromatin stability and teratozoospermia of the sperm head. Sperm with abnormal chromatin are incapable of fertilizing oocytes or they result in defective development of early-stage embryos. Chromatin fragmentation and defects in histone-protamine exchange may be attributed to an abnormal chromatin structure.

Defects of the sperm mid-piece

A lack of the mitochondrial sheath and an enlargement of the fibrous sheath constitute morphological deviations of the mid-piece. Cytoplasmic droplets are the most common defect, present at the neck region, or somewhere along the mid-piece or principal piece of the tail. In normal sperm, residual cytoplasm is released along the tail during spermiogenesis; therefore, a disturbance of maturation process causes persistent cytoplasmic droplets. Proximal cytoplasmic droplets are generally regarded as detrimental to fertility and the defect was considered as major defects of sperm. Currently, distal cytoplasmic droplets are also considered detrimental to fertility and embryonic development, due to ubiquitination. Pseudo-droplets are thickening of the mid-piece. Granularity of mitochondria is another abnormality of mitochondria, apparently under the electron microscope. Strong folding, coiling and fracture of the distal part of the mid-piece with or without retained distal cytoplasmic droplet were regarded as a "dag" defect. Mitochondrial sheath defects, the loss of single mitochondria and irregular axial fiber bundles are ultra-structural damages (viewed with an electron microscope). The "corkscrew" defect, another mid-piece defect, has also been described in bulls.

Structural abnormalities of the sperm tail

Abnormal tubular patterns in the tail are considered detrimental and sperm having defective patterns are immotile and unable reach the site of fertilization, resulting in sub-fertility and infertility. Simple coiled or broken tails and double tails are among the most common sperm defects seen on routine light microscopic examination. The "tail stump" defect was reported in bulls. Hyperplasia and marked disorganization of fibrous sheath, and axonemal and microtubule doublet distortions cause the "tail stump" defect. This defect results in sterility and it is thought to result from a genetic mutation. Most common observed defects are deviations from the normal tubular structure 9 + 2 + 2 structure; some tails have only three or four microtubules at the distal part. The flagella sometimes lack the central pair microtubules ("9 + 0" structure; this defect causes immotility). Mutations in sperm-associated antigen 6 gene are known to cause this defect. Additionally, reduced motility is caused by defects of peri-axonemal structures. Deformed or incomplete arrangements of the axial filaments, vacuolization of the axoneme, as well as an abnormal arrangement of the mitochondrial helix, have all been described in mammalian sperm. However, disorganized mitochondria, abnormal position of outer dense fiber and abnormal size of outer dense fiber are rarely present in sperm. Anomalies such as invagination or vacuolization of the outer plasma membrane can also be present. The immotile-cilia syndrome (primary ciliary dyskinesia) is caused by a lack of the dynein arms is also reported. Dogs with fucosidosis (deficiency of the enzyme fucosidase, which metabolizes the sugar fucose) resulted in abnormal spermatogenesis and sperm maturation (retention of proximal droplets), with morphologically abnormal sperm and poor motility.
Approaches to determining sire fertility

Male fertility can be estimated by applying the following:

1. Breeding soundness evaluation
2. Elucidation of sperm-specific organelles and their association with reproductive outcome
3. Correlation of mRNA expression of genes which are important for sperm structural and functional parameters with fertility outcome

In addition, micro RNA and associated gene regulators and genomics could also contribute to predict fertility potential. Each of these approaches has advantages and disadvantages. Even though these methods have merits over one another when applied individually, it is advisable to use combination of these tests to predict sire fertility (Fig. 2).

Application of breeding soundness evaluation

Breeding soundness evaluations (BSE) are commonly used for identifying males that have satisfactory breeding potential and those that are clearly unsatisfactory. The male should meet minimum standards (e.g. those of the Society for Theriogenology). It is important that BSE of a male should be done in a highly professional manner. Even though a BSE is the most commonly used method in the clinical field, the determination of fertility is limited to the test day.

Application of laboratory methods to determine associations of sperm organelles function and fertility

The ultimate goal of semen evaluation is to predict the fertilizing capacity of an ejaculate. Unfortunately, conventional sperm characteristics are not well correlated with the fertilizing capacity of sperm and both inter- and intra-assay variability of these characteristics are high. Furthermore, it is challenging to predict fertilizing capacity, as there is no single sperm parameter that accurately predicts fertility in vivo. Therefore, advanced techniques for semen evaluation are needed to increase the odds of achieving an accurate prediction. Researchers have used additional laboratory assays to accurately predict the fertilizing potential of a semen sample. Among them are assays that evaluate sperm DNA fragmentation index (DFI), membrane integrity of sperm and other organelles. Studies that determine the association of intactness of sperm organelles with fertility outcome, concluded that

(i) the chance of siring offspring was low for a male with higher sperm lipid peroxidation
(ii) the chance of siring offspring was low for a male with higher DFI
(iii) the chance of siring offspring was high for a male with a higher plasma membrane integrity (PMI) and
(iv) males with higher sperm lipid peroxidation were more likely to have a high DFI and low PMI

Semen cryopreservation is important for application of advanced reproductive technologies. Despite its usefulness, cryopreservation may cause deleterious changes in sperm structure and function. It is well-documented cryopreservation affects motility, morphology, viability and DNA integrity to certain extent. In addition, cryopreservation induced premature capacitation of sperm reduces its epithelial cell attachment capacity at the fertilization site and impairs fertilization capacity. Thus objective morphological evaluation of cryopreserved sperm is warranted.

Sperm-oocyte interaction tests are useful for diagnosis of subtle sperm defects that cause infertility without causing severe abnormalities detected during routine semen analysis. The availability of viable oocytes still remains an important limiting factor and thus warrants clinical laboratories to apply methodology that examines sperm-oocyte interaction. Sperm-zona pellucida binding is an essential requisite during fertilization. The sensitivity and specificity of sperm-zona binding results indicated the assay to be positively and significantly correlated with in vitro fertilization outcome in several species. Furthermore, there were highly significant correlations between normal sperm morphology, hyperactivated motility, sperm creatine kinase activity and the zona binding capacity of a given sperm sample. In dogs, sperm binding capacity was significantly greater in fresh versus stored oocytes. Furthermore, deep freezing of ovaries appeared to be a better method than salt storage of oocytes.
Application of sperm mRNA expression

Proteins present in sperm have distinctive functions and are essential for preparing sperm for fertilization in a timely manner. Understanding the function of individual sperm protein may explain male infertility. Males with these biomarkers may possess improved fertility. We conducted several studies to determine the association of sperm mRNA expression of genes with functions related to male fertility. It was noteworthy that these mRNAs were expressed more abundantly in high fertility compared to low fertility males (Table 4).

Conclusion

Evaluation of sperm morphology is an important component of semen evaluation. Sperm morphology provides evidence of normality or deviations in spermatogenesis and sperm maturation in the epididymis. Therefore, its results, if correctly assessed, are useful to predict male fertility. Although there are not many studies that used advanced techniques for assessment of sperm morphology in dogs, recent studies in other species using advanced techniques demonstrated a positive correlation of morphologically normal sperm with fertility. It should be noted that though total numbers of morphologically normal and progressively motile sperm per ejaculate is more important in predicting fertility, in clinical practice, advanced semen evaluation techniques may provide more information for predicting male breeding potential and for prospectively predicting infertility.

References

Table 1: Length (in μM) of sperm in various animal species

<table>
<thead>
<tr>
<th>Species</th>
<th>Length (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Man</td>
<td>50 to 60</td>
</tr>
<tr>
<td>Stallion</td>
<td>60</td>
</tr>
<tr>
<td>Bull</td>
<td>75 to 90</td>
</tr>
<tr>
<td>Boar</td>
<td>50 to 60</td>
</tr>
<tr>
<td>Ram</td>
<td>70 to 80</td>
</tr>
<tr>
<td>Buck</td>
<td>60 to 70</td>
</tr>
<tr>
<td>Dog</td>
<td>60</td>
</tr>
<tr>
<td>Tom cat</td>
<td>60</td>
</tr>
</tbody>
</table>

Table 2. Stains used for the determination of sperm structural and functional parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Stain used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm viability or plasma membrane integrity</td>
<td>Membrane-impermeable dyes: EB, EH, PI, YoPro-1, ToPro-3, TOTO and Hoechst 3358</td>
</tr>
<tr>
<td>Acylated membrane dyes: CFDA, CAM, SYTO-1 and SYBR-14</td>
<td>Double staining to distinguish live, apoptic and dead sperm: SYBR-14/PI, YO-PRO-1/PI, AnnexinV-FITC/PI and AnnexinV-PE/7-ADD</td>
</tr>
<tr>
<td>Plasma membrane fluidity</td>
<td>Merocyanine 540/Yo-Pro-1</td>
</tr>
<tr>
<td>Acrosome</td>
<td>Non Fluorescent: eosin/nigrosin, Giemsa, Papanicolaou and brilliant blue. Fluorescent: Fluorescein isothiocyanate (FITC)-labeled lectins are FITC-PSA, FITC-PNA, FITC-ConA and FITC-RCA-II.</td>
</tr>
<tr>
<td>Mitochondria activity</td>
<td>Rhodamine 123 (R123), Mitotracker Green TM, Mito-tracker Red CMXROs, Mitotracker Deep Red 633 (M-22426), Mitotracker Orange TM, DiOD6 and JC-1 Double staining: R123/PI; DiOD6(3)/PI</td>
</tr>
<tr>
<td>Sperm DNA</td>
<td>Chromomycin A3, toluidine blue or aniline blue. Sperm chromatin structure assay (SCSA) - Acridine orange; sperm chromatin dispersion (SCD) test - DAPI or Diff-Quik; Comet Assay – DAPI or EB; TUNEL – FITC or Texas Red; FISH- fluorescent labeled probe</td>
</tr>
</tbody>
</table>
Table 3. Abnormal sperm morphology (location, origin, and classification)

<table>
<thead>
<tr>
<th>Location</th>
<th>Abnormality</th>
<th>Origin</th>
<th>Classification</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head</td>
<td>Acrosome defect</td>
<td>Testicular</td>
<td>Y</td>
<td>Dominant or sex-linked recessive; defective spermatogenesis. Normal fertility in bulls.⁶⁹</td>
</tr>
<tr>
<td></td>
<td>(knobbed, ruffled or incomplete)</td>
<td></td>
<td>- Y/N</td>
<td></td>
</tr>
<tr>
<td>Head</td>
<td>Pin or small head</td>
<td>Testicular</td>
<td>Y</td>
<td>Defective spermatogenesis; defective sperm DNA compaction.</td>
</tr>
<tr>
<td>Head</td>
<td>Detached head</td>
<td>Testicular/Epididymal</td>
<td>Y Y Y/N</td>
<td>Sex-limited recessive; Impaired spermiogenesis or maturation. Senescence following sexual rest, acute stress, testicular degeneration.⁵⁰</td>
</tr>
<tr>
<td>Head</td>
<td>Round head</td>
<td>Testicular</td>
<td>Y</td>
<td>Defective spermatogenesis; with defective sperm DNA condensation and/or with no acrosome.</td>
</tr>
<tr>
<td>Head</td>
<td>Microcephalic and macrocephalic</td>
<td>Testicular</td>
<td>Y Y Y/N</td>
<td>Abnormal spermiogenesis; greater number affects fertility.</td>
</tr>
<tr>
<td>Head</td>
<td>Diadem/Crater Defect</td>
<td>Testicular</td>
<td>Y</td>
<td>Abnormal spermatogenesis; inherited?</td>
</tr>
<tr>
<td>Head</td>
<td>Nuclear vacuole defect</td>
<td>Testicular</td>
<td>Y</td>
<td>Abnormal spermatogenesis; number and size impacts fertility.</td>
</tr>
<tr>
<td>Head</td>
<td>Pyriform-shape head</td>
<td>Testicular</td>
<td>Y Y/N</td>
<td>Abnormal spermiogenesis; heat stress; defective thermoregulation; greater number affects fertility.</td>
</tr>
<tr>
<td>Head/Midpiece</td>
<td>Abaxial tail</td>
<td>Testicular</td>
<td>Y Y Y Y</td>
<td>Normal fertility.</td>
</tr>
<tr>
<td>Midpiece</td>
<td>Abnormal midpiece</td>
<td>Testicular</td>
<td>Y Y Y Y</td>
<td>Abnormal spermiogenesis; affects fertility with gossypol toxicity in bulls.</td>
</tr>
<tr>
<td>Defect</td>
<td>Region</td>
<td>Location</td>
<td>Y</td>
<td>Y/N</td>
</tr>
<tr>
<td>--------</td>
<td>--------</td>
<td>----------</td>
<td>---</td>
<td>-----</td>
</tr>
<tr>
<td>Midpiece Proximal cytoplasmic droplet</td>
<td>Testicular/Epididymal</td>
<td>Y</td>
<td>Y/N</td>
<td>Abnormal spermiogenesis; immaturity or testicular degeneration; affects fertility with greater degree of DNA fragmentation.</td>
</tr>
<tr>
<td>Midpiece Distal cytoplasmic droplet</td>
<td>Testicular/epididymal</td>
<td>Y</td>
<td>Y/N</td>
<td>Immaturity; Defective epididymal transit; cause infertility due to ubiquitination in boars.38</td>
</tr>
<tr>
<td>Tail Distal midpiece reflex</td>
<td>Epididymal</td>
<td>Y</td>
<td>Y</td>
<td>Thermal insult; exogenous estrogen; hypothyroidism, heritable?</td>
</tr>
<tr>
<td>Tail Bent tail</td>
<td>Epididymal or staining effect</td>
<td>Y</td>
<td>Y</td>
<td>May be real defect or caused by old stain (hypotonic effect).</td>
</tr>
<tr>
<td>Tail Folded or coiled tail (Dag defect)</td>
<td>Testicular, Epididymal?</td>
<td>Y</td>
<td>Y/N</td>
<td>Heritable;71 heat stress; zinc imbalance; greater ROS.</td>
</tr>
<tr>
<td>Tail Stump tail defect</td>
<td>Testicular/epididymal</td>
<td>Y</td>
<td>Y</td>
<td>Abnormal spermiogenesis; Heritable (chromosomal t(5;12) (p15.1; q21) translocation in human;72 gossypol toxicity in bulls; greater numbers cause infertility.</td>
</tr>
<tr>
<td>Tail Terminally coiled tail</td>
<td>Testicular/epididymal</td>
<td>Y</td>
<td>Y</td>
<td>Heat stress; gossypol toxicity in bulls.</td>
</tr>
</tbody>
</table>

Y-Yes; N-No;
Table 4. mRNA abundances of sperm functional and structural biomarkers and their association with fertility

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
<th>Association with fertility*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRISP2</td>
<td>Sperm capacitation and sperm-egg fusion</td>
<td>Positive</td>
</tr>
<tr>
<td>PEBP1</td>
<td>Sperm capacitation and sperm-egg fusion</td>
<td>Positive</td>
</tr>
<tr>
<td>CCT8</td>
<td>Indicator for the presence of immature cells</td>
<td>Negative</td>
</tr>
<tr>
<td>AK1</td>
<td>Motility</td>
<td>Positive</td>
</tr>
<tr>
<td>IB5</td>
<td>Fertilization and early embryo development</td>
<td>Positive</td>
</tr>
<tr>
<td>Doppel</td>
<td>Acrosome function and fertilization</td>
<td>Positive</td>
</tr>
<tr>
<td>TIMP2</td>
<td>Acrosome function and fertilization</td>
<td>Positive</td>
</tr>
<tr>
<td>AQP7</td>
<td>Membrane water channel</td>
<td>Positive</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>Fatty acid oxidation; membrane integrity</td>
<td>Positive</td>
</tr>
</tbody>
</table>

CRISP2, Cysteine-Rich Secretory Protein 2; CCT8, Chaperonin containing T complex protein 1, sub unit 8; PEBP1, Phosphatidylethanolamine binding protein 1; AK1 - Adenylate kinase 1; IB5 - Integrin beta 5; TIMP2 - Tissue inhibitors of metalloproteinases 2; AQP7 – Aquaporin 7;

*High fertile males showed increased mRNA expression compared to low fertile males;
Figure. 1. Schematic representation of sperm and the ultrastructure of the flagellum: (i) Sperm (ii) Schematic cross-section of the mid-piece showing the plasma membrane and mitochondrial sheath surrounding the 9 outer dense fibers (ODFs). The ODFs has 9 outer microtubule doublets of the axoneme associated with dynein arms and radial spokes and the central pair of microtubule doublets. (iii) Schematic cross-section of the principal piece showing the plasma membrane surrounding 7 ODFs. ODFs 3 and 8 have been replaced by the two longitudinal columns of the fibrous sheath. The two columns are connected by transverse ribs. (iv) Schematic cross-section of the end piece. 73
Figure 2. Hierarchy of sire fertility evaluation methods

- Genomics
- Competitive index
- Correlation of sperm mRNA with fertility outcome
- Correlation of sperm organelles functions with fertility outcome
- Breeding soundness evaluation