Cultivation of Viruses

- To isolate and identify the viruses,
- In order to establish inter-vital relationships,
- To prepare viruses for vaccines
- For use in serological tests,
- To obtain hyperimmune or type-specific serum,
- In order to demonstrate epidemiological and pathogenetic properties,

- Viruses are produced only in live systems. This system is called host. Host systems are examined in two basic groups, in vivo or in vitro.

- In vivo system, Laboratory animals, Embryonated Chicken Egg (ETY)

- In vitro system → Cell culture

In vivo Systems -1

Animals

Animals are used for studying viruses which do not grow in cell cultures or eggs, and for testing vaccines

- Conventional Animals: Animals that do not have any special care be feeding program. Obtainig Hyperimmun sera

- Spesific Pathogen Free (SPF) animals: Animals that lack the pathogen and serological response to it. Special care and feeding is applied. They are routinely checked for virological and serologic aspects of the pathogen being studied. They are used for potency and sterility control of prophylactic products.

- Germ Free (GF) animals: Animals that do not carry any pathogenic or apathetic microorganisms. They are obtained from parents who are GF like themselves. Feed and ambient air are sterilized. Constantly blood and whole body secretions and excretions are controlled for all pathogens. They are used in special pathogenesis studies.

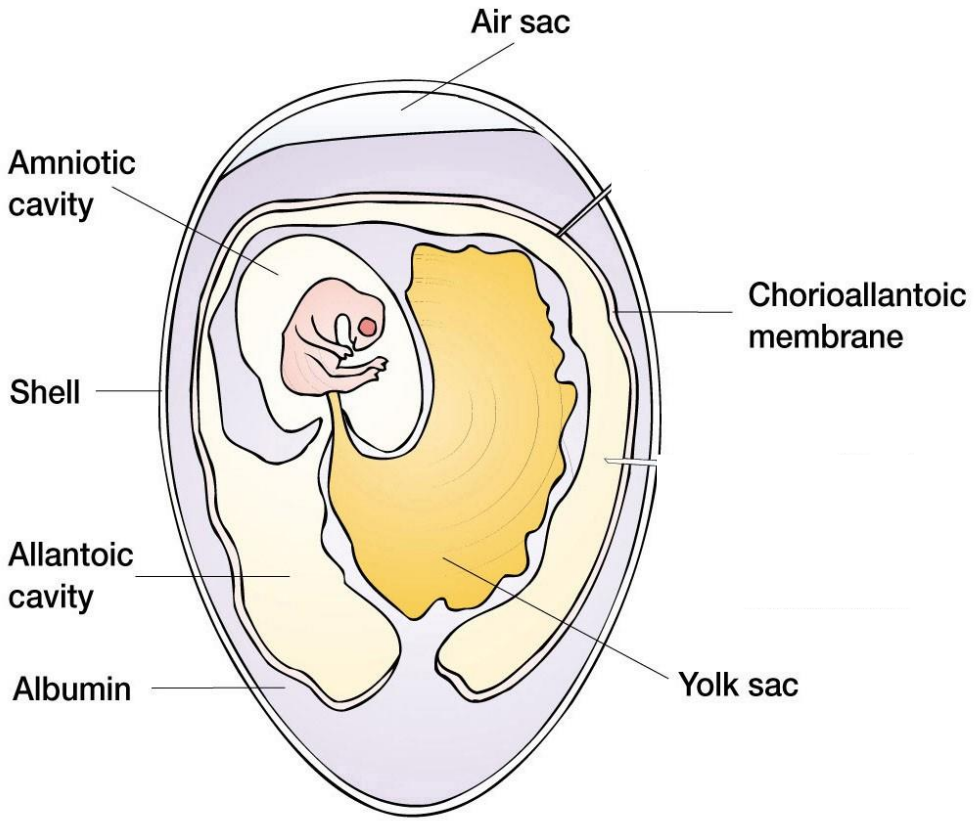
Inoculation of Virus in Animals

- Intradermal, Subcutan, Dermal scarification, Intramuscular, Intravenous, Intracerebral, Intraperitoneal, Intracardiac, Intranasal, Intratracheal, Subconjuctival, Conjuctival scarification, Rectal,Peros (Oral)

- After the animal is inoculated with the virus suspension, the animal is:
  - observed for signs of disease
  - visible lesions
  - or is killed so that infected tissues can be examined for virus

Inoculation of Virus in Embryonated Eggs

Embryonated Egg



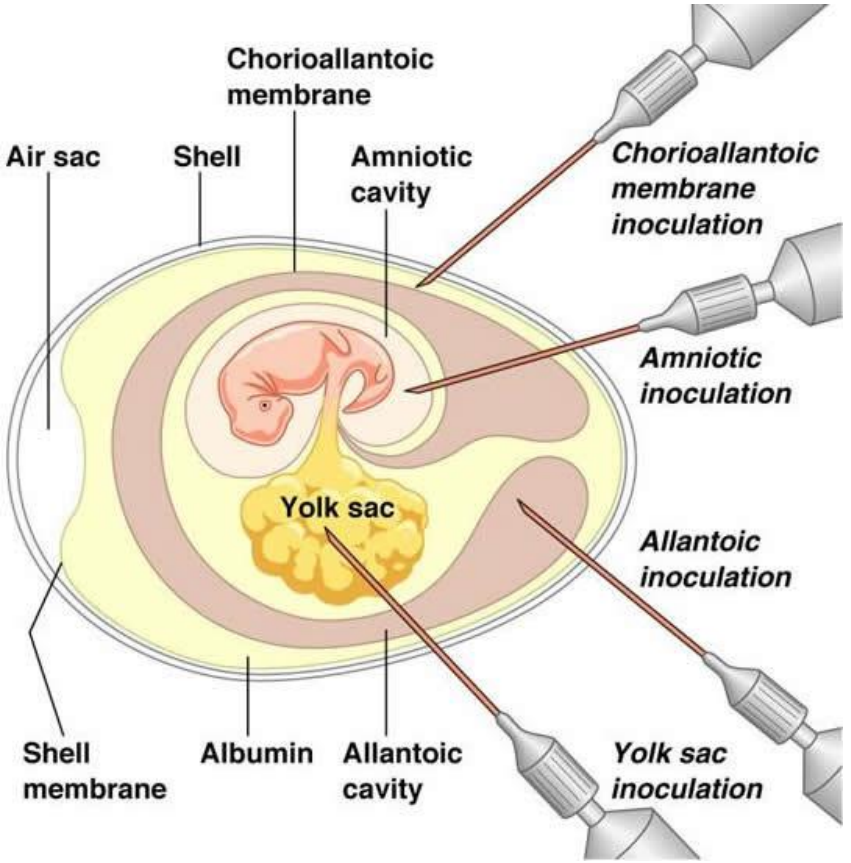
- The process of cultivation of viruses in embryonated eggs depends on the type of egg being used.

  - Embryonated: having an embryo
  - Unembryonated: not having an embryo
  - De-embryonated: having lost an embryo

- Incubation: They are stored in special incubators (incubator). They have 35-37 ° C heat and relative humidity of 40-70%. Every day is checked for viability during incubation.

Routes of Viral Inoculation

- The different sites of viral inoculation in embryonated eggs are:
    1. Chorioallantoic membrane(CAM)
    2. Amniotic Cavity
    3. Allantoic Cavity
    4. Yolk sac

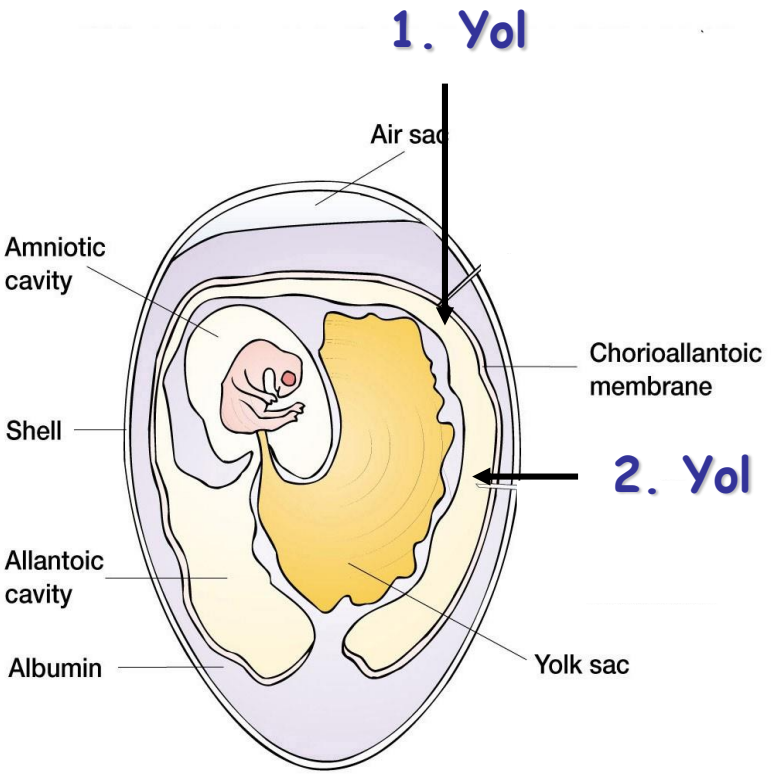


Chorioallantoic Membrane (CAM)

- Many viruses can be adapted to grow on the CAM
- Viruses produce visible foci or ‘pocks’, inclusion bodies, oedema or other abnormalities
- Viruses which can be grown include:
  - Herpes viruses
  - and poxviruses
  - Inoculation time 9-11 day old embryo

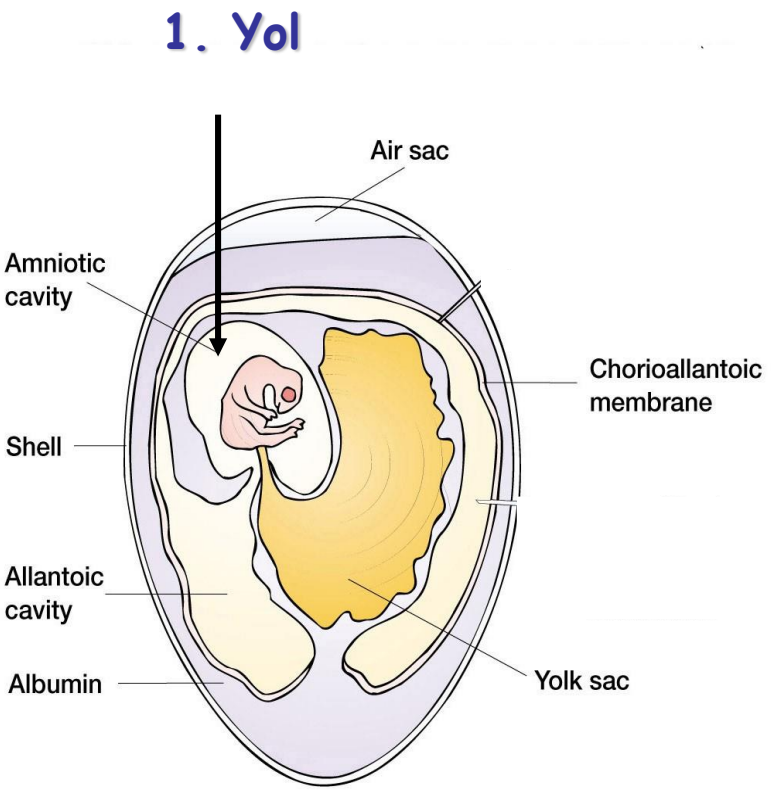
Chorioallantoic Cavity

- 9-12 day embryo
- New Castle virus,
- Viral replication is detected by HA test on allantoic fluid.



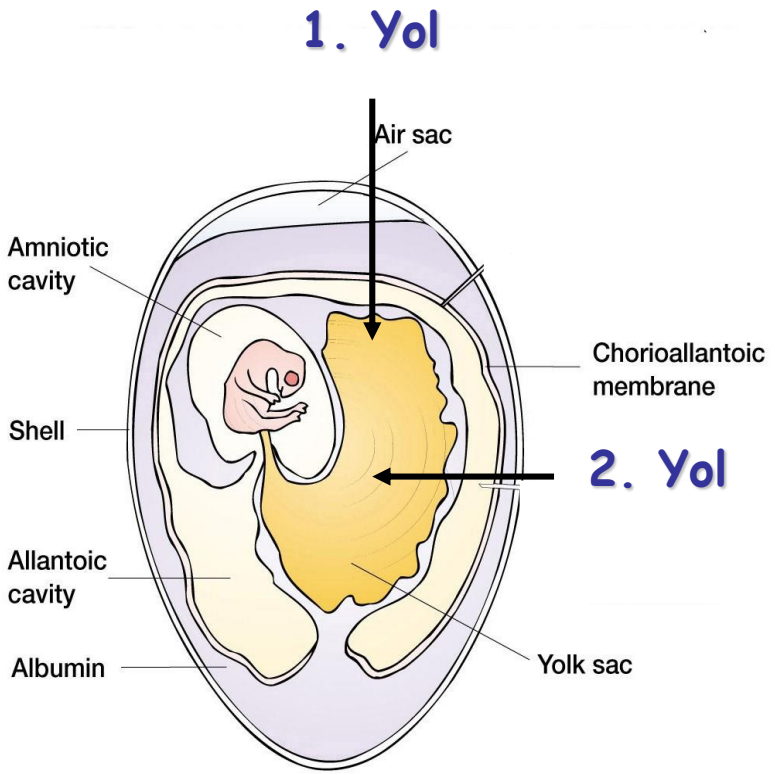
Amniotic Cavity Inoculation

- 9-12 day embryo
- Influenza virus, Mumps virus
- Virus infection is detected by HA test on amnion fluid.



Yolk Sac Inoculation

- Mostly mammalian viruses are isolated using this method.
- 6-8 day embryo
- Rabies virus, Blue tongue virus, EHV-1 and EHV-4
- Viral replication, egg yolk and, if necessary, yellow dyeing are painted with special dyes to follow the principle of detection of inclusion bodies.



In Vitro Systems

Cell cultures

Genel Tanımlar

- Organ Culture: They must be stored in vitro, provided that the functions and structures of certain parts or all of the organs are preserved.

- Tissue Culture: The functions and structures of tissues must be preserved and produced in vitro without deterioration.

- Cell Culture: Producing cells in vitro, multiplying from a single cell. Here, cells never display an organization that reaches tissue.

Cell Culture Definitions

- Primer Cell Culture: The form in which cells are originally adhered to in vitro conditions of the tissue or organs they originate from. This definition is valid until the first subculture. They are usually mortal (finite) cells.

- Permanent (Continuous) Cell Culture: Cell cultures that can reproduce indefinitely and subculture.

- Diploid Cell Cultures: Primer is obtained as a result of subcultures of cultures.

- Fibroblast Cell Culture: Cells that originate from embryonic tissues and exhibit shuttle (spindle) -like morphology.

- Subculture: Cells that have been replicated in a culture medium are transfused or transplanted into another culture medium, either with or without dilution.

- Cells are cultured in a plastic flask in Minimum Essential Media (MEM). As the cells divide, they cover the plastic surface.

- Media is supplemented with antibiotics viz. penicillin, streptomycin etc.
- Prepared media is filtered and incubated at 4 C

- The medium is added to the medium to give a volume of 10% of the volume of the bottle and contains inactivated calf serum in the range of 5-20%.

- According to their relationship with the surface of the cabin they are in;

- Built-in, (attached)

- Stationer (single layer or stratified)
- Roller (turns)

- Suspension, (which does not hold in the rough)
- Transformed cell lines either grows as monolayer or as suspension

Primer Cell Culture Preparation

1. The membrane or capsule on the tissue is lyophilized,
2. Tissue pieces are taken with scissors in a petri dish,
3. It is reduced by scissors and double bistuli method,
4. The tissues transferred to the Erlenmeyer flasks are washed with PBS.
5. The connective tissue between the cells is separated with heated trypsin
6. The cell trypsin mixture is taken every 20 minutes and the heat is reduced
7. The tripsinism lasts until the tissues are thoroughly disintegrated.
8. The resultant cell + trypsin mixture is separated by centrifugation.
9. Cells are counted, diluted in medium (calf serum) and transferred to culture flask.
10. It is incubated at 37oC. Each day, tissue culture is followed by microscopy.

Sub-culturing and freezing

Cells to be kept in healthy & in growing state have to be sub-cultured or passaged.

We mkae passage of cells when they reach to 80-90% confluency in flask/dishes/plates.

We can freeze our cells for further use.