Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXVIII

From Osteoblasts to Spermatozoa: Exploring the role of cannabinoid receptor 1 (CB1)

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THE ENDOCANNABINOID SYSTEM

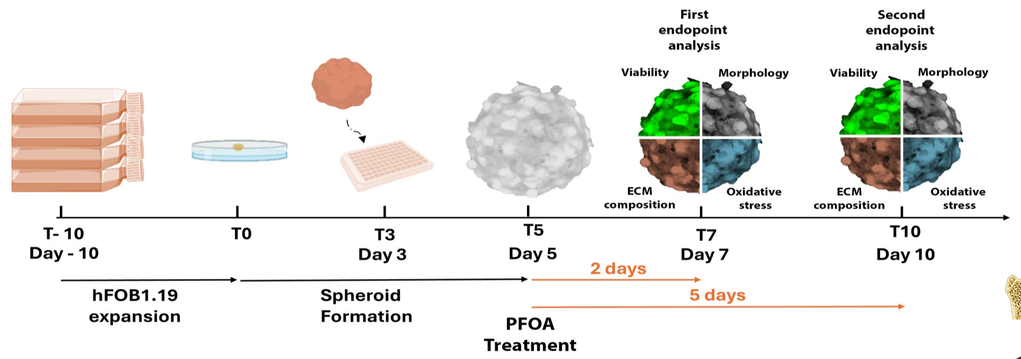
The endocannabinoid system (ECS) is a system composed of endocannabinoids (Anandamide, AEA and 2-arachidonoyl glycerol**,** 2AG), their receptors (cannabinoid receptor 1, CB1 and cannabinoid receptor 2, CB2), and the enzymes involved in their synthesis and degradation. It is present in bone where it plays a key role in regulating bone formation and turnover. ECS components are also found in the male reproductive system, from spermatogonia to spermatozoa, and in reproductive fluids and tracts. Despite its widespread presence and physiological relevance, much about ECS remains unknown.

BACKGROUND

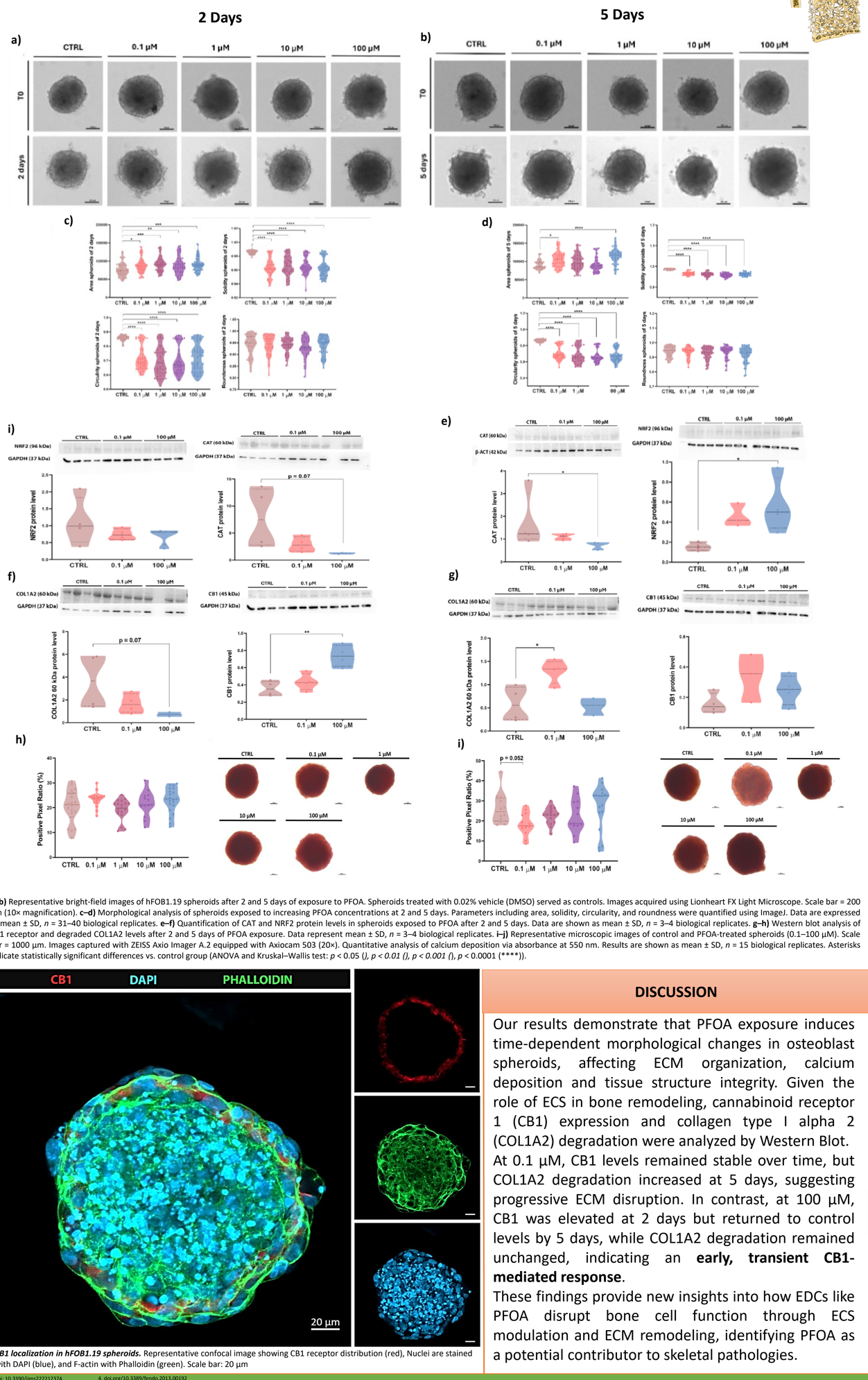
Emerging evidence supports a key role of ECS in bone homeostasis, influencing bone formation, remodeling and fracture healing [1-2]. Recently, the ECS has been identified as a target for endocrine-disrupting chemicals (EDCs)[3], such as perfluorooctanoic acid (PFOA), a widespread industrial contaminant. PFOA is associated with several health issues and is now under scrutiny for its potential to interfere with bone homeostasis.

This study aims to investigate the effects of PFOA on osteoblast differentiation using the human fetal osteoblast cell line (hFOB1.19) in 3D spheroid which better mimics the *in vivo* bone microenvironment compared to traditional 2D cultures. Especial focus is placed on the role of the endocannabinoid system (ECS) in mediating bone tissue responses to EDCs exposure.

EXPERIMENTAL DESIGN



RESULTS



a–b) Representative bright-field images of hFOB1.19 spheroids after 2 and 5 days of exposure to PFOA. Spheroids treated with 0.02% vehicle (DMSO) served as controls. Images acquired using Lionheart FX Light Microscope. Scale bar = 200 µm (10× magnification). **c–d)** Morphological analysis of spheroids exposed to increasing PFOA concentrations at 2 and 5 days. Parameters including area, solidity, circularity, and roundness were quantified using ImageJ. Data are expressed as mean ± SD, *n* = 31–40 biological replicates. **e–f)** Quantification of CAT and NRF2 protein levels in spheroids exposed to PFOA after 2 and 5 days. Data are shown as mean ± SD, *n* = 3–4 biological replicates. **g–h)** Western blot analysis of CB1 receptor and degraded COL1A2 levels after 2 and 5 days of PFOA exposure. Data represent mean ± SD, *n* = 3–4 biological replicates. **i–j)** Representative microscopic images of control and PFOA-treated spheroids (0.1–100 µM). Scale bar = 1000 µm. Images captured with ZEISS Axio Imager A.2 equipped with Axiocam 503 (20×). Quantitative analysis of calcium deposition via absorbance at 550 nm. Results are shown as mean ± SD, *n* = 15 biological replicates. Asterisks indicate statistically significant differences vs. control group (ANOVA and Kruskal–Wallis test: $p < 0.05$ (), $p < 0.01$ (), $p < 0.001$ (), $p < 0.0001$ (****)).

CB1 localization in hFOB1.19 spheroids. Representative confocal image showing CB1 receptor distribution (red), Nuclei are stained with DAPI (blue), and F-actin with Phalloidin (green). Scale bar: 20 µm

DISCUSSION

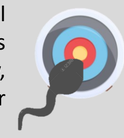
Our results demonstrate that PFOA exposure induces time-dependent morphological changes in osteoblast spheroids, affecting ECM organization, calcium deposition and tissue structure integrity. Given the role of ECS in bone remodeling, cannabinoid receptor 1 (CB1) expression and collagen type I alpha 2 (COL1A2) degradation were analyzed by Western Blot.

At 0.1 µM, CB1 levels remained stable over time, but COL1A2 degradation increased at 5 days, suggesting progressive ECM disruption. In contrast, at 100 µM, CB1 was elevated at 2 days but returned to control levels by 5 days, while COL1A2 degradation remained unchanged, indicating an **early, transient CB1-mediated response**.

These findings provide new insights into how EDCs like PFOA disrupt bone cell function through ECS modulation and ECM remodeling, identifying PFOA as a potential contributor to skeletal pathologies.

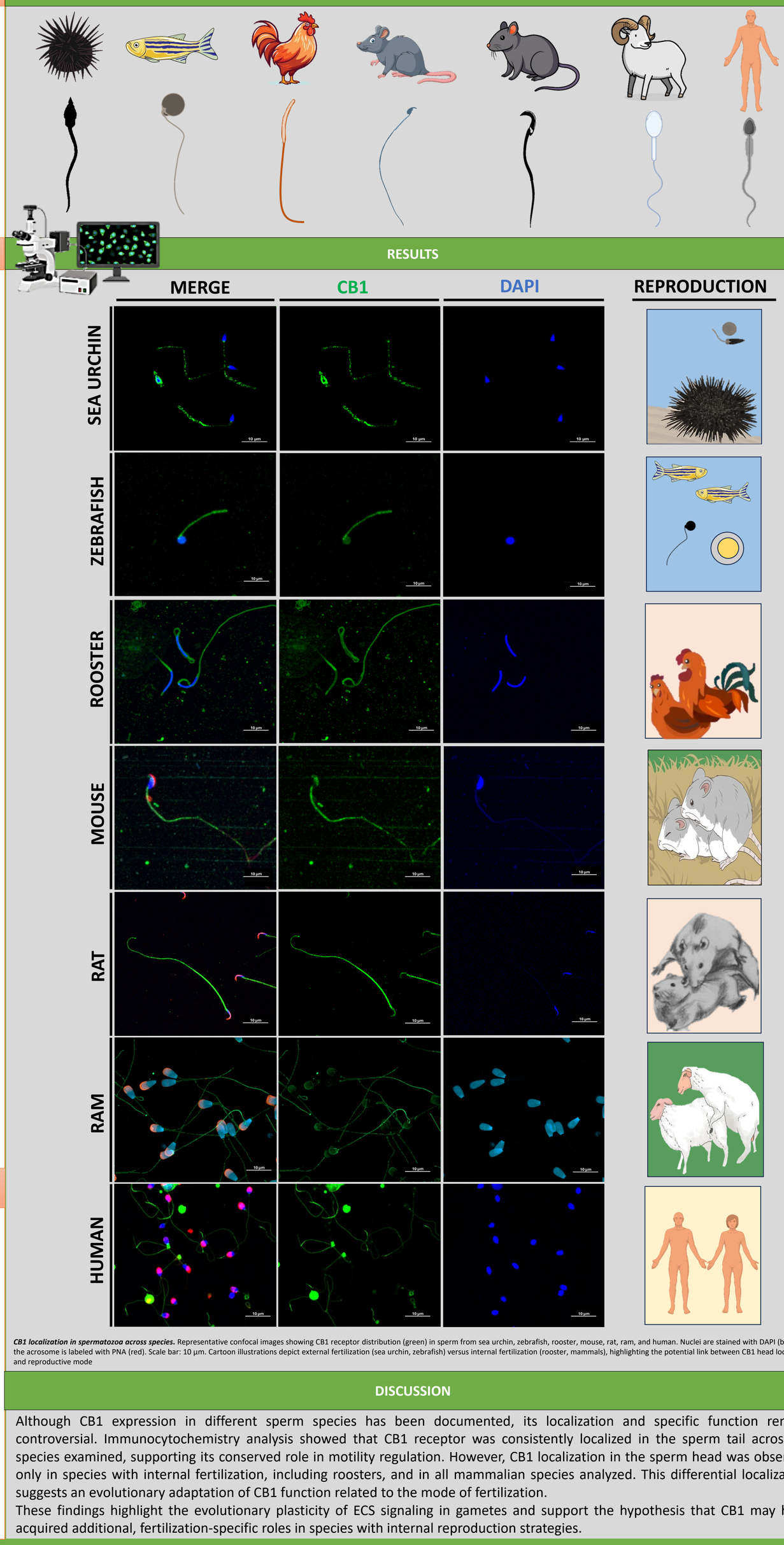
BACKROUND AND AIM

The endocannabinoid system (ECS) is an evolutionarily conserved signaling network that regulates key physiological processes across the animal kingdom, including reproduction, cell communication, and motility control [4-5]. Among its components, cannabinoid receptor 1 (CB1) plays a central role in sperm function, influencing events such as motility, capacitation, and fertilization [6]. Given the wide distribution of the ECS in animals, from Cnidaria to mammals, except for the insects, it is essential to explore how CB1 localization may reflect species-specific reproductive strategies.



The aim of this study is to investigate the evolutionary localization pattern of cannabinoid receptor 1 (CB1) in spermatozoa from vertebrates (mammals, birds, fish) to invertebrates (sea urchins), in order to clarify the conserved and divergent roles of ECS signaling in sperm architecture across phyla.

EXPERIMENTAL MODEL



CB1 localization in spermatozoa across species. Representative confocal images showing CB1 receptor distribution (green) in sperm from sea urchin, zebrafish, rooster, mouse, rat, ram, and human. Nuclei are stained with DAPI (blue), and the acrosome is labeled with PNA (red). Scale bar: 10 µm. Cartoon illustrations depict external fertilization (sea urchin, zebrafish) versus internal fertilization (rooster, mammals), highlighting the potential link between CB1 head localization and reproductive mode

DISCUSSION

Although CB1 expression in different sperm species has been documented, its localization and specific function remain controversial. Immunocytochemistry analysis showed that CB1 receptor was consistently localized in the sperm tail across all species examined, supporting its conserved role in motility regulation. However, CB1 localization in the sperm head was observed only in species with internal fertilization, including roosters, and in all mammalian species analyzed. This differential localization suggests an evolutionary adaptation of CB1 function related to the mode of fertilization.

These findings highlight the evolutionary plasticity of ECS signaling in gametes and support the hypothesis that CB1 may have acquired additional, fertilization-specific roles in species with internal reproduction strategies.

1. doi: 10.3390/ijms222212374
2. doi.org/10.3389/fendo.2012.00136
3. doi.org/10.1016/j.reprotox.2024.108822
4. doi.org/10.3389/fendo.2013.00192
5. doi.org/10.1093/humrep/det232
6. 10.1016/j.mce.2011.01.022