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Extending the limits of avian embryo culture with the modified Cornish pasty and whole-embryo transplantation methods

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ABSTRACT

The New and Early Chick (EC) methods, two commonly used techniques for ex ovo culture of early-stage avian embryos, are limited by poor survivability after initiation of circulation. This limitation is circumvented with two recent technical advancements: the modified Cornish pasty culture and whole-embryo transplantation. The former supports optimal ex ovo growth till stage HH18, and the latter allows ex-ovo-manipulated embryos to have long-term in ovo-survivability. Here we provide step-by-step instructions for both methods. These two new techniques can also be combined to achieve targeted labeling, imaging and electroporation in early-stage embryos ex ovo, and phenotypic and functional analyses at more advanced developmental stages in ovo.

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1. Introduction

Early stages of avian embryos are not well-suited for in ovo manipulations. Several types of ex ovo culture systems have been devised for various experimental purposes. Among them the modified New culture [1,2] and the Early Chick (EC) culture [3] are the most common ones used to manipulate pre-streak and gastrulation stage chicken embryos. Both methods are known to be reliable and reproducible, and can be easily combined with classical and advanced experimental techniques such as tissue graft/transplantation, cell labeling, gain-of-/loss-of-function analysis and live imaging. In both culture systems embryos are explanted together with the vitelline membrane and grown with their ventral side facing up. Partly due to these unique culture conditions and partly due to a lack of continuous yolk nutrient supply, the maximal growth limit for the New culture and EC culture is stage HH13-15, depending on the initial stage of explanted embryos.

In contrast, the Cornish pasty method was designed under a different concept from the New and EC culture systems. It allows chick embryo to grow without a vitelline membrane by cutting and resealing it into a fish-like embryonic vesicle. The vesicle extends as a consequence of the inward water-transporting ability of epiblast cells, ensuring tension in the epiblast required for proper embryonic development. The original Cornish pasty culture [4] used a tissue culture medium and the roller bottle method for culturing mammalian pre-implantation embryos [5,6] (invented by the same Dennis New whom the avian New culture system was named after). With thin albumen replacing the original tissue culture medium, embryos grown in Cornish pasty culture can survive up to HH16 with some abnormalities [7].

We recently reported a modified protocol for the Cornish pasty culture [8]. In this modified version (called MC culture for modified Cornish pasty culture), we used a mixture of Pannett–Compton saline and thin albumen as the culture medium and replaced the roller bottle culture condition with simple petri dish culture without rotation. It is very easy to set up and allows normal development up to stage HH18. Established techniques for embryo manipulation (electroporation, labeling, imaging, etc) are all compatible with the MC culture. We have since tried to extend the growth limit of MC culture by intra-vesicular injection of yolk materials. This led to faster growth and higher percentage of embryos reaching HH18 with normal morphology, but did not extend maximal developmental stage by much, suggesting that there is a yet unknown "checkpoint" for embryonic development. We therefore sought to extend the growth potential of MC-cultured embryos by combining this method with a recently invented whole-embryo transplantation technique [9].

The whole-embryo transplantation technique [9] was based on the interspecific transplantation technique reported over forty years ago [10]. These two techniques however are somewhat different because in the whole-embryo transplantation the entire area pellucida and area vasculosa (the vascularized part of the area opaca) of a graft embryo is sutured to the area vitellina (the two-germ-layered, non-vascularized part of the area opaca) of a host embryo. After healing, extraembryonic mesoderm of the graft

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embryo extends beyond the fusion boundary and contributes to the growing yolk sac vasculature, whereas the host area vitellina contributes to the extraembryonic ectoderm and yolk sac endoderm partially, but not to any mesoderm lineage. Using the whole-embryo transplantation method, embryos can be grown and manipulated ex ovo using New culture, EC culture or MC culture, and studied later on in ovo for developmental effects. Maximal growth after whole-embryo transplantation was reported to be E5.5 [9], but unpublished data from our labs showed that operated embryos can survive at least to E10.

In this article, we will first describe the basic procedures for the MC culture and whole-embryo transplantation methods. We will next demonstrate the advantages of the MC culture as a tool for embryological manipulation. Lastly, we will introduce how to achieve longer survival of MC culture embryos by combining it with the whole-embryo transplantation technique.

2. MC culture and whole-embryo transplantation

In this section, we review the principles of MC culture and whole-embryo transplantation followed by basic procedures for each technique.

2.1. Preparation of MC culture

The MC culture system is the most convenient and cost-effective ex ovo culture method and produces the best ex ovo embryo growth among all existing culture systems. Required experimental set-up includes fertilized chicken eggs (for getting embryos and thin albumen), tweezers, micro-scissors, forceps commonly used for embryo dissection, petri dishes to culture embryos, Pannett–Compton saline solution and a simple humidified incubator without any gas regulation.

2.1.1. Embryo collection

Eggs are incubated at 38.5 °C to desired stages in accordance with the Hamburger and Hamilton staging system [11]. A small crack is made at the sharp end of the egg by tapping with the handle of tweezers and the egg shell is opened from this point [13]. Thick albumen is carefully removed from the vitelline membrane surface on top of embryo with tweezers while thin albumen is collected and kept for later use. The embryo together with the vitelline membrane is cut from the yolk surface and a spoon/spatula is used to scoop the embryo into a petri dish filled with Pannett–Compton saline (with antibiotics) sufficient enough for the embryo to be immersed. Thick forceps are used to carefully peel the membrane from the yolk. Care should be taken to disperse the force by pulling slowly from various edges. Briefly wash any excess yolk by holding the edge of an embryo with a pair of thick forceps and gently moving the embryo back and forth in saline. Alternatively, pipettes can be used to gently flush saline over the embryo to remove big patches of adhering yolk. Embryos are transferred to fresh Pannett–Compton saline and placed ventral-side up. Fine forceps are used to peel the embryo from the vitelline membrane (Fig. 1A). Collected embryos are kept in Pannett–Compton saline at room temperature for later use.

2.1.2. Procedure of MC culture

Ideal stages of chicken embryos for the MC culture are between HH3 and 7. Collected embryos are folded along the axial midline. The folded embryo is gently pressed at the peripheral edge with

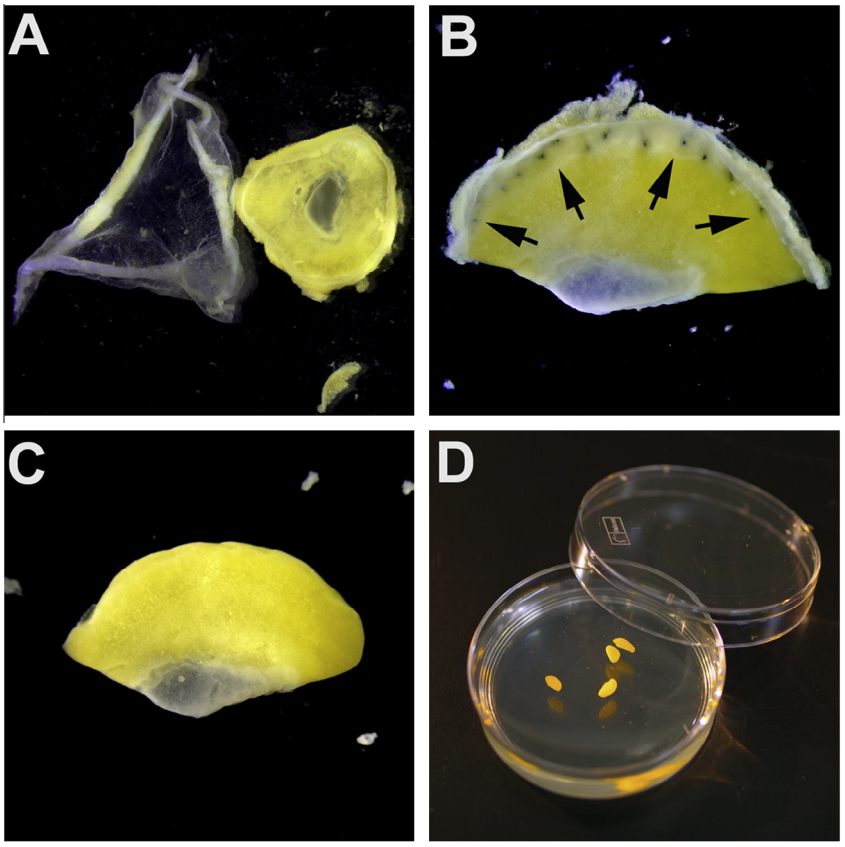

Fig. 1. MC culture preparation. Collected embryo is placed in Pannett–Compton saline and the vitelline membrane is removed (A). Embryo is folded along the medial axis and forceps are used to mark the peripheral edges (B, black arrows). Micro-scissors are used to cut the periphery (C) resulting in a partially "sealed" embryo. After 1–2 h of healing at room temperature MC-prepared embryos are transferred to thin albumen and fresh Pannett-Compton saline in a ratio of 2:1 and incubated at 38.5 °C in a humid environment (D).

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