GRAPHENE OXIDE AFFECTS SPERM MEMBRANE FEATURES

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INTRODUCTION

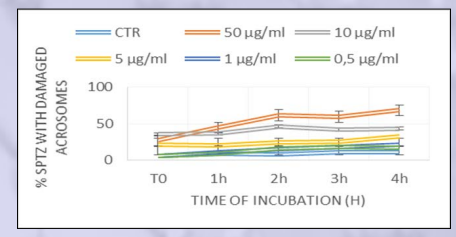
GRAPHENE IS A TWO DIMENSIONAL ALLOTROPE OF CARBON CONSISTING OF A PLANAR SHEET OF CONDENSED BENZENE RINGS. THIS STRUCTURE GIVES TO GRAPHENE EXTRAORDINARY PROPERTIES THAT MAKE IT IDEAL FOR USE IN MANY FIELDS OF RESEARCH SUCH AS BIOMEDICINE. FOR APPLICATION IN BIOMEDICINE, ONE OF THE MAJOR LIMITATIONS IN THE USE OF GRAPHENE IS ITS POOR WATER SOLUBILITY. THE RESEARCHERS OVERCOME THIS DRAWBACK BY COVALENTLY FUNCTIONALIZING[1-3] IT WITH HYDROPHILIC MOIETIES. A WIDELY USED AND STUDIED HYDROPHILIC GRAPHENE DERIVATIVE IS GRAPHENE OXIDE (GO). THE TOXICITY OF GRAPHENE OXIDE (GO) HAS BEEN EXTENSIVELY STUDIED BUT, UP TO NOW, DATA ARE CONFLICTING[4,5].

DUE TO THE LACK AND CONTROVERSY OF DATA ABOUT GO TOXICITY ON SPERM[6,7], WE STUDIED THE EFFECTS OF GO ON MATURE MAMMALIAN SPERMATOZOA BY ANALYZING THE INTERACTION BETWEEN GO AND BOAR SPERM MEMBRANES DURING CAPACITATION (I.E. THE PROCESS LEADING THE SPERM TO OBTAIN ITS FERTILIZATION ABILITY).

MATHERIALS AND METHODS

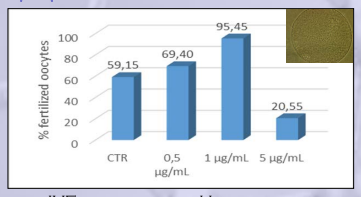
- AQUEOUS GO SOLUTION, OBTAINED BY MODIFIED HUMMERS METHOD, WAS DILUTED AT THE ELECTED CONCENTRATION, BATH ULTRASONICATED AND STERILIZED UNDER UV LAMP. THE CONCENTRATION OF GO WAS CHECKED BY UV-VIS SPECTROPHOTOMETRY AT λ_{MAX} 230 NM.
- SPERMATOZOA WERE EXPOSED TO DIFFERENT GO CONCENTRATIONS (50, 10, 5, 1 AND 0.5 µG/ML) IN A VALIDATED *IN VITRO* SYSTEM CAPABLE OF PROMOTING CAPACITATION.
- SEVERAL PARAMETERS WERE EVALUATED SUCH AS: (I) STRUCTURAL DAMAGES AT THE ACROSOMES, (II) FERTILIZING ABILITY OF SPERMATOZOA, (III) MEMBRANE FLUIDITY, (IV) SPERMATOZOA ADHESION PROPERTIES.
- SAMPLES WERE ANALYSED BY DIFFERENT TECHNIQUES: (I) FLUORESCENCE MICROSCOPY, (II) FRAP, (III) AFM AND (IV) IVF.

1 GO TOXICITY ON ACROSOME INTEGRITY



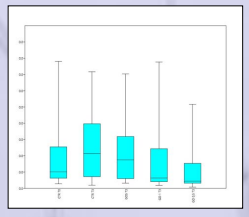
DIFFERENT CONCENTRATIONS OF GO WERE EVALUATED (50, 10, 5, 1, 0.5 µG/ML) AT DIFFERENT CAPACITATION TIMES, CONFIRMING A CONCENTRATION-DEPENDENT INCREASE IN LOSS OF ACROSOMES. THE PERCENTAGE OF CELLS SHOWING STRUCTURAL DAMAGES IN THE ACROSOME WERE EVALUATED USING PSA STAINING AND FLUORESCENCE MICROSCOPY.

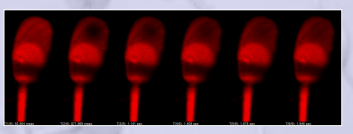
2 FERTILIZING ABILITY

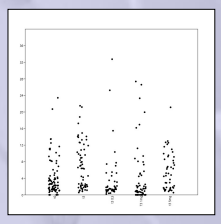


GO EFFECTS ON IVF EXPERIMENTS. HIGH CONCENTRATIONS OF GO WERE CONSIDERED AS TOXIC WHEREAS SPERMATOZOA CO-INCUBATED WITH A CONCENTRATION OF 1 µG/ML OF GO SHOWED AN INTERESTING INCREASE IN THE NUMBER OF FERTILIZED OOCYTES. OOCYTES WERE ANALYZED USING HOECHST STAINING AND FLUORESCENCE MICROSCOPY.

3 MEMBRANE FLUIDITY

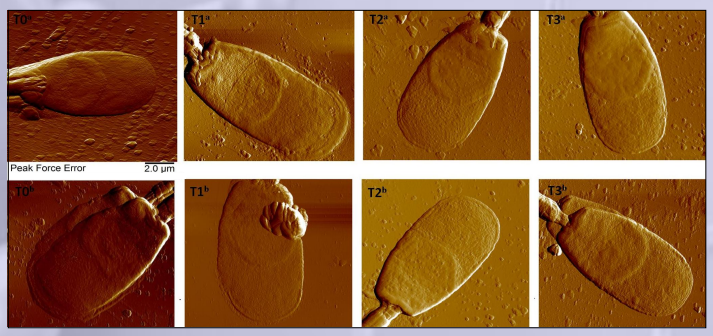






FLUIDITY CHANGES ON SPERMATOZOA MEMBRANE CAUSED BY GO. THE PRESENCE OF GO ALTERED THE SPERMATOZOA POPULATION FLUIDITY BY FAVORING AN EXTRA FLUIDITY IN A SMALL NUMBER OF SPERMATOZOA.

4 AFM



AFM IMAGES OF BOAR SPERMATOZOA AT DIFFERENT TIME OF CAPACITATION (T0, T1, T2, T3) BEFORE (a) AND AFTER (b) TREATMENT WITH GO 1µG/ML

5 CONCLUSIONS

- FOR THE FIRST TIME GO WAS USED IN A PROTEIN FREE SYSTEM OF *IN VITRO* CAPACITATION OF SPERMATOZOA;
- THE CONCENTRATIONS OF GO ABLE TO PROMOTE A TOXIC EFFECT ON CELL VIABILITY (>5 µG/ML) AND ON ACROSOME INTEGRITY (>1 µG/ML) WERE DETERMINED;
- GO INTERACTION WITH SPERM MEMBRANE WAS INVESTIGATED REVEALING A GO INTERFERENCE WITH THE DYNAMIC OF MEMBRANE REMODELING AND A MODIFICATION IN MEMBRANE FLUIDITY;
- SPERMATOZOA ADHESION TO SUBSTRATE WITHOUT AND WITH GO AT DIFFERENT CONCENTRATION REVEALED A CHANGE WITH THE TIME IN A DOSE DEPENDENT MANNER;
- GO INCUBATION WITH SPERMATOZOA PRODUCED A DOSE-DEPENDENT EFFECT ON FERTILIZING ABILITY.

REFERENCE

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