Graphene Oxide as an agent for personalized ARTs

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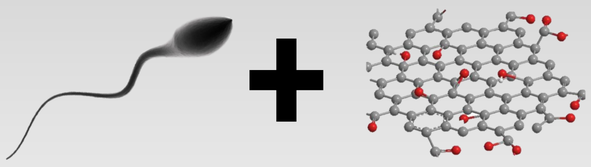
Introduction

Graphene Oxide (GO) has shown great promise in many field of study. However few studies have reported its effect in reproduction. Although the consensus is that in high dosage it can be harmful for the reproductive tract and the reproductive cells an interesting effect surges when controlled small amounts are used. Previous studies in boar (Bernabò, Fontana et al. 2018) and bull (Ramal Sánchez, Valbonetti et al. 2019) have shown that in a concentration of 1µg/mL GO it can facilitate spermatozoa in vitro capacitation which leads to higher rates of *in vitro* Fertilization. This appears to be mostly due to its ability to extract cholesterol from the membrane, therefore helping with the capacitation-dependent membrane remodeling (Bernabo, Machado-Simoes et al. 2019). However GO effect on human spermatozoa is still poorly studied, which is an essential step in ensuring its safety for use in ARTs.

Methodology



- ✓ Mobility
- ✓ Viability
- ✓ Tyrosine Phosphorylation
- ✓ Acrosome Reaction
- ✓ Mitochondria Superoxide formation

- ✓ **Mobility** was assessed by light microscopy.
- ✓ **Viability** analyzed by counter staining with eosin.
- ✓ **Tyrosine Phosphorylation** analyzed by immunocytochemistry with anti
- ✓ **Acrosome Reaction** analyzed by immunocytochemistry with PSA-FITC
- ✓ **Mitochondria Superoxide formation** evaluated by flow cytometry using MitoSox Red

Results

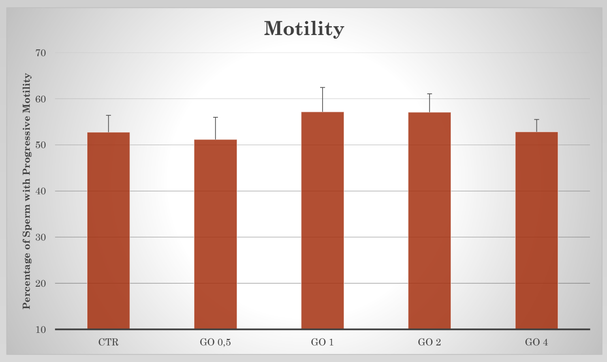


Fig. 1: Motility measured after 3h of incubation with GO. There is no significant effect of GO in the motility of spermatozoa.

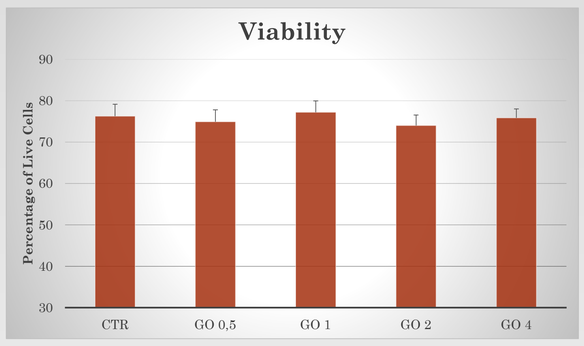


Fig. 2: Viability after 3h of incubation with GO measured by counterstaining with eosin. GO does not significantly decrease the survival of spermatozoa.

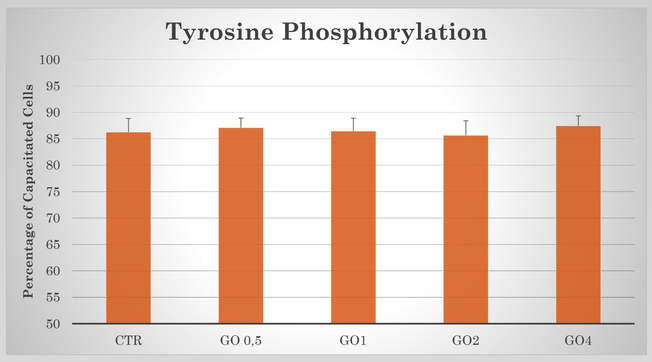


Fig. 3: Tyrosine Phosphorylation after 3h of incubation with GO. There are no significative difference in the percentage of spermatozoa with phosphorylated tyrosine in the tail.

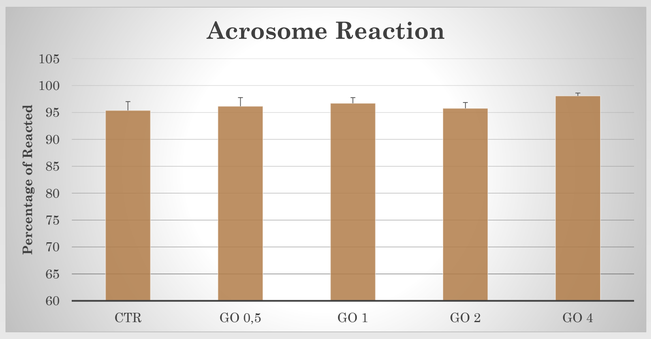


Fig. 4: Acrosome Reaction after 3h of incubation with GO. There are no significative difference in the percentage of spermatozoa which underwent acrosome reaction.

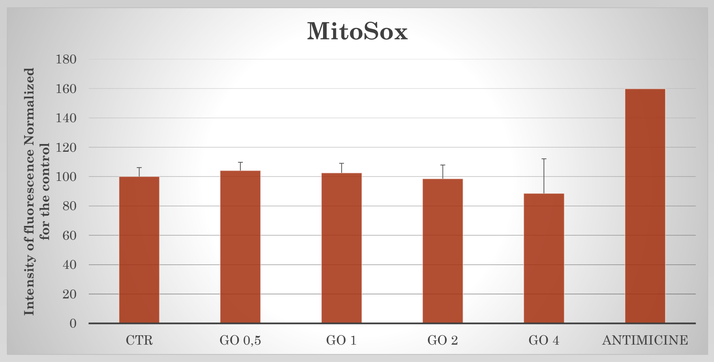


Fig. 4: Effect of GO in the production of superoxide by the mitochondria. MitoSox staining shows that GO is not affecting the production of ROS by the mitochondria.

It is evident that GO does not affect sperm's viability or motility. Also, the percentage of cells that undergo tyrosine phosphorylation and acrosome reaction after 3h of capacitation has no variation in relation to control. Also there is no alteration in the production of superoxide by the mitochondria. Put together these indicate that GO could be sagely applied to human ART's.

Discussion/Conclusion

In studies with animal models, Graphene Oxide has shown great potential has a facilitator of capacitation and fertilization. In humans, there are not enough conclusive studies to either support or deny this possibility. Although some studies report a toxic effect of GO, these effects occur only when high concentration of GO are used(Asghar, Shafiee et al. 2016). The concentrations that have a positive effect in the capacitation of boar and bull spermatozoa have not been previously studied in human. Our results show that in this range of concentration, GO can be safely applied to human spermatozoa without affecting its normal functions, such as their ability to undergo Tyrosine phosphorylation in the tail or acrosome reaction. Further studies are required to confirm if the effect GO has on the remodeling of the membrane evident in boar spermatozoa are also in effect in human. Particularly, to understand if GO is facilitating the cholesterol efflux form the spermatozoa membrane in a way that can benefit IVF rates in human.

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