

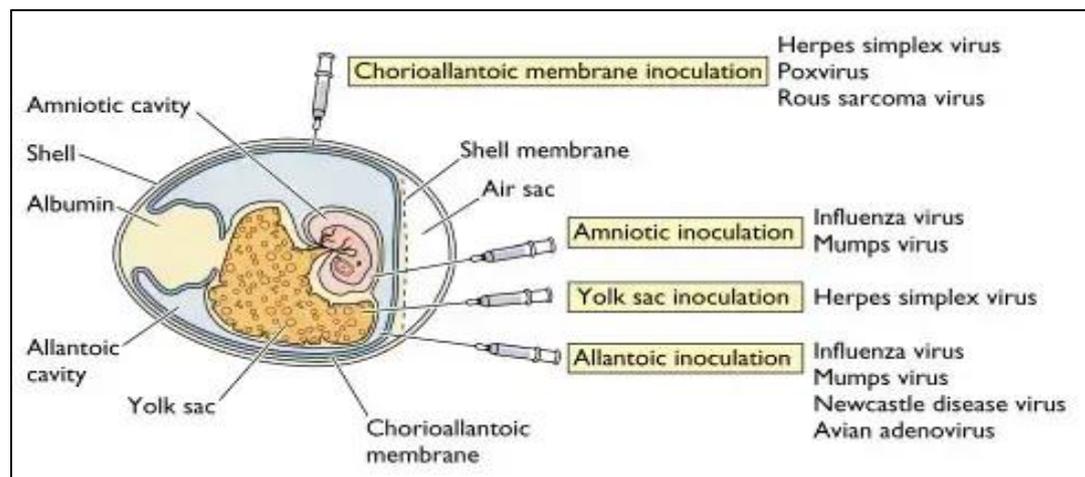
Embryonated Egg Cultures for Virus Propagation

Introduction

The cultivation of viruses using embryonated chicken eggs has been a cornerstone technique in virology since its inception in the early 20th century. Pioneered by Woodruff and Goodpasture in 1931, this method facilitated significant advancements in the study and production of viral vaccines . Despite the advent of cell culture systems, embryonated eggs remain integral to the propagation of various viruses, notably in the production of influenza vaccines .

Egg Inoculation Sites and Associated Viruses

The selection of the inoculation site within the embryonated egg is critical, as different viruses exhibit tropism for specific embryonic membranes or cavities .



- **Allantoic Cavity:** Preferred for influenza viruses, Newcastle disease virus, and mumps virus.
- **Amniotic Cavity:** Utilized for primary isolation of influenza viruses.
- **Chorioallantoic Membrane (CAM):** Suitable for poxviruses and herpes simplex virus, where viral replication leads to visible pock lesions.
- **Yolk Sac:** Employed for the cultivation of certain arboviruses and rickettsiae .

Allantoic Cavity Inoculation Procedure

The allantoic cavity is a fluid-filled space that supports the replication of several viruses, including influenza. The standard procedure involves:

1. Preparation: Pathogen-free fertilized chicken eggs are incubated for 10–12 days.
2. Candling: Eggs are examined under a light source to identify the allantoic cavity's location.

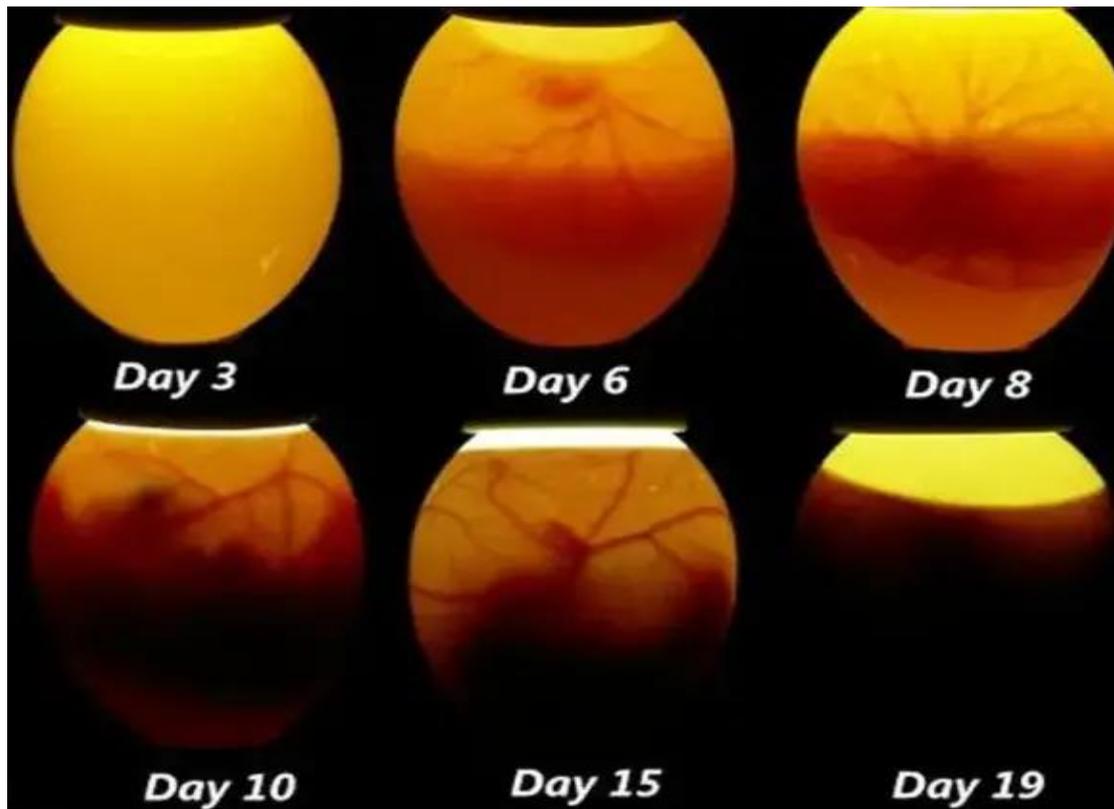


Figure 1 : Candling of embryonated eggs to see chicken embryo inside

3. Disinfection: The shell surface is disinfected with 70% alcohol.
4. Drilling: A small hole is made at the identified site.
5. Inoculation: A specific volume of viral suspension is injected into the allantoic cavity using a sterile syringe.
6. Sealing and Incubation: The hole is sealed, and the egg is incubated at 37°C for several days.
7. Harvesting: Post-incubation, the allantoic fluid is collected for virus isolation and analysis .

Applications in Influenza Vaccine Production

Embryonated eggs are extensively used in the production of both inactivated and live attenuated influenza vaccines. The process involves the inoculation of the virus into the allantoic cavity, followed by incubation, harvesting of the allantoic fluid, and subsequent purification and inactivation steps to produce the vaccine .

Advantages of Using Embryonated Eggs

Cost-Effectiveness: Eggs are relatively inexpensive and readily available.

High Yield: They support the replication of high titers of virus.

Versatility: Suitable for a wide range of viruses.

Sterility: The closed system minimizes contamination risks .

Limitations and Modern Alternatives

Despite their advantages, the use of embryonated eggs has limitations, including:

Allergenicity: Residual egg proteins can cause allergic reactions in sensitive individuals. **Adaptation:** Some human viruses may undergo adaptive mutations when propagated in eggs, potentially affecting vaccine efficacy .

Conclusion

Embryonated egg cultures have played a pivotal role in virology, particularly in vaccine development. While modern techniques offer alternatives, the method remains a valuable tool for the propagation of various viruses and the production of vaccines.

Table 1: viral signs in the Embryonated Eggs

Inoculation Site	Associated Viruses	Indicators of Viral Growth
Yolk Sac	Arboviruses	Embryonic death
Amniotic Sac	Influenza viruses, Mumps virus	Detection of viral antigens in amniotic fluid, embryonic death
Chorioallantoic Membrane	Poxviruses, Herpes simplex virus	Formation of pocks on the membrane
Allantoic Cavity	Influenza viruses, Newcastle disease virus, Mumps virus	Presence of virus in allantoic fluid, embryonic death

References

- Woodruff, A. M., & Goodpasture, E. W. (1931). The Cultivation of Vaccine and Other Viruses in the Chorioallantoic Membrane of Chick Embryos. *American Journal of Pathology*, 7(2), 209–222.
- Centers for Disease Control and Prevention. (2019). How Influenza (Flu) Vaccines Are Made. Retrieved from <https://www.cdc.gov/flu/vaccine-process/index.html>
- Microbe Online. (n.d.). Embryonated Egg Cultures for Viruses. Retrieved from <https://microbeonline.com/embryonated-egg-cultures-viruses/>
- Food and Agriculture Organization. (n.d.). Inoculation of embryonated eggs by the allantoic cavity route. Retrieved from <https://www.fao.org/4/ac802e/ac802e09.htm>
- Centers for Disease Control and Prevention. (2023). Cell-Based Flu Vaccines. Retrieved from <https://www.cdc.gov/flu/vaccine-types/cell-based.html>
- Centers for Disease Control and Prevention. (2023). Key Facts About Seasonal Flu Vaccine. Retrieved from <https://www.cdc.gov/flu/vaccines/keyfacts.html>
- CDC