

# Isolation of extracellular vesicles from turkey (*Meleagris galopavo*) seminal plasma



Ewa Drzewiecka<sup>1</sup>, Izabela Siemińska<sup>2</sup>, Laura Pardyak<sup>2</sup>, Andrzej Ciereszko<sup>1</sup>, Mariola Słowińska<sup>1</sup>

<sup>1</sup>Institute of Animal Reproduction and Food Research, Polish Academy of Science in Olsztyn, Olsztyn, Poland  
e.drzewiecka@pan.olsztyn.pl

<sup>2</sup> University of Agriculture in Krakow, Kraków, Poland

## Introduction

Extracellular vesicles (EVs) in seminal plasma may play a significant role in maintaining sperm function. Previously, proteomic analysis of turkey seminal plasma revealed the presence of a large group of proteins assigned to EVs. So far, little is known regarding the role of EVs in processes determining good semen quality in turkeys. For this reason, effective methods of EVs isolation from turkey semen are required for further research.

## Hypothesis and objectives

We hypothesize that using different methods of EVs isolation from turkey seminal plasma affects EVs recovery.

We aimed to test different EVs isolation approaches to find the most accurate method for further research on turkeys' seminal plasma EVs.

## Materials & Methods



### Fresh semen

#### Method I

(Sequential Centrifugation)

1. 7000 x g, 10 min
2. 15200 x g, 15 min
3. 100 000 x g, 90 min
4. 100 000 x g, 60 min

#### Method II

(Sequential Centrifugation with PBS)

1. 959 x g, 10 min
2. +500 uL PBS to sperm;
3. 959 x g, 10 min
4. +500 uL PBS to sperm; agitation
5. 959 x g, 10 min
6. Centrifugation of pooled seminal plasma and supernatants
7. 15200 x g, 15 min
8. 100 000 x g, 90 min
9. 100 000 x g, 60 min

#### Method III

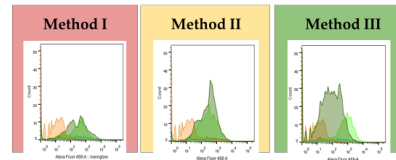
(Sequential Centrifugation with PBS and proteases\*)

1. 959 x g, 10 min
2. +500 uL PBS to sperm;
3. 959 x g, 10 min
4. +500 uL PBS to sperm; agitation
5. 959 x g, 10 min
6. Centrifugation of pooled seminal plasma and supernatants
7. 15200 x g, 15 min
8. 100 000 x g, 90 min
9. 100 000 x g, 60 min

\*protease inhibitor, EDTA-free (04693159001, Roche Diagnostics)

## Results

### MemGlow 488 – Flow Cytometry

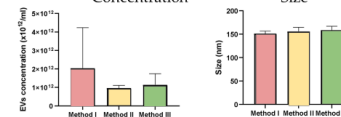
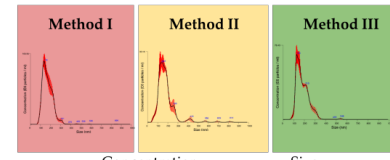


Evaluation of turkey EVs protein markers (anti-HSP70 IgG, anti-CD9 IgG)

HSP70 (70k Da)

CD9 (25-30 kDa)

### Nanoparticle tracking analysis (NTA)



## Conclusion

Method II and III might be used in further studies.

## Acknowledgement

This work was supported by funds appropriated to the Institute of Animal Reproduction and Food Research [20/FBW/2023]