Chicken-derived airway organoids: a model to study viral dynamics for IBV, NDV, and H5N8 HPAIV

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INTRODUCTION

Organoids are extensively used in biomedical research as alternative models to animals for the study of human diseases[1], but fewer efforts are invested in animal organoids[2].

Animal organoids can help studying epizootic and panzootic infectious diseases, to understand which animals are susceptible and/or can act as reservoirs of pathogens, understand viral dynamics or screen for novel treatments.

See poster by G. Ceada on FARMBANK, an animal organoid biobank).

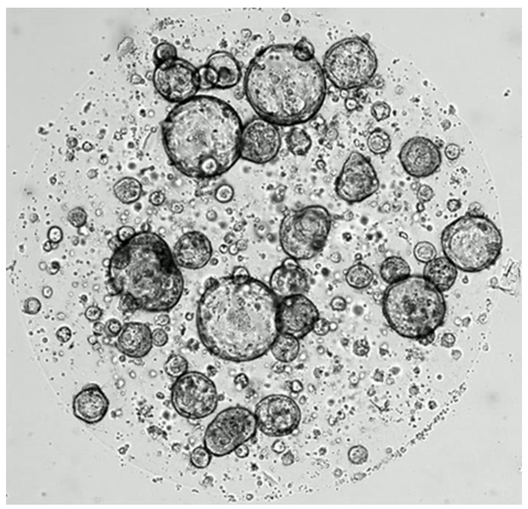

Figure 1. Optical microscopy image at 4X of 3D-cultured chicken airway organoids generated from adult layer hens.

In this work, we present the generation of organoids from adult chickens (**Figure 1**) and how they can be used to study the pathophysiology and viral dynamics of three avian viruses of high importance in the poultry sector: Newcastle Disease Virus (**NDV**)[3], Infectious Bronchitis Virus (**IBV**)[4], and an H5N8 highly pathogenic avian influenza virus (**H5N8 HPAIV**)[5].

METHODS

- Lung organoids were isolated from adult leghorn laying hens (**Figure 2**).
- Organoids were 3D cultured with proliferation media adapted from: **6**.
- Air-liquid interface (ALI) systems were cultured for 21 days with STEMCELL Organoid Differentiation Medium (**Figure 2, steps 5-6**).
- ALI cultures were infected with NDV, IBV, and H5N8 HPAIV (MOI=0.01).
- Supernatants were analysed at 24h and 48h by qPCR and histology staining performed on the cell fraction at 48h post-infection.

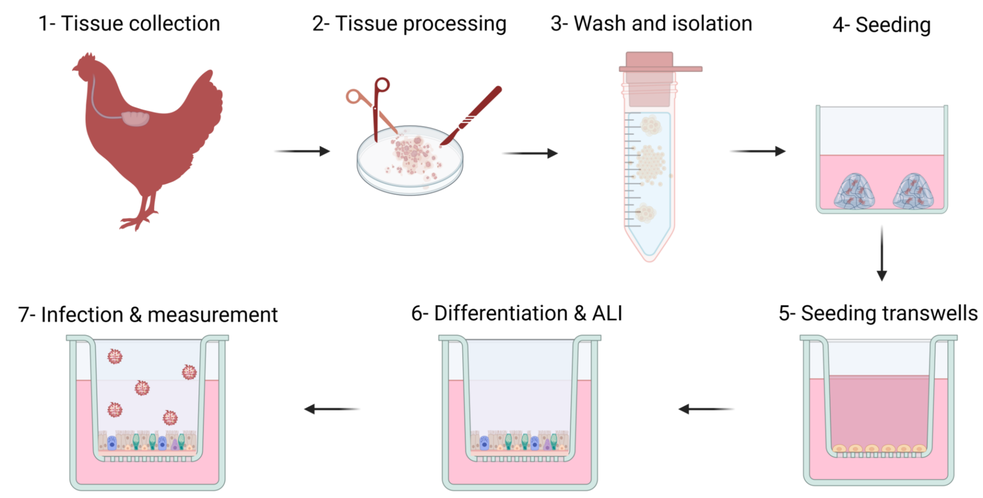

Figure 2. Schematic representation of the experimental procedure for the organoid generation and ALI culture. Created with BioRender.com.

MOLECULAR CHARACTERISATION OF AIRWAY ORGANOIDS

Airway organoids cultured in 3D for passaging were analysed by conventional PCR to assess the cellular composition using specific markers and compared to the tissue of origin (**Table 1**).

Marker expression analysed by conventional PCR of the airway organoids matched the tissue of origin (+: expressed; +/-: dim bands; -: not detected).

Table 1. Identification of the cell types in lung tissue and orgnaoids by conventional PCR.

Sample	Media	Basal (tp63)	Ciliated (FOXJ1)	Goblet (MUC5AC)	Alveolar (Sftpa1)	HK gene (GAPDH)
Tissue	-	+	-/+	-	+	+
Organoids	OGM	+	-/+	-/+	+	+
	ODM	+	+	+	+	+

EXPERIMENTAL INFECTION with IBV, NDV, and H5N8 HPAIV

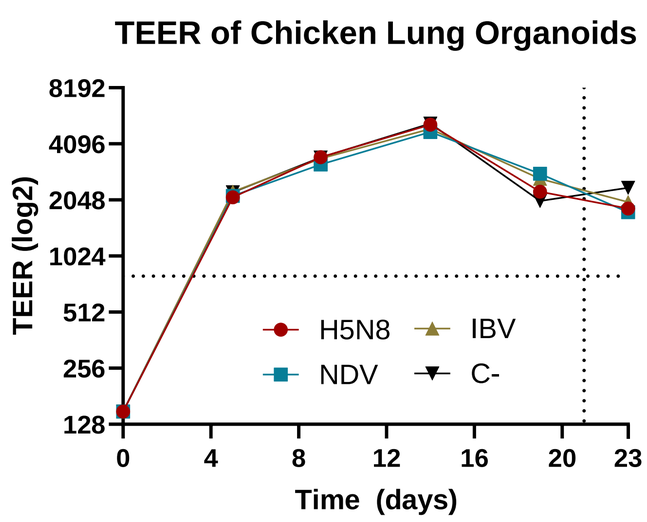

Figure 3. TEER measurements in chicken-airway ALI cultures. The integrity of the epithelium was maintained throughout the experiment, even after infection.

Table 2. Viral detection by qPCR in ALI cultures

Virus	Site	Ct (24h)	Ct (48h)
C-	Apical	N.D.	N.D.
	Basal	N.D.	N.D.
H5N8	Apical	32,06	24,68
	Basal	35,63	30,91
NDV	Apical	24,52	15,38
	Basal	39,95	25,92
CoV-IBV	Apical	28,42	23,63
	Basal	N.D.	N.D.

All viruses were detected on the apical chamber; H5N8 HPAIV and NDV were also detected apically.

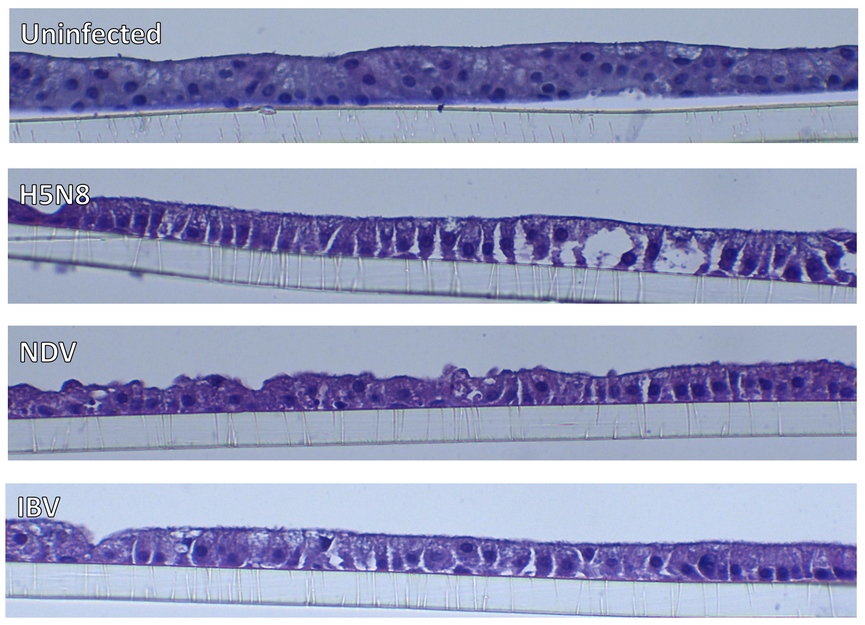

Figure 4. Histopathological analysis of chicken-airway organoids in ALI cultures infected with H5N8, NDV and IBV (or uninfected). Infected had intercellular oedema; NDV: apoptotic vacuolisation; H5N8: deciliation.

CONCLUSIONS

- Animal-derived organoids can be useful tools to study animal diseases.
- Chicken-airway organoids marker expression matches tissue of origin.
- Infection of chicken-airway organoids cultured in an ALI system with NDV, IBV, or H5N8 HPAIV reproduced *in vivo* lesions.
- **Only H5N8 and NDV were detected in the basal chamber of ALI cultures, mimicking the *in vivo* capacity to produce viremia.**

Funding:

INFLUOMA: Proyecto PID2020-114060RR-C31 financiado por MCIN/ AEI /10.13039/501100011033

FARMBANK: Proyecto de I+D+i PLEC2022-009171, financiado por MCIN/AEI/10.13039/501100011033 y UE NextGenerationEU/PRTR.

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Acknowledgements: the authors want to thank all personnel involved in these experiments, from the administrative and laboratory (BSL2 and BSL3) management units. Additionally, we want to acknowledge all the people that has contributed to the growth of the organoid field which represents a pillar stone for the reduction of the use of animal models in research.