

# Chick chorioallantoic membrane model

*Commonly used acronym: CAM*

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## Organisation

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**Geographical Area** Brussels Region

## SCOPE OF THE METHOD

<b>The Method relates to</b>	Human health, Other
<b>The Method is situated in</b>	Translational - Applied Research
<b>Type of method</b>	In vivo
<b>Used species</b>	Chick embryo
<b>Targeted organ system or type of research</b>	Chick chorioallantoic membrane

## DESCRIPTION

### Method keywords

xenografting

revascularization

ovarian tissue transplantation

follicle survival

follicle activation

### **Scientific area keywords**

short term xenotransplantation

angiogenesis

vascularization

natural immunodeficiency

### **Method description**

The chick chorioallantoic membrane (CAM) model is particularly attractive to study short-term xenografting of human ovarian tissue. Its angiogenic potential and natural immunodeficiency allow scrutiny of early follicle activation and loss and graft revascularization mechanisms. Chick embryo development takes 21 days until hatching, and the CAM is formed within the first 4-5 days through the fusion of the allantois and chorion. Notably, the chick embryo is a naturally immunodeficient host until day 17, so xenografting experiments can be performed without any risk of graft rejection. Fertilized eggs are incubated from day 0 at 37°C in 40%–50% relative air humidity. On day 3 of egg incubation, a puncture is made with a sterile 19G needle and 1.5-2 ml of egg albumen is aspirated to detach CMA from the shell, after having located the air pocket and the yolk sac using a focal cold light source. Then, the egg is placed horizontally and a rectangular window is made in the eggshell, which is then covered with adhesive tape. On day 7 of egg incubation, CAM is traumatized by gently and quickly touching it with a 1-cm<sup>2</sup> strip of sterile ether-extracted lens paper, allowing the impenetrable upper peridermal part of its double epithelial layer to be disrupted. Then, one ovarian cortical strip per egg (4x2x1 mm<sup>3</sup>) is deposited onto traumatized CAM. After one day of grafting, ovarian cortical strips are partially adherent and show signs of revascularization. Ovarian cortical strips have to be removed before activation of chick immune system (day 17 from egg fertilization).

### **Lab equipment**

- Egg incubator,
- Focal cold light source,
- Sterile kit for egg manipulation (straight pin, 19 G needle, 5 ml syringe, scroll saw blade, forceps).

## Method status

Internally validated

Published in peer reviewed journal

## PROS, CONS & FUTURE POTENTIAL

### Advantages

- Inexpensive method;
- quick learning curve to apply successfully;
- ideal for short-term xenografting and investigation on vasculogenesis.

### Challenges

The most challenging part of the protocol described here is making the small hole required to aspirate the albumen in order to detach the CAM from the eggshell prior to creating a window. Applying too much pressure can result in overpenetration or may even crack and destroy the egg, causing irrevocable damage to the CAM and its vasculature. To keep mistakes to a minimum during initial attempts to separate the CAM, it is strongly advised to practice making small holes in the eggshell of non-fertilized, grocery-bought eggs using a straight pin.

## REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

### References

1. Hossay C, Tramacere F, Cacciottola L, et al. Follicle outcomes in human ovarian tissue: effect of freezing, culture, and grafting. *Fertil Steril*. 2023;119(1):135-145.
2. Hossay C, Cacciottola L, Storder S, Van Kerk O, Dolmans MM. The Chick Chorioallantoic Membrane (CAM) Model as a Tool to Study Ovarian Tissue Transplantation. *J Vis Exp*. 2023;(196):10.3791/64867.

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