Dr. Hussein Abbas Al-Rishawi, *PhD. Theriogenology*
Department of Surgery and Obstetrics, College of Veterinary Medicine
University of Al-Muthanna, Samawa, Iraq
hussinabbaskhamees@mu.edu.iq

FERTILIZATION

Fertilization: process in which the male gamete (Sperm) union with the female gamete (Ovum) to form a fertilized egg called (Zygote). Following deposition of semen during copulation, sperms are exposed to a series of different environments that significantly alter their number and their function:

1. After insemination, much amount of semen is lost from the female reproductive tract by retrograde transport.
2. The high acidity of the vagina and uterus may be killing the inactive sperms.
3. During estrus, the cervix produces mucus consists of two types:

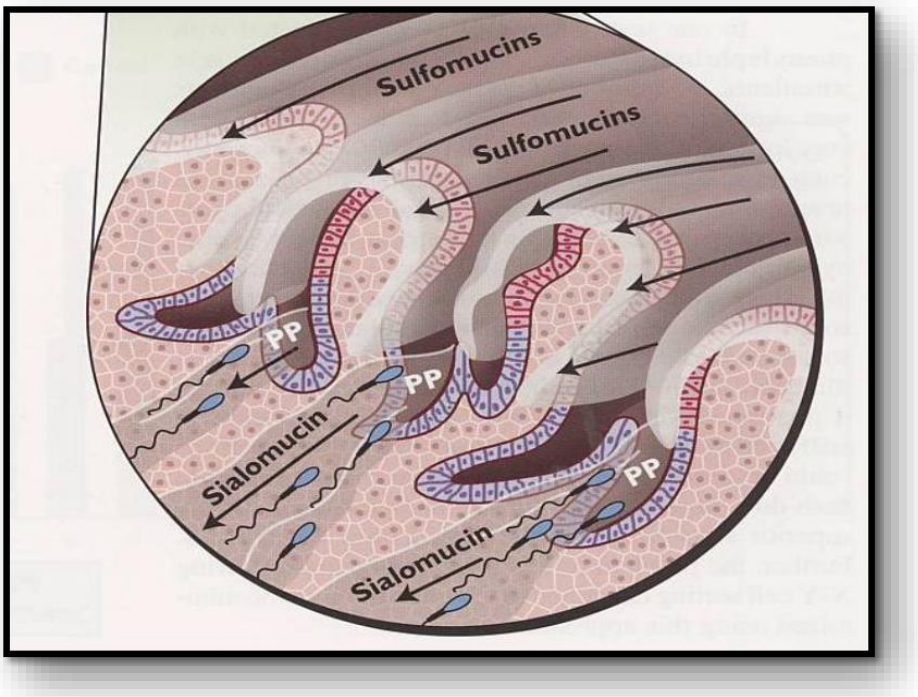
- **Sialomucin**: a mucous of low viscosity produced by cells in the basal areas of cervical crypts, this mucous is low viscosity and the sperms are swim into it and create "privileged pathways" through which the sperms can move.
- **Sulfomucin**: a mucous by the cells in the apical portions of the cervical crypts and it is a high viscosity that the sperms encountering the viscous sulfomucin are washed out of the tract.

4. When the female reproductive tract is under the influence of **estrogen** during estrus, neutrophils (powerful phagocytic white blood cells) sequester in the mucosa of the tract, especially in the vagina and uterus. These neutrophils are poised to attack foreign materials that are introduced into the tract at insemination. It should be recognized that, in addition to sperms, microorganisms are introduced into the tract during copulation. Thus, the neutrophil population is important in preventing

Dr. Hussein Abbas Al-Rishawi, *PhD. Theriogenology*
Department of Surgery and Obstetrics, College of Veterinary Medicine
University of Al-Muthanna, Samawa, Iraq
hussinabbaskhamees@mu.edu.iq

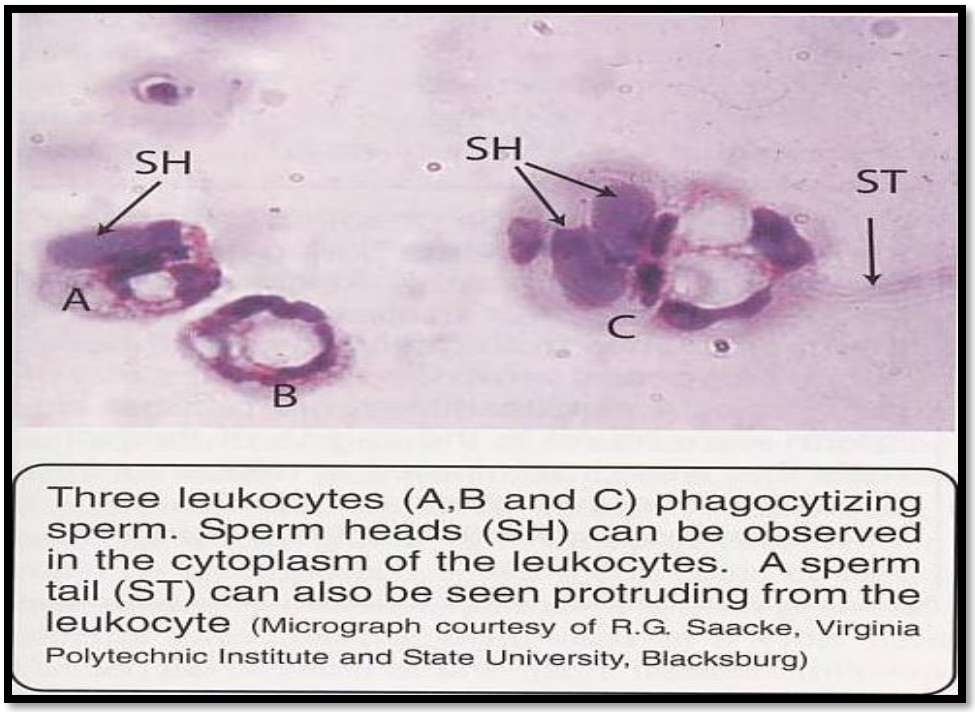
these microorganisms from colonizing the tract from an immunologic perspective; sperms are foreign to the female. As a result, neutrophil activity phagocytizes sperms. They do not differentiate between live and dead sperm. A single neutrophil is capable of engulfing several motile sperms.

5. Microvilli (fimbria) of the oviduct act as a trap for some sperms.



Cervical secretion in cow

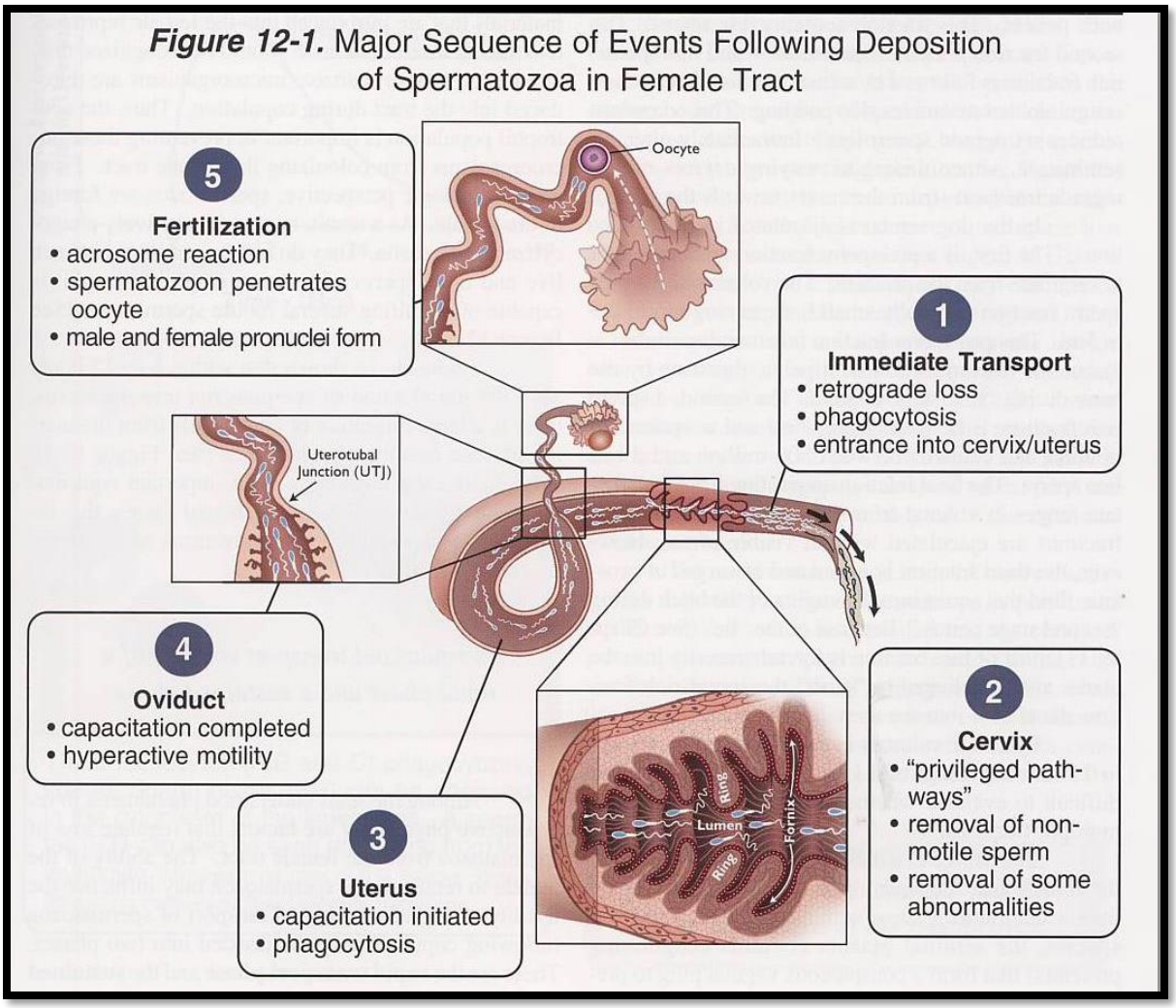
Dr. Hussein Abbas Al-Rishawi, *PhD. Theriogenology*
Department of Surgery and Obstetrics, College of Veterinary Medicine
University of Al-Muthanna, Samawa, Iraq
hussinabbaskhamees@mu.edu.iq



Three leukocytes (A,B and C) phagocytizing sperm. Sperm heads (SH) can be observed in the cytoplasm of the leukocytes. A sperm tail (ST) can also be seen protruding from the leukocyte (Micrograph courtesy of R.G. Saacke, Virginia Polytechnic Institute and State University, Blacksburg)

Phagocytosis of sperms by leucocytes

Dr. Hussein Abbas Al-Rishawi, *PhD. Theriogenology*
Department of Surgery and Obstetrics, College of Veterinary Medicine
University of Al-Muthanna, Samawa, Iraq
hussinabbaskhamees@mu.edu.iq



Transport the sperms after insemination

Location of semen ejaculation

1. Cow, sheep, rabbit, primates, dog, and cat: the male ejaculates the semen into the cranial vagina.
2. Pig: directly into the cervix.
3. Horse: squirted through the cervical lumen.

The **stallion** ejaculates in two series of "jets":

Dr. Hussein Abbas Al-Rishawi, *PhD. Theriogenology*
Department of Surgery and Obstetrics, College of Veterinary Medicine
University of Al-Muthanna, Samawa, Iraq
hussinabbaskhamees@mu.edu.iq

1. The first includes 3-4 high-pressure squirts. This fraction contains about 80% of sperms.
2. The last includes 5-8 lower pressure jets and contains fewer sperms. The seminal plasma in the last jets is highly viscous and may serve to minimize retrograde sperm loss from the mare's tract.

In the **boar**, the ejaculation occurs in three series:

1. Consist of accessory fluids and gelatinous pellets. This fraction contains few sperm.
2. Rich in sperms.
3. The final fraction forms a coagulum that resembles rice pudding. This coagulum reduces retrograde sperm loss.

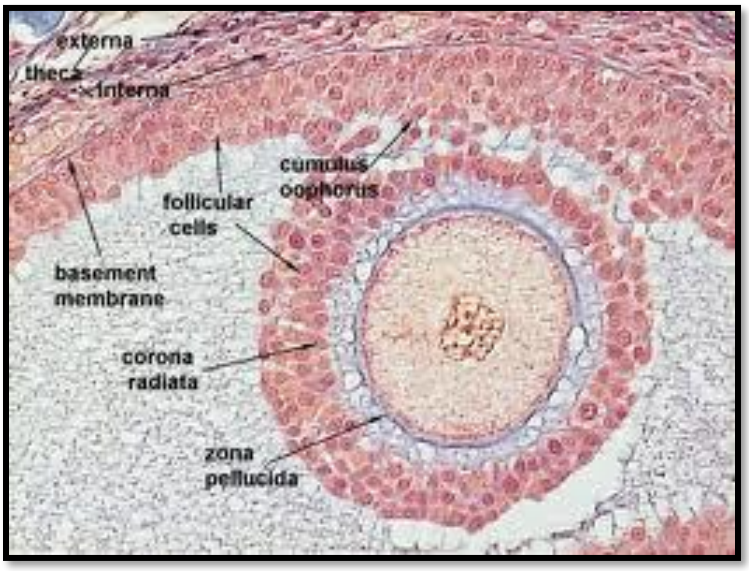
In the **dog**, the semen is ejaculated in three fractions:

1. The pre-sperm fraction is thought to originate from the prostate (0.5-5 ml). It is clear and acellular.
2. Sperm-rich fraction (1-4 ml), is opalescent in color and contains 300 million-2 billion sperm.
3. Final: originated from the prostate (1-8 ml).

The ovum after ovulation has many layers:

1. Cumulus oophorus (Oolema)
2. Corona radiate.
3. Zona pellucida.
4. Cytoplasmic membrane.
5. Cortical granules
6. Nucleus

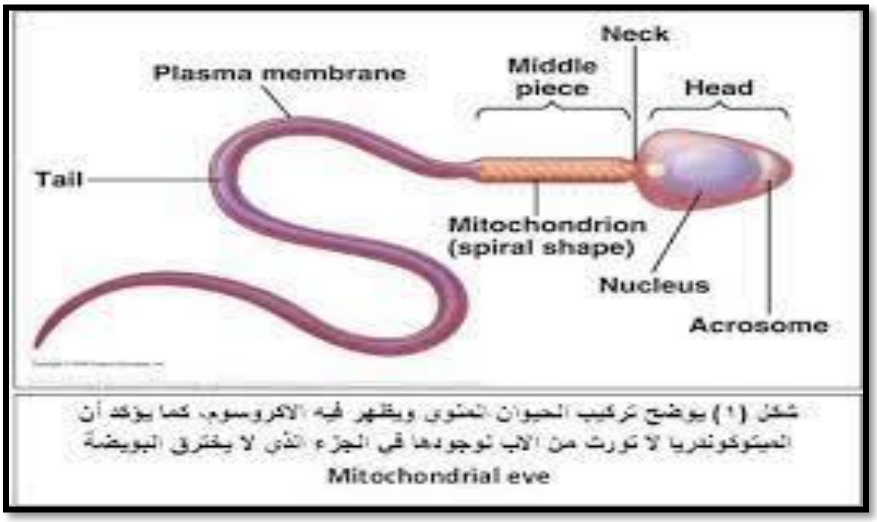
Dr. Hussein Abbas Al-Rishawi, *PhD. Theriogenology*
Department of Surgery and Obstetrics, College of Veterinary Medicine
University of Al-Muthanna, Samawa, Iraq
hussinabbaskhamees@mu.edu.iq



Layers of the ovum

The sperms (sperm) morphology is:

1. Head (which includes the Acrosome at the tip) and contains a nucleus.
2. Middle piece (contain mitochondria).
3. Tail (source of the sperm movement).



Sperm shape

Dr. Hussein Abbas Al-Rishawi, *PhD. Theriogenology*
Department of Surgery and Obstetrics, College of Veterinary Medicine
University of Al-Muthanna, Samawa, Iraq
hussinabbaskhamees@mu.edu.iq

Steps of fertilization:

There are four steps of fertilization:

A. Sperm transport and capacitation.

B. Sperm-ovum binding.

C. Acrosome reaction.

D. Plasma membrane fusion.

A. Sperm transport and capacitation:

Sperm transport: transport of sperms following copulation can divide into two phases:

1. **Rapid transport:**

Within a few minutes after copulation, sperms can find in the oviducts. This phase is very important because it delivered sperms to the site of the fertilization very shortly after copulation. This transport may simply represent a burst of transport activity brought about by the contraction of the muscles of the tract in conjunction with copulation.

2. **Sustained transport:**

It is a very important phase in which sperms are transported to the oviducts in a "trickle-like" effect from a so-called reservoir in the cervix and the utero-tubal junction. In the oviduct, the motility patterns of sperms become hyperactive.

The motility pattern changes from progressive, linear motility in which they swim in a relatively straight line (like an Olympic swimmer), into a frenzied, dancing motion that is not linear and localized in a small area (like dancers in a disco). In general, hyperactive motility occurs in the ampulla of the oviduct and is believed

Dr. Hussein Abbas Al-Rishawi, *PhD. Theriogenology*
Department of Surgery and Obstetrics, College of Veterinary Medicine
University of Al-Muthanna, Samawa, Iraq
hussinabbaskhamees@mu.edu.iq

to be brought about by specific molecules produced by the epithelium there. Hyperactive motility is believed to facilitate sperm-oocyte contact.

Sperm capacitation:

The process by which the sperm becomes capable for fertilize the ovum. After ejaculation of the semen, the sperms are movable, mature but aren't capable of fertilization because of the presence of **"t,s, de-capacitating factors**" which are protein substances found in the seminal plasma and surrounding a sperm as a coat.

As well as the capacitation includes re-arrangement of the cytoplasmic proteins.

There are four steps of capacitation are:

1. Removed the de-capacitation factors.
2. Spermatic motility is activated and tails change beat frequency.
3. Sperm cell surface antigens are lost. The loss of these proteins renders the sperm more receptive to binding to the ovum.
4. Re-arrangement of the cytoplasmic proteins.

B. Sperm-ovum binding:

The sperm head binds directly to the ovum's outer surface and this triggers the acrosome reaction. The acrosome contents are released and there is a balanced Na^+ influx and H^+ efflux, causing an increase in pH. The increased pH triggers the dissociation of the profilactin complex (actin and profilin) and released actin monomers polymerize to form a filament called the acrosome process. This acrosome process penetrates the ovum coatings to allow fusion of the sperm and ovum plasma membrane.

Dr. Hussein Abbas Al-Rishawi, *PhD. Theriogenology*
Department of Surgery and Obstetrics, College of Veterinary Medicine
University of Al-Muthanna, Samawa, Iraq
hussinabbaskhamees@mu.edu.iq

- **Sperm receptors on the ovum:**

The zona pellucida of the ovum has 3-zona pellucida glycoproteins (ZPGPs) called: ZPGPI, ZPGPII, and ZPGPIII. Only ZPGPIII prevents sperm binding to the ovum which shows that ZPGPI and ZPGPII are considered as receptors for the sperms.

- **Ovum receptors on sperm:**

A Golgi enzyme known as "galactosyl transferase" found in the cytoplasmic membrane of sperm serves as a receptor for the ovum.

C. Acrosome reaction:

The acrosome reaction is a regulated exocytotic event in which an apical vesicle in the sperm head fuses with the sperm plasma membrane. The purpose of the acrosome reaction is tow fold:

- The reaction enables sperms to penetrate the zona pellucida.
- Exposes the equatorial segments that it can later fuse with the plasma membrane of the oocyte. The acrosome reaction begins when the plasma membrane of the spermatozoon forms multiple fusion sites with the outer acrosome membrane. When the two membranes fuse; many small vesicles are formed and this process is called vesiculation. After vesiculation has occurred, the acrosome contents are dispersed and the sperm nucleus is left with an inner acrosome membrane surrounding it. The penetration of the zona pellucida by a spermatozoon is believed to be a rapid process and takes no more than a few minutes. After attachment to the zona pellucida, the acrosome reaction allows the release of a variety of enzymes:

1. **Hyaluronidase:** analysis of the hyaluronic acid in the cumulus oophorus.

Dr. Hussein Abbas Al-Rishawi, *PhD. Theriogenology*
Department of Surgery and Obstetrics, College of Veterinary Medicine
University of Al-Muthanna, Samawa, Iraq
hussinabbaskhamees@mu.edu.iq

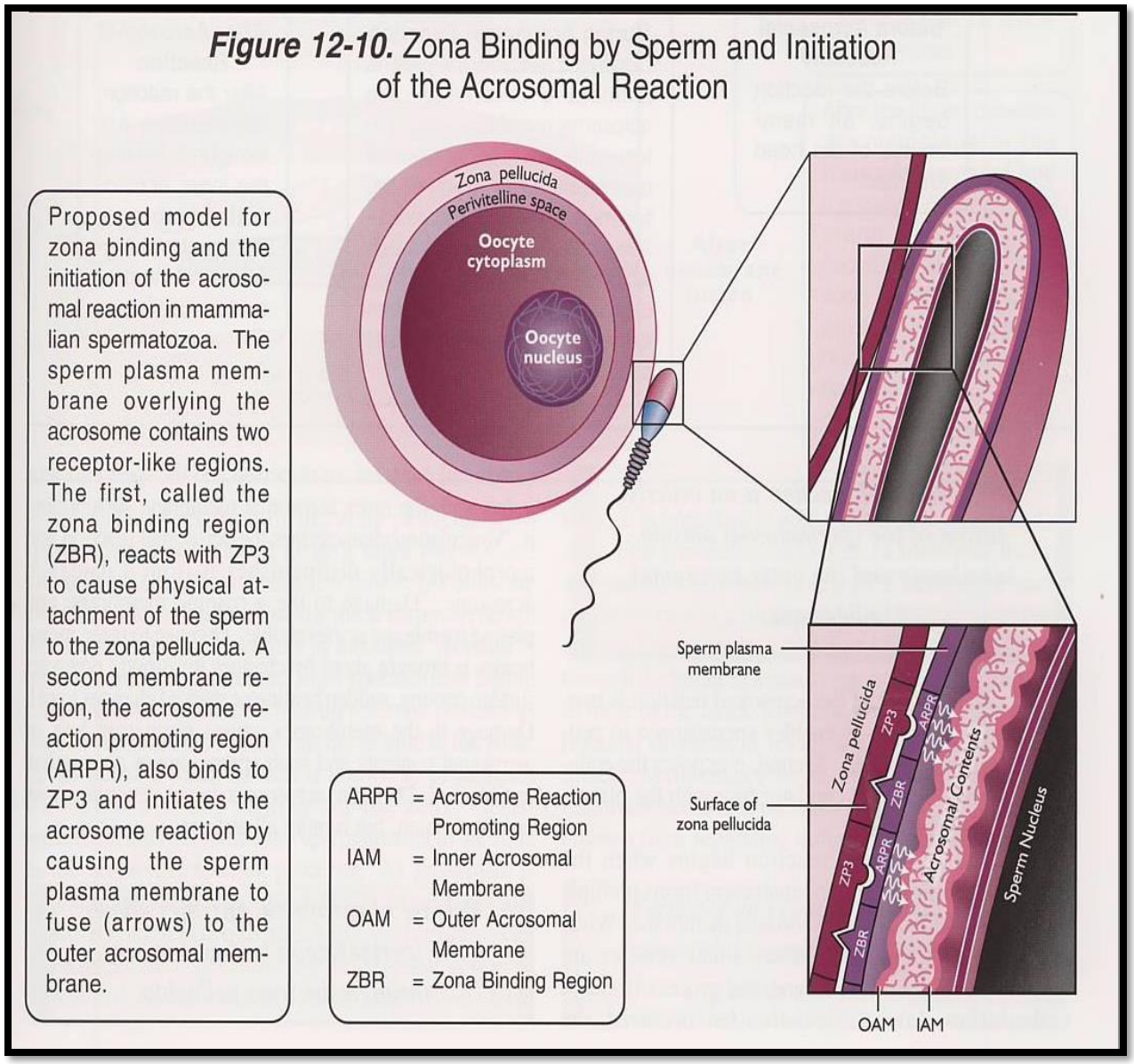
2. **Corona penetration enzyme**: analysis of the matrix cells in the corona radiate.
3. **Acrocin**: it hydrolyzes zona proteins as well as enhances the sperm's ability to bind to the zona. This allows the spermatozoon to digest a small hole through the zona through which it can pass.

D. Plasma membrane fusion:

When the penetration is complete, the sperm reach the perivitelline space (the space between the zona pellucida and plasma membrane), the plasma membrane of the oocyte fuses with the sperm plasma membrane. Then the oocyte undergoes a series of changes that prepare it for early embryogenesis. The contents of the cortical granules consist of mucopolysaccharides, proteases, plasminogen activators, acid phosphatase, and peroxidase. After membrane fusion; the corticle granules undergo "exocytosis" and their contents are released into the perivitelline space, resulting in the "zona block" a process whereby the zona pellucida undergoes biochemical changes so that further sperm cannot penetrate it. Polyspermy is prevented by the zona block.

The final step of fertilization is the fusion of the male and female pronuclei. This fusion is referred to as "**Syngamy**". Following syngamy, the zygote enters the first stage of embryogenesis.

Dr. Hussein Abbas Al-Rishawi, *PhD. Theriogenology*
Department of Surgery and Obstetrics, College of Veterinary Medicine
University of Al-Muthanna, Samawa, Iraq
hussinabbaskhamees@mu.edu.iq



Plasma membranes fusion