STRESS REDUCES SPERMATOZOA FUNCTIONALITY THROUGH ADRENERGIC-MEDIATED DISTURBANCE OF MITOCHONDRIAL DYNAMICS MARKERS

Isidora M Starovlah, Sava M Radovic Pletikosic, Tatjana S Kostic, Silvana A Andric

Laboratory for Reproductive Endocrinology and Signaling, Laboratory for Chronobiology and Aging, CeRES, DBE, Faculty of Sciences
University of Novi Sad, Novi Sad, Serbia

INTRODUCTION:
A growing body of evidence, states the increasing rate of male infertility in humans, an increasing number of unexplained cases of infertile males in the peak of the reproductive period, and a decrease of the fertility rate in men younger than age 30. Many studies discussed the correlation between stress and/or stressful life and male (in/sub)fertility. Yet, the mechanisms are not described.
OBJECTIVES:
Here we investigate the stress-signaling responsible for the effects of acute/repeated psychological stresses (the most common stresses in human society) on spermatozoa number and functionality, as well as the transcriptional profile of mitochondrial dynamics markers by using the in vivo and ex vivo approaches.
METHOD / DESIGN:
In the search for the possible mechanism(s) causing the reduced spermatozoa functionality during/after psychological stress, two approaches (*in vivo* and *ex vivo*) were applied. The *in vivo* approach was designed to mimic the situations in the human population exposed to acute as well as repeated psychological stress, the most common stress in human society, by using the immobilization of the adult male rats. The *ex vivo* approach was performed on epididymal spermatozoa isolated from the undisturbed adult male rats and exposed to stress hormones, adrenaline and hydrocortisone, and the agonists/antagonists of their receptors.
RESULTS:
Acute and repeated stress inhibit spermatozoa functionality (acute–>3.2-fold, repeated–>2.5-fold), while only repeated stress reduces the spermatozoa number (1.7-fold). Stress hormones mimic these effects and decrease the spermatozoa functionality (adrenaline: 10 µM–>2.4-fold, 100 µM–>2.8-fold; hydrocortisone: 50 pM–>2.7-fold, 500 pM–>8.5-fold). They also significantly disturb the transcriptional profile of all main mitochondrial dynamics markers in spermatozoa. *Ex vivo* manipulation of stress signaling in spermatozoa reveals that most of these effects are mediated through α1-and/or-β-adrenergic receptors.
CONCLUSIONS:
Stress-hormones-triggered changes in the transcriptional profile of mitochondrial dynamics markers, as well as adrenergic receptors and adrenergic receptors kinases are important molecular markers of spermatozoa functionality representing an adaptive mechanism regulated by stress signaling and does not only correlate-with but also are essential for spermatozoa functionality, being all events depend on the same regulators. The stress mimetics disturb (mostly increase) sixteen out of nineteen mitochondrial dynamics markers in spermatozoa with adrenergic signaling being more effective, suggesting the importance of these spermatozoa markers in response on high energy demand during stress. Accordingly, the above mentioned molecular markers can be used as a test for spermatozoa functionality and for a better understanding of the correlation between stress as well as any other life-style-environmental-one-health-factors and male (in)fertility.

KEYWORDS: acute/repeated psychological stress; mitochondrial biogenesis markers; mitochondrial fusion/architecture markers; mitochondrial fission markers; mitophagy; spermatozoa functionality.

Results

The functionality of spermatozoa decreases after *in vivo* psychophysical stress and *ex vivo* stimulation of spermatozoa with stress hormones adrenaline and hydrocortisone

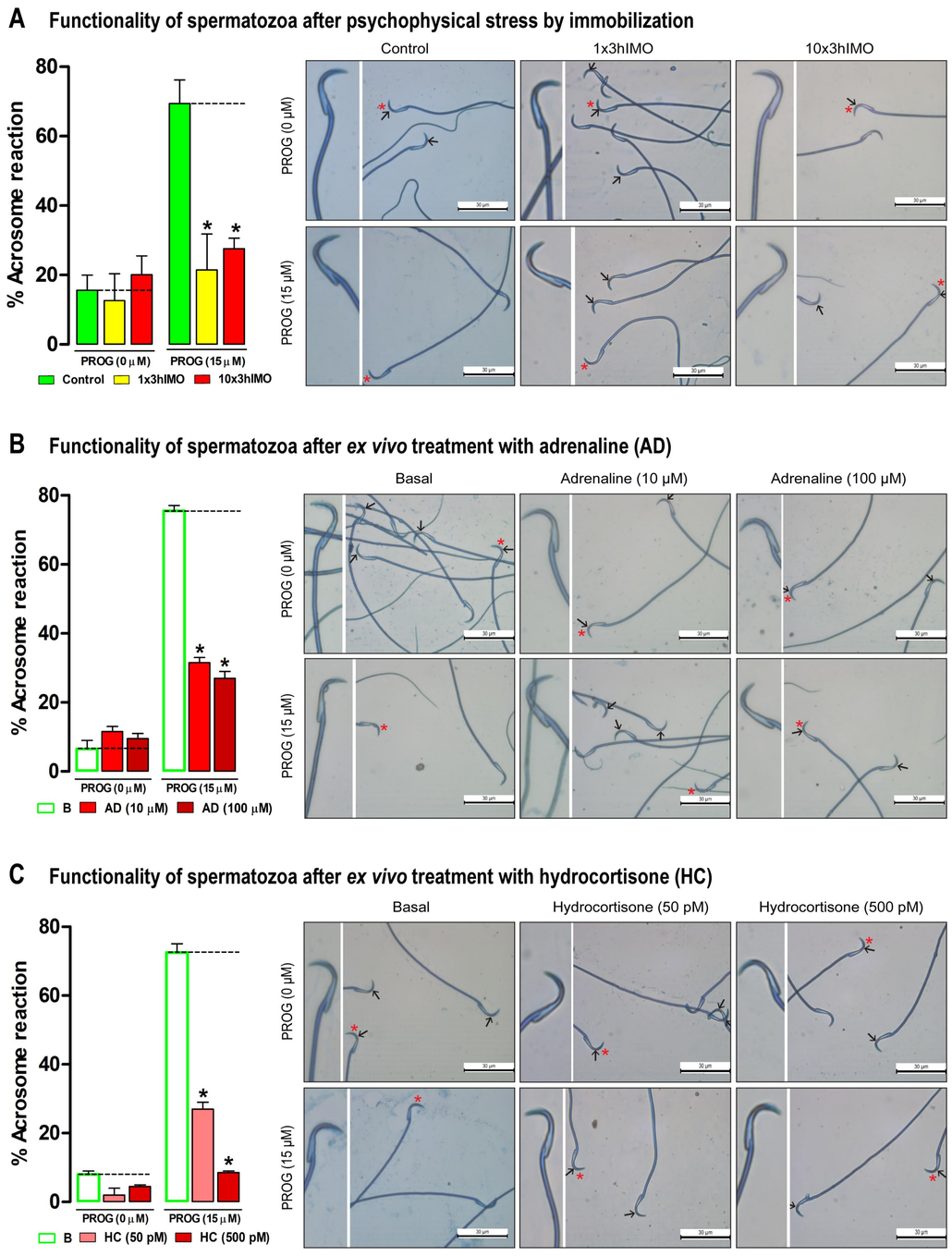


Figure 2. The functionality of spermatozoa (% acrosome reacted spermatozoa) after psychophysical stress by immobilization **(a)**, or ***ex vivo*** treatment for 30 min with adrenaline - AD **(b)** or hydrocortisone - HC **(c)**. Capacitated spermatozoa were stimulated with progesterone (PROG 15 µM) in parallel with spermatozoa not treated with progesterone (PROG 0 µM). Blue staining in the acrosome region of the head indicated intact acrosome, whereas spermatozoa without blue staining in the acrosome region were considered to be acrosome reacted. Arrows indicate acrosome intact spermatozoa; scale bar 30 µm. Star indicate spermatozoa magnified on the right panel. Data are presented as the percentage of acrosome reacted spermatozoa ± SEM values of four independent *in vivo* experiments and individual isolation and capacitation/acrosome-reaction-processing of spermatozoa from each rat (please see the number of rats in the brackets), while for *ex vivo* experiments SEM values of three independent experiments involving six rats per experiment (eighteen in total). Statistical significance was set at level p <0.05: * vs. control group (a - *in vivo*); or basal group (b, c – *ex vivo*).

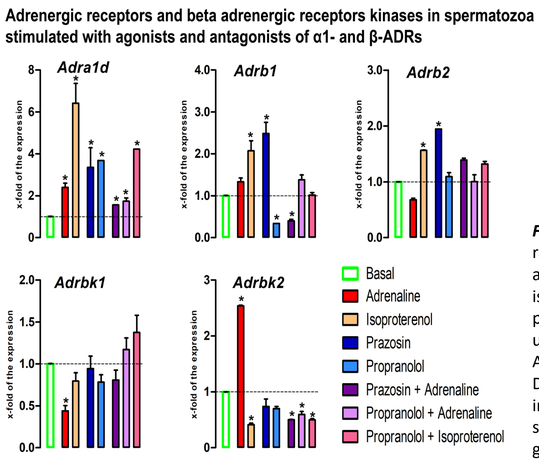
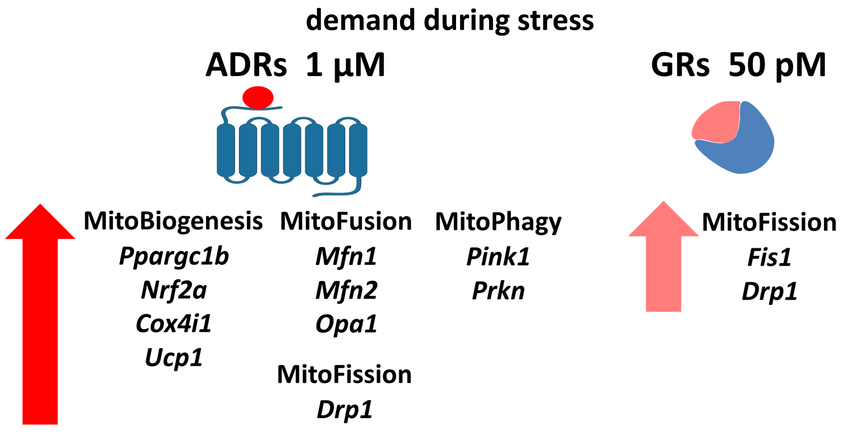


Figure 7. Spermatozoa isolated from undisturbed rats were treated *ex vivo*, for 6 hrs, either with adrenergic receptors (ADRs) agonists (adrenaline, isoproterenol) or/and antagonists (α1-ADRs agonist prazosin; β-ADRs agonist propranolol). The RNA was used for analyses of the transcriptional profile of ADRs and β-ADRs kinases.
Data bars are mean ± SEM values of three independent *ex vivo* experiments. Statistical significance was set at level p <0.05: * vs. basal group.

Conclusion

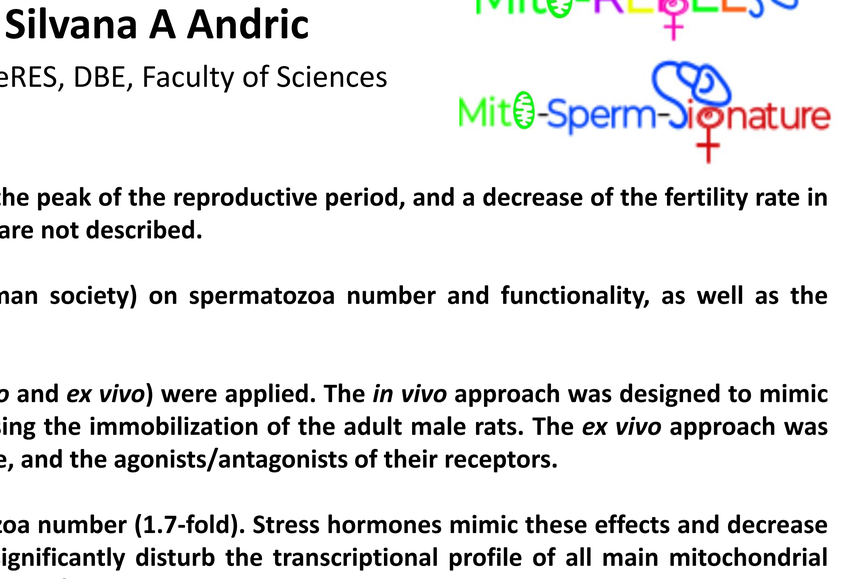
The stress mimetics disturb (mostly increase) sixteen out of nineteen mitochondrial dynamics markers in spermatozoa with adrenergic signaling being more effective, suggesting the importance of these spermatozoa markers in response on high energy demand during stress



Contact: *silvana.andric@dbe.uns.ac.rs*, *isidora.starovlah@dbe.uns.ac.rs*

This work was supported by the grant no. 451-03-904/2021 for Centre of Excellence CeRES and grant no. 451-03-9/2021-14/ 200125, both from the Ministry of Education, Science and Technological Development Republic of Serbia.

Experimental design



Scheme 1. Experimental design used to asses levels of hormones, spermatozoa number and functionality as well as transcriptional profiles of mitochondrial dynamics markers and related signaling molecules.

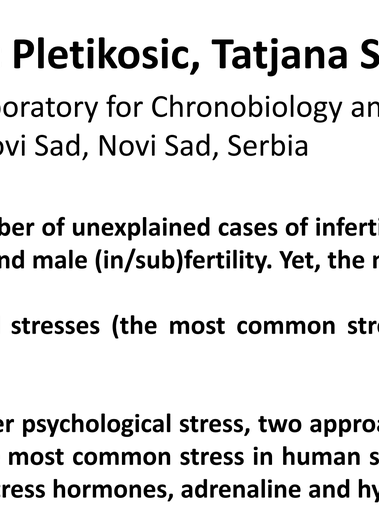


Figure 1. The circulating (serum) levels of (a) stress hormones adrenaline and corticosterone (CORT), as well as, (b) androgens (testosterone+dihydrotestosterone, T+DHT) and number of spermatozoa isolated from caudal epididymides (c) after psychophysical stress by immobilization.

The stress hormones change the transcriptional profile of mitochondrial dynamics markers in spermatozoa

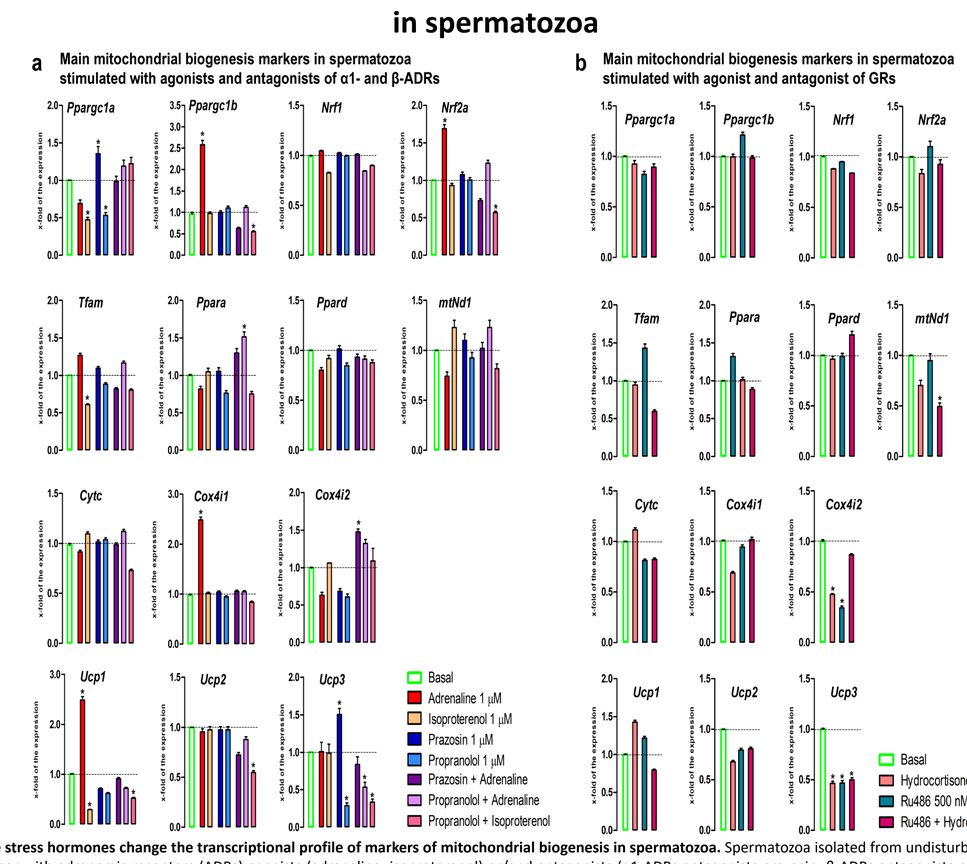


Figure 3. **The stress hormones change the transcriptional profile of markers of mitochondrial biogenesis in spermatozoa.** Spermatozoa isolated from undisturbed rats were treated ex vivo, for 6 hrs, either with adrenergic receptors (ADRs) agonists (adrenaline, isoproterenol) or/and antagonists (α1-ADRs antagonists prazosin; β-ADRs antagonists propranolol) or glucocorticoid receptors (GRs) agonist (hydrocortisone) or/and antagonist (RU486). The RNA was used for analyses of the transcriptional profile of mitochondrial biogenesis markers (a, b). Data bars are mean ± SEM values of three independent ex vivo experiments involving six rats per experiment (eighteen in total). Statistical significance was set at level p <0.05: * vs. basal group.

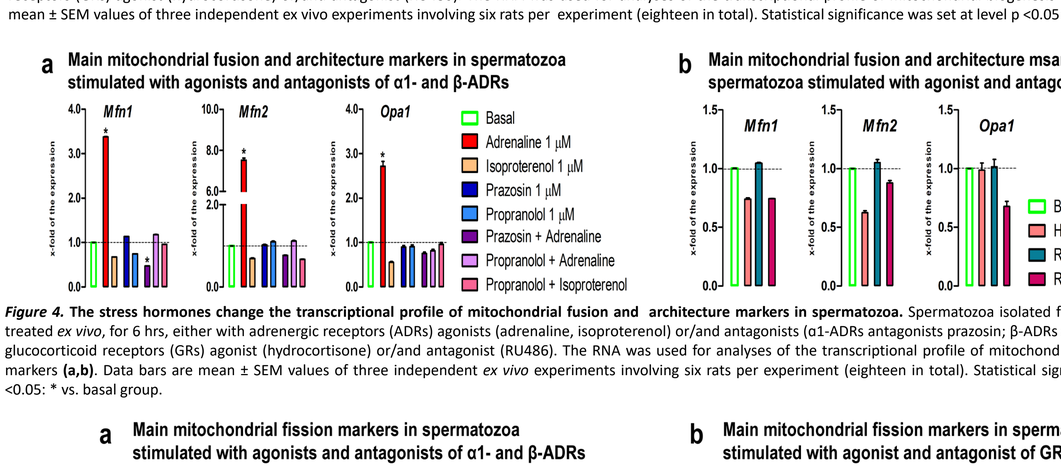


Figure 4. **The stress hormones change the transcriptional profile of mitochondrial fusion and architecture markers in spermatozoa.** Spermatozoa isolated from undisturbed rats were treated *ex vivo*, for 6 hrs, either with adrenergic receptors (ADRs) agonists (adrenaline, isoproterenol) or/and antagonists (α1-ADRs antagonists prazosin; β-ADRs antagonists propranolol) or glucocorticoid receptors (GRs) agonist (hydrocortisone) or/and antagonist (RU486). The RNA was used for analyses of the transcriptional profile of mitochondrial fusion and architecture markers **(a,b)**. Data bars are mean ± SEM values of three independent *ex vivo* experiments involving six rats per experiment (eighteen in total). Statistical significance was set at level p <0.05: * vs. basal group.

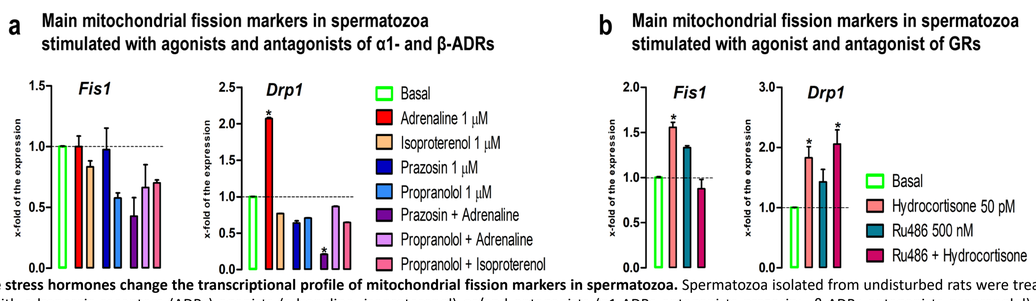


Figure 5. **The stress hormones change the transcriptional profile of mitochondrial fission markers in spermatozoa.** Spermatozoa isolated from undisturbed rats were treated ex vivo, for 6 hrs, either with adrenergic receptors (ADRs) agonists (adrenaline, isoproterenol) or/and antagonists (α1-ADRs antagonists prazosin; β-ADRs antagonists propranolol) or glucocorticoid receptors (GRs) agonist (hydrocortisone) or/and antagonist (RU486). The RNA was used for analyses of the transcriptional profile of mitochondrial fission markers (a, b). Data bars are mean ± SEM values of three independent ex vivo experiments involving six rats per experiment (eighteen in total). Statistical significance was set at level p <0.05: * vs. basal group.

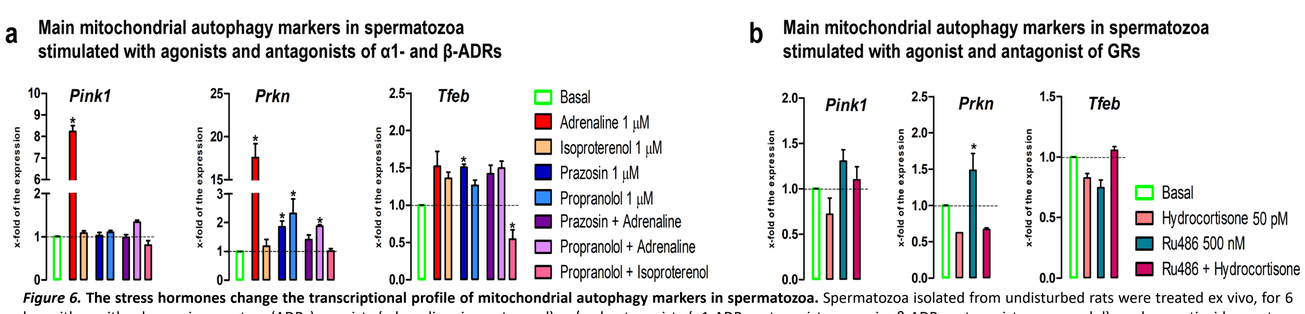


Figure 6. **The stress hormones change the transcriptional profile of mitochondrial autophagy markers in spermatozoa.** Spermatozoa isolated from undisturbed rats were treated ex vivo, for 6 hrs, either with adrenergic receptors (ADRs) agonists (adrenaline, isoproterenol) or/and antagonists (α1-ADRs antagonists prazosin; β-ADRs antagonists propranolol) or glucocorticoid receptors (GRs) agonist (hydrocortisone) or/and antagonist (RU486). The RNA was used for analyses of the transcriptional profile of mitochondrial autophagy markers (a, b). Data bars are mean ± SEM values of three independent ex vivo experiments involving six rats per experiment (eighteen in total). Statistical significance was set at level p <0.05: * vs. basal group.