Graphene oxide induces sperm release from bovine oviductal epithelial cells by modifying sperm membrane fluidity and binding proteins expression

Marina Ramal Sánchez[1,2]
Marie Saint-Dizier[2], Marie-Claire Blanche[2], Guillaume Tsikis[2], Emilie Corbin[2], Antonella Fontana[3], Romina Zappacosta[3], Luca Valbonetti[1], Barbara Barboni[1], Pascal Mermillod[2], Nicola Bernabò[1].

1 Faculty of Veterinary Medicine, University of Teramo, Via Renato Balzarini 1, 64100 Teramo, Italy
2 INRA-Centre Val de Loire, Physiology of Reproduction and Behaviours, 37380, Nouzilly, France
3 Department of Pharmacy, University "G. D' Annunzio" of Chieti-Pescara, Via dei Vestini, 66100, Chieti, Italy
Contact: mramalsanchez@unite.it

Graphene Oxide (GO) has acquired a scattered use in regenerative medicine and biotechnology during the last years. However, data about toxicity on reproduction are controversial. For this reason, our group recently studied the effects of GO on different aspects of swine sperm capacitation, finding out a positive effect in the fertilizing ability of GO treated spermatozoa in vitro, explained by the extraction of cholesterol present in the membrane [1]. With the aim of studying the interaction between GO and spermatozoa in other mammalian species and in a more physiological condition, bovine sperm were co-incubated with bovine oviductal epithelial cells (BOEC) in the presence and absence of GO (1µg/mL). In this in vitro model, the attachment of sperm to BOEC simulate the "sperm reservoir" in the oviduct, where sperm storage, capacitation, fertilization and early embryo development take place [2].

After 30 min of co-incubation with BOEC, spermatozoa were treated 1h with GO, whereupon they were collected and both cells and bound spermatozoa were stained and counted by confocal microscopy, finding a high number of sperm released after GO treatment (data not shown). FRAP analysis was performed on released spermatozoa to evaluate the changes in membrane fluidity, an event directly related to capacitation. The released from BOEC by GO caused an increase in membrane fluidity (Figure 1). To study the expression of the Binder of Sperm Proteins (BSP-1,-3,-5), major proteins of the bovine seminal plasma and keys in the event of capacitation [3], Western Blotting experiments were carried out with the control, unbound and GO-released spermatozoa. A lessen or lacked expression of BSP was seen on GO-released spermatozoa compared to control and unbound sperm (Figure 2).

In conclusion, GO effects were studied in bovine spermatozoa, finding out similar effects to those produced on boar sperm and with an interesting role in the process of capacitation that leads to fertilizing ability acquisition.

References

[1] N. Bernabò et al. Carbon 129:428-437 (2018).
[2] Lamy et al . Reproduction 154(4):497-508 (2017).
[3] Plante et al. Andrology 3(5):817-24 (2015).

Figures

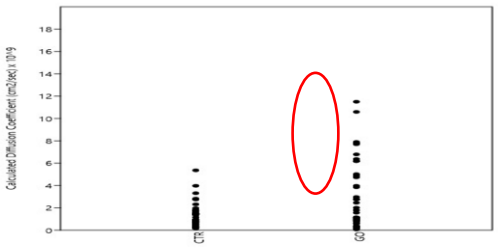

Figure 1: FRAP analysis of GO treated spermatozoa. GO causes an increase in membrane fluidity (p=0.01443).

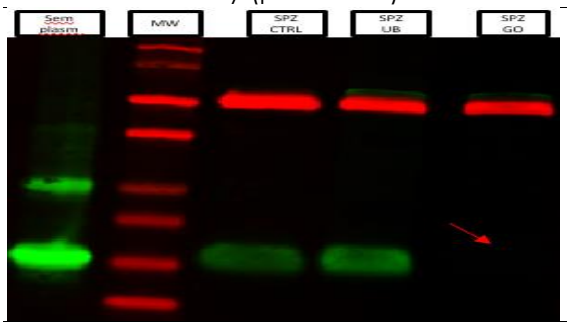

Figure 2: Western Blotting representative experiment shows the lack of BSP-1 (15 kDa) on GO-released sperm (C+: seminal plasma).