Lab: 5

Asst.prof
Hanan
Sami

Cultivation of Viruses

"Embryonated Egg"

After completing this practical, you will be able to: Know the routes of inoculation of eggs for propagation of viruses.

INTRODUCTION:

First used by Rous & Murphy, 1911, but Goodpsture 1930, He played a major role in the development of this technology.Prior to 1950s, the technique of propagation of viruses in embryonated eggs was popular because of non-availability of cell culture techniques during those times.

Uses:

1. Isolation and diagnosis of viruses such as: Cow pox & Influenza viruses.
2. Production of diagnostic antigens.
3. Production of many vaccines.

Advantages of this method:

1. Eggs do not need special care.
2. Availability, cheap price, ease of laboratory handling.
3. The inner of the egg is sterile.
4. It does not cause infection.
5. Ease of noticing the symptoms resulting from the injection of the virus.
6. The viruses can grow in different compartments of the egg.
7. Eggs lack a well-developed defense mechanism of their own, they do not interfere with growth of viruses.

Disadvantages of this method:

1. It cannot be used for all types of viruses.
2. The need for special incubation equipment.
3. Allergy to the prepared vaccines due to the presence of albumin.
4. It takes days or weeks for results to appear
5. Some viruses do not show symptoms when injected.

Conditions of eggs used for cultivation and reproduction of viruses:

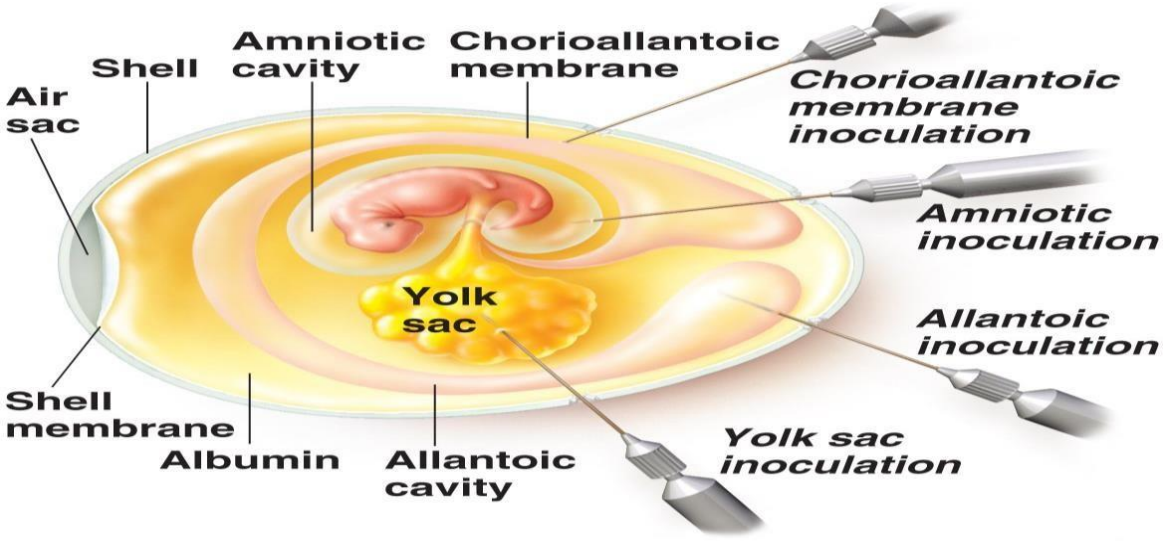
1. The eggs are fertilized.
2. The weight of the egg is about 70 gm.
3. To be germ free.
4. To be homogeneous in shape and color.
5. It has a rough shell that is easy to write on.
6. Clean and dirt free.

Fertilized egg care:

1. Fertilized eggs are placed in special incubators (37-39 c) before the virus is injected
2. Humidity ranged between 55-65%.
3. Turn the eggs at least twice
4. After injection, it is preferable to incubate it at 37 c.
5. The wide part of the egg should be upwards when incubating.

Fertilized egg components:

1. Shell: The exchange of gases and liquids, providing mechanical protection.
2. Shell membrane.
3. Air sac: Gas exchange, maintaining pressure inside the egg.
4. Chorio allantoic membrane (CAM)
5. Amniotic sac: The membranous system that surrounds the fetus.
6. Yolk sac.
7. Albumin.



Distinguish between fertilized and unfertilized eggs:

The eggs are examined before the virus is injected using the Candling lighting method, which is in front of a light source and in a dark room using a Candler device. It consists of a simple box equipped with a light with a barrier containing a circular opening in front of it and the shell becomes transparent. The contents of the egg can be observed as the location of the air sac, the location of the fetus and some large blood vessels are determined. Three types of eggs can be distinguished:

1. Fertilized eggs and the fetus is less than four days old: The area of the air sac appears in the form of a more transparent area than the rest of the egg parts, and the yolk sac is noted in the form of a dark halo.
2. Fertilized eggs and the fetus is more than four days old: The appearance of blood vessels on the shell from the inside, As the fetus ages, it begins to spread and expand on the surface of the shell ,The fetus appears as a dark body, By moving the egg in front of the light source, a dark spot appears that moves according to the movement of the egg, Which forms the head of the fetus, and with the advancing age, it increases in size and appears in the form of a dark area that includes all the size of the egg.
3. Fertilized eggs and dead fetus: The blood vessels begin to decay and a red ring appears at the pointed end of the egg formed by the deposition of blood cells. In advanced stages, the fetus appears as a black mass, and we do not notice the movement of the eyespot in front of the light source when moving the egg.

PRINCIPLE:

Usually 8-11 days old chick embryos are used. Age of the egg chosen depends on route of inoculation. These eggs are inoculated by one of the following routes:

1. yolk sac: Yolk sac is mainly used for culture of some viruses(measles, Rabies), certain bacteria(Chlamydia and Rickettsia) and parasite, 5-8 day old embryo.
2. amniotic sac: Amniotic sac is used for the primary isolation of influenza virus, 10-12 day old embryo.
3. allantoic cavity: Allantoic cavity is mainly employed for harvesting influenza virus for vaccine production , and also for yellow fever, 9-11 day old embryo.
4. chorioallantoic membrane (CAM): CAM is mainly used for growing pox viruses. These producecharacteristic visible lesions such as pocks. Pocks produced by different viruses vary in their morphology.

PROCEDURE:

1. Disinfect the eggshell.
2. The area of the air sac is determined with a pencil, and then we mark its center with a pencil as well.
3. An opening is made in the eggshell and in the center of the air sac using a needle.
4. The area of the opening is sterilized, after which a needle containing the viral suspension is inserted, and perpendicular to the egg, Then the virus is injected.
5. Inoculate the specimen in the embryonated egg through appropriate route. Note: Age of the embryonated egg is chosen depending on the route of inoculation since various membranes and their contents vary in size as embryo matures.
6. After the injection, we remove the needle and cover the hole with melted wax.
7. After 2–5 days post injection, viral growth in the egg is recognized by death of embryo, pocks, or hemagglutination.

Note: Age of the embryonated egg is chosen depending on the route of inoculation since various membranes and their contents vary in size as embryo matures.

QUALITY CONTROL:

1. Before inoculation, the eggs are candled to determine the position of the embryo and its viability.
2. Age of the embryonated egg is chosen depending on the route of inoculation since various membranes and their contents vary in size as embryo matures.
3. Sterile precautions should be taken throughout the procedure.

OBSERVATIONS: The inoculated part of embryonated egg is observed for changes due to viral infection.

RESULTS AND INTERPRETATION: Viral growth in the inoculated egg is recognized by death of the embryo, pock formation in CAM, haemagglutination, etc.

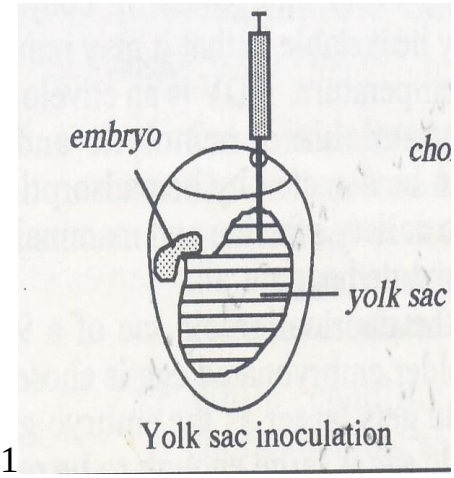
Signs of viral growth in embryonated eggs:

1. Death of Embryo.
2. Dwarfing of Embryo.
3. Observing of Inclusion bodies.
4. Presence of blood clots in Embryonic fluid.
5. Bleeding and congestion.

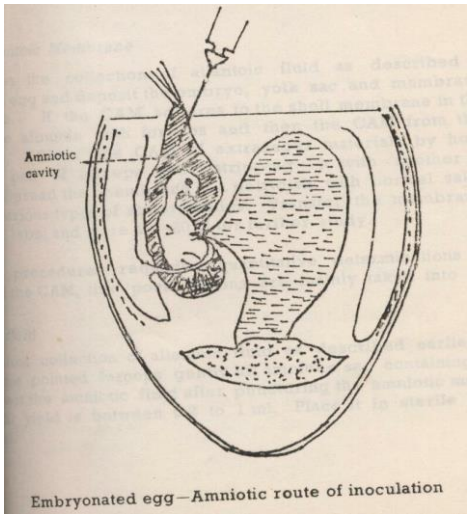
Table 83-1 The routes of inoculation of the egg and the viruses isolated

Route of inoculation	Age of embryo	Virus	Use
Chorio allantoic membrane.	10-14 days.	Variola virus. Herpes simplex virus. Vaccinia virus.	Isolation. Isolation and typing. Vaccine titration.
Amniotic sac.	10-12 days.	Influenza A virus. Mumps virus.	Isolation. Isolation.
Allantoic sac.	9-12 days.	Mumps virus. Influenza A, Influenza B. Parainfluenza viruses.	HA antigen preparation. Hybrid vaccine production. Isolation.
Yolk sac.	6-8 days.	Flavi viruses.	Isolation, vaccine production.

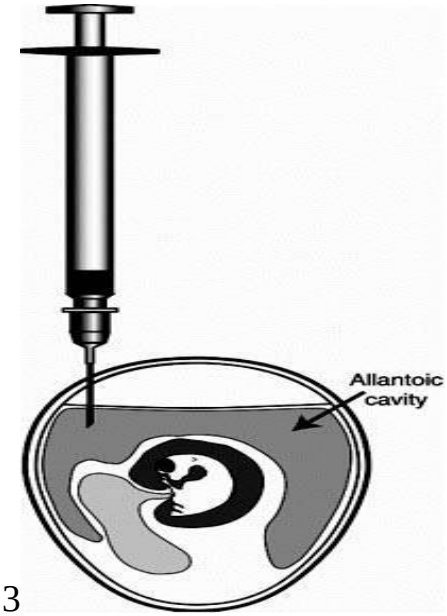
1



2



3



4