PROGESTERONE INDUCES SPERM RELEASE FROM BOVINE OVIDUCTAL EPITHELIAL CELLS BY MODIFICATION OF THE SPERM PROTEIN AND LIPID COMPOSITIONS

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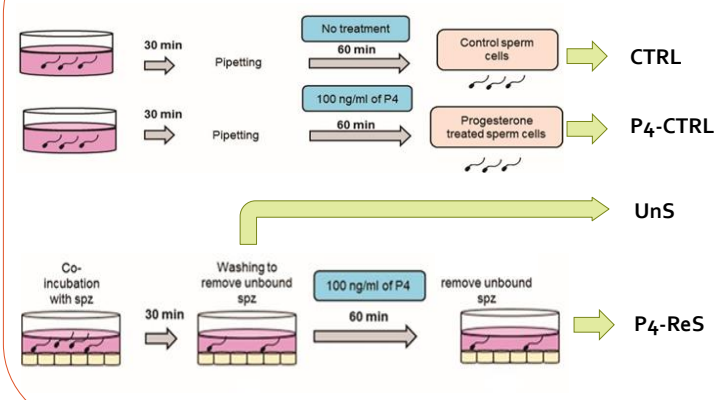
INTRODUCTION AND AIM OF THE STUDY

After mating or insemination, spermatozoa reach the oviduct where they bind to oviductal epithelial cells (OEC) for hours to days in the so called "functional sperm reservoir" before moving towards the fertilization site. During this storage, the interactions between spermatozoa and OEC are believed to play an important role in sperm selection and capacitation. Recently, after measuring progesterone (P_4) concentrations in the post-ovulatory bovine tubal fluid (Lamy et al. Theriogenology 86:1409-1420, 2016) our group evidenced that P_4(100 ng/mL) was able to trigger sperm release from bovine OEC (BOEC) in vitro, similar to what occurs in vivo, selecting a population of spermatozoa with a higher fertilizing competence (Lamy et al. Reproduction 154:497-508,2017). The aim of this study was to elucidate the underlying mechanisms of action.

MATERIALS AND METHODS

- **Frozen-thawed bovine spermatozoa** ($4x10^6$/mL) were subjected to Percoll density gradient (45/90%) and incubated with frozen-thawed **BOEC** obtained from healthy cows and cultured until confluence.
- Proteomic and lipidomic profiles were assessed on Intact Cells by **Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry** (ICM-MS) (Bruker Daltonics, Bremen, Germany).
- DilC12(3) staining and **FRAP** (fluorescence recovery after photobleaching) analysis coupled with confocal microscopy were carried out on CTRL and P_4-ReS spermatozoa to assess changes in **membrane fluidity.**
- Western blotting analysis were performed on sperm protein homogenates using **anti-Binder of Sperm Protein (BSP)-1, -3, -5** and Odyssey® Imager System for signal detection and quantification.

EXPERIMENTAL DESIGN



RESULTS

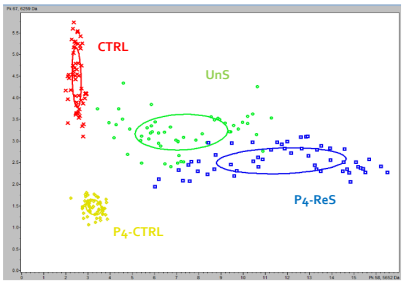


Figure 1. Principal Component Analysis. The 2 most different peaks are represented, showing the 4 different populations of spermatozoa (CTRL, P_4-CTRL, UnS and P_4-ReS).

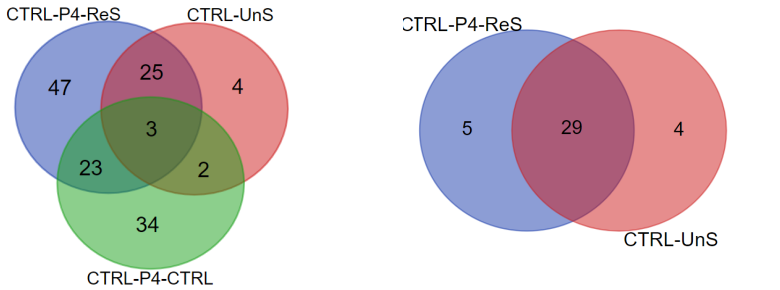


Figure 2. Proteomic and lipidomic analysis. 139 m/z (mass/charge) peaks were found as differential m/z on spermatozoa proteomic profiles (left) by ICM-MS. The number of differential peaks was highest between P_4-ReS and CTRL (94), followed by P_4-CTRL vs.CTRL (62), and UnS vs.CTRL (34). On the other hand, only 38 peaks were found as differential m/z on lipidomic profiles (right), all of them from P_4-ReS vs.CTRL (34) and UnS vs.CTRL (33) comparisons.

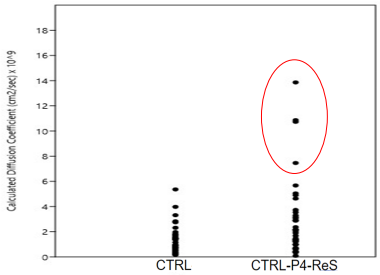


Figure 3. FRAP analysis. After staining with DilC12 and bleaching the alive sperm cells, the Calculated Diffusion Coefficient was measured. The results showed a higher membrane fluidity on P_4-ReS compared to CTRL (KW,p=0.014).

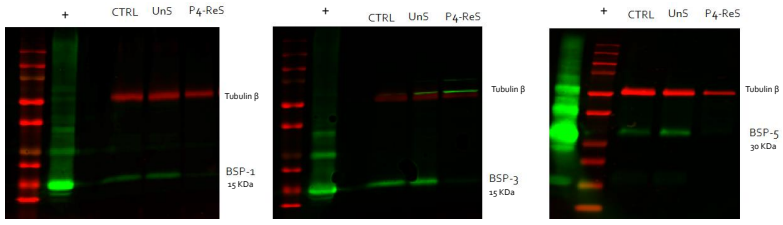


Figure 4. Western Blotting Analysis. Antibodies recognizing BSP-1, -3, -5 were used to study the abundance of these proteins in all the sperm groups and to detect the differences on bands intensity. A 3 to 4-fold decrease in the abundance of BSP-1, -3 and -5 was seen on P_4-ReS compared to CTRL ($p<0.05$), but not in UnS or P_4-CTRL groups (data not shown). (Positive control "+"= bovine seminal plasma proteins).

CONCLUSIONS

- ❑ After binding to BOEC, P_4-induced release from BOEC triggers major changes in sperm protein and lipid composition
- ❑ Unbound spermatozoa show moderate changes in protein and lipid compositions, suggesting a "BOEC effect" that may be due to a short time binding-release process
- ❑ Spermatozoa released by P_4 evidenced a loss of BSPs at their surface and an increase in membrane fluidity, suggesting a membrane destabilization probably involved in the increase of fertilizing competence of this sub-population

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