



## Formation of the Male Sex Cells: Spermiogenesis

### Lecture Outline

- Four Phases of Gametogenesis
- Spermatogenesis
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- Spermiogenesis
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- Cell Differentiation
- Cell Differentiation: A Simple Diagram
- Sperm Membrane is specialized for Fertilization
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- Structure of the Normal Sperm Axoneme
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First, let's take a step back and understand that, although there are major differences, the four phases of gametogenesis are fundamental to both oogenesis and spermatogenesis.

### Four Phases of Gametogenesis

- Formation & Migration of PGCs
- Mitotic Increase in Germ Cell Numbers
- Meiotic Reduction in DNA/Chromosome Content
- Differentiation & Maturation

While the early events are similar in males and females (phase 1 & 2), the later stages differ dramatically. First, the germ cell number does not undergo dramatic apoptosis as occurs in the female but instead stem cells (spermatogonia) remain proliferative (mitotic) throughout life. Meiosis is a sequential and continuous process and doesn't stop and start in response to hormonal and other influences as occurs in the egg. Like the egg the mature sperm cells will be haploid. Unlike the egg. Differentiation occurs after meiosis is complete and maturation occurs during the latter stage of spermatogenesis.

### Spermatogenesis

- Process of sperm formation
- Occurs in testes
- Begins at puberty
- Continuous process
- Continues throughout life: Males always fertile (80+ year olds have become fathers)

### The Events of Spermatogenesis

As with oogenesis, there are a number of sequential stages that have been identified during spermatogenesis. They are listed here with some of the attributes that define each stage:

Spermatogonia (2N): somatic like cells; stem cells; reproduce by mitosis

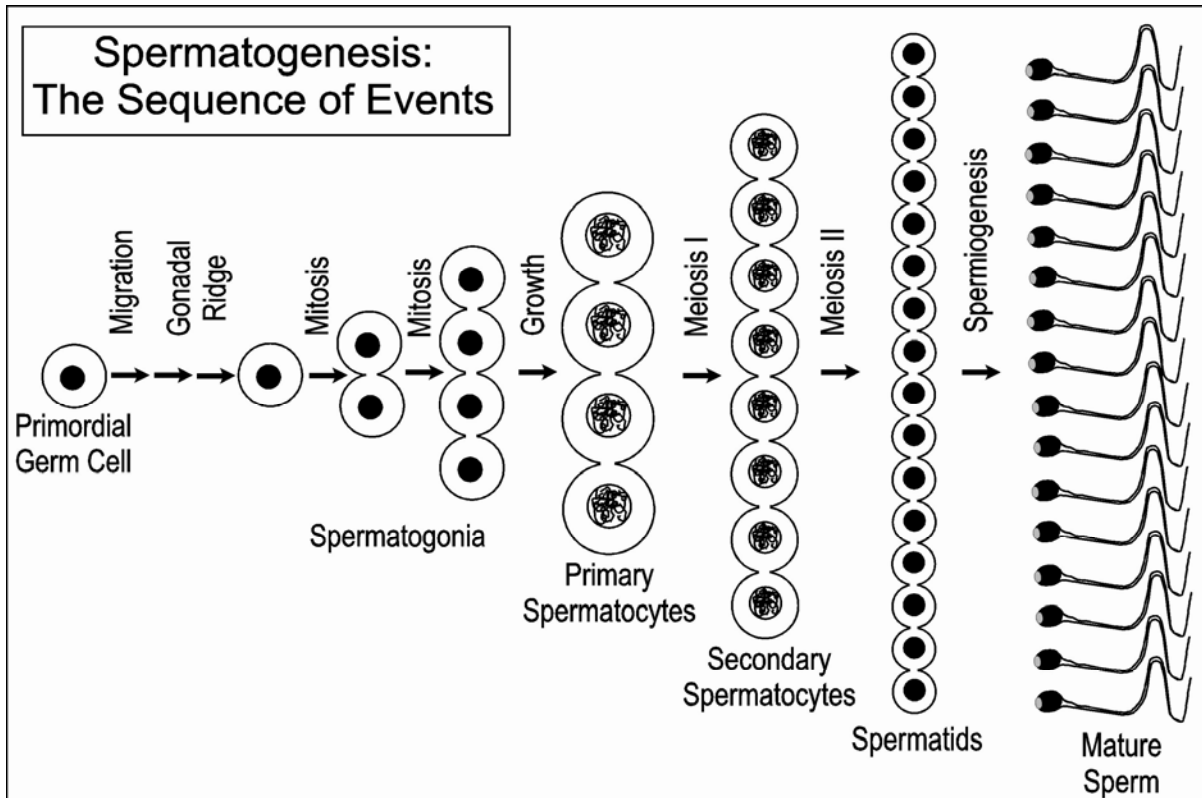
**1° Spermatocytes:** Growth phase; the cells increase ~2x in size; double their DNA content to 4C; this stage represents the start meiosis

**2° Spermatocytes:** result of 1st Meiotic Division; smaller than 1o spermatocytes

**Spermatid:** result of 2nd Meiotic Division; smaller than 2o spermatocytes; dense nucleus; differentiate into sperm

**Spermatozoa:** fully differentiated sperm

Now, let's look at a diagram starting with the migration of primordial germ cells and ending with sperm.

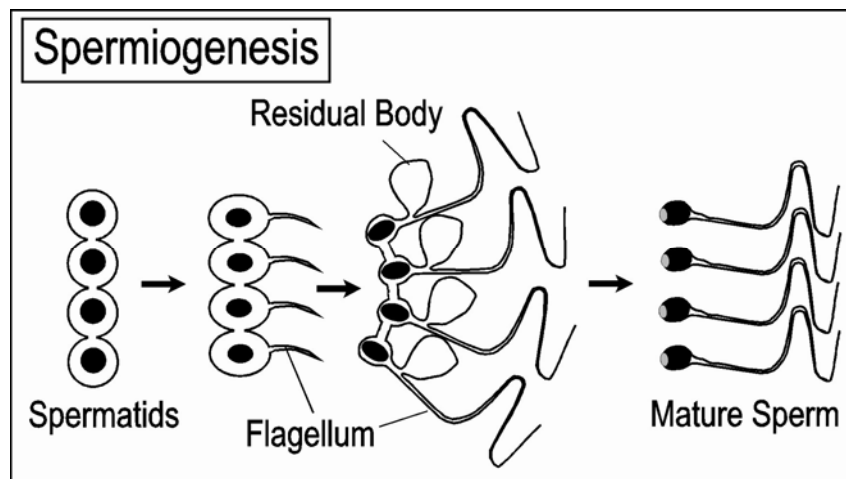


### Spermiogenesis

Spermiogenesis refer to the differentiation of the spermatid into the functional spermatozoan

**Spermatid:** non-motile, round, non-specialized

**Spermatozoan:** motile, elongate, specialized components, special surface molecules and characteristics

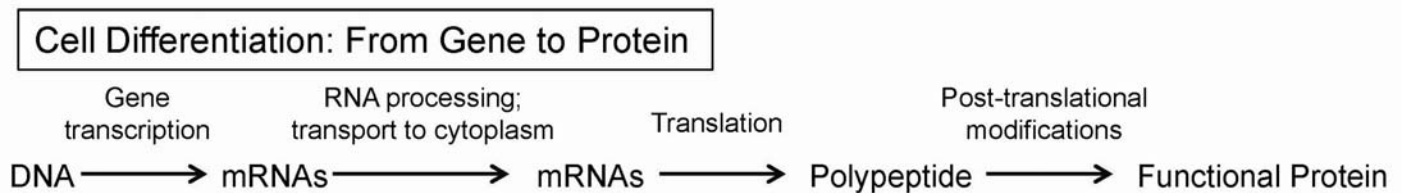


## Events of Spermiogenesis

- Nuclear Shaping & Condensation occurs due to changes in chromatin packaging (change from somatic histones to sperm-specific histones).
- Formation of flagellar tail results due to elongation of microtubules (9+2 axoneme) from distal centriole at the base of the nucleus.
- Formation of Acrosome (from the golgi) at the front end of the sperm
- Rearrangement of organelles (e.g., mitochondria, centrioles); Mitochondria fuse and form spiral around anterior portion of axoneme of flagellum in the midpiece region.
- Most of the cytoplasm is lost. It is shed as the Residual Body

## Cell Differentiation

While there are many ways to define "cell differentiation" one of the simplest, yet most accurate is simply the specialization of a cell for a specific role. Thus the gametes are cells that are specialized for the role of fertilization. The end product of cell differentiation is commonly the result of processes that begin with changes in gene activity leading to alterations in cellular structure and function which then are usually visible (e.g., under a microscope) as physical changes in the cell.



Thus it is important to understand the following terms and to use them accurately when you are discussing the process of cell differentiation. Let's use the simple red blood cell (RBC) as an example since we'll also be using these terms to discuss sperm differentiation shortly.

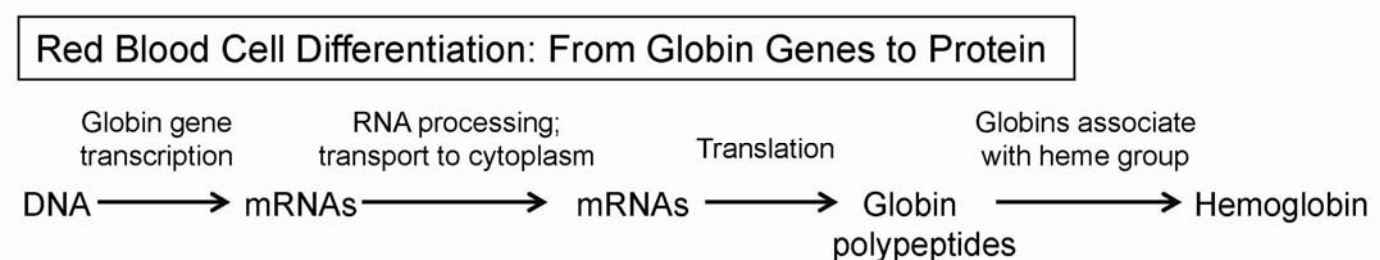
**Morphological Differentiation:** changes in the way the cell looks (e.g., RBC is round)

**Biochemical Differentiation:** appearance of new biochemical components such as proteins (e.g., Hemoglobin in RBC)

**Molecular Differentiation:** regulation of specific genes that leads to biochemical changes and then morphological changes (e.g., transcription of Globin genes in RBC)

It should be noted that you could also discuss other types of differentiation (e.g., physiological differentiation if you were discussing changes in physiology of the cells in question).

## Cell Differentiation: A Simple Diagram



So, let's summarize how this would apply to Sperm Cell Differentiation as shown in the table that follows:

## Sperm Cell Differentiation: Components & Gene Activity

CELLULAR STRUCTURE	PROTEIN COMPONENTS	GENE ACTIVITY
Flagellum	Tubulin; Dynein	Tubulin, dynein genes activated prior to flagellum formation
Acrosome	Acrosin	Acrosin gene activates for acrosome formation
Nucleus	Protamines	Protamine genes activate for chromosome gene condensation

Now let's look in a bit more detail at a few of the events that occur during spermiogenesis so we can apply this information to a deeper understanding the actual processes and complexity of differentiation.

### **Sperm Membrane is specialized for Fertilization**

The sperm must fuse with the egg, this involves at least three sequential events:

- Sperm-egg recognition
- Sperm-egg binding
- Sperm-egg fusion

Thus, the sperm and the egg must have complementary recognition molecules (receptor & ligand) for these events. Since the acrosome will exocytose from the front of the sperm this region of the sperm cell membrane must also have special attributes for fusion of the acrosome with cell membrane.

### **Nuclear Morphogenesis**

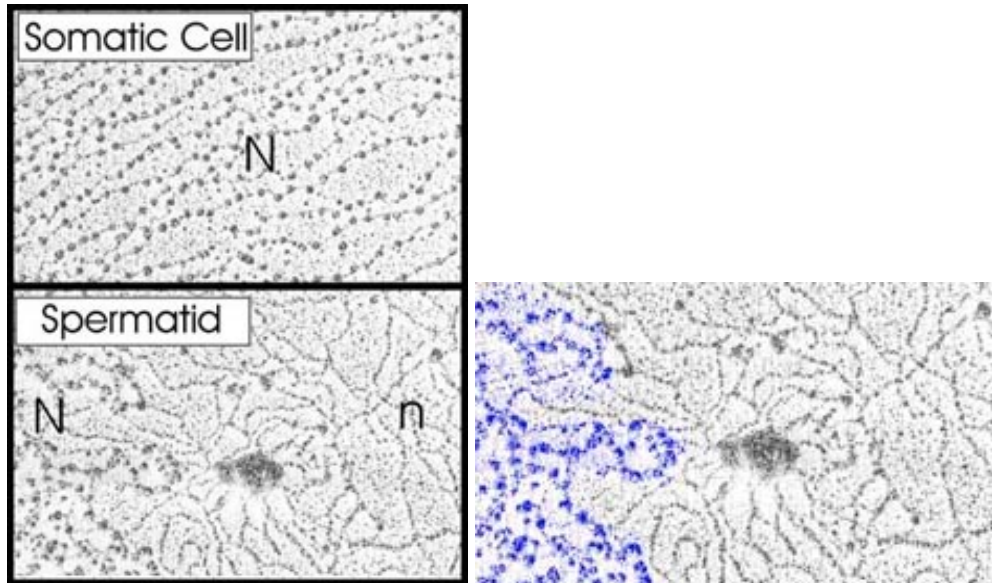
You should have noticed that the nucleus underwent some changes as well. It changed shape (elongated) and became more dense due to chromatin condensation.

Each species of animal has a species-specific sperm morphology which often involves the shape, size and density of the nucleus. The change in nuclear morphology is referred to as nuclear morphogenesis. In humans spermatogenesis, nuclear morphogenesis involves a number of changes, the significance of which are still under analysis. The following are important points to remember:

- The Nucleus changes shape and density giving it a specific morphology
- Chromatin is repackaged to protect it from environment (UV, chemicals)
- Histone & DNA constitute chromatin of somatic cells; nucleosomal organization
- Sperm specific histone proteins appear during spermiogenesis; non-nucleosomal organization allows tighter DNA packing

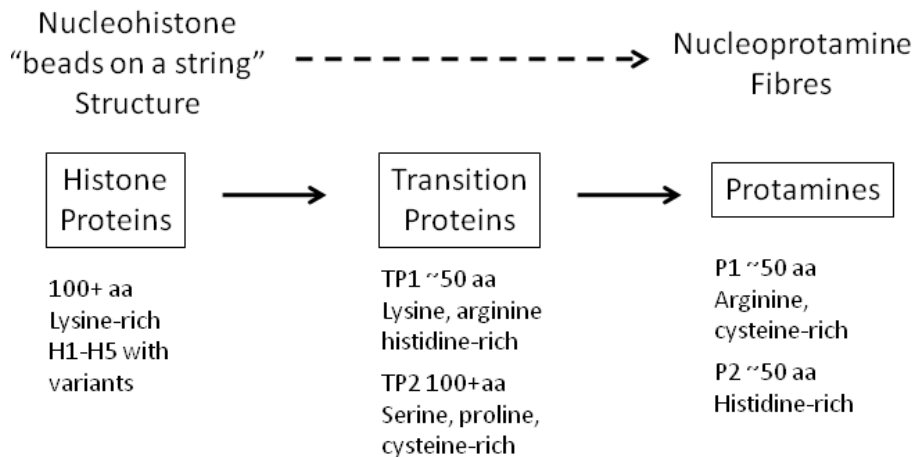
### **Basic Nucleoprotein Changes During Spermatogenesis**

Nuclear morphogenesis is driven, at least in part, by changes in the way the sperm chromatin is packaged in the nucleus. The following ultrastructural pictures reveal this change. In the early stages of spermatogenesis, somatic cells package their DNA with somatic histones (basic proteins) that results in the formation of nucleosomes (N) which are seen throughout the nucleus. This gives chromatin the appearance of "beads on a string" (like a pearl necklace). During spermiogenesis, the somatic histones (N) are replaced with protamines (n) which don't form nucleosomes and so allow a tighter packing of the DNA.



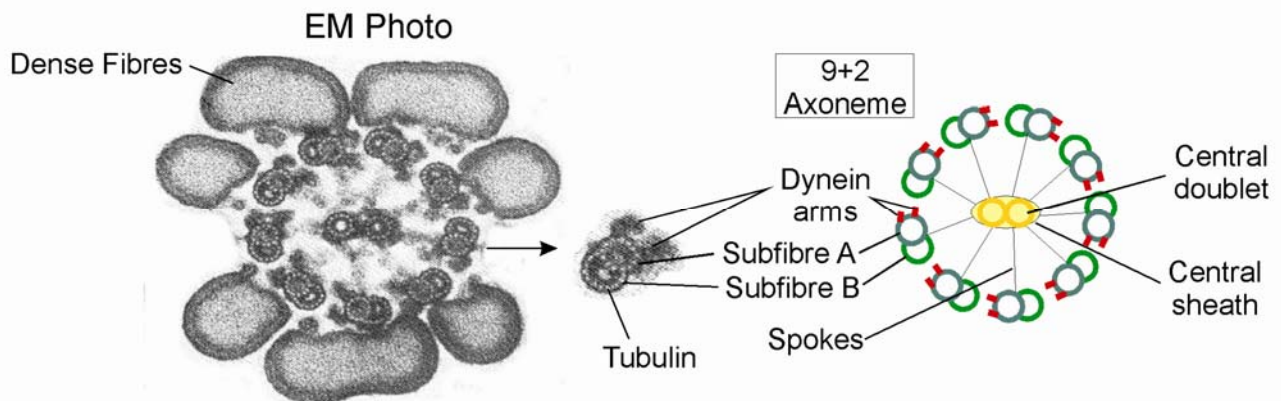
If that isn't clear, the false-coloured image on the right reveals the shift that occurs in the spermatid nucleus. The nucleosomal region is shown in blue while the region of the nucleus that has already incorporated protamines (no nucleosomes) is shown uncoloured.

### Basic Nucleoprotein Changes during Spermiogenesis



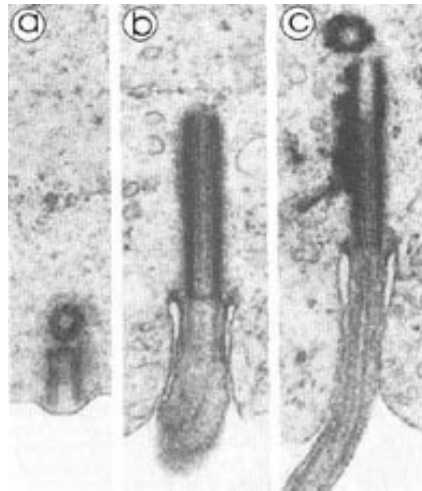
### The Sperm Tail

The sperm tail provides motility for the sperm. The tail consists of a central axoneme of microtubules and accessory proteins essential for flagellar movement.

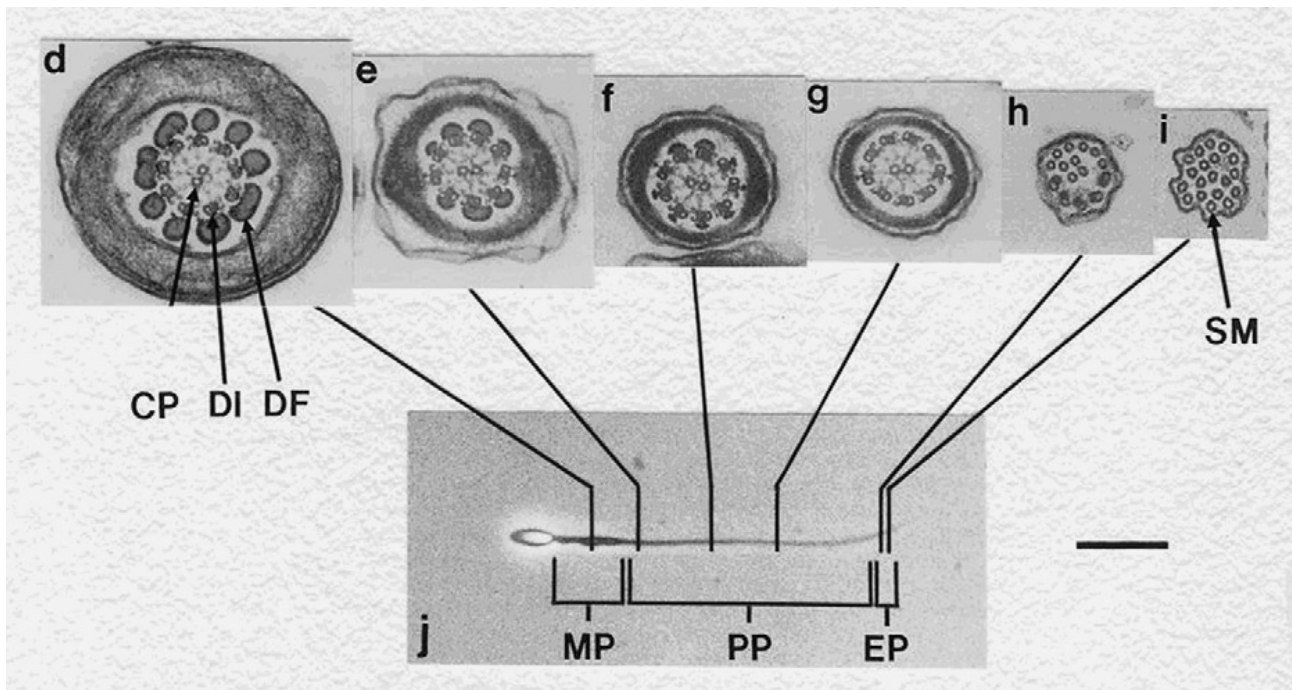


Surrounding the axoneme are some dense structures (at least they appear that way in the EM) called accessory fibers. The following picture shows a mammalian sperm cell with the typical 9+2 structure of microtubules that make up the axoneme (Phillips & Fawcett, 1955). Because this material was negatively stained you can actually see the outlines of the tubulin molecules in each microtubule. The flagellar membrane is not visible.

As seen in the figure following this paragraph, since the sperm tail is primarily comprised of an organized pattern of microtubules (the axoneme) then its formation is dictated by the presence of a microtubule organizing center (MTOC). In this case the MTOC is the pair of sperm centrioles. The distal centriole starts to organize (direct the polymerization of tubulin) microtubules just below the spermatid cell membrane (a). As the axoneme grows, this pushes out the membrane as the flagellar membrane (b). The process continues (c) until a fully formed sperm tail results. At this time the distal centriole will be associated with the posterior region of the nucleus.



Cross-sections of a horse sperm tail reveal its organization from midpiece to end-piece.



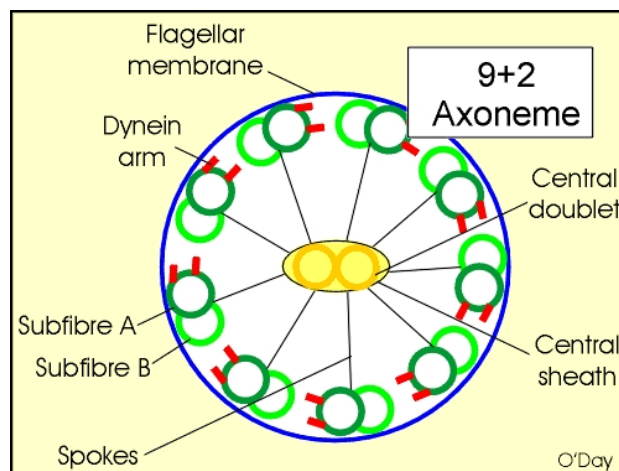
The human sperm tail would have a similar organization. (From Fig. 4. Larry Johnson et al, 2008. Animal Reprod. Sci. 105: 23-51)

### Kartagener's Syndrome

- Also known as Immotile cilia syndrome
- Patients have problems with respiratory tract (since it is lined with cilia)
- Patients produce normal numbers of sperm but the sperm are not motile--they can't swim
- Morphologically nothing appeared to be wrong with these sperm cells until they were examined under the electron microscope (EM).
- EM: Sperm lacking dynein arms of A subfibres in flagellar axoneme

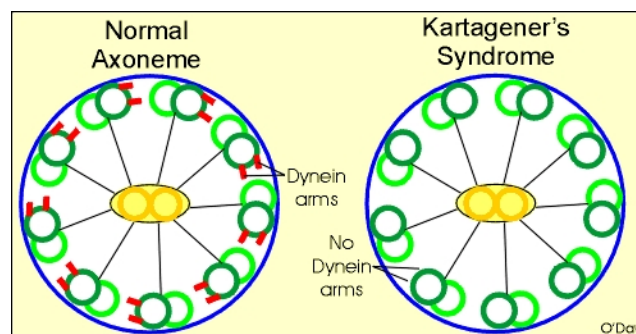
### Structure of the Normal Sperm Axoneme

The sperm axoneme is surrounded by the cell membrane which is referred to as the flagellar membrane in this part of the sperm. The axoneme consists of the 9 + 2 arrangement of microtubules plus associated molecules (e.g., dynein) that in the presence of ATP allows the microtubules to slide thus making the microtubule bend. Dynein is a motor protein that is an ATPase (breaks down ATP to release the energy stored in the terminal phosphate bond). The dynein molecules hydrolyze ATP and undergo shape changes in the presence of ATP to move the microtubules past each other. The central sheath and doublet and the spokes, as well as other yet uncharacterized molecules, all seem to play a role in how the tail moves when the microtubules slide.

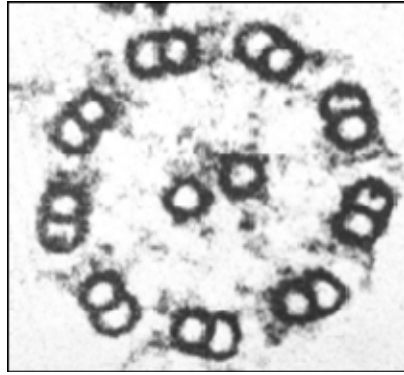


### Sperm Axoneme: Normal Vs. Kartagener's Syndrome

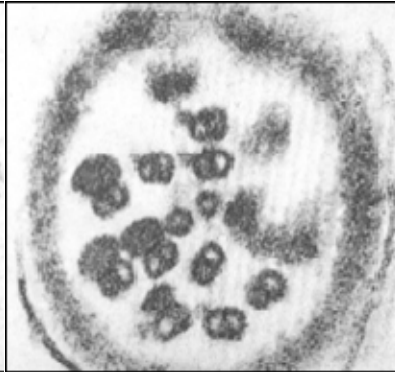
Patients with Kartagener's Syndrome lack the dynein arms and thus cannot use ATP to cause the sliding of the microtubules. Thus the sperm are immotile.



**Electron Micrograph of the cross-section of a sperm tail**



**Cross-section of sperm tail with defective dynein**



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