Laboratory Manual: Developmental Biology (ZOO501)
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Virtual University

Virtual University of Pakistan

Contents

S. No	Practical	P. No.
1	Study of the structure of gametes in some representative cases, i.e. frog, fish and mammal	2
2	Hen's egg internal and external structural details	5
3	Microscopic analysis of hen's egg yolk, albumin and shell membranes	8
4	Study of cleavage and subsequent development from prepared slides and/or models in various animals i.e., frog, mammals and chick etc.	11
5	Study of fertilization, early development of frog/fish through induced spawning under laboratory conditions.	16
6	Study of developmental stages of nematodes through microscopic analysis of animal dung	19
7	Semen analysis	23
8	Dactylography and its uses in developmental biology	28

Practical No: 1 Study of the structure of gametes in some representative cases, i.e. frog, fish and mammal

Male Gametes

The male gamete (sperm) is small and motile and only contributes the male's haploid nucleus to the zygote.

Sperm

A typical human spermatozoon can be divided into three sections,

1. Head,
2. Mid-piece
3. Tail

Head region:

The head region contains three structures,

- A haploid nucleus
- An acrosome cap
- Paired centrioles

The haploid nucleus contains the paternal DNA (this will combine with maternal DNA if fertilization is successful)

- The acrosome cap contains hydrolytic enzymes which help the sperm to penetrate the jelly coat of the egg
- The centrioles are needed by a zygote in order to divide (egg cells expel their centrioles within their polar bodies)

Mid-piece:

The mid-piece contains high numbers of mitochondria which provide the energy (ATP) needed for the tail to move

Tail:

The tail (flagellum) is composed of a microtubule structure called the axoneme, which bends to facilitate movement.

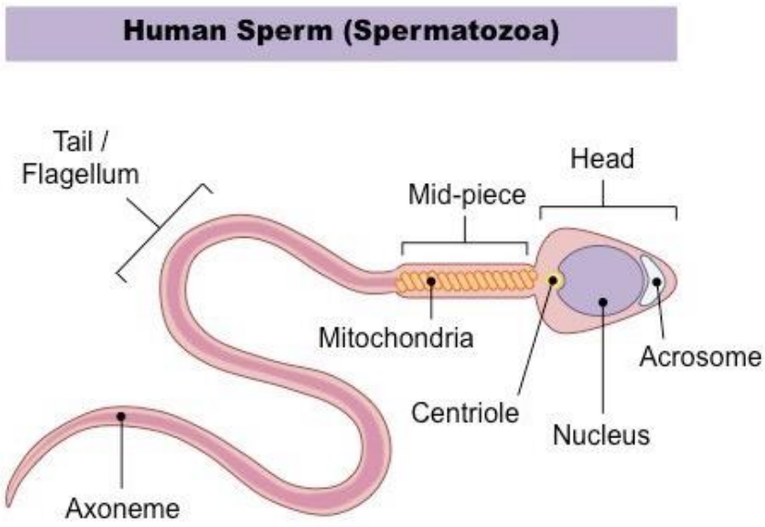


Fig 1.1: Detail of sperm

Female Gametes

A typical egg cell is surrounded by two distinct layers

1. The zone pellucida (jelly coat)
2. Corona radiate

Zone or Zona pellucida:

The zona pellucida is a glycoprotein matrix which acts as a barrier to sperm entry.

Corona radiate:

The corona radiata is an external layer of follicular cells which provide support and nourishment to the egg cell. Within the egg cell are numerous cortical granules, which release their contents upon fertilization to prevent polyspermy.

Egg cells commonly include a haploid nucleus. The cytoplasm of the egg is called ooplasm. It contains a very little mount of yolk in human female and therefore it is alecithal. In animals where huge amount of yolk is present, the cytoplasm of egg consists of lipoproteins, pigment granules, water and along with other cytoplasmic organelles.

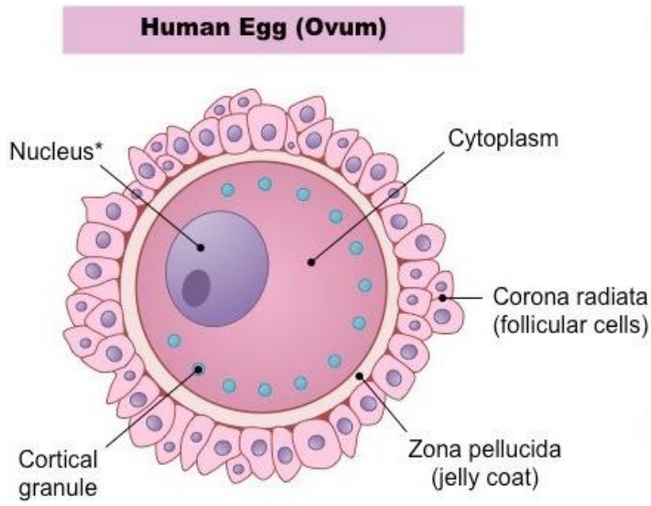


Fig 1.2: Detail of human egg

Practical No: 2 **Hen's egg**

internal and external structural details

Chicken egg quality has been defined by Kramer (1951) as the properties of any given food that have an influence on the acceptance or rejection of this food by the consumer. Egg quality is a general term which refers to several standards which define both internal and external quality. External quality is focused on shell cleanliness, texture and shape, whereas internal quality refers to egg white (albumen) cleanliness and viscosity, size of the air cell, yolk shape and yolk strength.

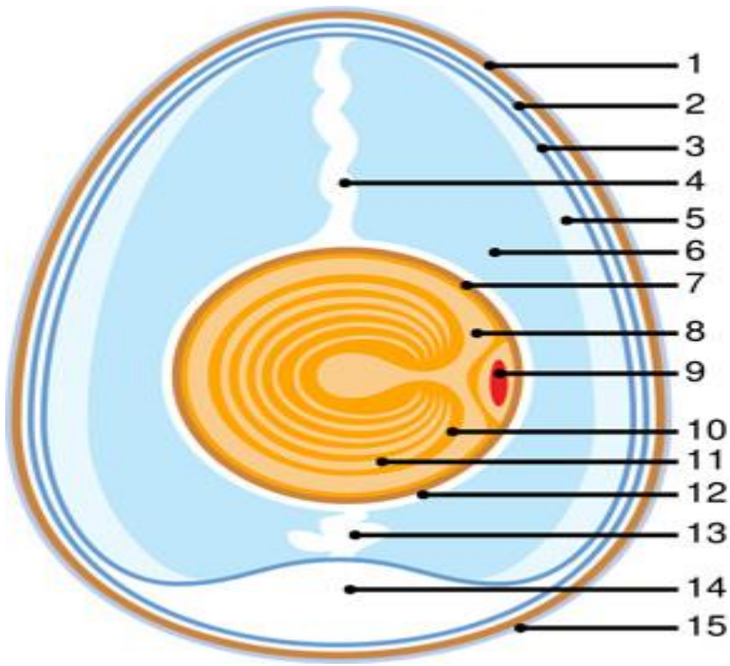


Fig 2.1: Detail of egg

1. Eggshell

The outer eggshell is made almost entirely of calcium carbonate (CaCO3) and is covered with as many as 17,000 tiny pores. It is a semipermeable membrane, which allows air and moisture to pass through its pores. The shell also has a thin outermost coating called the bloom or cuticle that helps keep out bacteria and dust.

2. Outer shell membrane

3. Inner shell membrane

These two membranes – outer and inner – are just inside the shell surrounding the albumen (white). The two membranes provide an efficient defense against bacterial invasion and are made partly of keratin. The outer membrane sticks to the egg shell while the inner membrane sticks to the albumen. When an egg is first laid, it is warm. As it cools, the contents contract and the inner shell membrane separates from the outer shell membrane to form the air cell (see 14 below).

4. Chalaza

Chalaza is twisted in opposite directions and serves to keep the yolk centered. The more prominent the chalazae, the fresher the egg.

5. Exterior albumen (outer thin albumen)

The outer thin albumen is a narrow fluid layer next to the shell membrane.

6. Middle albumen (inner thick albumen)

The inner thick white (chalaziferous layer) is a dense, matted, fibrous capsule of albumen around the vitelline membrane of the yolk. The matted fibrous capsule terminates on each end in the chalazae, which are twisted in

opposite directions and serve to keep the yolk centered. This part of the egg is a excellent source of riboflavin and protein. In high-quality eggs, the inner thick albumen stands higher and spreads less than thin white. In low-quality eggs, it appears thin white.

7. Vitelline membrane

The clear casing that encloses the egg yolk. When an egg is said to be “mottled”, the yolk surface is covered with many pale spots or blotches. The strength and integrity of the vitelline membrane are very important in preventing egg yolk mottling.

8. Nucleus of pander

Nucleus of pander a plug of whitish yolk, with no particular significance for development and whose function is purely a nutritive one, like the rest of the yolk.

9. Germinal disk (blastoderm)

A small, circular, white spot (2-3 mm across) on the surface of the yolk; it is where the sperm enters the egg. The nucleus of the egg is in the blastodisc. The embryo develops from this disk, and gradually sends blood vessels into the yolk to use it for nutrition as the embryo develops.

10. Yellow yolk

A major source of vitamins, minerals, almost half of the protein, and all of the fat and cholesterol. The yolk contains less water and more protein than the white, some fat, and most of the vitamins and minerals of the egg. These include iron, vitamin A, vitamin D, phosphorus, calcium, thiamine, and riboflavin. The yolk is also a source of lecithin, an effective emulsifier. Yolk color ranges from just a hint of yellow to a magnificent deep orange, according to the feed and breed of the hen.

11. White yolk

Also known as, the latebra is an area of white yolk located in the center of the yolk. It is lower in fat and therefore stands out as a bright white area in many Magnetic Resonance Images. The specific function of the latebra is uncertain but it may act as a central structure around which the additional layers of the yolk are formed.

12. Internal albumen (Chalaziferous albumen)

The inner thick white (chalaziferous layer) is a dense, matted, fibrous capsule of albumen around the vitelline membrane of the yolk. The matted fibrous capsule terminates on each end in the chalazae, which are twisted in opposite directions and serve to keep the yolk centered.

13. Chalaza chalazae

It is twisted in opposite directions and serves to keep the yolk centered. The more prominent the chalazae, the fresher the egg. Chalazae, which are twisted in opposite directions and serve to keep the yolk centered.

14. Air cell

An air space forms when the contents of the egg cool and contract after the egg is laid. The air cell usually rests between the outer and inner membranes at the eggs larger end. As the egg ages, moisture and carbon dioxide leave through the pores of the shell, air enters to replace them and the air cell becomes larger.

15. Cuticle or bloom

The shell is produced by the shell gland (uterus) of the oviduct, and has an outer coating, the bloom or cuticle. The cuticle somewhat seals the pores and is useful in reducing moisture losses and in preventing bacterial penetration of the egg shell. Most of cuticle is removed from table eggs when they are mechanically washed.

Practical No: 3 Microscopic analysis of hen's egg yolk, albumin and shell membranes

Analysis of egg yolk

Egg yolk has a lipid to protein ratio of 2:1. The major lipid components are triacylglycerols, phospholipids and cholesterol. The major protein components are low-density lipoprotein, high density lipoprotein, and livetin (globular, water soluble), phosvitin (a phospho protein). Yolk from one large egg usually contains about 213 mg of cholesterol. In this experiment, you will qualitatively determine the lipids present in the yolk. You will then quantify the amount of cholesterol in the yolk.

Extraction Procedure:

1. Gently crack open the egg. DO NOT BUST THE YOLK.
2. Using the shell, separate the yolk from the white. Discard the white.
3. Add the yolk to a beaker (you can bust it now) and record its mass and volume. If you can, remove the white protein layer that the yolk is in. Scramble the egg.
4. Split the yolk into to 2 equal parts and perform the following steps

Acetone Extraction

5. Add an equal volume of acetone to each tube. This must be done in a glass centrifuge tubes!!
6. Shake vigorously with vertex for 2 minutes
7. Centrifuge
8. Decant acetone
9. Repeat 3 x more
10. Pool acetone fractions and allow acetone to evaporate in the hood.
11. The acetone extract should contain the cholesterol and yolk pigments. Save for your cholesterol assay.

Cholesterol analysis:

The cholesterol reagent contains cholesterol esterase, cholesterol oxidase and horse radish peroxidase. The reaction for the production of the colored complex is:

$$cholesterol + O_2 \xrightarrow{cholesterol\ oxidase} cholestenone + H_2O_2$$

$$H_2O_2 + phenol + 4-aminoantipyrine \xrightarrow{HRP} colored\ product + H_2O$$

1.Since only free cholesterol is a substrate for cholesterol oxidase, any cholesterol esters present in the sample must first be hydrolyzed with cholesterol esterase.

2.Redissolve the acetone fraction in a few mLs of isopropanol (you should know the amount)

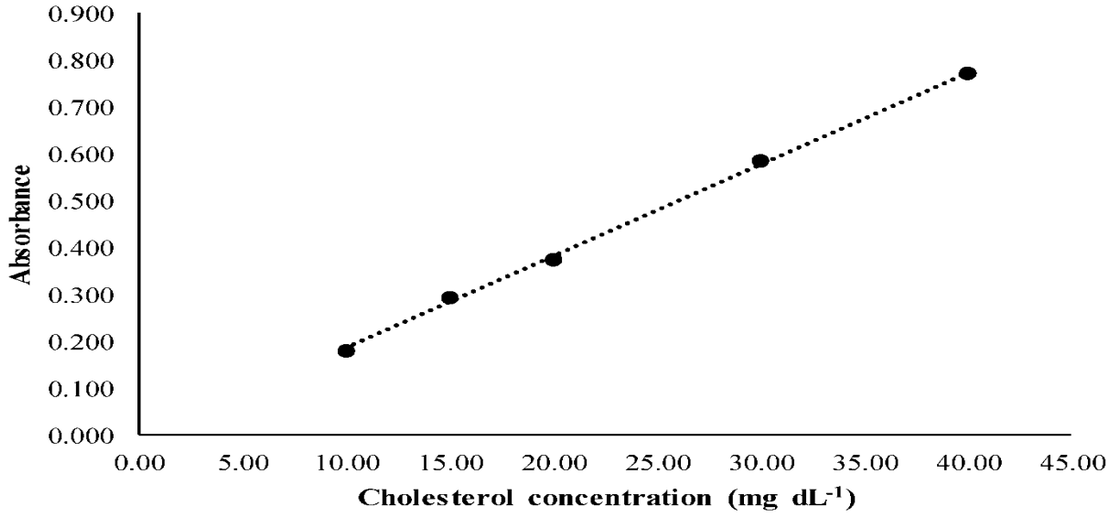
3.Vortex the isopropanol sample.

4.Remove 1 mL of sample and place it into another tube.

5.Add 5 mL of isopropanol.

6.Vortex

7.Use the table below to construct your calibration curve and cholesterol assay. Put the reagents directly into semi-micro cuvettes.



Read the absorbance of the tubes at 500 nm on the UV-VIS. Use the graph to determine the mg/mL of cholesterol in your sample. Use the 2 dilutions you made to calculate the mass of cholesterol in the original extract.

Analysis of albumin

1. Prepare phosphate buffer of 0.5 M in 100 ml at 7 pH.

	1M		0.1M	0.02M	
1a	→	61.0% →	61/10= 6.1	→	1.22
1b	→	39.0% →	39/10= 3.9	→	0.78
			10ml	**2ml in 98ml**	**100ml total**

BSA

2. Prepare phosphate buffer of 0.5 M in 100 ml at 7 pH.

	1M		0.5M	
1a	→	61.0% →	61/2= 30.5	
1b	→	39.0% →	39/2= 19.5	
			50ml →	In 50 ml distilled water. 100ml total.

3. Above solution will be maintained at 7 pH by diluted HCL with help of dropper.
4. After that 100 ul of supernatant (sample).
5. 1ml Bradford dye reagent will be added.

Coomassive blue B →	Ethanol (95%) →	Phosphoric acid (85%) →	Filter it.
100mg	50ml	85ml in 15 ml water	100ml

6. Mix for 10 min at 37C.
7. Check the solution absorbance at 595 nm.

Practical No: 4 Study of cleavage and subsequent development from prepared slides and/or models in various animals i.e., frog, mammals and chick

Cleavage of Chick

The zygote nucleus undergoes a series of mitoses, with the resulting daughter nuclei becoming partitioned off, by cytokinesis, in separate, and ever-smaller, cells. The first cleavage occurs shortly after the zygote nucleus forms. A furrow appears that runs longitudinally through the poles of the egg, passing through the point at which the sperm entered and bisecting the gray crescent. This divides the egg into two halves forming the 2-cell stage. The second cleavage forms the 4-cell stage. The cleavage furrow again runs through the poles but at right angles to the first furrow. The furrow in the third cleavage runs horizontally but in a plane closer to the animal than to the vegetal pole. It produces the 8-cell stage.

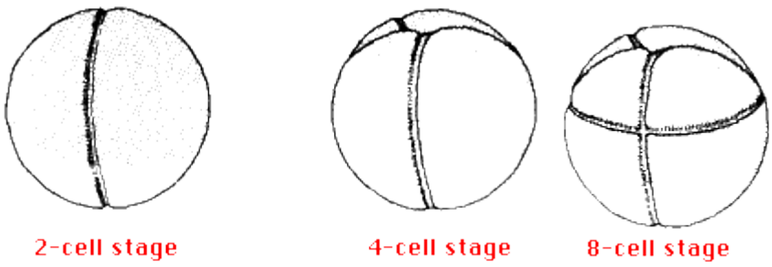


Fig 4.1: Detail of zygote development

Various stages of cleavage in a frog zygote

The next few cleavages also proceed in synchrony, producing a 16-cell and then a 32-cell embryo. However, as cleavage continues, the cells in the animal pole begin dividing more rapidly than those in the vegetal pole and thus become smaller and more numerous. By the next day, continued cleavage has produced a hollow ball of thousands of cells called the blastula. A fluid-filled cavity, the blastocoel, forms within it.

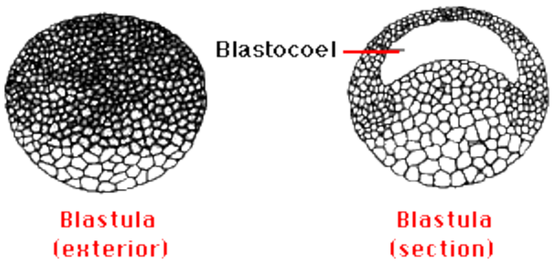


Fig 4.2: Stages of blastula

Frog Bastula

During this entire process there has been no growth of the embryo. In fact, because the cells of the blastula are so small, the blastula looks just like the original egg to the unaided eye. Not until the blastula contains some 4,000 cells is there any transcription of zygote genes. All of the activities up to now have been run by gene products (mRNA and proteins) deposited by the mother when she formed the egg.

Cleavage of Amphibian

Cleavage in most frog and salamander embryos is radially symmetrical and holoblastic, just like echinoderm cleavage. The amphibian egg, however, contains much more yolk. This yolk, which is concentrated in the vegetal hemisphere, is an impediment to cleavage. Thus, the first division begins at the animal pole and slowly extends down into the vegetal region as shown in Fig. In the axolotl salamander, the cleavage furrow extends through the animal hemisphere at a rate close to 1 mm per minute. The cleavage furrow bisects the gray crescent and then slows down to a mere 0.02–0.03 mm per minute as it approaches the vegetal pole.

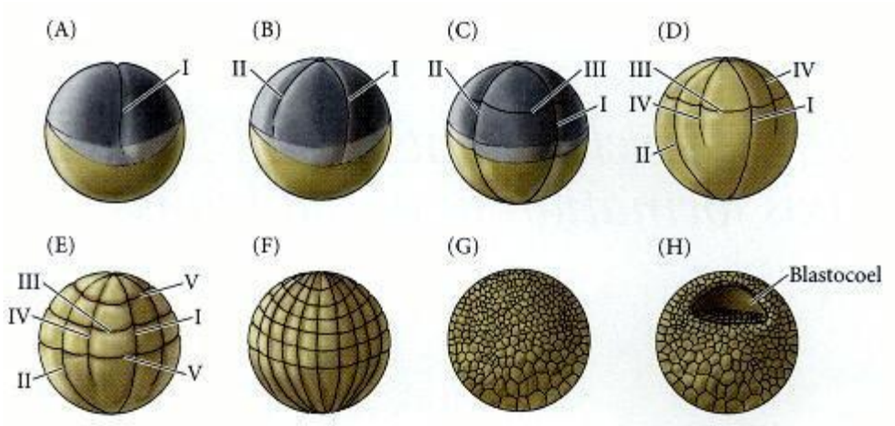


Fig 4.3: Cleavage of a frog egg

Cleavage furrows, designated by Roman numerals, are numbered in order of appearance. (A, B) Because the vegetal yolk impedes cleavage, the second division begins in the animal region of the egg before the first division has divided the vegetal cytoplasm. (C) The third division is displaced toward the animal pole. (D-H) The vegetal hemisphere ultimately contains larger and fewer blastomeres than the animal half. H represents a cross section through a midgastrula stage embryo.

Cleavage in Mammals

It is not surprising that mammalian cleavage has been the most difficult to study. Mammalian eggs are among the smallest in the animal kingdom, making them hard to manipulate experimentally. The human zygote, for instance, is only 100 μm in diameter—barely visible to the eye and less than one-thousandth the volume of a *Xenopus* egg. Also, mammalian zygotes are not produced in numbers comparable to sea urchin or frog zygotes, so it is difficult to obtain enough material for biochemical studies. Usually, fewer than ten eggs are ovulated by a female at a given time. As a final hurdle, the development of mammalian embryos is accomplished within another organism, rather than in the external environment. Only recently has it been possible to duplicate some of these internal conditions and observe development in vitro.

With all these difficulties, knowledge of mammalian cleavage was worth waiting for, as mammalian cleavage turned out to be strikingly different from most other patterns of embryonic cell division. The mammalian oocyte is released from the ovary and swept by the fimbriae into the oviduct (Fig). Fertilization occurs in the ampulla of the oviduct, a region close to the ovary. Meiosis is completed at this time, and first cleavage begins about a day later. Cleavages in mammalian eggs are among the slowest in the animal kingdom—about 12–24 hours apart. Meanwhile, the cilia in the oviduct push the embryo toward the uterus; the first cleavages occur along this journey.

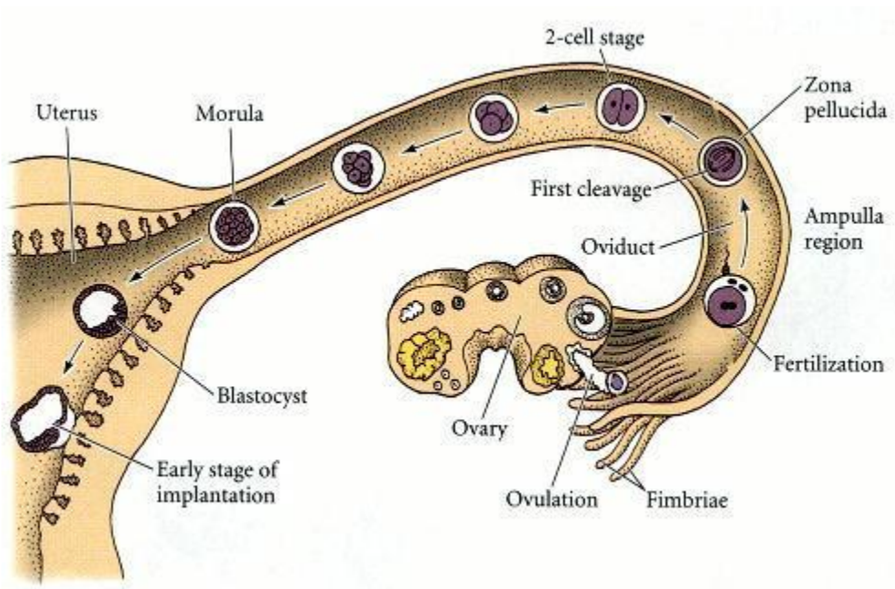


Fig 4.4: Development of a human embryo from fertilization to implantation

Compaction of the human embryo occurs on day 4 when it is at the 10-cell stage. The embryo "hatches" from the zona pellucida upon reaching the uterus. During its migration to the uterus, the zona prevents the embryo from prematurely adhering to the oviduct rather than traveling to the uterus.

In addition to the slowness of cell division, there are several other features of mammalian cleavage that distinguish it from other cleavage types. The second of these differences is the unique orientation of mammalian blastomeres with relation to one another. The first cleavage is a normal meridional division; however, in the second cleavage, one of the two blastomeres divides meridionally and the other divides equatorially. This type of cleavage is called rotational cleavage.

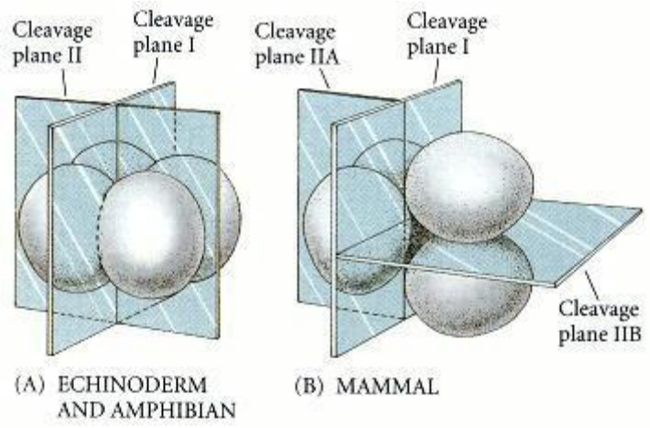


Fig 4.5: Comparison of early cleavage A) echinoderms, B) mammals

Comparison of early cleavage in

(A) Echinoderms and amphibians (radial cleavage) and

(B) Mammals (rotational cleavage). Nematodes also have a rotational form of cleavage, but they do not form the blastocyst structure characteristic of mammals.

The third major difference between mammalian cleavage and that of most other embryos is the marked asynchrony of early cell division. Mammalian blastomeres do not all divide at the same time. Thus, mammalian embryos do not increase exponentially from 2- to 4- to 8-cell stages, but frequently contain odd numbers of cells. Fourth, unlike almost all other animal genomes, the mammalian genome is activated during early cleavage, and produces the proteins necessary for cleavage to occur. In the mouse and goat, the switch from maternal to zygotic control occurs at the 2-cell stage.

Practical No: 5 Study of fertilization, early development of frog/fish through induced spawning under laboratory conditions

Induced spawning is one of the common methods to stimulate ovulation of the fish in the hatchery. Several natural and artificial hormones have been used to **induce** breeding of fish, while Ovaprim is one of the most popular and effective solutions to stimulate the maturation of male and female broodfish.

Hormone administration

Three dosages of Ovaprim were tested in this study: for females, 0.3 ml kg $^{-1}$ body weight (BW), 0.5 ml kg $^{-1}$BW, and 0.7 ml kg $^{-1}$BW; for males, 0.15 ml kg $^{-1}$BW. The broodfish in control groups were injected with physiological solution (0.9% NaCL) at the dosage of 0.25 ml kg $^{-1}$BW.

Sperm and egg collection

The female broodfish were injected with their respective dosage two times; the first injection with half of the tested dosage, and the second injection was conducted 6 hours after the first injection, with the remainder of the tested dosage. The female broodfish was ovulated 6 hours after the second injection. The eggs were collected by gentle finger pressure to the abdomen. The collected eggs were put in a plastic jar and kept in an ice box at 4°C.

The males were injected with a single dose of 0.25 ml kg $^{-1}$ BW. The male siban fish was sacrificed with MS-222 (Merck), prepared by dissolving 4g of MS-222 in 5L tap water.The testes were removed and washed with physiological solution and then perforated and chopped with scissors. The semen was gently squeezed out and put in a tube, and kept in an icebox (4°C). The semen was mixed with physiological solution (0.9% NaCl) at dilution ratio 1: 100 (v/v).

Fertilization and incubation

A total of 1 ml eggs and 1 ml of diluted sperm were mixed homogenously in a plastic basin (Calista, Volume 500 mL) and with approximately 1 ml of fertilization solution (contains 4 g urea and 3 g NaCl L $^{-1}$). The resulting mixture was left in contact for 5 minutes. The incubation basin was installed with 24-hour portable aerator and LED lamp.

A total of 100 eggs were taken randomly and then incubated in a plastic basin (Calista, volume: 2L), with three replicates at a water temperature of 25–27°C in a water heater. Successful fertilization was observed 8 hours after incubation. Unfertilized eggs were identified by their opacity; the unfertilized eggs were removed from the jar, while hatching rate was monitored at two-hour intervals. The larvae were fed on *Tubifex* sp. *ad libitum* on day 5 after being hatched and reared in the same jar for 40 days.

Measured parameters and data analysis

The latency period, the relative number of ovulated eggs, egg diameter, fertilization rate, hatching rate, and survival rate of larvae on day 40 after hatching were measured. The latency period was determined by calculating the time between the second injection and ovulation. The relative number of ovulated eggs was calculated by dividing the total number of released eggs after Ovaprim injection by the total body weight of the female broodfish. Fertilization, hatching, and survival rates were calculated.

Results

Table 5.1: Latency period, fertilization, hatching, and survival rate of siban fish *Cyclocheilichthys apogon* based on Ovaprim dosages

No	Parameter	Ovaprim dosage (ml kg $^{-1}$ body weight)			
		0	3	5	7
1	Latency period (hour)	10.01±0.01 ^{d}	7.27±0.06 ^{c}	7.18±0.04 ^{b}	7.07±0.04 ^{a}
2	The relative number of ovulated eggs (eggs g $^{-1}$ of BW)	5.5±0.71 ^{a}	10.67±6.03 ^{b}	11.00±0.00 ^{b}	19.00±11.53 ^{c}
3	Fertilization rate (%)	40.00±1.41 ^{a}	60.00±8.19 ^{b}	60.00±7.55 ^{b}	76.67±9.07 ^{c}
4	Hatching rate (%)	25.55±2.12 ^{a}	62.94±2.05 ^{b}	56.95±5.31 ^{b}	76.00±2.92 ^{c}
5	Survival rate on day 40	40.00±2.83 ^{a}	61.95±1.80 ^{b}	58.60±1.08 ^{b}	79.99±9.13 ^{c}

No	Parameter	Ovaprim dosage (ml kg $^{-1}$ body weight)			
		0	3	5	7
	after hatched (%)				

In general, the relative number of ovulated eggs, fertilization, hatching, and survival rates were increased with increasing Ovaprim dosage. However, there were no significant differences between the values seen at 3 ml kg $^{-1}$ BW and 5 ml kg $^{-1}$ BW dosage.

Practical No: 6 Study of developmental stages of nematodes through microscopic analysis of animal dung

Material required

- Zinc sulphate
- Centrifuge tube
- Fecal samples
- Centrifugation machine
- Microscope
- Cover slip
- Slides

Procedure

1. Fecal samples were collected from the rectum, and examined by standard parasitological method of zinc sulphate flotation. For this method, approximately 1 g of fecal material was diluted with tap water and passed through a sieve (No 150) into a centrifuge tube.
2. The tube was centrifuged at 200 ×g for 3 min, the supernatant fluid was discharged down to approximately 1 cm above the sediment and zinc sulphate (ZnSO4•7H2O) solution 33.2 % (w/v) was added to the sediment.
3. After thorough dilution of the sediment, zinc sulphate solution was added to just over the top of the tube and a cover slip was placed on the top of the tube.
4. After centrifugation at 150 ×g for 1 min, the cover slip was carefully removed and placed on a microscope slide.
5. All the preparations were examined under the optical micro-scope at 100×, 400× and 1000× magnification.

Observation

1. The most prevalent nematode was *Trichostrongylus retortaeformis* (50%) (Fig. 6.1), followed by *Trichuris leporis* found 18 (21.42 %) (Fig 6.2) animals and *Passalurus ambiguus* in 4 (4.76 %).
2. Mixed infections (Fig. 3) were significantly more common than single infections

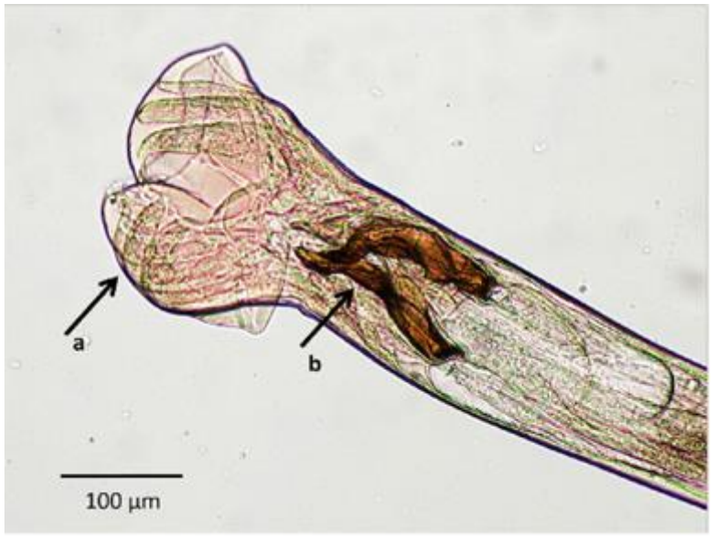


Fig 6.1: Posterior end of male *Trichostrongylus retortaeformis*

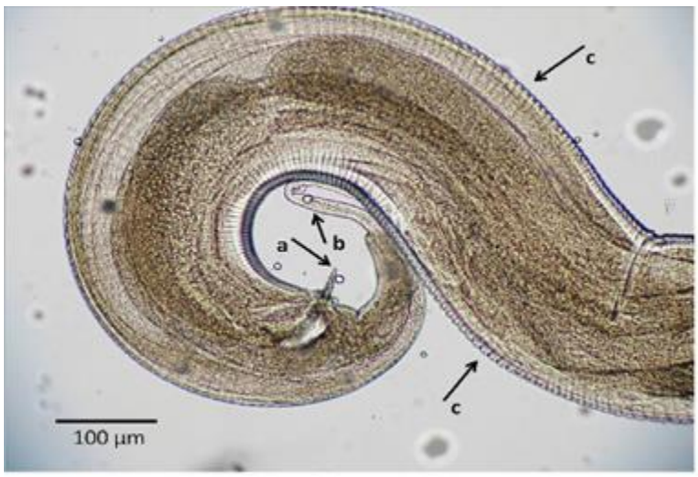


Fig 6.2: Posterior end of male *Passalurus ambiguus*

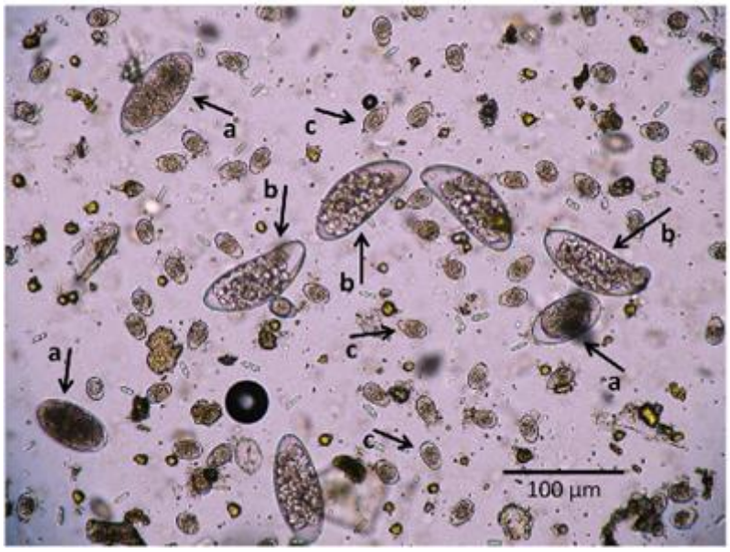


Fig 6.3: Nematode eggs

***Trichostrongylus* species**

1. *Trichostrongylus* species are nematodes (round worms), which are ubiquitous among herbivores worldwide, including cattle, sheep, donkeys, goats, deer, and rabbits.
2. At least 10 *Trichostrongylus* species have been associated with human infections.
3. Infections occur via ingestion of infective larvae from contaminated vegetables or water.
4. Human infections are most prevalent in the Middle East and Asia, with a worldwide estimated prevalence of 5.5 million people.

Life cycle

1. *Trichostrongylus* spp. are infective as a third larval stage and are ingested along with plant material on infected pastures.
2. From this stage they develop through another stage to an adult stage. The adult parasite sheds eggs into the intestine of the host which are passed into the environment through the feces.
3. The eggs are strongyle type and they hatch releasing a free-living larval stage of the parasite.
4. The trichostrongyle larvae develop through a second larval stage to the third larval stage which is the infective stage.

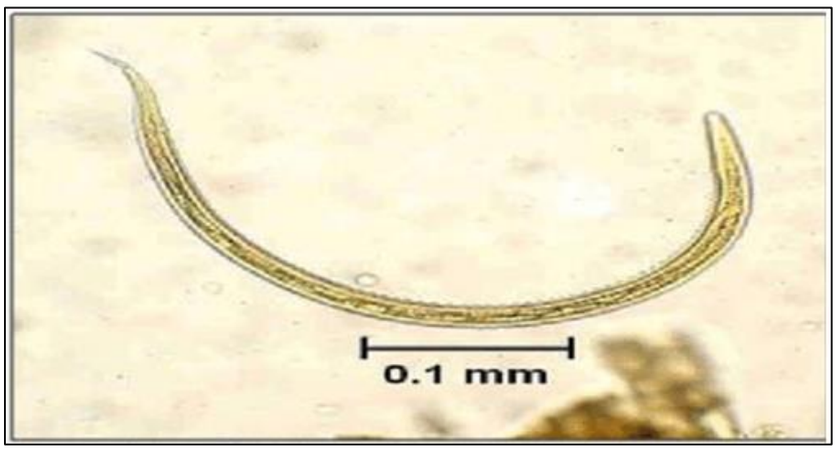


Fig 6.4: Trichostrongylus species

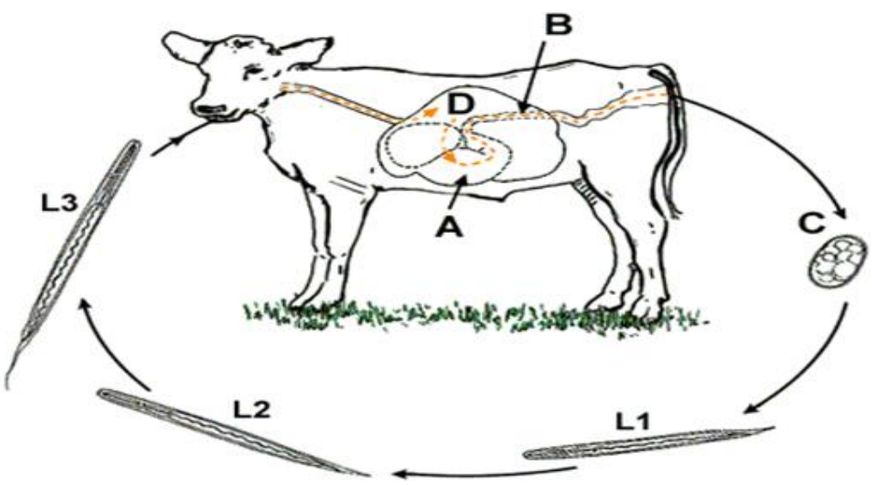


Fig 6.5: Trichostrongylus species – life cycle

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1. https://www.researchgate.net/publication/267761569_Endoparasites_found_in_European_brown_hares_Lepus_europaeus_hunted_in_Macedonia_Greece
2. https://en.wikipedia.org/wiki/Trichostrongylus

Practical No: 7 Semen analysis

Semen analysis

A semen analysis (plural: semen analyses), also called "seminogram" evaluates certain characteristics of a male's semen and the sperm contained therein. It is done to help evaluate male fertility, whether for those seeking pregnancy or verifying the success of vasectomy. Depending on the measurement method, just a few

characteristics may be evaluated (such as with a home kit) or many characteristics may be evaluated (generally by a diagnostic laboratory). Collection techniques and precise measurement method may influence results.

Semen analysis is a complex test that should be performed in andrology laboratories by experienced technicians with quality control and validation of test systems. A routine semen analysis should include: physical characteristics of semen (color, odor, pH, viscosity and liquefaction), volume, concentration, morphology and sperm motility and progression. To provide a correct result it is necessary to perform at least two, preferably three, separate seminal analyses with an interval between them of 7 days to 3 months.

Reasons for testing

The most common reasons for laboratory semen analysis in humans are as part of a couple's infertility investigation and after a vasectomy to verify that the procedure was successful. It is also commonly used for testing human donors for sperm donation, and for animals semen analysis is commonly used in stud farming and farm animal breeding.

Parameters

Some of semen analysis is: sperm count, motility, morphology, volume, fructose level and pH.

Sperm count

Sperm count, or sperm concentration to avoid confusion with total sperm count, measures the concentration of sperm in a man's ejaculate, distinguished from total sperm count, which is the sperm count multiplied with volume. Over 15 million sperm per milliliter is considered normal, according to the WHO in 2010. Older definitions state 20 million. A lower sperm count is considered oligozoospermia. A vasectomy is considered successful if the sample is azoospermic (zero sperm of any kind found). Some define success as when rare/occasional non-motile sperm are observed (fewer than 100,000 per millilitre). Others advocate obtaining a second semen analysis to verify the counts are not increasing (as can happen with re-canalization) and others still may perform a repeat vasectomy for this situation.

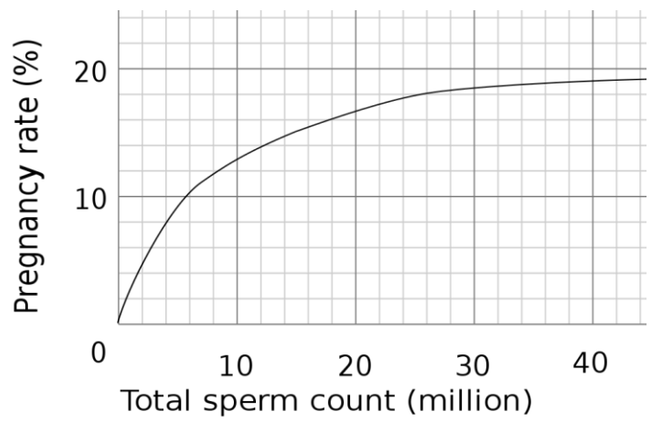

Fig 7.1: Approximate pregnancy rate varies with amount of sperm used in an artificial insemination cycle

Motility

The World Health Organization has a value of 50% and this must be measured within 60 minutes of collection. WHO also has a parameter of *vitality*, with a lower reference limit of 60% live spermatozoa. A man can have a total number of sperm far over the limit of 20 million sperm cells per milliliter, but still have bad quality because too few of them are motile. However, if the sperm count is very high, then a low motility (for example, less than 60%) might not matter, because the fraction might still be more than 8 million per millilitre. The other way around, a man can have a sperm count far less than 20 million sperm cells per millilitre and still have good motility, if more than 60% of those observed sperm cells show good forward movement - which is beneficial because nature favours quality over quantity.

A more specified measure is *motility grade*, where the motility of sperm are divided into four different grades:

- **Grade a**: Sperm with progressive motility. These are the strongest and swim fast in a straight line. Sometimes it is also denoted motility IV.
- **Grade b**: (non-linear motility): These also move forward but tend to travel in a curved or crooked motion. Sometimes also denoted motility III.
- **Grade c**: These have non-progressive motility because they do not move forward despite the fact that they move their tails. Sometimes also denoted motility II.
- **Grade d**: These are immotile and fail to move at all. Sometimes also denoted motility I.

Morphology

Regarding sperm morphology, the WHO criteria as described in 2010 state that a sample is normal (samples from men whose partners had a pregnancy in the last 12 months) if 4% (or 5th centile) or more of the observed sperm have normal morphology.

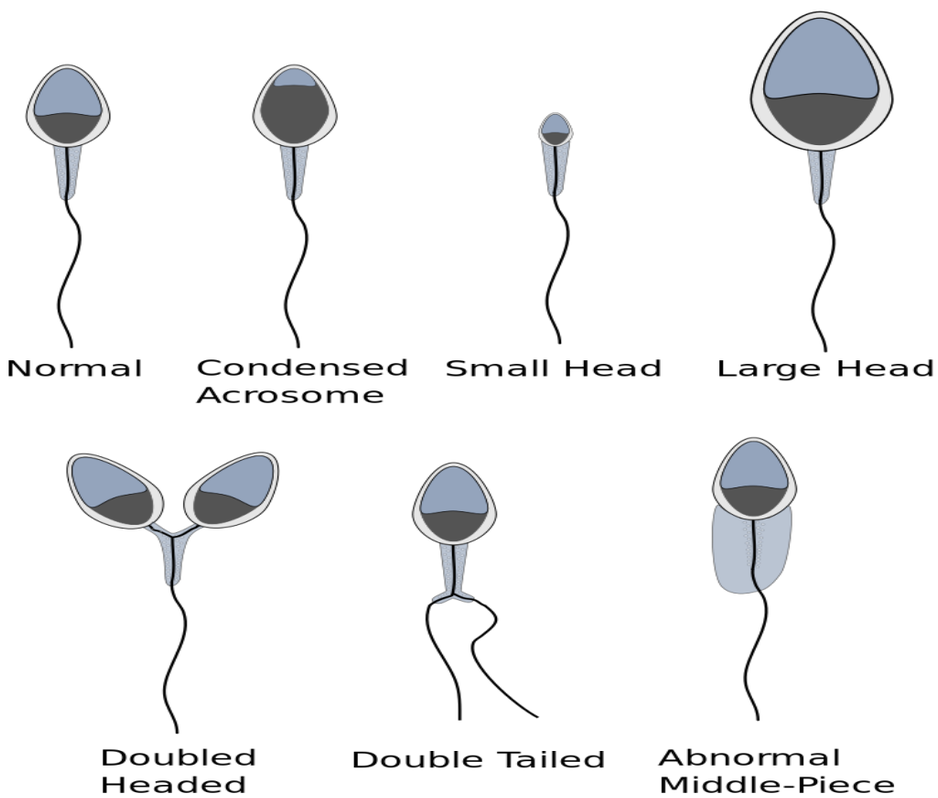

Fig 7.2: Morphology is a predictor of success in fertilizing oocytes during in vitro fertilization

Up to 10% of all spermatozoa have observable defects and as such are disadvantaged in terms of fertilising an oocyte.

Also, sperm cells with tail-tip swelling patterns generally have lower frequency of aneuploidy.

Volume

According to one lab test manual semen volumes between 2.0 mL and 5 mL are normal; WHO regards 1.5 ml as the lower reference limit. Low volume may indicate partial or complete blockage of the seminal vesicles, or that the man was born without seminal vesicles. In clinical practice, a volume of less than 2 mL in the setting of infertility and absent sperm should prompt an evaluation for obstructive azoospermia.

Color

Semen normally has a whitish-gray color. It tends to get a yellowish tint as a man ages. Semen color is also influenced by the food we eat: foods that are high in sulfur, such as garlic, may result in a man producing yellow semen. Presence of blood in semen (hematospermia) leads to a brownish or red colored ejaculate. Hematospermia is a rare condition.

Fructose level

Fructose level in the semen may be analysed to determine the amount of energy available to the semen for moving.WHO specifies a normal level of 13 μmol per sample. Absence of fructose may indicate a problem with the seminal vesicles.

pH

According to one lab test manual normal pH range is 7.1-8.0; WHO criteria specify normal as 7.2-7.8. Acidic ejaculate (lower pH value) may indicate one or both of the seminal vesicles are blocked. A basic ejaculate (higher pH value) may indicate an infection. A pH value outside of the normal range is harmful to sperm and affects their ability to penetrate the egg.

Liquefaction

The liquefaction is the process when the gel formed by proteins from the seminal vesicles is broken up and the semen becomes more liquid. It normally takes less than 20 minutes for the sample to change from a thick gel into a liquid. In the NICE guidelines, a liquefaction time within 60 minutes is regarded as within normal ranges.

Viscosity

After liquefaction, the viscosity of the sample can be estimated by gently aspirating into a wide-bore (approximately 1,5 mm of diameter) plastic disposable pipette, allowing the semen to drop by gravity and observing the length of any thread. A normal sample leaves the pipette in small discrete drops. If viscosity is abnormal, the drop will form a thread more than 2 cm long. High viscosity can interfere with determination of sperm motility, sperm concentration, detection of antibody-coated spermatozoa and measurement of biochemical markers.

MOT

MOT is a measure of how many million sperm cells per ml are highly motile, that is, approximately of grade a (>25 micrometer per 5 sek. at room temperature) and grade b (>25 micrometer per 25 sek. at room temperature). Thus, it is a combination of sperm count and motility.

With a straw or a vial volume of 0.5 milliliter, the general guideline is that, for intracervical insemination (ICI), straws or vials making a total of 20 million motile spermatozoa in total are recommended. This is equal to 8 straws or vials 0.5 ml with MOT5, or 2 straws or vials of MOT20. For intrauterine insemination (IUI), 1-2 MOT5 straws or vials is regarded sufficient. In WHO terms, it is thus recommended to use approximately 20 million grade a+b sperm in ICI, and 2 million grade a+b in IUI.

DNA damage

DNA damage in sperm cells that is related to infertility can be probed by analysis of DNA susceptibility to denaturation in response to heat or acid treatment and/or by detection of DNA fragmentation revealed by the presence of double-strand breaks detected by the TUNEL assay.

Total motile spermatozoa

Total motile spermatozoa (TMS) or *total motile sperm count* (TMSC)is a combination of sperm count, motility and volume, measuring how many million sperm cells in an entire ejaculate are motile.

Practical No: 8
Dactylography and its uses in developmental biology

Dactylography refers to the scientific study of fingerprints as a method of identification. Experts in dactylography recognize that palm prints have the characteristic of uniqueness and that they contain reference points that enable accurate and conclusive comparisons just as do fingerprints.

Fingerprints can be used in all sorts of ways:

- Providing biometric security (for example, to control access to secure areas or systems)
- Identifying amnesia victims and unknown deceased (such as victims of major disasters, if their fingerprints are on file)
- Conducting background checks (including applications for government employment, defense security clearance, concealed weapon permits, etc.).

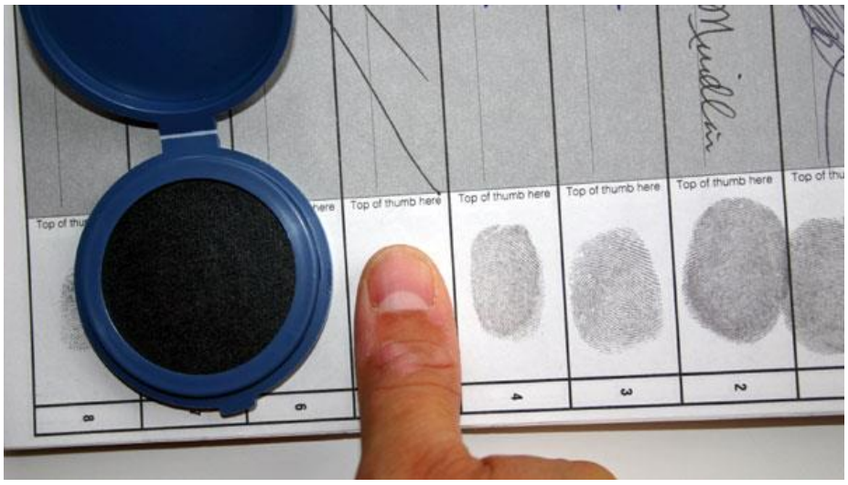

Fig 8.1: Dactylography

Fingerprints are especially important in the criminal justice realm. Investigators and analysts can compare unknown prints collected from a crime scene to the known prints of victims, witnesses and potential suspects to assist in criminal cases. For example:

- A killer may leave their fingerprints on the suspected murder weapon
- A bank robber's fingerprints may be found on a robbery note
- In an assault case, the perpetrator may have left fingerprints on the victim's skin
- A burglar may leave fingerprints on a broken window pane
- A thief's fingerprints may be found on a safe

In addition, fingerprints can link a perpetrator to other unsolved crimes if investigators have reason to compare them, or if prints from an unsolved crime turn up as a match during a database search. Sometimes these unknown prints linking multiple crimes can help investigators piece together enough information to zero in on the culprit.

In the absence of DNA, fingerprints are used by the criminal justice system to verify a convicted offender's identity and track their previous arrests and convictions, criminal tendencies, known associates and other useful information. Officers of the court can also use these records to help make decisions regarding a criminal's sentence, probation, parole or pardon.