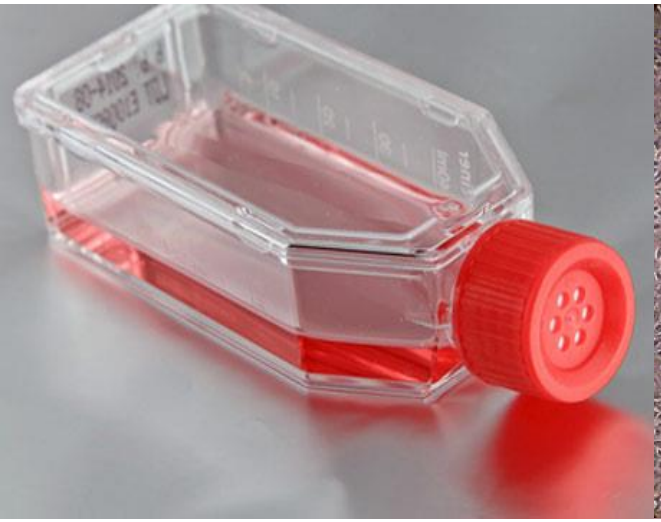
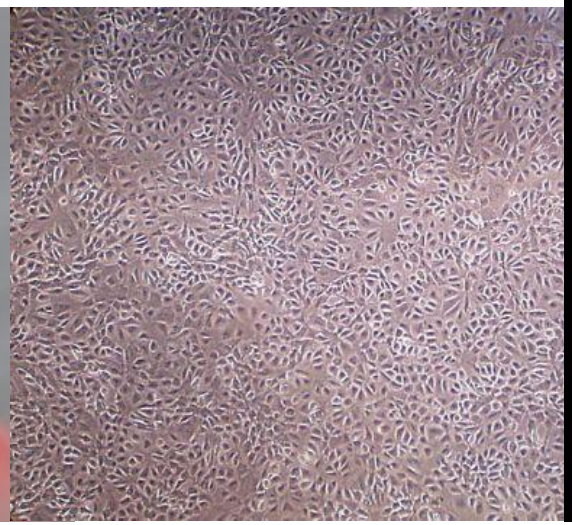
VIRUS INOCULATION IN EMBRYONATED CHICKEN EGG

Dr. İlke KARAYEL HACIOĞLU

Virus Cultivation Systems

- In-vivo
  - Experimental animals
  - Embryonated eggs
- In-vitro
  - Cell and tissue cultures

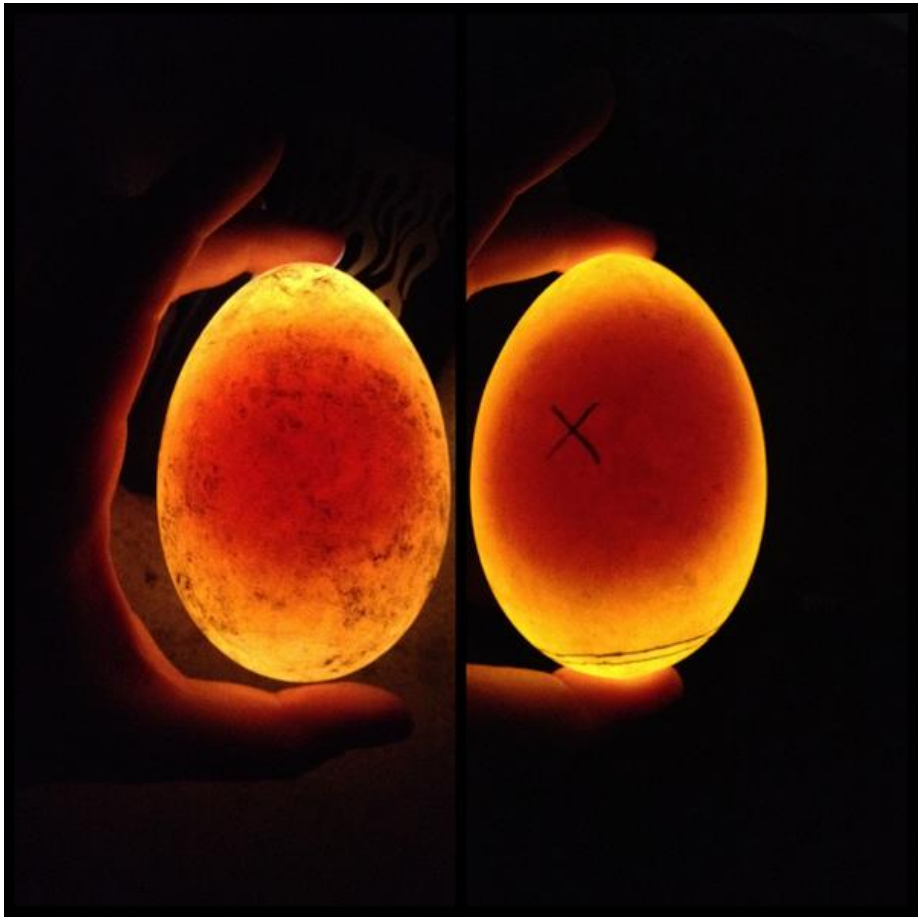




http://www.backyardchickens.com/t/662123/help-clueless-about-incubating-a-rescued-goose-egg-photos

Embryonated Eggs

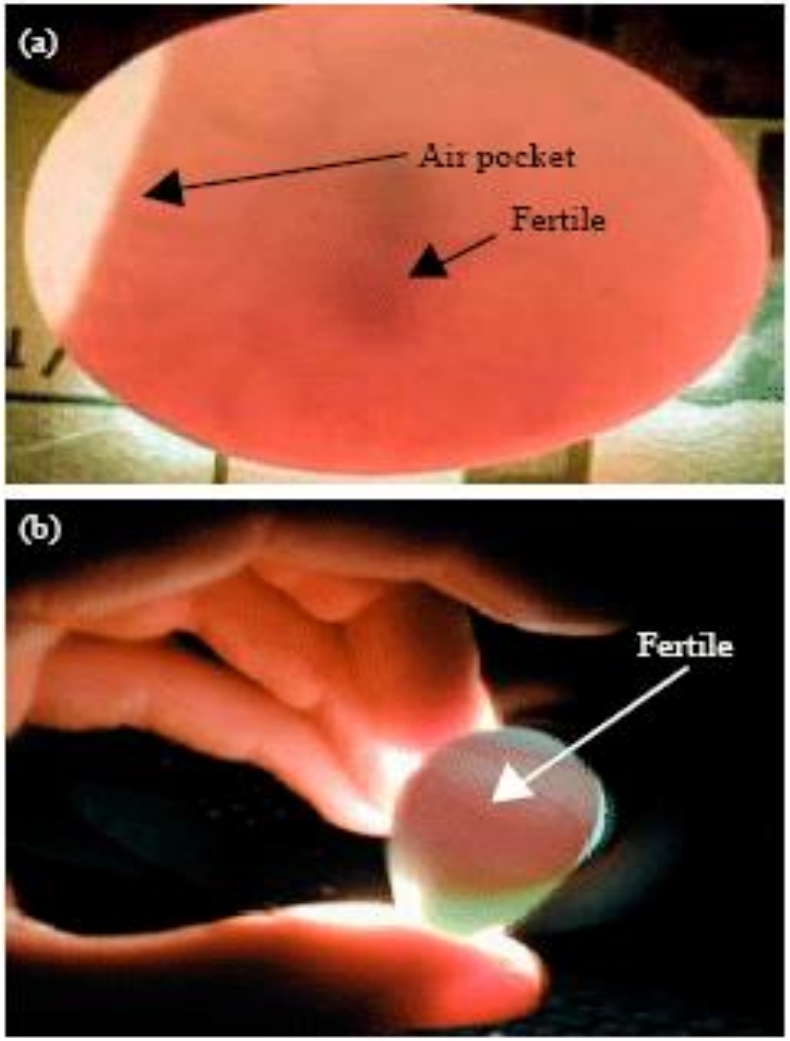
- Embryonated chicken eggs are most commonly used.
- Duck eggs are also preferred from time to time.
- Although ETYs have remained in the background due to the widespread use of cell cultures today, they are still the ideal breeding environment for some viruses. Such as,
  - many avian viruses,
  - Bluetongue virus
  - Influenza A



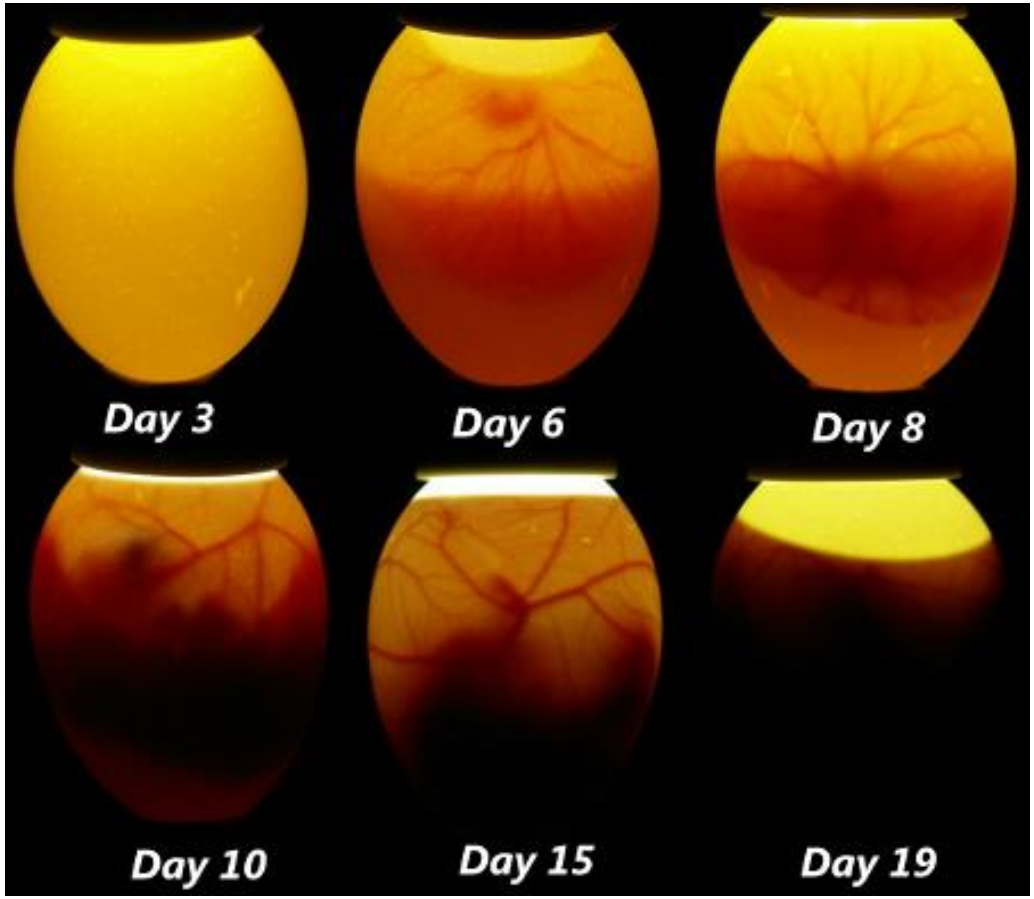
http://www.backyardchickens.com/t/662123/help-clueless-about-incubating-a-rescued-goose-egg-photos

- In order to be successful in ECE applications, hatcheries, where eggs are obtained;
  - should be constantly monitored,
  - should be free of important diseases,
  - should have a high fertility rate.

- Fertilized eggs taken from the coops are placed into an incubator at 35-37 C, and 40-70% humidity for pre-development.
- Eggs are turned by hand or mechanically every day.
- About a week later, they are taken to the dark room and checked for vitality.
- In this examination, the movement and veins of the embryo are observed under a strong light source.



A. Farhangi, A. Akbarzadeh, M.R. Mehrabi, M. Chiani, Z. Saffari, S. Ghassemi, M. Kheiri and R. Bashar, 2010. Safety of Human Therapeutic Morphine Vaccine Employing Lohmann Specific Pathogen Free Eggs. *Pakistan Journal of Biological Sciences, 13: 1047-1051.*

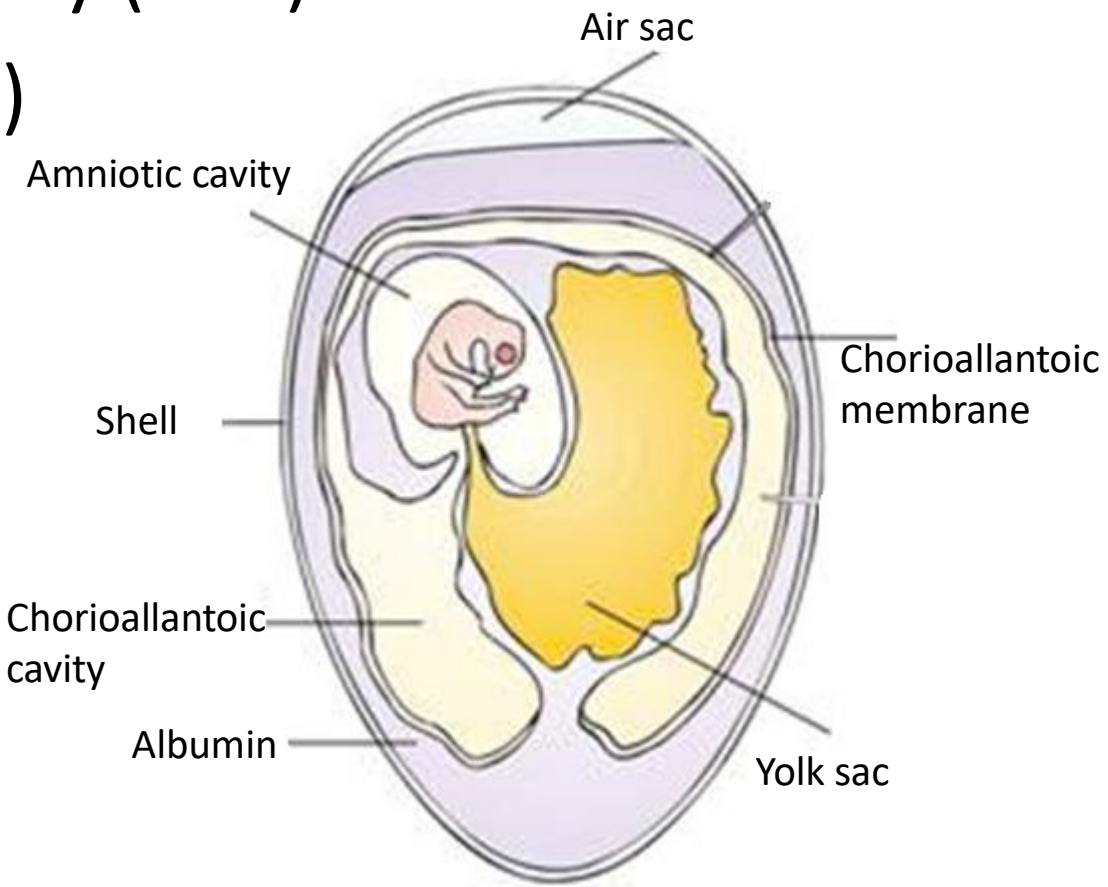


http://incubatorwarehouse.com/egg-candling

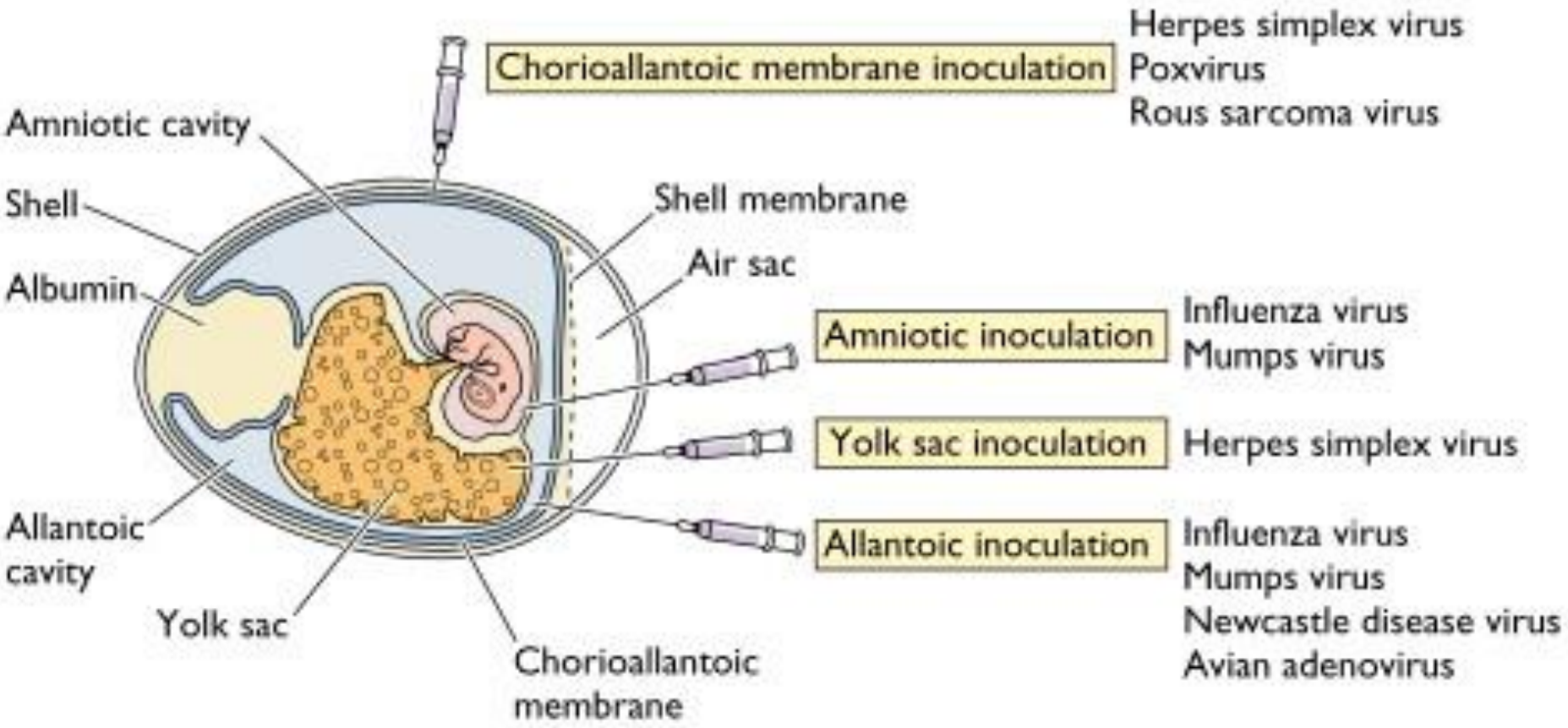
- Under light, the immobile and dark mass of the embryo indicates its dead.
- Live embryos are put back in the incubator for proper use.
- The inoculation process should be done immediately after the markings, before changing the location of the embryo.

Areas Frequently Used for Virus Inoculation to ECE

1. Chorio-allantoic membrane (CAM)
2. Chorio-allantoic cavity (CAC)
3. Amniotic cavity (AC)
4. Yolk sac (YS)

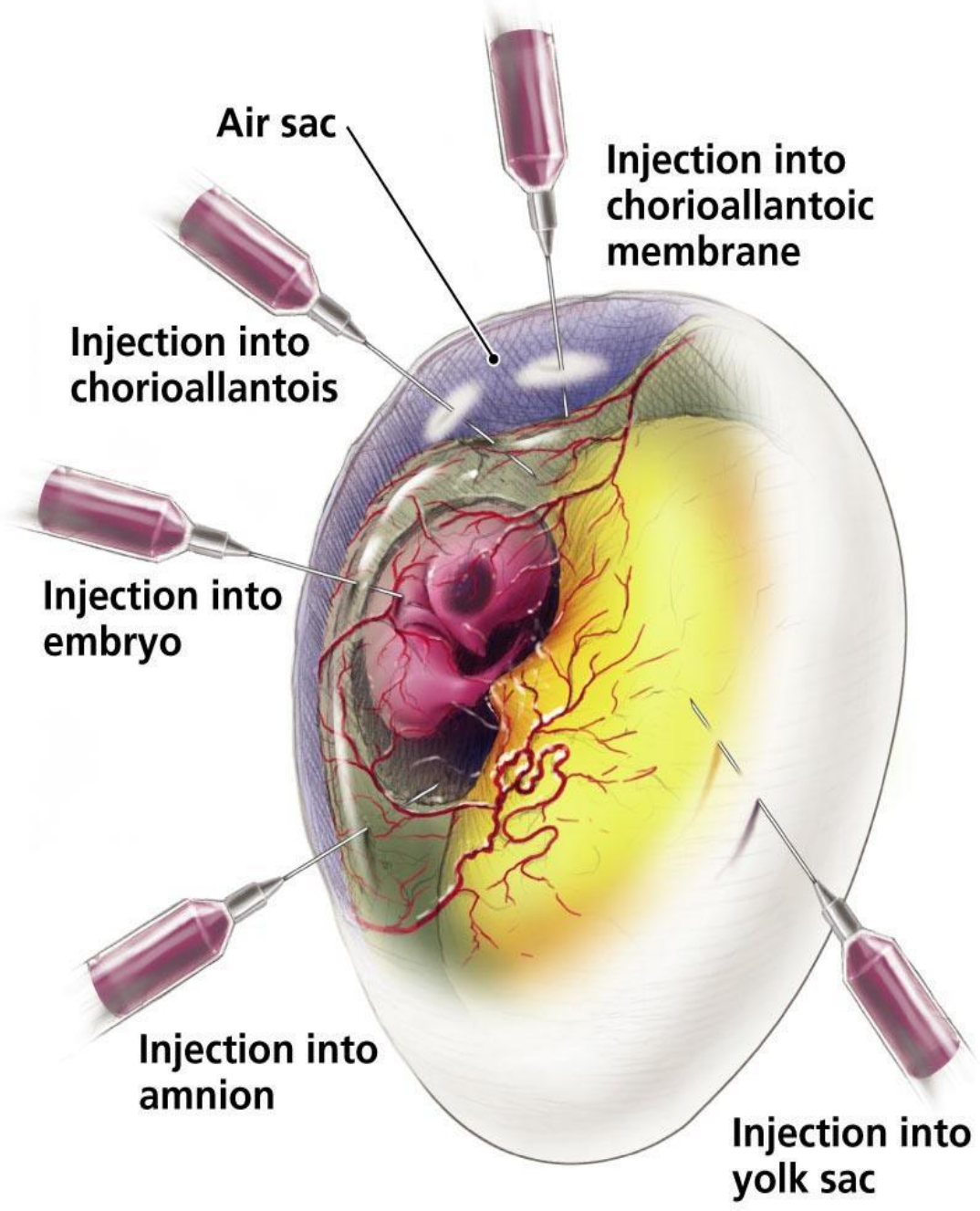


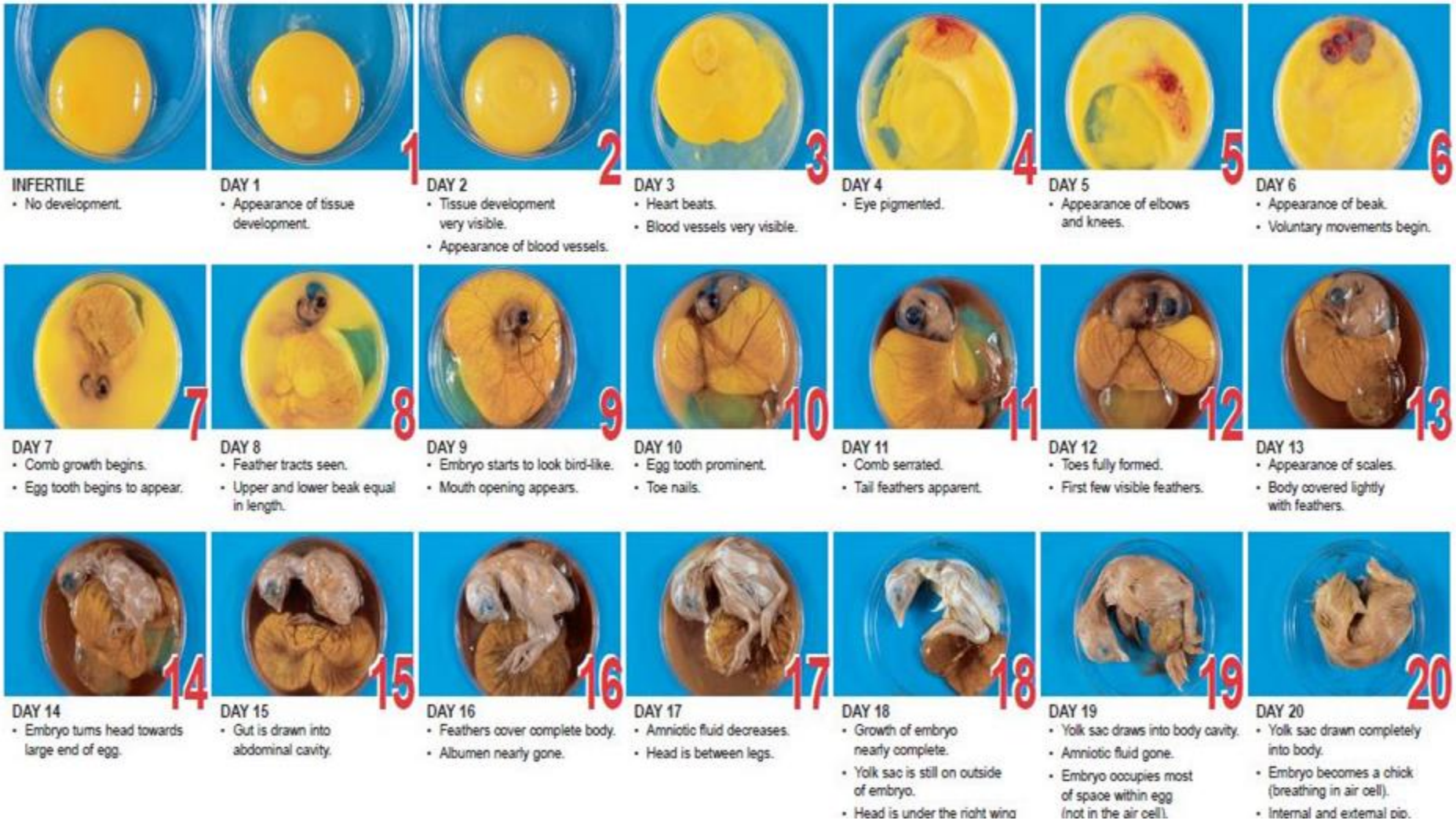
Вирусология: Методические рекомендации к лабораторным занятиям /Авт.-сост.А.Н. Евтушенков, Р.А. Желдакова, О.Б. Русь, А.М. Ходосовская. – Мн.: БГУ, 2006



http://www.virology.ws/2009/12/10/influenza-virus-growth-in-eggs/

Apart from these regions, implantation can also be made to embryonal vessels on the chorio-allantoic membrane and directly to the embryo. The virus replicates in the membrane cells that form these sacs, and mature virions accumulate in the sacs.

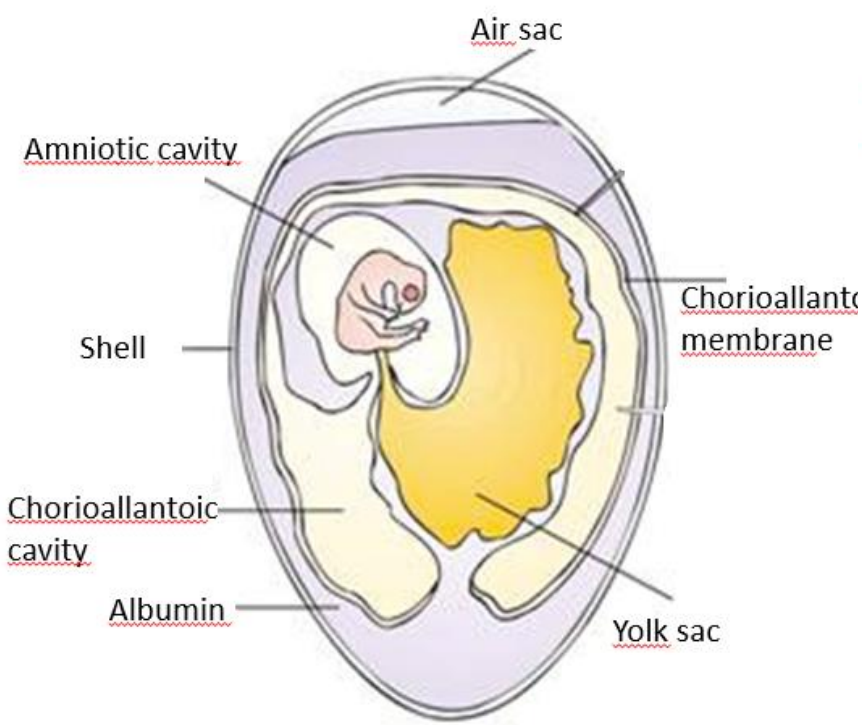


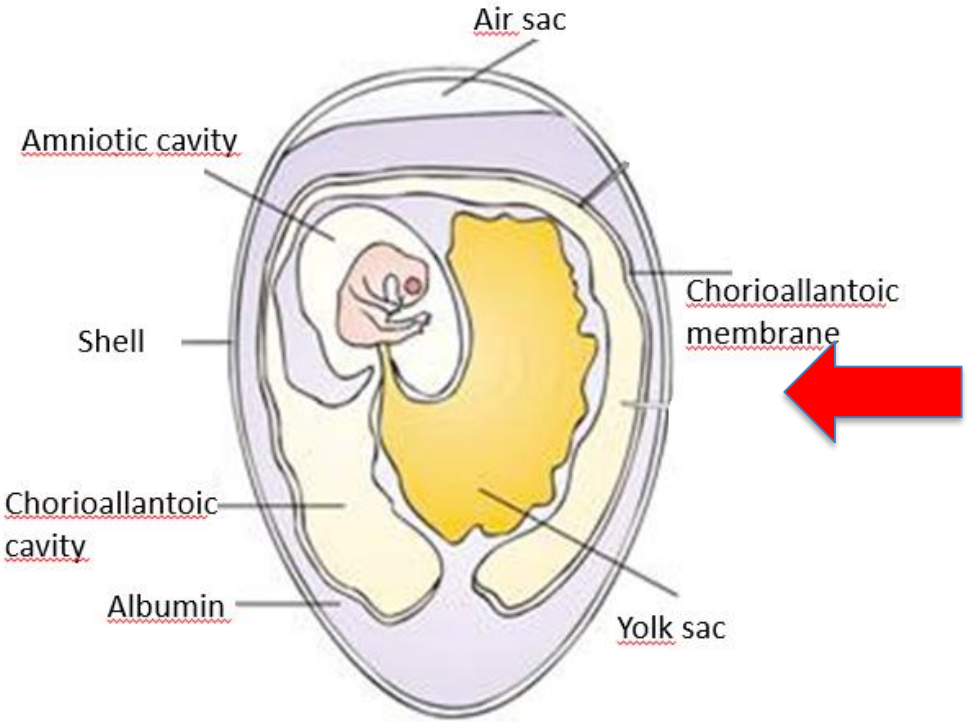


http://www.riverbendfarmtasmania.com/incubation-info

Routes of inoculation:

1. from the side of the air sac





2. from the side

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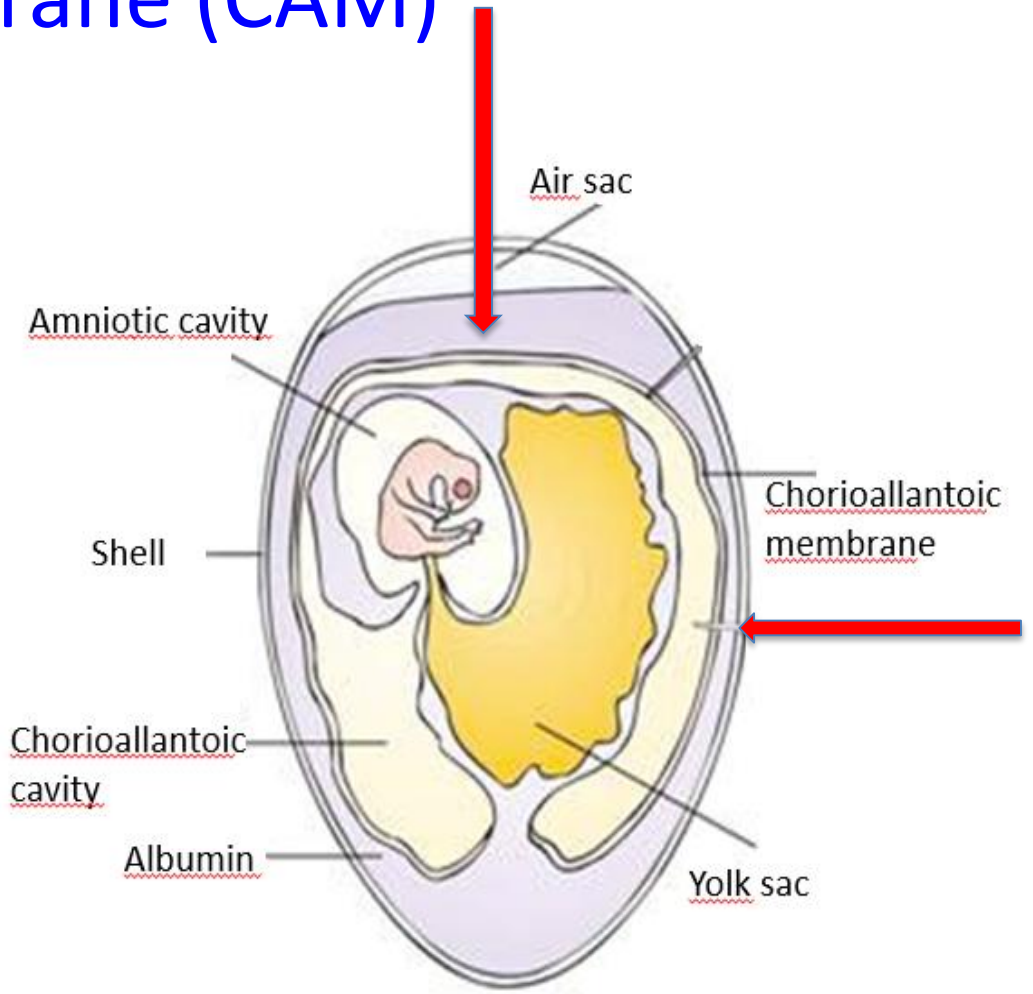
Materials Needed for Egg Inoculation

- Eggs: embryonated eggs. Candle the eggs and mark the inoculation sites. Eggs should be placed in an egg rack with the inoculation site uppermost.
- Egg shell punch.
- Cotton wool.
- A 70 percent alcohol solution in water or İodine.
- Syringe 1 mL.
- Needles preferably 25 gauge, 16 mm.
- Stationery tape (also called cello or sticky tape) or melted wax to seal the inoculation site.
- Inoculum. This must be free of microbial contamination.
- Discard tray.

1. Inoculation to Chorioallantoic membrane (CAM)

- 10-12 days old embryo
- Poxvirus and some epitheliotropic virus cultivation



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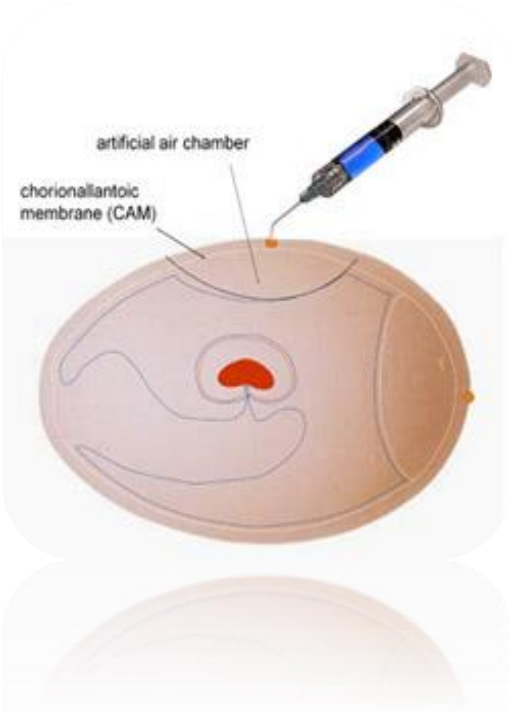
from the side of the air sac

- mark the position of air cell.
- Drill a small hole through the eggshell just above the air cell.
- Open a bigger hole by scissors and forceps
- Deposit the inoculum directly on the dropped CAM, seal the holes and keep eggs positioned horizontally for a few hours. Eggs are usually incubated for 5-7 days.

Amie J Eisfeld, Gabriele Neumann , Yoshihiro Kawaoka, (2014) Nature Protocols 9, 2663–2681

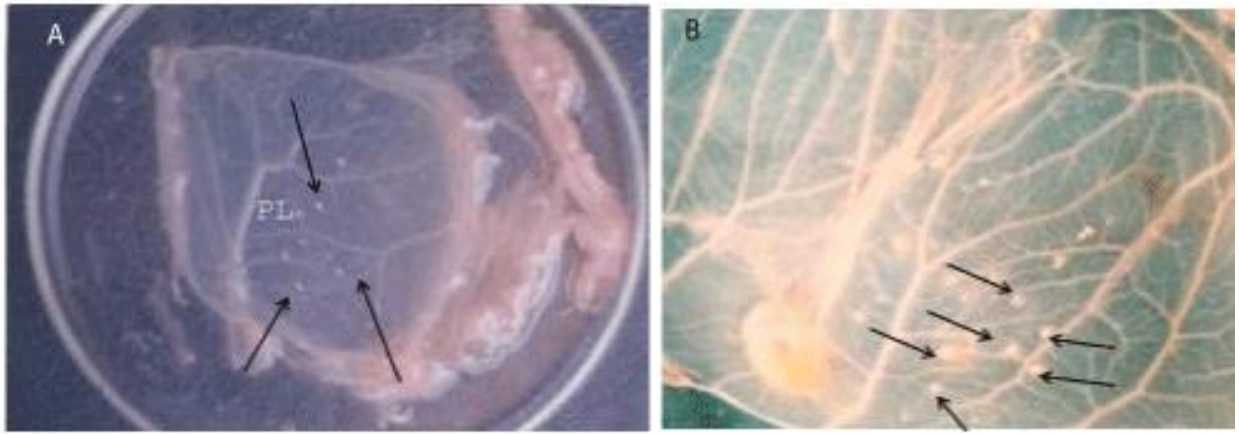
From the side

- mark the position of air cell.
- Drill a small hole through the eggshell just above the air cell.
- Place eggs horizontally on the egg flat and disinfect the eggshell.
- Drill a second hole on the side of egg in area devoid from blood vessels or between large blood vessels.
- Apply gentle vacuum to the hole in the air cell, this will cause the CAM to drop, thus forming a new false air cell directly over CAM.
- Open a bigger hole by scissors and forceps
- Deposit the inoculum directly on the dropped CAM, seal the holes and keep eggs positioned horizontally for a few hours. Eggs are usually incubated for 5-7 days.



Evaluation

- The deaths in the first 48 hours should be discarded as nonspecific or traumatic death due to inoculation.
- Death and pathological changes occurring in the later period are because of the virus.
- The shell on the air sac is removed and the membrane is taken into the petri dish.
- After washing with PBS, thickening and pox nodules are sought on the membrane.
- It is evaluated by looking at the control and making a comparison.



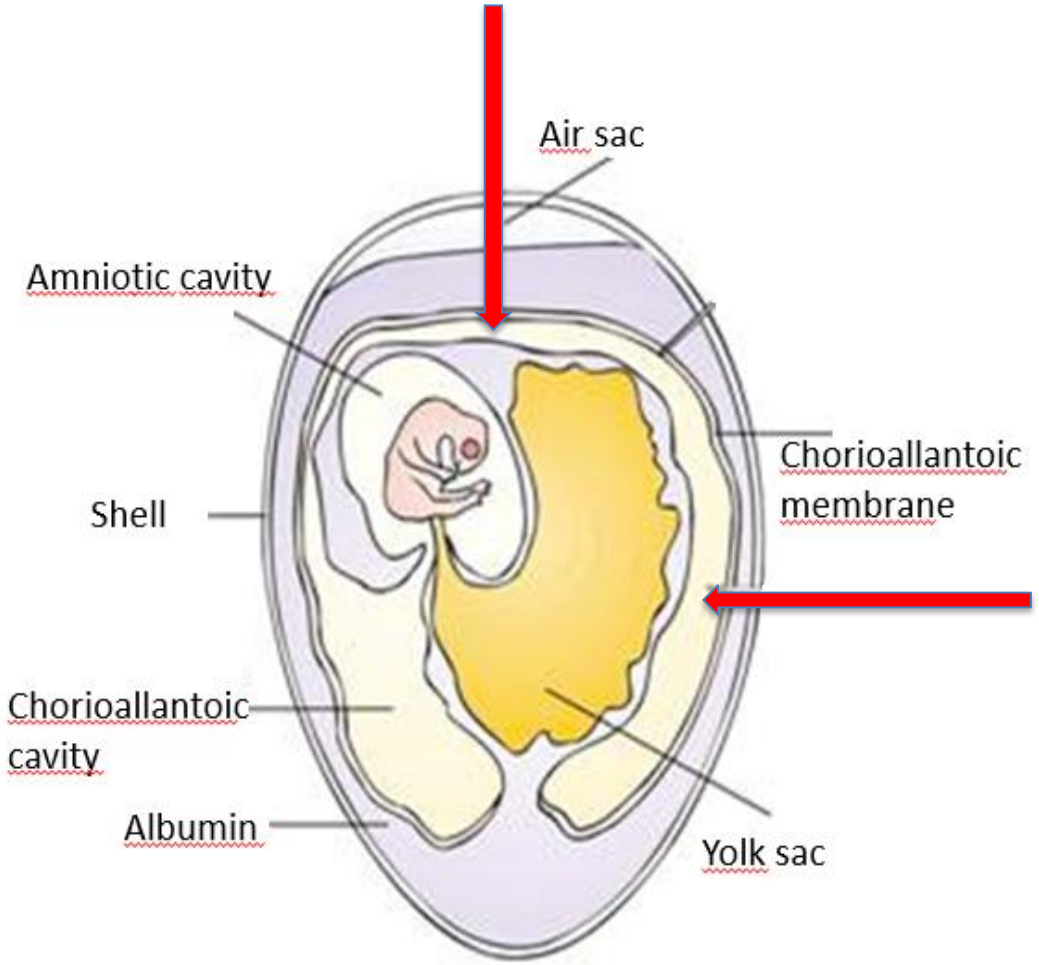
Chorio-allantoic membranes of the ECE inoculated with ORF virus produced the characteristic pock lesions (black arrows). **(A)** very small pock lesion, **(B)** well developed pock lesion

https://www.omicsonline.org/articles-images/JAA-06-154-g004.html

Nagasse-Sugahara TK, Kisielius JJ, Ueda-Ito M, Curti SP, Figueiredo CA, Cruz AS, Silva MM, Ramos CH, Silva MC, Sakurai T, Salles-Gomes LF (2004). Human vaccinia-like virus outbreaks in São Paulo and Goiás States, Brazil: virus detection, isolation and identification. Rev Inst Med Trop Sao Paulo. 46(6):315-22.

2. Inoculation to Chorioallantoic cavity

- It is routinely used in the production of Newcastle virus.
- 9-11 days old eggs are preferred.



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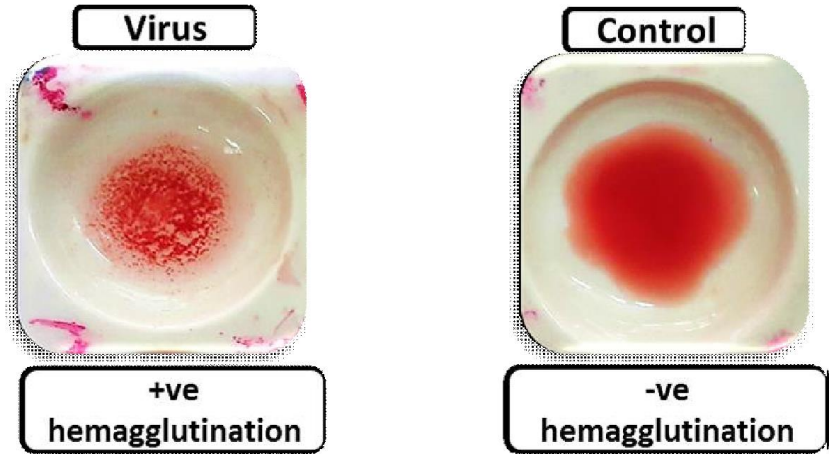
Evaluation

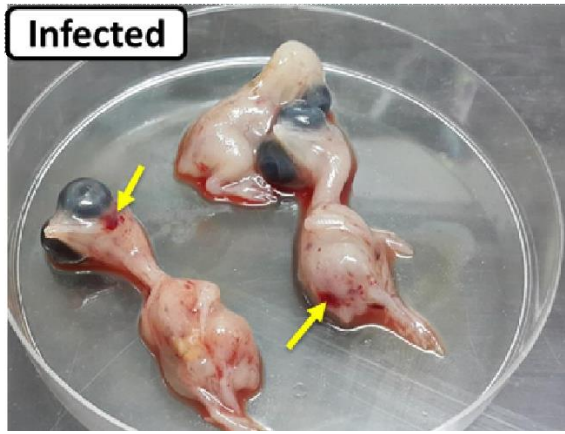
- The deaths in the first 48 hours should be discarded as nonspecific or traumatic death due to inoculation.
- Death and pathological changes occurring in the later period are because of the virus.
- If the embryo has not died during this period, it is kept for 2-4 hours at 4 ° C to die.
- Hemagglutination test is performed after the chorio-allatoic fluid is taken into a sterile tube.
- A positive result indicates that the virus has replicates.



NDV INFECTED EMBRYO

48 hours postinoculation

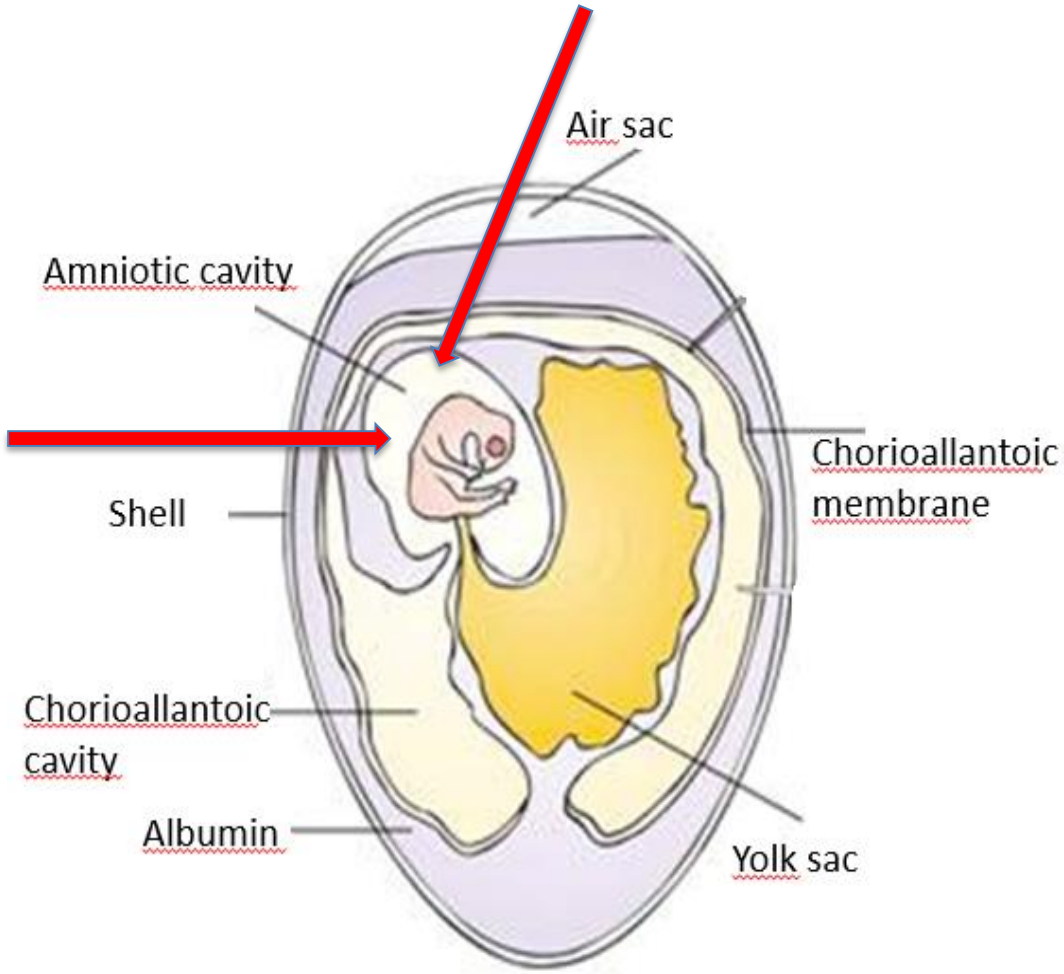
Strain - Cal. 11914



http://www.poultrydisease.ir/Atlases/avian-atlas/search/lesion/661.html

3. Inoculation to Amniotic cavity (AC)

- Measles, Mumps and Influenza viruses
- The embryo is large;
- 12-14 days old eggs



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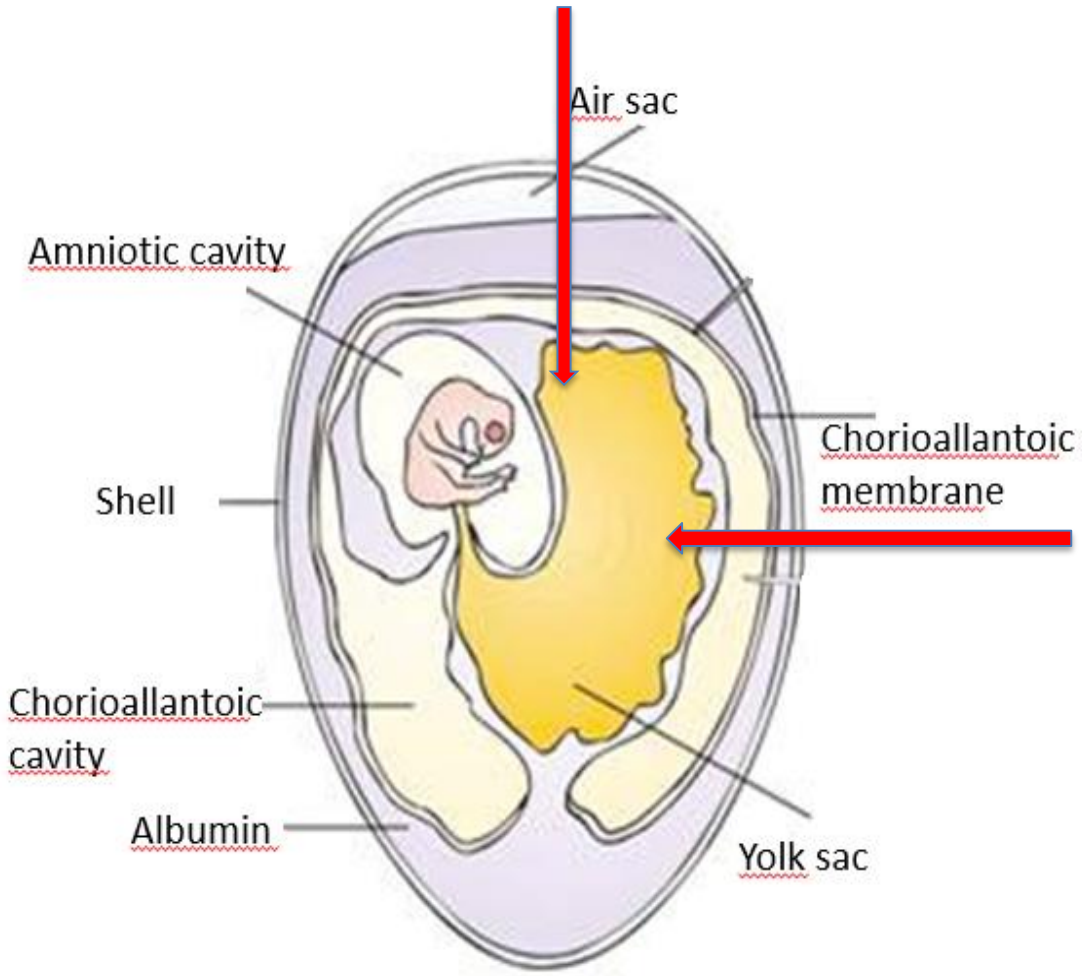
- Death and pathological changes occurring in the later period are because of the virus.
- If the embryo has not died during this period, it is kept for 2-4 hours at 4 ° C to die.
- Hemagglutination test is performed after the amniotic fluid is taken into a sterile tube.
- A positive result indicates that the virus has replicates.

4. Inoculation to Yolk Sac

- Bluetongue virus also Equine Herpesvirus and rabies virus
- When the yellow sac is largest, 6-8 days old eggs



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Evaluation

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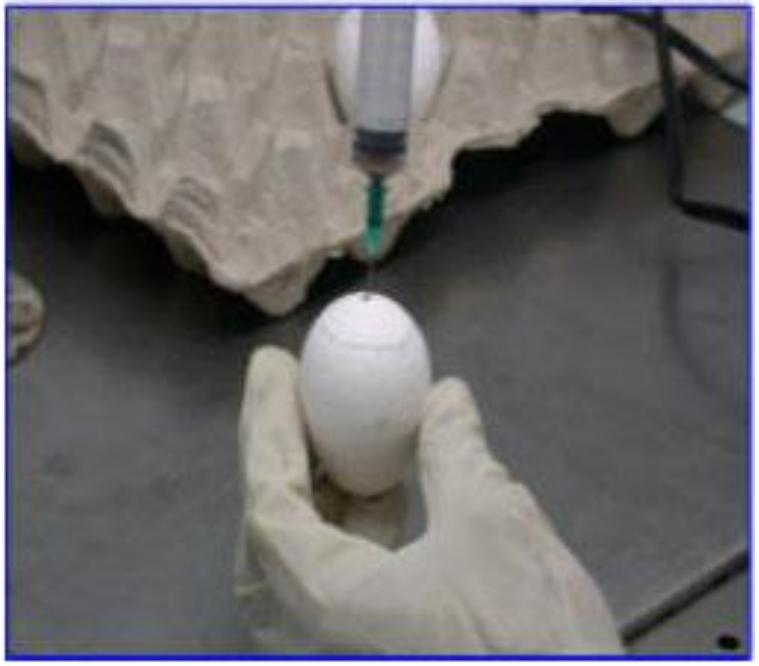
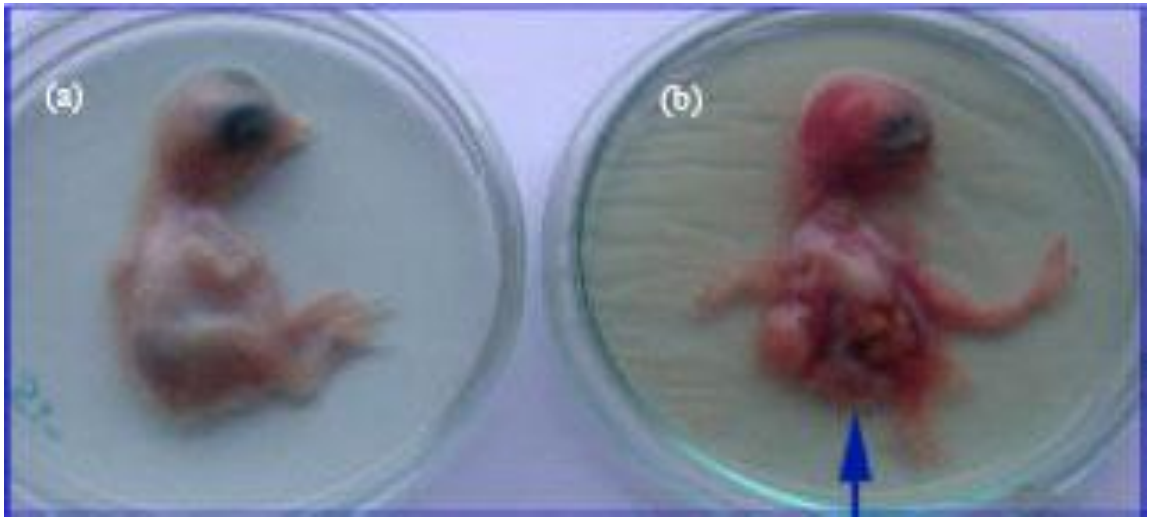
- Death and pathological changes occurring in the later period are because of the virus.
- Yumurta açılarak sarı kesesi çıkarılır.
- After the membrane is washed with PBS, they are placed on a slide and stained.
- Then, Inclusion bodies are searched under the microscope.





https://scialert.net/fulltext/?doi=jm.2009.186.193

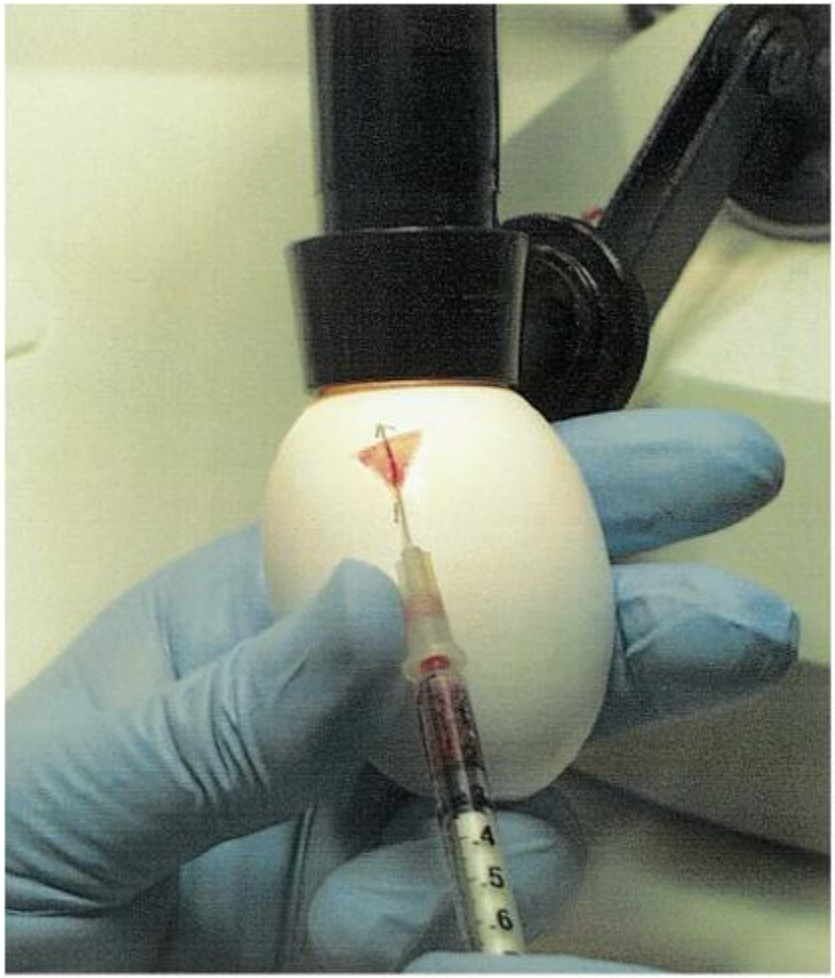


Fig. 1

Fig. 2

Clavijo, A., Heckert, R. A., Dulac, G. C., & Afshar, A. (2000). Isolation and identification of bluetongue virus. *Journal of virological methods*, *87*(1), 13-23.

- Fig 2. Mavidil ile enfekte embriyo (sol). Embriyo inokulasyonu takiben genelde 3-6 güun içerisinde ölür ve multiple hemoraji ve ödem gözlenir.

References

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- 2. Genel Viroloji (Yesilbag K., 2010, Bursa)
- 3. Veterinary Virology (Murphy ve ark., 1999), Academic Press
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