

## FERTILIZATION, CLEVEAGE AND IMPLANTATION

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### Abstract

Most of the livestock in Indonesia are still conventional or traditional farms, where the quality of the seeds, the use of technology and the skills of farmers are still relatively low, thus affecting the productivity and genetic quality of livestock. Therefore it is necessary to have knowledge about livestock reproduction that discusses fertilization, cleavage and implantation. Fertilization or fertilization (singami) is the fusion of two gametes which can be a nucleus or nucleus cells to form a single cell (zygote) or fusion of the nucleus. the process starts with the preparation of ovum cells and spermatozoa; penetration; core incorporation; and early zygote cleavage. Fertilization phase is the meeting between sperm cells and ovum cells and will produce zygote. Zygote will perform cell division (cleavage). The zygote then undergoes growth and development through stages, namely division, gastrulation, and organogenesis. Implantation or also known as oxidation is the process of implanting the embryo, which is the result of conception, into the uterine wall (endometrium) to further develop.

**Keyword : fertilization, cleavage, implantation, reproduction**

## 1. Introduction

Low productivity and genetic quality of livestock is still a problem in the world of livestock in Indonesia. This situation occurs because most of the livestock in Indonesia are still conventional or traditional farms, where the quality of the seeds, the use of technology and skills of farmers are still relatively low, livestock raising is done on an occasional basis (not a major economic source) with 1-3 livestock ownership. Reproduction is a physiological process in living things to produce offspring. Higher animals reproduce sexually and the reproduction process includes several physiological levels that include very complex functions and are integrated between one process and another. These physiological levels include the formation of sex cells (gametes), release of functionally differentiated gamete cells, mating to bring together male and female gametes, fertilization, fusion between the two pronuclei, growth, differentiation and development of the zygote until birth. Normal. The field of livestock productivity cannot be separated from the reproductive process. Reproductive success is a reflection of the success of a livestock business. Population development is highly dependent on broodstock and quality seedlings as well as the large number of cows born. This is of course highly supported by optimal reproductive management. Production and reproduction are closely related to the development and availability of livestock. Failure of a livestock to become pregnant in one or more matings will eliminate the product of conception in one or more gestation periods.

Based on the description above, it is necessary to have knowledge about the science of livestock reproduction, especially discussing the issue of fertilization, cleavage and implantation to help improve the genetic quality of an animal.

## 2. DISCUSSION

### 2.1 FERTILIZATION

#### 1. Definition of fertilization

Fertilization or fertilization (singami) is the fusion of two gametes which can be a nucleus or nucleus cells to form a single cell (zygote) or fusion of the nucleus. Usually involves the fusion of the cytoplasm (plasmogamy) and the fusion of nuclear material (karyogamy). With meiosis, the zygote forms a fundamental feature of most eukaryotic sexual cycles, and basically the fused gametgamete is haploid. When both are motile as in plants, then fertilization is called isogamy, if they differ in size but are similar in shape it is called anisogamy, if one is not motile (and usually larger) it is called oogamy. This is typical of some plants, animals, and most fungi. In some gymnohytes and all antophytes, the gametes are not flagellated, and the pollen tube is involved in the fertilization process.

#### 2. Types of Fertilization

1. **External fertilization (typical in aquatic animals):** the gametes are removed from the body before fertilization
2. **Internal fertilization (typical for adaptation to life on land):** sperm are inserted into the female reproductive area which is then followed by fertilization. After fertilization, the egg forms a fertilization membrane to prevent further sperm entry. Sometimes the sperm is needed only to activate the egg (Anonymous, 2008)

#### 3. Fertilization process

Fertilization occurs when spermatozoa cells are released and can fertilize the ovum in the ampulla of the fallopian tube. As many as 300 million spermatozoa are ejaculated into the female genital tract. About 1 million can swim through the cervix, hundreds of which can reach the fallopian tube and only 1 can fertilize an egg. Spermatozoa cells have a life span of about 48 hours (Cambridge, 1998). Before fertilizing an egg, spermatozoa must go through the

capacitation and acrosome correction stages first. Capacitation is a period of adjustment in the female reproductive tract, lasting about 7 hours. During that time a glycoprotein sheath from the cement plasma is removed from the plasma membrane which encloses the acrosome region of the spermatozoa. Meanwhile, the acrosome reaction occurs after the attachment of spermatozoa to the zona pellucida. This reaction causes the release of enzymes needed to penetrate the zona pellucida contained in the acrosome (Sadler, 1996). Oocytes (ovum) will reach the tube more than one hour after ovulation. The ovum is surrounded by the corona of small cells and the zona pellucida which will filter the existing spermatozoa cells so that only one cell can penetrate the ovum.

After the spermatozoa penetrates the ovum, it combines its core material and stores the usual double chromosome complement. This chromosome contains all the genetic information which will be passed on to the offspring (Canbridge, 1998).

The fertilized egg will form a zygote which continues to divide by mitosis into two, four, eight, sixteen and so on.

## 2.2 Cleavage

### 1. Definition of Cell Division

Cell division is the process of dividing a parent cell into two or more daughter cells. Cell division is usually a cycle of small cells which will lead to the next major cycle.

### 2. Types of cell division and their processes

#### a. Amitotic cell division (binary fission)

Amitotic cell division is also called direct cell division or does not go through certain stages, this process also takes place spontaneously, or is called binary fission. This process does not involve chromosomes why is that? Because the DNA is present in such a small amount and size that it cannot be packaged, most of this

division occurs in prokaryotic cells such as bacteria. The purpose of this division is to form new offspring.

### b. Mitotic Cell Division

Mitotic division, division which results in two daughter cells that are the same as the parent, meaning that these daughter cells can divide again. In Humans, this division occurs in somatic meristem cells (young body cells). This process takes place through structured and orderly stages, unlike Amitosis which occurs spontaneously.

This mitotic division goes through two stages, namely Kariokinesis and Cytokinesis.

#### 1. Kariokinesis

This process shows a striking difference in each phase and aims to divide the core material, as for the changes as follows:

##### • Interphase

At this stage the cells do not divide. The nucleus consists of ribosomal RNA and is the site of protein synthesis and dark matter known as chromatin or the form of chromosome strings so that the shape of the chromosomes cannot be seen clearly. At one end of the cell, there are 2 pairs of proteins called centrioles, but in plants, centrosomes do not appear.

##### • Prophase

At this stage the DNA begins to be packaged into chromosomes. The chromosomes begin to shorten and thicken. In animal cells the centrioles divide and each move to the opposite pole and a spindle thread is formed which is connected to the poles. Eventually the chromosome appears to consist of two chromatids attached to the centromere. The nucleolus is lost and the nuclear membrane is destroyed.

##### ▪ Metaphase

In this phase, the chromosomes move into a line called *the equator*.

In addition, threads called spindles appear and are attached to the centromere of each chromosome. This spindle connects the chromosomes to the 2 opposite poles of the centrioles

▪ **Anaphase**

Each centromere that binds the chromatid divides together and the chromatid moves towards the cleavage pole, producing paired copies of the chromosomes.

▪ **Telophase**

At this stage the chromosomes begin to organize to form a separate nucleus and are surrounded by members of the nucleus. Cleavage Burrow / groove division narrows and over time divides cells. In contrast, in plants, cleavage occurs with a *cell plate* rather than a *cleavage burrow*.

**2. Cytokinesis**

During cytokinesis, the cytoplasm of animal cells is divided into two by the formation of a contractile ring formed by actin and myosin in the center of the cell. This contractile ring causes the formation of a cleavage groove which will eventually produce two daughter cells. Each of these daughter cells that form contains a cell nucleus, along with cell organelles. In plants, cytokinesis is characterized by the formation of a dividing wall in the middle of the cell. This cytokinesis stage is usually included in the telophase stage.

c. **Meiosis Cell Division**

This division will produce gametes that cannot divide again until the fertilization stage. Meiotic division produces children who have half the number of chromosomes that their parents have, occur in the reproductive organs and directly between phase 1 followed by phase 2 without interphase interrupted. The stages are as follows:

a. **Meiosis I**

**Interphase**

At interphase, the cell is in the preparatory stage for division. The preparation is the DNA doubling of one copy into two copies (the same as in the mitotic interphase). The final stage of interphase is the presence of two DNA copies that are ready to be packed into chromosomes

- **Prophase 1.** At this stage the following process occurs
- **Leptotene** is the stage where the chromatin threads turn into chromosomes. This is done by self-compacting.
- **Zygotene / Zigonema**, at this stage, homologous chromatids pair up or synapses to form bivalents. The centrosome splits into 2 centrioles and moves to the opposite pole.
- **Pakiten / Pakinema**, the chromosomes are then duplicated into 4 at this stage and are called tetrads (homologous chromosomes that multiply so that there are 4 paired chromatids). At this stage, gene recombination often occurs through the cross-transfer process.
- **Diplotently**, homologous chromosomes that were bivalent are separated. When there is cross movement, there will be a chiasma as a sign.
- **Diakinesis**, in the diacinesis phase, the nucleolus (nuclear membrane) will disappear and the centrioles move to each pole and form spindle threads.
- **Metaphase 1.** Homologous chromosome pairs arrange themselves and face each other at the equator. Half of the homologous chromosome pairs point towards the other pole and the other half of the homologous chromosome pairs point toward

- the other pole
  - **Anaphase 1.** Each homologous chromosome begins to be pulled by the spindle thread to the opposite direction of the cleavage pole.
  - **Telophase 1.** The chromosome which still consists of two chromatids is at the poles. Furthermore, the nucleus membrane is formed which is followed by the cytokinesis process. At the end of telophase I two daughter cells are formed. Each daughter cell contains  $n$  chromosomes so that at the end of meiosis I two haploid daughter cells are formed.
  - **Cytokinesis 1.** In cytokinesis I, each homologous chromosome is separated by a divider so that cytokinesis produces two cells, each containing a chromosome with its twin chromatids
- b. Meiosis II**
- **Prophase II.** In prophase II, twin chromatids are still attached to each chromosome centromere. This stage sometimes occurs in a short time because it is followed by the next stage.
  - **Metaphase II.** In metaphase II each chromosome (which contains two chromatids) stretches out in the equatorial plane. Spindle threads are formed, one end is attached to the centromere, and the other end extends towards the poles of division in the opposite direction.
  - **Anaphase II.** In anaphase II the spindle thread begins to attract the chromatids towards the opposite poles of the cleavage. As a result, the chromosomes separate their two chromatids and move towards different poles. The separated chromatids are now called chromosomes.
  - **Telophase II.** In telophase II, the chromatids (or nowadays called chromosomes) have reached the poles of division. The total result of this stage is that four cores are formed. Each nucleus contains half a pair of chromosomes (haploid) and one DNA copy ( $1n, 1c$ ).  $\Rightarrow$  Cytokinesis II. There is cytokinesis II, each nucleus begins to be separated by a cell divider and eventually produces four haploid twin cells.
- 3. CLEAVEAGE (zygote cell division)**
- Fertilization phase is the meeting between sperm cells and ovum cells and will produce *zygote*. *Zygote* will perform cell division (cleavage). The zygote then undergoes growth and development through stages, namely division, gastrulation, and organogenesis.
- Cleavage is the rapid cleavage of the *zygote* into smaller units called blastomeres. The cleavage stage is a series of mitosis that takes place in succession immediately after fertilization to produce morula and blastomeres
- The initial process of embryo division in humans takes place along with the attachments of the embryo to the uterine wall of its mother. Human eggs in general do not have a yolk, are fertilized by the sperm as they move towards the uterus and the initial divisions last less than 24 hours. The cleavage is meridional not equal. Subsequent division is somewhat irregular, but quickly forms a dense sphere of cells, called the morulla.
- The zygote divides repeatedly until it consists of dozens of tiny cells, which are called blastomeres. This division can cover all parts, it can also be only in a small part of the zygote. This division occurs by mitosis, although sometimes it is also followed by continuous nuclear

division without cytoplasm. The area of division traveled by the direction of division when the zygote undergoes continuous mitosis into many cells, is called the division plane. There are 4 types of division, namely:

- **Meridians**, passing through the animal and vegetal polar axis.
- **Vertically**, passing upright from animal to vegetal poles does not pass through animal and vegetal poles.
- **Equator**, perpendicular to the axis of the animal-vegetal pole and halfway between the two poles.
- **Latitudinal**, parallel to the equatorial plane.

According to Orphans (1990: 155) in humans, division occurs holoblastically irregularly. Where the plane and time of the cleavage stages are not the same and are not simultaneously in various zygote regions. Initially the zygote divides into 2 cells, then occurs at the 3 cell level, then the 4 cell level, then the 5 cell, 6 cell, 7 cell, 8 cell level, and continuously until a blastomer is formed consisting of 60-70 cells, in the form of massive clumps called morula.

In animals, the first and second division of the fertilized ovary occurs while the ovary is in the fallopian tube. Normally, the embryo is at the 4-cell stage of its development when it enters the uterus. The embryo is not immediately distributed throughout the uterus but is temporarily held in the *anterior part of the uterine cornua*. Cleavage had reached the level of morula on day 5 to day 6 after the initiation of estrus and *blastocyst* formation with removal of the zona pellucida occurred on day 6 to day 8. *Blastocyst* elongation occurs prior to implantation. After chorion lengthening, the embryo is evenly distributed within the uterine horn. The length of the uterus increases rapidly from day 2 to day 6 after the onset of estrus. A linear weight gain of the uterus occurs during pregnancy. The space available in the uterus affects the number of children at

early gestation but is quite decisive at 105 days of gestation (Fenton et al., Cited in Toelihere, 1993).

#### a. Stages of cell division

##### 1. Stage Morulla

Morulla is a spherical (round) cell formation due to continuous cell division. Morula is a cell division that occurs after the cells number 32 cells and ends when the cell has produced a number of blastomeres of the same size but smaller in size. These cells condense to become small blastodisc forming two layers of cells. At this time the cell sizes began to vary. The cell divides transversely and begins to form a second layer of faintness at the animal cap. The morula stage ends when cell division has produced blastomeres. The blastomer then condenses into small blastodisc to form two layers of cells.

At the end of the division, two groups of cells will be produced. The first is the main cell group (blastoderm), which includes formative cells or inner mass cells, whose function is to form the body of the embryo. The second is the complement cell group, which includes trophoblast, periblast, and auxiliary cells. Its function is to protect and contact the embryo with the parent or the wider environment.

The trophoblast is attached to the uterine wall. The cells multiply rapidly and enter the uterine epithelium in the early stages of implantation. After 9 days, all blastocysts are retained in the uterine wall. When this takes place, the cells that are at the bottom of the cell mass organize themselves into a layer called the primary endoderm which will form the digestive tract of food. The remaining cells from the inner cell mass form a chip, namely the embryo piece. Between the embryo and the trophoblast that covers the emergence of a cavity (amniotic cavity) containing

a solution. The cavity wall, namely the amnion, spreads around the embryo and is surrounded by a cushion of amniotic fluid.

## 2. Blastula Stage

Blastulation is a process that produces blastula, which is a mixture of blastoderm cells that form a fluid-filled cavity as a blastocoel. At the end of blastulation, blastoderm cells will consist of neural, epidermal, notochordal, mesodermal, and endodermal which will form organs. It is characterized by two very distinct layers of flat cells forming the blastocoel and the blastodisc is in the transplanted vegetal hole covering most of the yolk. In the blastula there are already differentiated areas to form certain organs such as digestive tract cells, exoderm nerve notochords, ectoderm, mesoderm, and endoderm.

In humans, the cleavage results in the form of a solid ball (morulla). The outer layer of the blastula forms a layer surrounding the actual embryo, while the embryo is formed from the morulla (inner cells mass) / outer layer (trophoblast) on one side of the mass. cells in detach themselves, forming a shape similar to a blastula and this structure is known as a blastocyst. The embryo attaches and stays on the uterine wall for a certain period of time, where the embryo will get food until it is born

## 3. Gastrula Stadium

Gastrula is an advanced formation of the blastula whose curvature is more pronounced and has a layer of the embryo's body wall and body cavity. Gastrula in certain animals, such as lower animals and higher animals, differ in the number of layers of the embryo body wall. Triploblastic is an animal that has 3 layers of the embryo's body wall, namely the outer layer (ectoderm), the middle layer (mesoderm), and the inner layer

(endoderm). So gastrulation is the process of forming three embryonic layers. In further development the embryonic layer will experience growth and development to produce various organs of the body.

The three layers produced by gastrulation are embryonic tissue known as the ectoderm, endoderm and mesoderm which are collectively referred to as the embryonic germ tissue. The ectoderm forms the outer layer of the gastrula, the endoderm lines the digestive tract of the embryo and the mesoderm fills as the space between the ectoderm and endoderm. Eventually, these three layers develop into adult body parts. For example, the human nervous lining comes from the ectoderm, the outermost layer of our digestive tract and its organs come from the endoderm and most other organs and tissues, such as the kidneys, heart and muscles, come from the mesoderm.

## 2.3 IMPLANTATION

### 1. Definition of Implantation

Implantation or also known as oxidation is the process of implanting an embryo which is the result of conception into the uterine wall (endometrium) for further development. Implantation usually occurs on the 5<sup>th</sup> to 8<sup>th</sup> day of embryo development. And as for the sequence of events in the cell division in question is as follows:

Prior to implantation, the zona pellucida disappears by way of lysis. Prior to implantation, the blastosul fluid contained a lot of potassium and bicarbonate ions. This material comes from uterine fluids. After implantation, the amount of potassium and bicarbonate decreases, so that it is the same as the level in the main serum.

### 2. Implantation Process

The implantation process occurs after going through the fertilization process

and the cleavage process. Just when in the form of a morula (undergoing division into 32 cells), the embryo begins to enter the uterus. The division process is still happening. When going through implantation, the embryo is a blastocyst.

Pertama, zona pellucida akan terlepas sebagai aktivitas dari enzim proteolitik dari cairan uterus disebut proses hatching. Lalu bagian dari blastosit, yaitu tropoblast akan menempel pada endometrium dan berkembang menjadi plasenta yang berfungsi sebagai penyuplai zat-zat makanan kepada fetus.

When in contact with the endometrium, the tropoblast cells release protein digesting enzymes, allowing the tropoblast cells to penetrate into the endometrium. Apart from making a hole which is essential for implantation, the breakdown of the endometrial wall which is rich in nutrients is also important for a fuel source and metabolic feedstock. Furthermore, the tropoblast plasma membrane degenerates to form a multinucleated syncytium which later becomes the fetal placenta.

Endometrial tissue that undergoes modification at the implantation site is called the decidua. By responding to the chemical methods released by blastocysts, endometrial cells secrete prostaglandins which locally cause increased vascularity, edema and increased nutrient storage. When implantation is complete, all blastocysts are immersed into the endometrium and tropoblast cells continue to digest the surrounding decidual cells to provide energy for the embryo until the placenta is formed.

The endometrium around the implantation results will recover so that all implantation results are implanted in the endometrium. Along with the

embryo's invasion of the parent tissue, trophoblast cells then differentiate into 2 types of cells, namely:

1. Syncytiotrophoblast cells are large, multinuclear cells that develop from the cytotrophoblast layer. These cells actively secrete placental hormones and transfer nutrients from the mother to the fetus.
2. A group of cytotrophoblasts that are invasive, pass through the endometrial stroma to reach the main blood vessels, including the endometrial spiral artery.

In pigs, junction complexes begin to form between the tropoblast and the epithelial cells of the uterine wall. The next stage occurs the association of chorions and microvilli and adhesions with the uterine wall. In pigs and horses the association is an area which is the site of diffusion activity between chorions and villi (cotyledons).

### **3. Types of Implantation**

1. Interstitial implantation occurs in humans, chimpanzees and guinea pigs where the invasion of the embryo damages the uterine stromal tissue so deeply that the embryo enters the stroma and the surface of the uterus will cover the area where the embryo entered.
2. Implantation of eccentrics such as in rhesus monkeys, dogs, cats and mice, stromal damage occurs only partially and the developing embryo is still associated with the uterine lumen.
3. In superficial implantations such as in horses, pigs, cattle, sheep and goats, adhesions only occur on the surface of the uterus and relatively do not occur.





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Figure 1. *Fertilization*

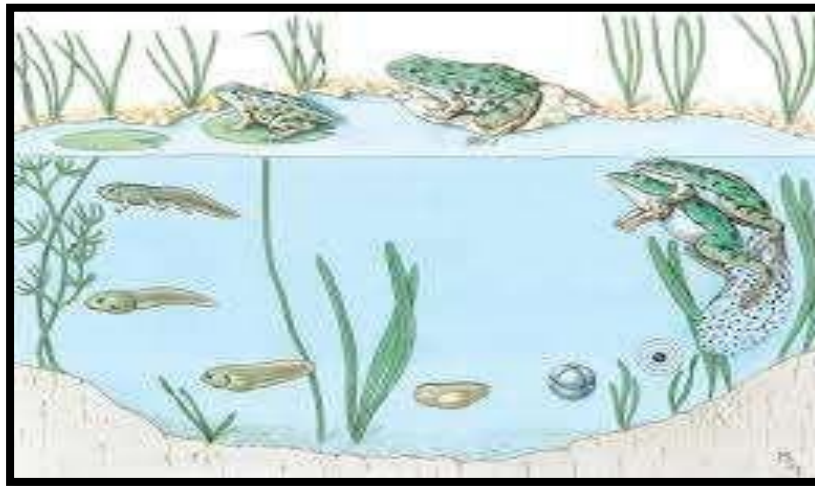


Figure 2. *External fertilization*



Figure 3. *Internal fertilization*

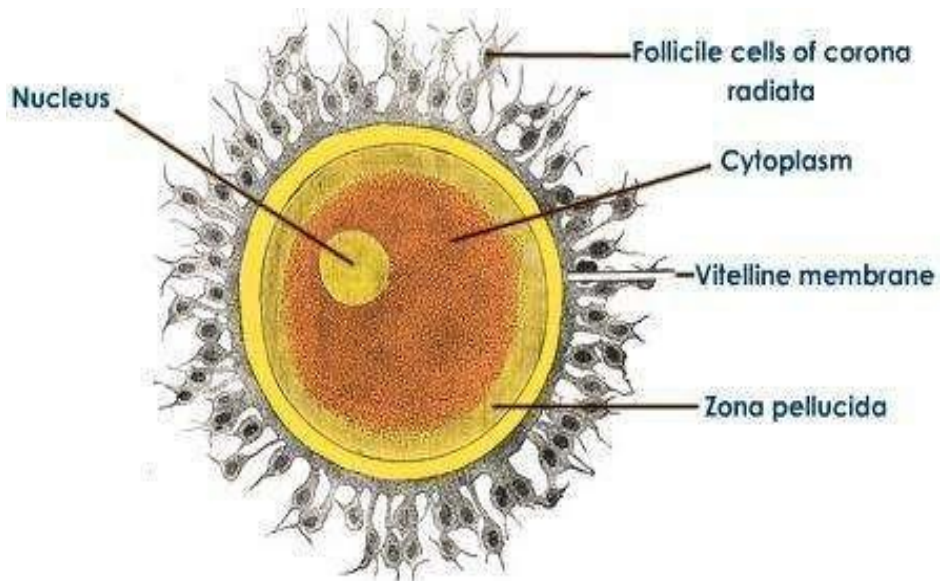


Figure 4. *Structure of the ovum*

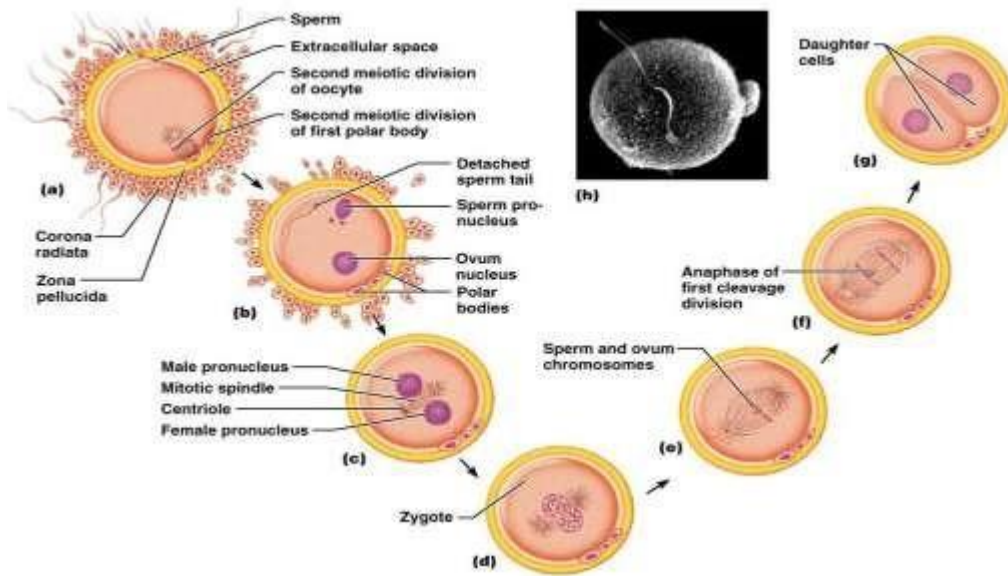


Figure 5. Fertilization Process

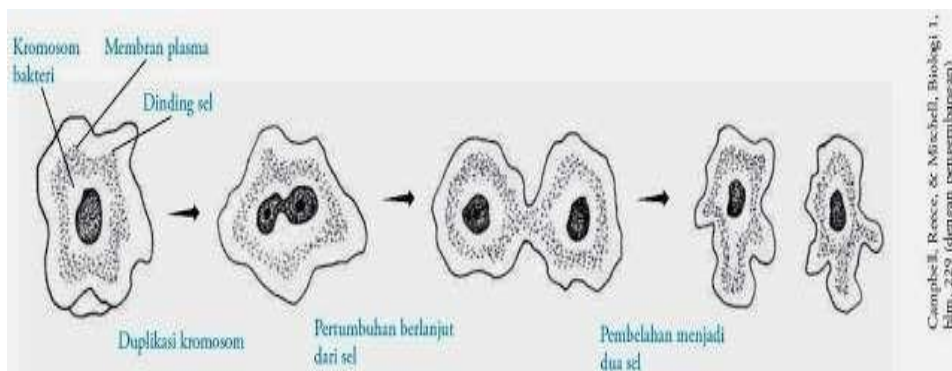


Figure 6. Division by amitosis

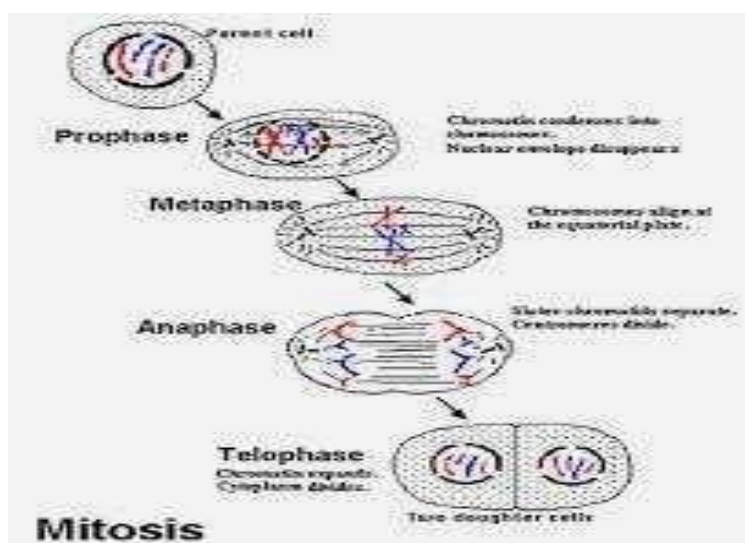
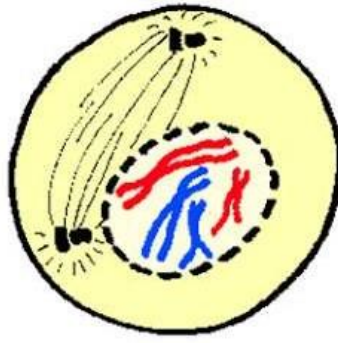
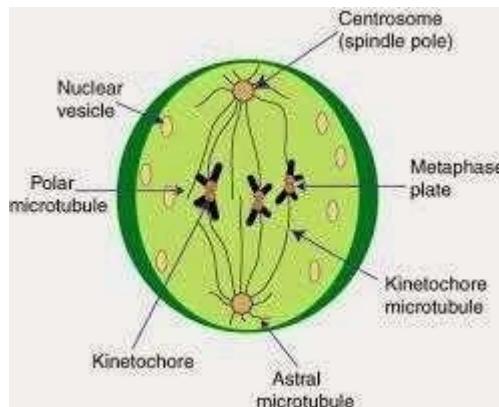


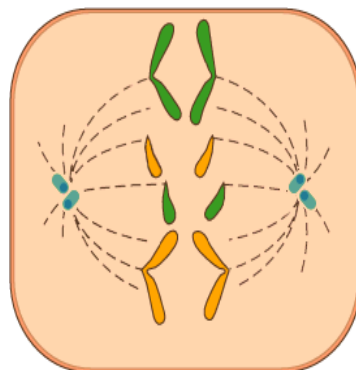
Figure 7. Mitotic Cleavage



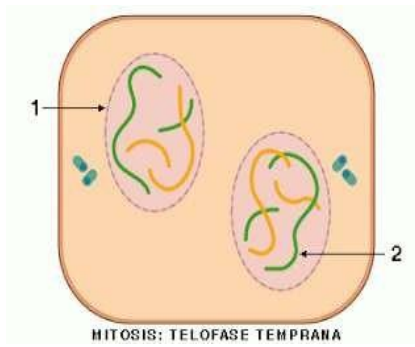
**Mitosis I: Prophase**  
**Figure 8. *The Profase Stage***



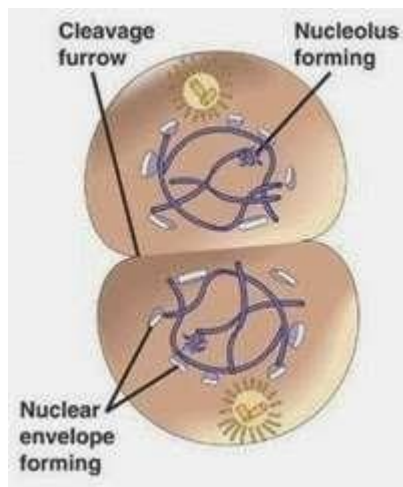
**Mitosis: Metaphase**  
**Figure 9. *Metaphase Stage***



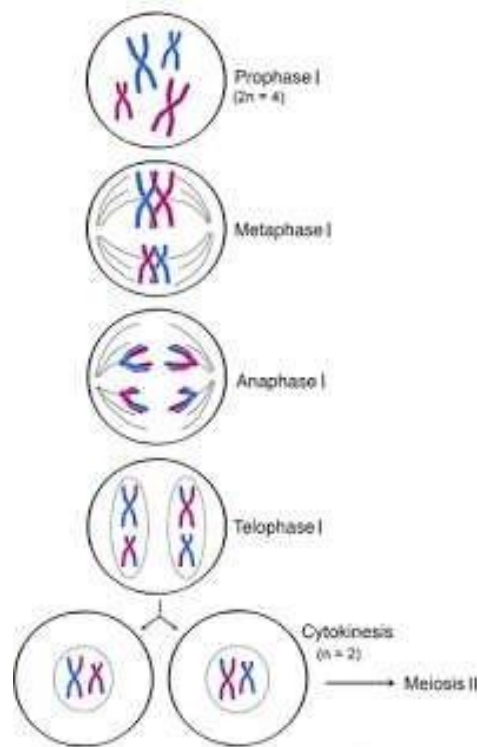
**MITOSIS: ANAFASE TEMPRANA**  
**Mitosis: Anaphase**  
**Figure 10. *Anaphase Stage***



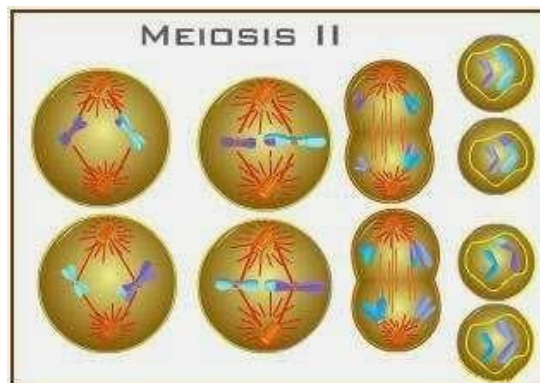
**Mitosis: Telophase**  
**Figure 11. *Telophase Stage***



**Mitosis: Cytokinesis**  
**Figure 12. *Cytokinesis***



**Meiosis I**  
**Figure 13. Meiosis I**



**Figure 14. Meiosis II**

Comparison Factor	Mitosis	Meiosis
The number of cleavages	One time cleavage	Twice cleavage
Number of daughter cells and genetic composition	two, each diploid (2n) and genetically identical to the parent	Four, each haploid (n) contains half of the chromosome number of the parent cell is not genetically identical to the parent cell and with each other
Homologous chromosome synapses	not occur	Synapses are unique to meiosis during prophase 1. Homologous chromosomes join their bodies, forming tetrads (groups of 4 kroatids), synapses cause crossovers

		between chromatids
Place Happened	Occurs in the cells of the body	Occurs in the reproductive organs
Role	For cell multiplication, growth, repair and cell reproduction	For the formation of sex cells

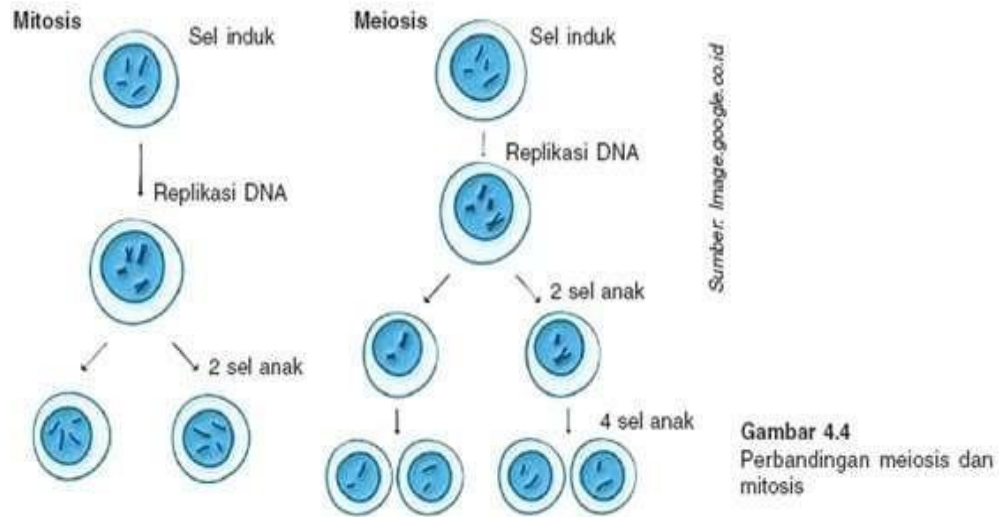


Figure 15. Comparison of Meiosis and Mitosis

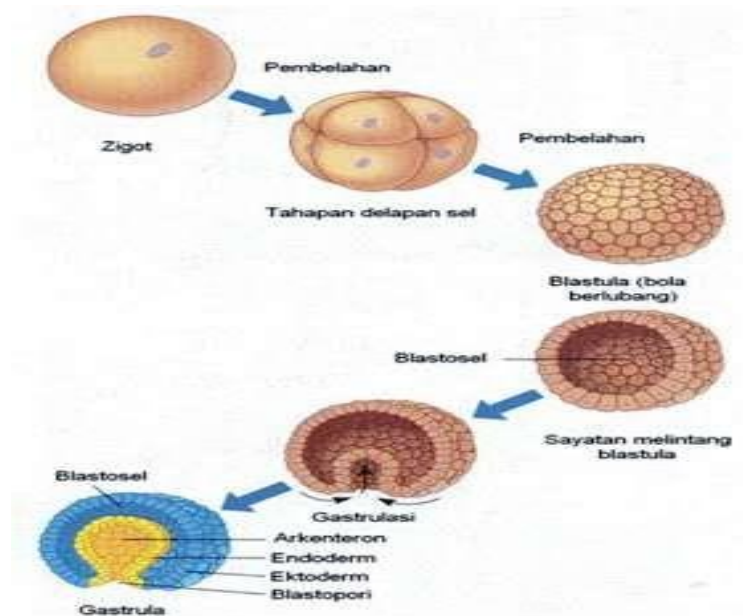


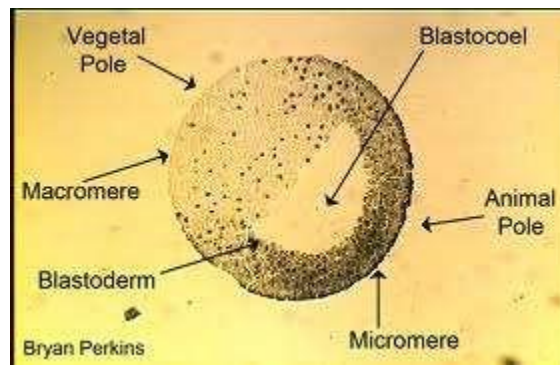
Figure 16. Cleavage



**Figure 17. Zygote cell division**



**Figure 18. Morula cells**



**Figure 19. Blastula Cells**



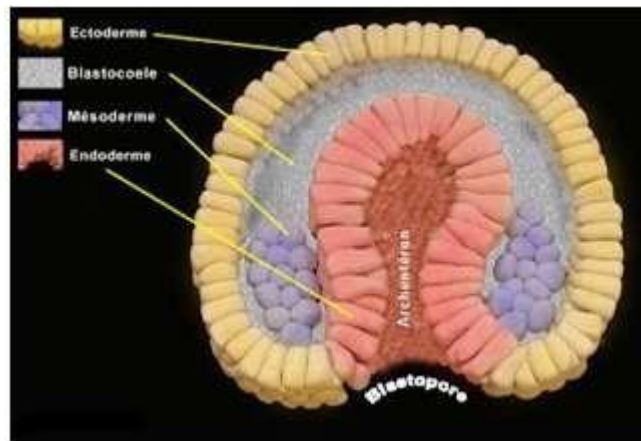


Figure 20. *Gastrula Cells*

And the sequence of cell division events in question is as follows:

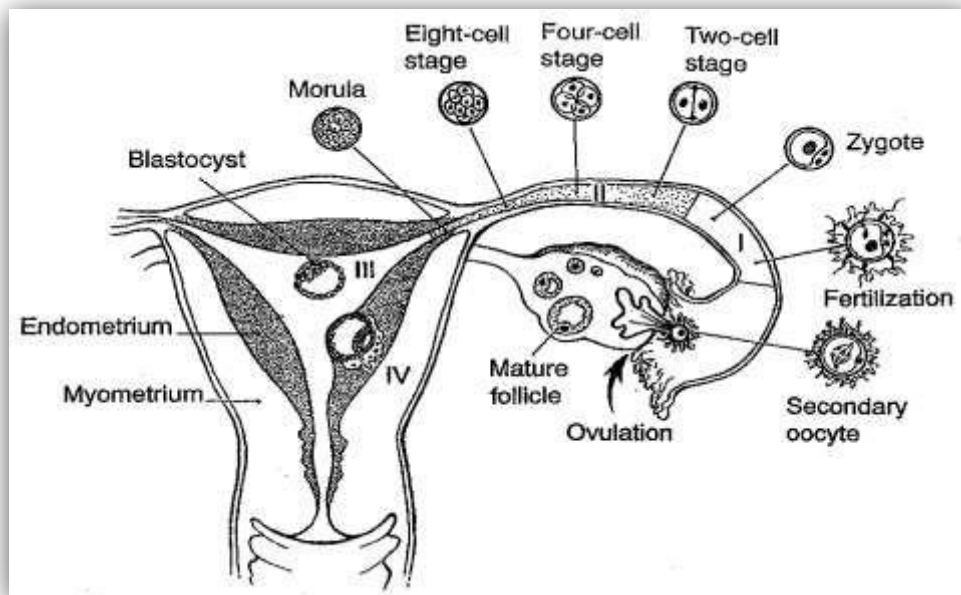
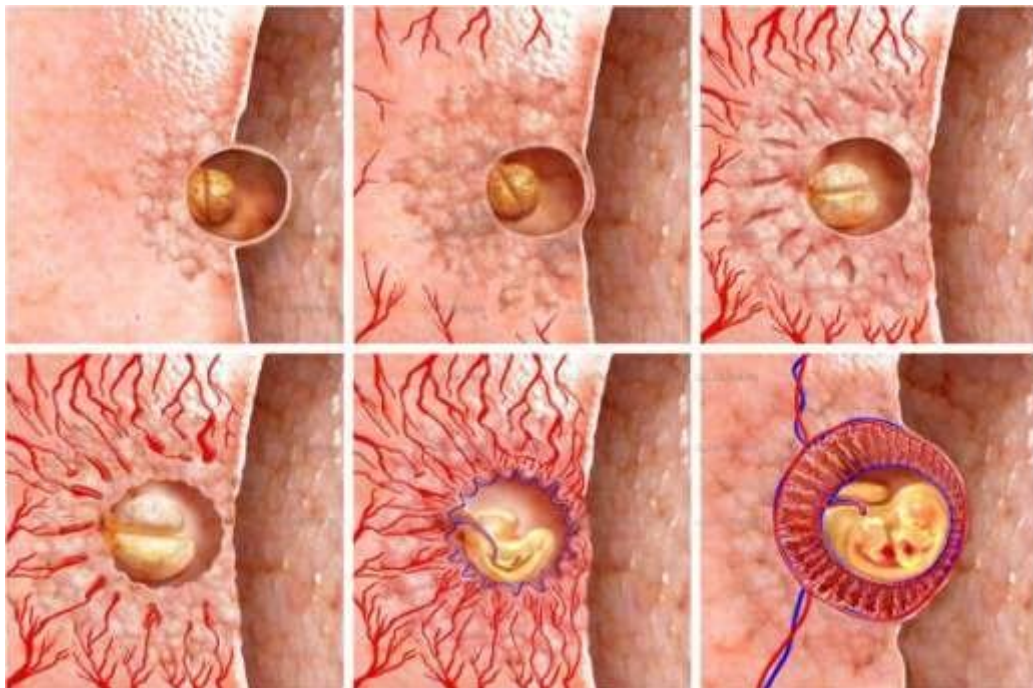
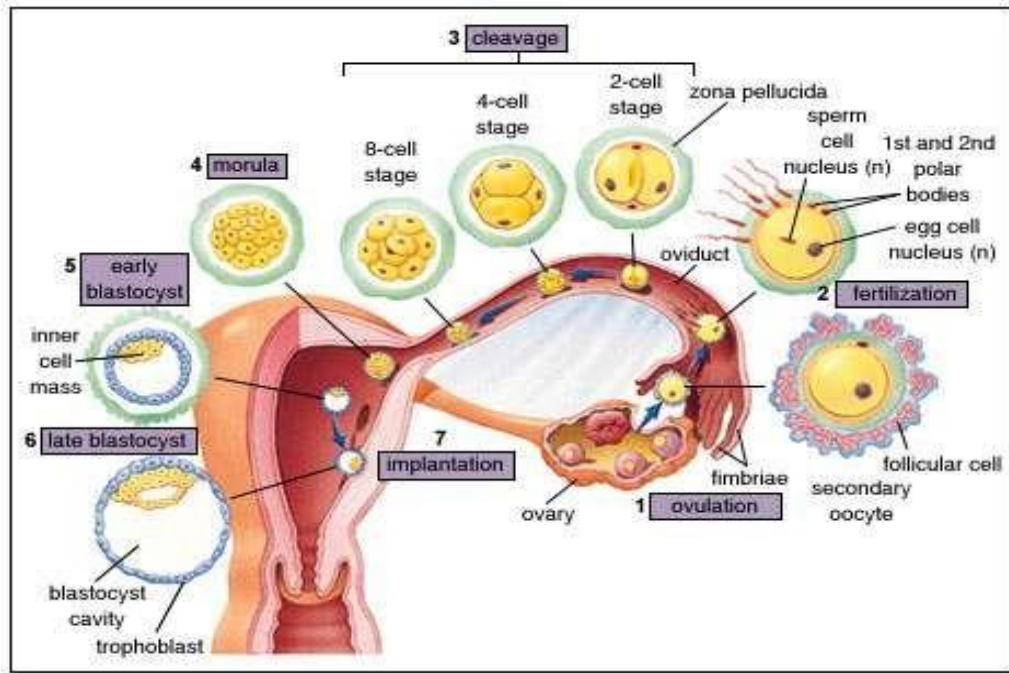


Figure 21. *Development of Cell Division*



#### 4. Conclusion

Conclusion From the above discussion it can be concluded that:

1. Fertilization or fertilization (singami) is the fusion of two gametes which can be either a nucleus or nucleated cells to form a single cell (zygote) or nucleus fusion. The process starts with the preparation of ovum and spermatozoa cells; penetration; core incorporation; and early zygote

cleavage.

2. Fertilization phase is the meeting between sperm cells and ovum cells and will produce zygote. Zygote will perform cell division (cleavage). The zygote then undergoes growth and development through stages, namely division, gastrulation, and organogenesis.
3. Implantation or also known as oxidation is the process of implanting the embryo, which is the result of conception, into the

uterine wall (endometrium) to further develop.

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