

Lisbon, August 18, 2018

Dr. Indrani Mukherjee
Science Editor of Journal of Visualized Experiments

Dear Dr. Indrani Musherjee,

We are submitting an article for publication in JoVE entitled "Isolation of embryonic tissues and formation of quail-chicken chimeric organs: the thymus example." by Marta Figueiredo and Hélia Neves.

This manuscript provides a method to isolate pure embryonic tissues from quail and chicken embryos that can be combined to form *ex vivo* chimeric organs. This method was developed by Hélia Neves at N. Le Douarin's Laboratory, INAF/CNRS, France and employed in Neves *et al.*, 2012¹ to study epithelial-mesenchymal interactions during early-stages of thymus formation.

Briefly, embryonic tissues are isolated by mechanical forces and subject to *in vitro* pancreatin digestion. In the described procedure, the conditions of enzymatic digestion, temperature and time of incubation, were optimized to preserve tissues biological properties.

With this method, the isolated tissues can be associated in an organotypic *in vitro* system for 48h. This system mimics the local cellular interactions in the embryo overcoming some restrictions of *in vivo* manipulation. Then, the capacity of the cultured tissues to form organs can be further tested using *in ovo* approaches as described in previous Jove's publication².

The method presents an important tool for studying complex tissue interactions in developmental processes with highly dynamic spatial modifications and can also be applied to explore the potential/contribution of embryonic territories in organ formation.

Additionally, the isolation protocol allows three-dimensional preservation of the embryonic tissues particularly useful for detailing *in situ* gene-expression patterns of embryonic territories otherwise inaccessible by conventional methods. The transcriptome analysis approaches, including RNA-seq or microarrays, can also be applied in the isolated tissues without requiring genetic markers while providing a tissue-specific high throughput "omics" analysis.

We would like to acknowledge the kind invitation to publish this experimental procedure in JoVE and we hope that you will find it worth considering for publication.

With my best regards,
Sincerely,



Hélia Neves
Assistant Professor, Medical School, University of Lisbon

¹Neves, H., *et al.* N. M. Modulation of Bmp4 signalling in the epithelial-mesenchymal interactions that take place in early thymus and parathyroid development in avian embryos. *Dev Biol.* **361** (2), 208–219 (2012).

²Figueiredo, M. & Neves, H. Two-step Approach to Explore Early-and Late-stages of Organ Formation in the Avian Model: The Thymus and Parathyroid Glands Organogenesis Paradigm Video Link. *J. Vis. Exp* **5711437915** (13610) (2018).