Cellular events in the process of fertilization

Nigatu Ebisa[1], Chala Mohammed[1], Umer Seid[1] and Awel Hussein[2]

[1] Wollega University, College of Health and Medical Sciences, School of Veterinary Medicine, Ethiopia
[2] Oda Bultum University, College of Agriculture, Department of Animal Science, Ethiopia
Corresponding Author: Dr. Chala Mohammed, Wollega University, School of Veterinary Medicine, P.O. Box: 395, Ethiopia. Tel: +251-913-11-5805.
chalamohammed@wollegauniversity.edu.et

Abstract: the fertilization process in mammals take place in complex microenvironment in the Female genital tract, and the oviduct fluid represent in which oocyte, spermatozoa, and embryo are suspended during oviductal transit. Early events in fertilization requires interactions between complementary molecules present on the different cell surfaces. In this way carbohydrate play a key role in different reproductive events such as sperm-oviduct cell interaction, a prerequisite for oocyte zona pellucida (ZP) interaction, and sperm-oocyte recognition, necessary for primary binding, among others. Fertilization process consist different sequence such as production of oocyte, production of spermatozoa, migration of sperm into reproductive tract, gamete adhesion and fusion, acrosomal reaction to zona pellucida and penetration sperm in side of oocyte. Even though some studies and literature review have been conducted on the cellular events of fertilization, the sequence events are not as such understand by many people. So to give the artificial insemination, veterinarians should be know the process of fertilization and get awareness regarding the molecules participating in fertilization.
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1 Introduction

Fertilization is a multistep and complex process of culminating in a merger of gamete membranes, cytoplasmic unity and fusion of genomes, initiating the development of a new individual. Even though membrane fusion is a key event in this process, there is still very little known about its mechanism or the molecules involved. Fusion shows less distinct species-specific than do the preceding steps in fertilization, like zona pellucida (ZP)-sperm interaction, which suggests that the mechanism and molecules involved in membrane fusion are more conserved [1].

During recent years, efforts have been made towards the identification of the molecular players and their function, and several molecules on the egg or the sperm side have been found to be essential or nearly essential. Although the concept of multiprotein complexes on both membranes has been accepted in recent years, the first known molecules of direct interaction in mammalian fertilization have only recently been discovered [2].

The fertilization process is when spermatozoon is attached and bound to the ZP after the successful penetration of the cumulus matrix. It is believed that the cumulus mass secrets chemoattractant for the spermatozoa to "locate" the ovulated oocyte. Soon after "locating" the oocyte, the acrosome-intact spermatozoa begin the acrosome reaction (AR) process. Molecules of the acrosomal matrix, such as zona adhesion and sp56, have been proposed to play significant, roles in both the binding and adhesion processes. From the oocyte side, cluster differentiation 9 (CD9) and α6β1Integrin have been shown to play a major role in the adhesion process. These are participating in the process of fertilization [3]. Even though some studies and literature review have been conducted on the cellular events of fertilization, the sequence events are not as such understand by many people.

Therefore the objectives of this seminar paper are:

- To review on the cellular events in the process of fertilization.
- To enumerates the sequence of events in the fertilization.

2 Cellular Events In The Process Of Fertilization

2.1 Production of the Mature Oocyte

Oocyte maturation is defined as the re initiation and completion of the first meiotic division subsequent progression to metaphase II and the completion of nuclear and cytoplasmic process which are essential for fertilization and early embryo development. Oocytes are arrested in prophase I of meiosis during the fetal period, and this nucleated stage persists until maturation begins. During this prolonged period, the oocytes enlarges and synthesizes RNA and protein as

the follicle grows. The oocyte and follicle become sensitive to the actions of gonadotrophins, and the follicle stimulating hormone (FSH) and luteinizing hormone (LH) surges in mid-cycle, or the external application of human chorionic gonadotrophins (HCG), initiate the onset of maturation in antral follicles and oocytes [4].

2.2 Production of the Mature Spermatozoa

Production of Spermatozoa is throughout post pubertal male reproductive life, spermatozoa are formed from spermatogonial cells by a complex process referred to as spermatogenesis. In the sexually mature male, the two testicles or the testes produce and store millions of tiny micro-scopic structures, the spermatozoa. The entire process of sperm formation consists of three sequential phases of cell proliferation and differentiation [5].

First, there is an extensive multiplication and proliferation of spermatogonial cells to produce optimal number of spermatogonia that give rise to early and late primary spermatocytes and also to maintain a pool of stem cells. Second, each primary spermatocyte undergoes a lengthy meiotic cell division that results in the formation of two secondary spermatocytes [6]. Third, each secondary spermatocyte undergoes the second meiotic cell division to produce two haploid rounds spermatid. The round spermatids are incapable of further division. These cells have spherical nucleus with dispersed chromatin and the absence of nucleoli. By a complex set of nuclear and cytoplasmic changes, the ordinary looking round spermatids gradually undergo remodeling of the nuclear and cellular components during their transformation into spermatozoa bya process referred to as spermiogenesis [7].

The net result is the formation of hydrodynamically shaped spermatozoa containing two part: the head with a nucleus and an acrosome (anteriorhead) and a postacrosomal region, the flagellum comprising of the middle, principal, and end pieces. Sperm development takes place within the germinal epithelium of seminiferous tubules in the testes where spermatogenic cells are arranged along the basal membrane in a well-defined combination of various developmental stages. the cycle of seminiferous epithelium the entire process of spermatogenesis is dependent on the specific environment provided by the somatic cells in the testes and requires endocrine and paracrine as well as cell-cell interactions [8].

Along each testicle is an epididymis and vas deferens (vas) that make up the network of the male reproductive system. The epididymides are a set of two coiled tubes, one from each testicle, where testicular spermatozoa undergo many biochemical and morphological changes collectively called epididymal maturation [9,10,11]). The convoluted tubules are connected to vas, a pair of muscular tubes that transport sperm containing fuid to uretha and semen out of the body through penis. These properties develop over a period of time as spermatozoa traverse through the epididymis. The tubule is divided into three regions: Proximal (caput), Middle (corpus), and Distal (cauda). The precise region of the epididymis where the sperm cells undergo most of the modifications remains evasive. However, the caudal spermatozoa are motile and fertilization-competent cell [11].

During epididymis passage, spermatozoa interact with epididymal luminal secretions. the epithelial cells lining the epididymal duct form a luminal fluid environment by actively secreting and absorbing small molecules (sugars electrolytes, etc.) and macromolecules (proteins, glycoproteins, etc.). Thus epididymis duct secretions mixed with the testicular content, provide a specific environment in which functionally immature spermatozoa undergo multiple modifications. The net result is the production of the self-propelled and functionally competent spermatozoa. It is important to emphasize that spermatozoa are surrounded by the plasma membrane (PM) that mediates many of the early events leading to sperm-egg adhesion and fertilization [9, 10].

2.3 Sperm Migration into the Reproductive Tract

At coitus, sperm are deposited into the anterior vagina, where, to avoid vaginal acid and immune responses, they quickly contact cervical mucus and enter the cervix. Cervical mucus filters out sperm with poor morphology and motility and as such only a minority of ejaculated sperm actually enters the cervix. In the uterus, muscular contractions may enhance passage of sperm through the uterine cavity. A few thousand sperm swim through the uterotubal junctions to reach the Fallopian tubes (uterine tubes, oviducts) where sperm are stored in a reservoir, or at least maintained in a fertile state, by interacting with endosalpingeal (oviductal) epithelium [12].

As the time of ovulation approaches, sperm become capacitated and hyper activated, which enables them to proceed towards the tubal ampulla. Sperm may be guided to the oocyte by a combination of thermo taxis and chemotaxis. Motility hyperactivation assists sperm in penetrating mucus in the tubes and the cumulus oophorous and zona pellucida of the oocyte, so that they may finally fuse with the oocyte PM [13].

Capacitation is the process by which sperm become competent to fertilize an egg. Capacitation takes place after ejaculation, in the female reproductive tract, and is required only by mammalian sperm. Cholesterol is particularly abundant in seminal plasma and has an inhibitory effect on sperm

capacitation. During capacitation, cholesterol and other sterols are removed from the sperm surface, and non-covalently attached glycoproteins acquired in the epididymis are released from the sperm surface together, these modifications create a more fluid membrane environment, making the sperm competent for subsequent fertilization cues. Mammalian sperm have to travel a long distance through the female reproductive tract to the oviduct, in which fertilization takes place [12].

To accomplish the journey to the egg-sperm interaction.

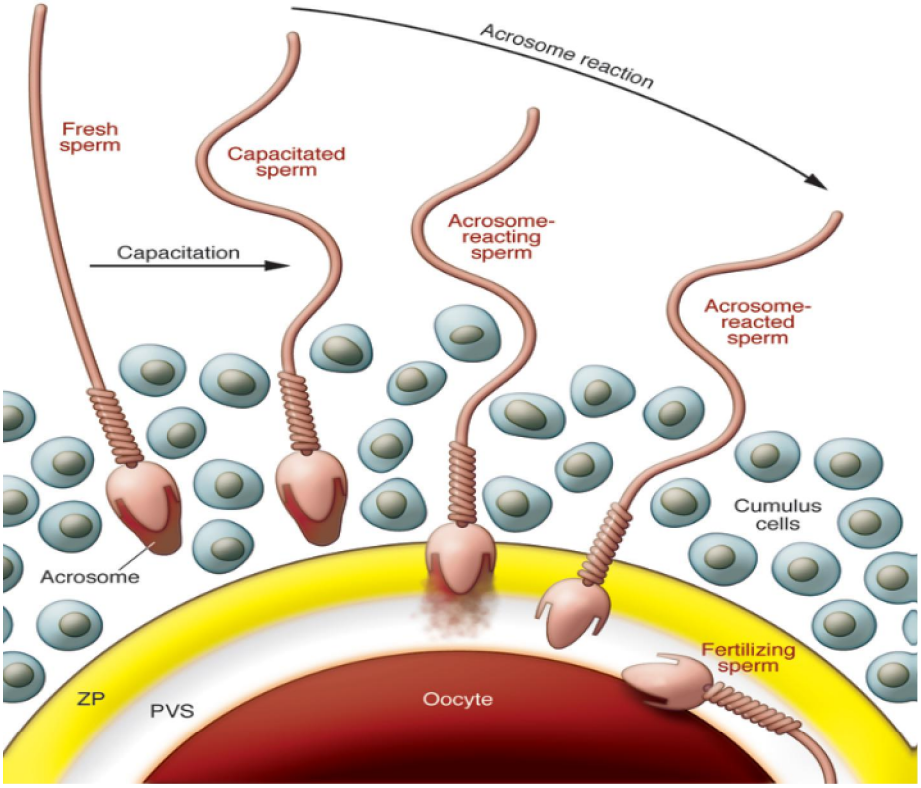

Figure: 1 Mechanism of sperm-egg interaction.
Source (Yano, 2009)

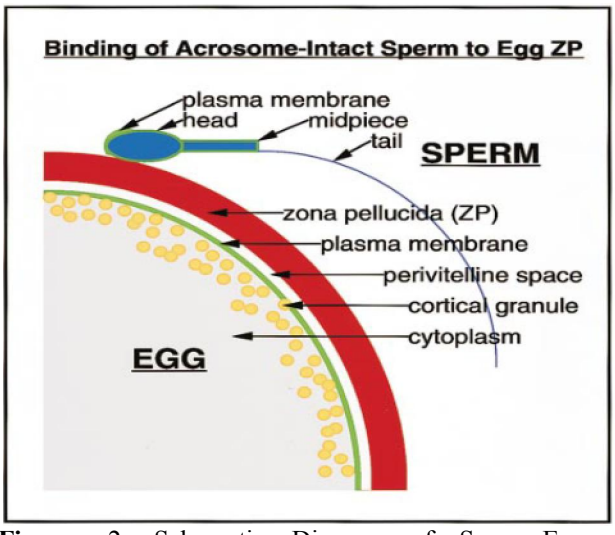

Figure: 2. Schematic Diagram of Sperm–Egg Interaction in Mammals
Source (Paul, 1999)

Over the nucleus of each mammalian sperm is a membranous sac known as the acrosome, which is filled with many kinds of hydrolytic enzymes. Sperm undergo capacitation, which permits the acrosome reaction. Near the eggs, probably stimulated by the cumulus cells and the ZP, sperm release their acrosomal contents by exocytosis and penetrate the ZP. Only acrosome-reacted sperm fuse with eggs, but their competency for fusion does not last long. During sperm storage, the isthmic epithelium creates a microenvironment that delays capacitation and stabilizes sperm for a period of approximately 24 hours, at least in animal [14, 15].

2.4 Molecular Aspects of Gametes Adhesion Exocytosis and Fusion

2.4.1 Specific aspects of mammalian fertilization

The mammalian oogenesis and spermatogenesis prepare eggs and sperm, respectively, for fertilization. During ovulation, fully grown oocytes from antral (Graafian) follicles undergo "meiotic maturation," a

process that transforms fully grown oocytes into unfertilized eggs prepared to interact with sperm. Similarly, following deposition into and migration up the female reproductive tract; sperm undergo "capacitation," a process that enables sperm to-bind to eggs and to undergo the acrosome reaction [16, 17].

2.4.2 Egg zona pellucida glycoprotein

The plasma membrane of all mammalian eggs is completely surrounded by a ZP

The morphological features of an acrosome-intact mammalian sperm bound to the ZP of an unfertilized egg by PM overlying the anterior region of the sperm head **[18].**

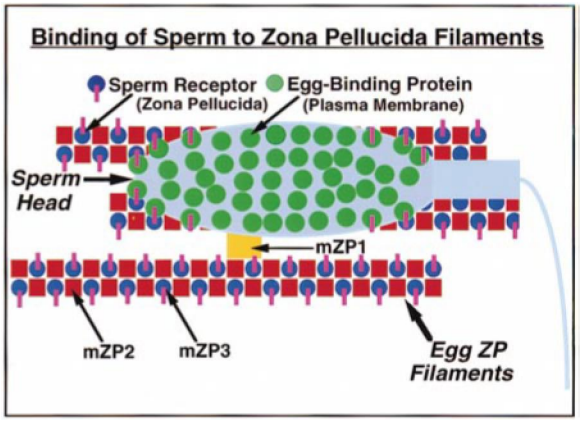

Figure: 3. Schematic diagram of some molecular features of a mammalian sperm bound to the ZP of an unfertilized Egg
Source (Paul, 1999)

Each sperm is bound to O-linked oligosaccharides of thousands of ZP3 molecules that are located periodically along ZP filaments. The filaments are composed of ZP2 and ZP3 and are cross linked by ZP1. The active O-linked oligosaccharides of ZP3 that are recognized by sperm are colored pink [19,20].

2.5 Molecular Aspects of the Sperm Acrosome Reaction with Egg

The acrosome is a Golgi-derived exocytosis organelle that covers the tip of the sperm head. Acrosomal exocytosis, the so called acrosome reaction, happens only in capacitated sperm and is a prerequisite for a sperm to fuse with an egg **[21].**

The acrosome is a large secretory vesicle that overlies the nucleus in the apical region of the sperm head [22, 23].

Acrosomal membrane just underlying the PM is referred to as "outer" acrosomal membrane, and that overlying the nucleus is referred to as "inner" acrosomal membrane. Morphologically, the acrosome reaction is seen as multiple fusions between outer acrosomal membrane and PM at the anterior region of sperm head, extensive formation of hybrid membrane vesicles and exposure of inner acrosomal membrane and acrosome, somatic contents [24]. Again, only acrosome-reacted sperm can penetrate the ZP and fuse with egg PM**.** In somatic cells, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor proteins (SNAREs) are involved in the membrane-fusion machinery responsible for secretion [25]. SNAREs required for exocytosis have been identified in mammalian sperm [26].

These identified in rat and porcine sperm (also reported in human and mouse sperm) all SNAREs that are required to form the SNARE complex involved in membrane fusion [27]. In addition, the vesicle-associated membrane proteins (VAMPs; the vesicle, in our case the acrosomal SNAREs) aggregated at the apical tip of the outer acrosome membrane at the same time. These also demonstrated that SNAREs of the PM are not interacting with SNAREs in the acrosome in ejaculated sperm and that sperm activation is required for this interaction (preliminary results). The regulation of the formation of SNARE protein fusion complexes (and additional adhering proteins participating in the (AR)) is currently under study and, to a large extent, remains unknown. The AR is relatively easy to detect on live sperm [28].

Due to the extraordinary size of the surface area where membrane docking and the multiple fusions take place during this secretion event. This makes it easier to study whether or not post-translational modifications of the relevant proteins are involved in regulating the sperm AR. Another interesting hypothesis worthy of investigation is the possibility that the zona-binding protein complex is orchestrating the assembly of the acrosome–fusion protein complex. In this respect, a putative K+ channel has been identified in the zona-binding complex which may be indirectly involved in the influx of calcium into the sperm head which is, in turn, required for SNARE zippering and conformational changes of the SNARE fusion complex to establish acrosomal fusions [29].

2.6 Molecules Involved in Sperm –Oocyte Interaction

2.6.1 Sperm and cumulus mass interaction

After the successful detachment from the ishmus, the spermatozoa are moving to the last segment of the oviduct, the ampulla, where the spermatozoa seem to "identify" the presence of the oocyte by "locating" the cumulus oocyte complex (COC). This "identification" seems to act in a chemoattractant manner, since it has been reported that follicular fluid factors and COCs include and secrete, respectively, chemoattractants [30,31]. Promoting in this way the capacitation process. Moreover, olfactory family1 proteins that are present as receptors on the COCs have been suggested to act as chemoattractants [32].

The sperm, through some molecules, "smell" the molecules presented or secreted by the COCs. The ovulated oocytes are surrounded by two distinct layers of cells, the outer layer of cumulus cells and the ZP. Beneath these two substantial layers the spermatozoon should reach and interact also with the PM of the oocyte in order for the fusion process to take place. Cumulus oocyte complex have been proposed to have a beneficial role in the fertilization procedure, as it has been found that disruption of genes involving the synthesizing or stabilization of COCs, result in a phenotype with reduced fertilization performance [33, 34].

The cumulus matrix has been reported to promote sperm acrosome reaction [35]. Since the cells of cumulus oophorus are embedded in an extracellular matrix which consists mainly of proteins and carbohydrates, among which hyaluronan is the most known as a no-sulfated glycosaminoglycan. Sperm acrosome reaction, part of the process that is known as capacitation, has been characterized quite well. Briefly, acrosome reaction is an exocytosis procedure and occurs only in capacitated spermatozoa. Soon after ejaculation, capacitation takes place and is characterized by the removal of cholesterol and other sterols from the sperm surface. Following capacitation, the acrosome releases enzymes, among of which hyaluronidase seems to be the most responsible for the degradation/hydrolation of the hyaluronan of the cumulus oophorous Hyalouronidase has been found to exist in two isoforms in epididymal spermatozoa, the Ph _20glycosylphosphatidylinositol (GPI)-anchored protein and Hyal5 [36,37].

Ph-20 is present on the PM of acrosome-intact spermatozoa, while Hyal5 is located on both acrosomal and plasma sperm membrane. Both molecules appear to play an important role in the degradation of the cumulus matrix. Specifically, Ph-20 is initially located on the PM of acrosome-intact spermatozoa, and as the acrosome reaction proceeds, it moves onto the inner acrosomal membrane of the acrosome-reacted spermatozoa [38].

This migration of Ph-20 during the AR is thought to explain the protease activity of Ph-20 through the cumulus mass. Large amounts of Hyal5 are secreted during the AR, thus implying that Hyal5 functions mostly for the hyaluronan hydrolysis of the cumulus mass. Suggesting that Ph-20 contributes most to the penetration of the cumulus matrix and barely to the late stages of fertilization. The reduced fertilization rate in Ph-20-deficient spermatozoa appear to be attributed to the difficulty of these spermatozoa to penetrate the cumulus oophorous. Last, given the co-operation of the two molecules, it has been suggested that Hyal5 disperse the cells from the cumulus matrix in order for Ph-20 to digest the cumulus cells [37].

2.6.2 Sperm Binding, Adhesion and Penetration of the Zona Pellucida (ZP)

The second obstacle that spermatozoa should overcome is the ZP. Among the three major components of the ZP (*ZP1*, *ZP2* and *ZP3*), *ZP3* has been proposed to interact with sperm surface molecules. There are many reports that have investigated the role of *ZP3*. Laboratory experiments revealed that *ZP3* knockout female mice failed to have a ZP around their growing oocytes, thus resulting in infertility [39]. Also, it seems that the absence of the ZP impairs oocyte growth and follicle development, reducing in that way the number of fully-grown oocytes [40].

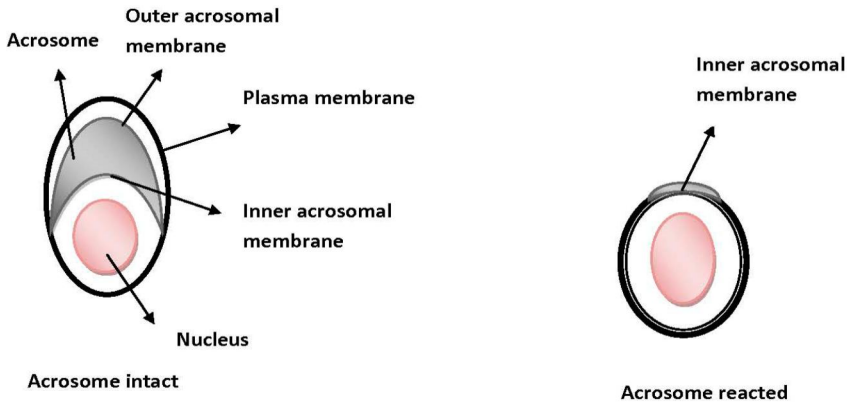

Figure: 4. The process where an acrosome intact spermatozoon becomes acrosome-reacted.
Source (Jovine *et al.*, 2007)

It is believed that the acrosome intact spermatozoa reach the ZP. The latter and especially *ZP3* has been proposed to promote the acrosome reaction. Following binding, the outer acrosomal membrane fuses with the sperm plasma membrane for the acrosomal contents (enzymes, such as acrosin) to be released for penetration of the ZP. *ZP3* is composed of a polypeptide region of 424 amino acids (AA) to

which are added a serine /threonine- (-*O*) and an asparagine- (-*N*) linked oligosaccharides [41]. *ZP3*, as also the other ZPs, has another region, which is called the ZP domain. There have been a variety of experimental studies to find out in which site of the *ZP3* the spermatozoon binds. This site was called the sperm combining-site and it is the epitope where a molecule (s) from the spermatozoon side matches with specific domains of *ZP3*. Investigation of this site revealed that acrosome-intact spermatozoa are adhered and bound to the *ZP3* O-linked oligosaccharides [42].

The binding of spermatozoa to *ZP3* appears to activate a cascade of intracellular signals that culminate in the alteration of the intracellular Ca2+ concentrations and pH, something that is essential for acrosome exocytosis. In contrast to reports indicating that *ZP2* has no direct role in the binding process [43]. Here are reports suggesting that *ZP2* gains affinity to acrosome-reacted spermatozoa [44].

Collectively, there is no consensus regarding the exact mechanism of AR by the ZP, but it is very possible that particular epitopes of the ZP (*ZP1*, *ZP2* and *ZP3*) initiate the AR and the carboxy-terminal region of the *ZP3* is responsible for the completion of acrosomal exocytosis. Moreover, the acrosomal matrix consists of proteins that during or following capacitation are exposed to the sperm surface interacting with the ZP of the oocytes. Among these proteins, sp56 and zonadhesin have been characterized [45]. Zonadhesin has gained much attention. Is a multiple domain protein that is produced during spermatogenesis and is located at the outer acrosomal membrane of spermatozoa [46]. but as soon as an acrosome reaction takes place, zonadhesin disappears. Recent evidence indicates that the adhesion, between spermatozoa and oocytes, is significantly reduced when zonadhesin antibodies a represent, implying that following capacitation, exposure of zonadhesin during acrosomal exocytosis is essential for ZP binding and adhesion [47].

2.6. 3 Sperm and Oocyte Plasma Membrane Interactions

After penetration of the ZP, the spermatozoon should overcome another obstacle, the oocyte plasma membrane. The spermatozoon plasma membrane fuses with the respective plasma membrane of the oocyte. It has been reported that only acrosome-reacted spermatozoa can fuse with the oocyte plasma membrane, implying that sperm molecules involved in membrane fusion are not yet active and become active upon exposure to the oocyte plasma membrane. It is very possible that acrosome reaction and penetration of the ZP triggers the initiation of cascade signals that promote the activation of the so far inactivated molecules that contribute to sperm fusion [48].

Cluster differentiation (CD) molecules are sperm and oocyte membrane surface clusters of differentiation molecules. These some CD molecules are involved in the fusion process since these molecules are expressed in cells of the male and female genital track. CD molecules are members of the tetraspanins superfamily, introduced as the "tetraspanins web'', because they form a multi-molecular "network", a characteristic of all tetraspanins, by interacting with other proteins of the oocyte's plasma membrane. After interacting it forms fertilization [49].

3. Conclusion And Recommendations

This paper covers many aspects of mammalian sperm-egg recognition and adhesion that lead to fertilization. It is apparent from the discussion in the paper that extensive progress has been made in this field of reproductive biology and physiology. The attempted to highlight molecular processes thought to be important in sperm function and fertilization. Although the molecule that initiates sperm egg recognition and adhesion. However, the significance of these molecules should not be undermined during *in vivo* fertilization. An understanding of the function of various sperm molecules in the process of *in vivo* fertilization will allow new strategies to regulate these events and alter sperm function and fertility.

Based on the above conclusion, the following recommendations are forwarded:

- To give the artificial insemination, veterinarians should know the process of fertilization.
- All peoples should get awareness regarding the molecules participating in fertilization.

Corresponding Author:
Dr. Chala Mohammed, Wollega University, School of Veterinary Medicine, P.O. Box: 395, Ethiopia.
Tel: +251-913-11-5805.

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