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Glucocorticoids improve sperm performance in physiological and pathological conditions: their role in sperm fight/flight response

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Abstract: Glucocorticoids play a physiologic role in the adult male reproductive functions, modulating gonadal steroid synthesis and spermatogenesis, through the glucocorticoid receptor (GR). The expression of GR has been described in several key testicular cell types, including somatic cells and early germ cell populations. Nothing is known on GR in human spermatozoa. Herein, we explored the GR expression and its possible role in normal and testicular varicocele semen samples from volunteer donors. After semen parameter evaluation by macro- and microscopic analysis, samples were centrifuged; then spermatozoa and culture media were recovered for further investigations. By western blotting and immunofluorescence analyses we evidenced for the first time in spermatozoa the presence of GR-D3 isoform which was reduced in sperm from varicocele patients. By treating sperm with the synthetic glucocorticoid dexamethasone (DEXA), we found that survival, motility, capacitation, and acrosome reaction were increased in both healthy and varicocele samples. GR involvement in mediating DEXA effects, was confirmed by using the GR inhibitor mifepristone (M2F). Worthy, we also discovered that sperm secretes different cortisol amounts depending on its physio-pathological status, suggesting a defence mechanism to escape the immune system attach in the female genital tract thus maintaining the immune-privilege as in the testis. Collectively, our data suggests a role for glucocorticoids in determining semen quality and function, as well as in participating on sperm immune defensive mechanisms. The novelty of this study may be beneficial and needs to take into account in artificial insemination/drug discovery aimed to enhancing sperm quality.

Key words: Human sperm, Varicocele, Glucocorticoid receptor, Male infertility

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Introduction

Glucocorticoids are steroid hormones produced and secreted by the cortex of adrenal glands. These hormones play critical roles in several physiological processes, including immune/inflammatory responses, stress-related homeostasis and reproduction [1]. Most actions of glucocorticoid are mediated by the glucocorticoid receptor (GR, also known as nuclear receptor subfamily 3, group C, member 1 or NR3C1), a member of the nuclear hormone receptor superfamily of ligand-activated transcription factors [2]. The consequences of GR activation for cell function are highly dependent on the tissue, cell and target gene analyzed [3]. Multiple GR isoforms, have been identified in different species, playing an active role in glucocorticoid biology [4]. These isoforms include GRα-A (94 kDa) and GRα-B (91 kDa), GRα C1-C3

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(82–84 kDa) and GRα D1-D3 (53–56 kDa) [5, 6]. Interestingly, the GRα-C isoforms are the most biologically active, while the GRα-D isoforms are the most deficient in glucocorticoid mediated functions. Pancreas and colon have the highest amount of the GR-C isoforms, whereas spleen and lungs have the highest amount of the GR-D isoforms [7].

The GR-D3 (a representative of the D1, D2, and D3) isoform-expressing cells even though not sensitive to glucocorticoid killing, are also capable of undergoing apoptosis [8].

GR may also act via non-genomic mechanisms to elicit rapid cellular responses that occur within a few seconds to minutes and do not require changes in gene expression [9]. Multiple mechanisms appear to be involved in these signaling events that ultimately impinge on the activity of various kinases, such as PI3-kinase (PI3K), AKT, protein kinases (MAPKs), and SAP/c-jun N-terminal kinase (JNK) [8].

Detectable GR expression has been described in several key testicular cell types, including the somatic Sertoli, Leydig, and peritubular cells, as well as early germ cell populations [10-12]. The specific role of GR within most of these cell types remains unknown. Studies in rats [13], boars [14], equine [15] and humans [16] have shown that glucocorticoids may influence testicular function. At physiological levels, glucocorticoids play a critical role in testicular morphogenesis [17], onset and maintenance of spermatogenesis [17, 18] and erectile function [19]. Increased plasma levels of glucocorticoids induced by stress and a long-term glucocorticoid therapy has been related with impairment of reproductive function [20].

Varicocele, the leading cause of male infertility, can impair spermatogenesis through several pathophysiological mechanisms, including increased testicular temperature [21]. In particular, the augmented testicular temperature caused by varicocele may damage Leydig and Sertoli cell's function, impaire sex hormone production, as well as determine changes in microenvironment concentration of renal and adrenal metabolites [22]. Moreover, in patients with varicocele, most studies have revealed an abnormal increase in the levels of pro-inflammatory cytokines in the seminal plasma and testicular tissue [23]. The stress-activated SAPK/JNK Thr183/Tyr185 (JNK) mediates many of the effects of cellular stress associated with inflammation as it occurs in varicocele patients [23].

Considering the involvement of glucocorticoids in testicular function, in this work we aimed to investigate the presence of GR in human ejaculated spermatozoa and to evaluate whether differences of its expression, between normal and varicocele patients, occur. Moreover, the ability of GR to influence the main features of the male gamete such as survival, motility, capacitation, and acrosome reaction, as well as the potential role for GR in sperm fight or flight response in both samples types, were studied.

Materials and Methods

Chemicals

Acrylamide/bisacrylamide (A6050), Triton X-100 (T8787), eosin Y (E4009), bovine serum albumin (810533), Laemmli sample buffer (S3401), prestained molecular weight markers (SDS7B2), dimethylsulfoxide (D8418), Earle's balanced salt solution (EBSS) (E2888), Hepes sodium salt (C-40020), synthetic glucocorticoid dexamethasone (DEXA) (D2915), GR inhibitor, mifepristone (M2F) (M8046) [24], Cholesterol quantitation kit (MAK043), and all other chemicals were from Merck Life Science. Bradford Protein Assay kit (5000201) was from Bio-Rad Laboratories. Clarity Wester enhanced chemiluminescence (ECL) Substrate (1705061) was purchases from Bio-Rad Laboratories, Amersham Hybond ECL Nitrocellulose Membrane (RPN303D) was from VWR International. GR (G-5 sc-393232), p-Akt (B-12: sc-377556) and β-actin (C-4, sc-47778) antibodies were from Santa Cruz Biotechnology (DBA). p-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (Cell Signaling# 9101) and anti p-SAPK/JNK (Thr183/Tyr185) (Cell Signaling# 9251) antibodies were from Euroclone. R&D Systems® cortisol immunoassay (cat # KGE008B) was from Bio-Techne SRL.

Semen samples and spermatozoa processing

Normal and varicocele semen samples were collected according to the World Health Organization [25] from healthy volunteer donors. Varicocele sperm samples were from patients with diagnosed varicocele of grade III (visible without palpation) on the left testis. Ejaculates were chosen to have similar parameters 16×10^6/ml of sperm cells, progressive motility >32%, normally formed features >5% and survival of 75% both for normal and varicocele patients. Importantly, samples with the presence of leucocytes, round cells, eritrocytes were excluded by the study. The study has been approved by the Ethical-Comittee of the University of Calabria (CEA) with protocol n° 6774 of 11 December 2020. The approval of the ethics committee was obtained by following the procedures governing people experimentation. All patients were trained on the project and signed informed consent before taking the exam.

After liquefaction, in each sample three different ejaculates were pooled, to compensate for individual variation, both for normozoospermic and varicocele patients. 30 normozoospermic ejaculates and 57 patients with varicocele were used, forming 10 normozoospermic and 19 varicocele samples. After then each sample was purified and recovered by the swim-up method [25]. The upper fraction was examined using an optical microscope to ensure that a pure sample of sperm was obtained. In the examination of semen samples three different technicians assessed all the parameters. Only standard dilutions were used (1:10, 1:20, 1:50). Sperm concentration, obtained counting at least 200 spermatozoa, was assessed using haemocytometers with improved Neubauer ruling and an optic microscope (200–400×).

For all experiments in this study, a concentration of 1×10^7 cells/tube were resuspended with un-supplemented EBSS medium to reach a volume of 0.5 ml/tube. The tubes were untreated and used as a control (–) or exposed to a specific amount of the relative drug, according to the experimental design. After then, the samples were incubated for 30 minutes at 37°C and 5% CO_2. 1 nM and 100 nM DEXA, were chosen respectively as a low and a high concentration of DEXA; furthermore, some samples were incubated with 5 μM M2F alone or combined with 100 nM DEXA. In the acrosome reaction assay also capacitated samples were used as positive control. After the swim-up, the purified human spermatozoa were then diluted in EBSS supplemented on the day of use with 3 mg/100 ml sodium pyruvate, 10 mM/100 ml $NaHCO_3$ and 0.37 ml/100 ml of 60% (v/v) sodium lactate syrup, and antibiotics.

Western blot analysis of sperm proteins

Sperm samples were centrifuged at 5,000 g for 5 minutes, later the pellets were resuspended in lysis buffer (62.5 mmol/L Tris-HCl, pH 6.8; 150 mm NaCl; 2% SDS; 1% Triton X-100; 10% glycerol; 1 mm phenylmethylsulfonylfluoride (PMSF); 10 μg/ml leupeptin; 10 μg/ml aprotinin; 2 μg/ml pepstatin), then centrifuged to obtain sperm proteins. Protein concentration was determined by Bradford Protein Assay. 70 μg of proteins were boiled for 5 minutes, separated on an 11% polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes and probed with an appropriate dilution of the indicated primary antibody. The binding of the secondary antibody was revealed with the Clarity Wester ECL Substrate, according to the manufacturer's instructions. The specificity of anti-GR antibody was tested by pre-absorption of primary antibody with an excess of the blocking peptide at 4°C (negative control, data not shown). β-Actin served as a control for equal loading. Specifically, the intensity of the p-AKT, p-MAPK and p-SAPK/JNK bands were normalized to the intensity of the actin band by densitometric analysis using ImageJ 1.53e software. p-MAPK intensity was analyzed only for the 42 kDa band.

Immunofluorescence labelling of GR

Sperm cells were rinsed three times with 0.5 mM Tris–HCl buffer, pH 7.5 and allowed to settle onto slides in a humid chamber. The overlying solution was carefully pipetted off and replaced by absolute methanol for 7 minutes at –20°C. After methanol removal, sperm cells were washed in Tris-buffered saline (TBS) containing 0.1% Triton X-100 and were treated for immunofluorescence. The anti-human GR (1:200) was utilized as primary antibody and the anti-rabbit FITC conjugated IgG (1:80) as secondary antibody, the slides were counterstaining with DAPI. The specificity of anti-GR antibody was tested by pre-absorption controls [26, 27]. The slides were examined with an epifluorescence microscope (Olympus BX41) and the images were taken with CSV1.14 software, using a CAM XC-30 for image acquisition, observing a minimum of 200 spermatozoa for nine slides.

Evaluation of sperm motility and survival

Sperm motility and survival were assessed by means of light microscopy examining an aliquot of each sperm sample, which had been incubated in the absence (–) or in the presence of the indicated treatments. Sperm motility was expressed as percentage of total motile sperm including the rapid progressive (PR) plus slow progressive (NP) sperm (normal values: PR+NP>42% as reported by World Health Organization 2021 [25]. Survival was assessed by red-eosin exclusion test using eosin Y scoring 200 cells for stain uptake (dead cells) or exclusion (live cells). Sperm survival was expressed as percentage of total live sperm. Motility and survival were evaluated before and after pooling the samples and there were no adverse effects among the different treatments on human sperm survival [28, 29].

Measurement of cholesterol in the sperm culture medium

Cholesterol was measured in duplicate by an enzymatic

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colorimetric method according to manufacturer's instructions in the incubation medium from human spermatozoa. Sperm samples were washed twice with un-capacitating medium, and incubated for 30 minutes at 37°C and 5% CO_2. Thereafter, the culture media were recovered by centrifugation, lyophilized and dissolved in 200 L of cholesterol assay buffer reaction. The reaction mixes were added to plate wells and incubated for 60 minutes at 37°C in the dark, then the cholesterol content was measured at 570 nm. The concentration of cortisol in the samples, was calculated by a calibration curve calibration curve obtained from standard concentrations. Cholesterol results are presented as mg per 1×10^7 number of spermatozoa.

Acrosome reaction

The FITC-PNA analysis was used to assess the acrosome reaction [30]. The slides were immediately evaluated, according to a published scoring system [31], by Olympus BX41 microscope. For each treatment, were examined a minimum of 200 live sperm, and successively classified into two categories on the basis of the staining: 1) spermatozoa brilliant green stained were classified as live acrosome-reacted cells; 2) spermatozoa without any fluorescence were considered as acrosome-non-reacted live cells; 3) spermatozoa stained with PI were considered as dead cells. Values are expressed as percentage of acrosome-reacted cells.

Determination of cortisol in the sperm culture medium

Cortisol was determined by an enzyme immunoassay based on the competitive binding for sites on mouse monoclonal antibodies to the cortisol present in the culture medium of spermatozoa and a fixed amount of horseradish peroxidase-labeled cortisol. Spermatozoa were washed twice and incubated in un-capacitating medium for 30 minutes at 37°C and 5% CO_2. Thereafter, the culture media were recovered by centrifugation, diluted 1:10 and add to the plate in the appropriate wells, following all the instructions indicated by manufacturer. After incubation at 37°C for 2 hours on a horizontal orbital microplate shaker (0.12" orbit) set at 500±50 rpm, wells were washed whit the appropriate buffer and the plate was incubated with substrate solution for further 30 minutes at T.A, in the dark. Finally, 50 μl of stop solution were added to each well and the optical density was measured, using a microplate reader set to 450 nm. The color intensity is inversely proportional to the concentration of cortisol in the samples, calculated by a calibration curve calibration curve obtained from standard concentrations. Values are expressed as pg per 1×10^7 number of spermatozoa.

Statistical analysis

All data were presented as the mean±SEM. The differences in mean values were calculated by the one-way analysis of variance (ANOVA). The Wilcoxson test was used after ANOVA as *post-hoc* test.

Results

In human ejaculated spermatozoa the only GR-D3 isoform of GR is expressed

By using western blot analysis, we first verified the expression of GR in ejaculated sperm from normozoospermic and patients affected by varicocele. In both normozoospermic and varicocele samples, we detected only one band at 55 kDa, corresponding to the GR-D3 isoform of GR (Fig. 1) [5-8]. Of note, in MCF7 cells, used as control two bands at 91–94 kDa were detected [32], while the band 55 kDa was not present, indicating the sperm specificity for GR-D3 (Fig. 1).

Compartmentalization of a molecule in human sperm, may be indicative of its function, since sperm have a very little amount of cytoplasm, then it is not possible the translocation of a molecule from the head to the tail. The tail is the region where metabolism and motility happen, while the head is important for capacitation and acrosome Immunofluorescence assay, performed with the same antibody

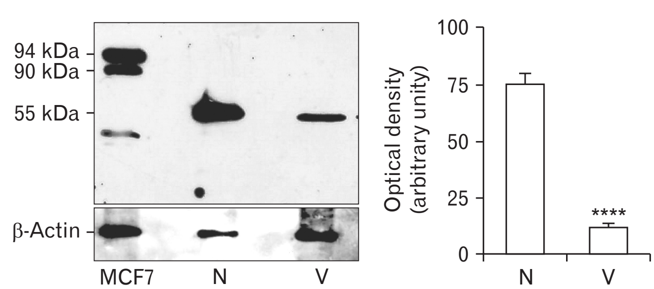

Fig. 1. GR-D3 is present in human ejaculated spermatozoa. Western blot of GR-D3 protein in human sperm from healthy and varicocele patients. MCF-7 extract was used as expression control. N: sperm lysate of tree pooled ejaculates from healthy men; V: sperm lysate of tree pooled ejaculates from patients with varicocele. The experiments were repeated at least four times and the blot shows the results of one representative assay. β-actin was used as loading control. The optical density of the GR-D3 band in N and V samples is reported on the right. GR, glucocorticoid receptor; N, normozoospermic; V, varicocele. ****P<0.001.

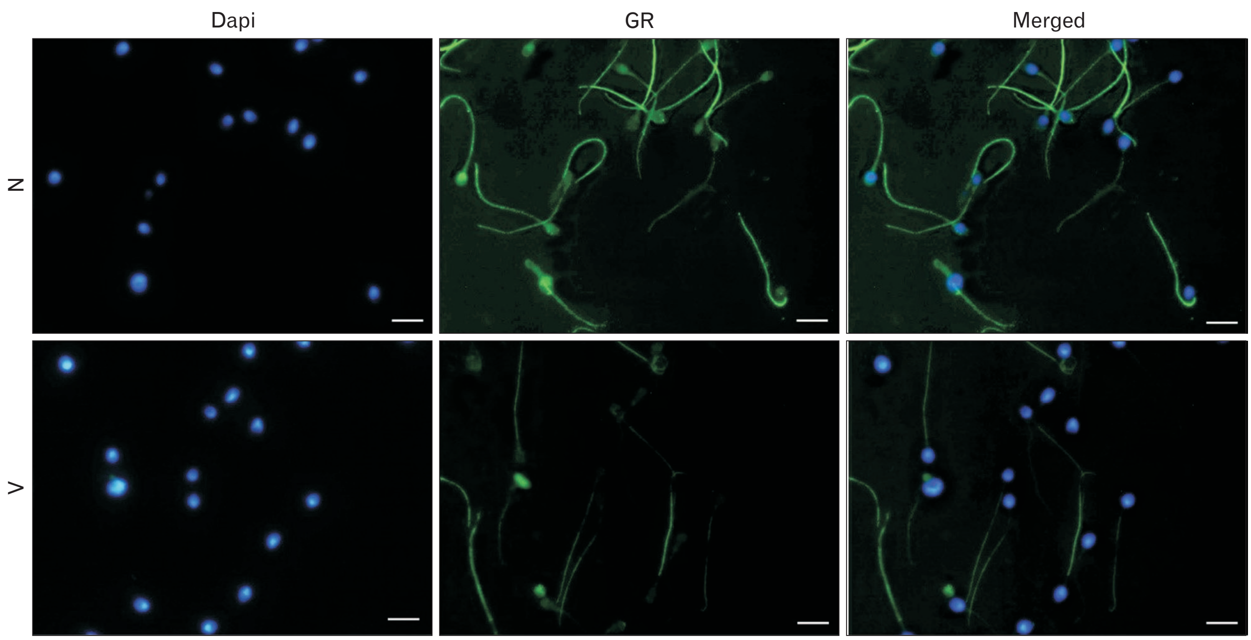

Fig. 2. Compartimentalization of GR-D3 in human spermatozoa from healthy and varicocele patients. Representative images of immunofluorescence assay in human spermatozoa by using the GR primary antibody (green). Nuclei were counterstained with DAPI (blue). N: sperm of three pooled ejaculates from healthy men; V: sperm of tree pooled ejaculates from patients with varicocele grade III. Scale bars: 12,5 μm. varicocele; N, normozoospermic; V, varicocele.

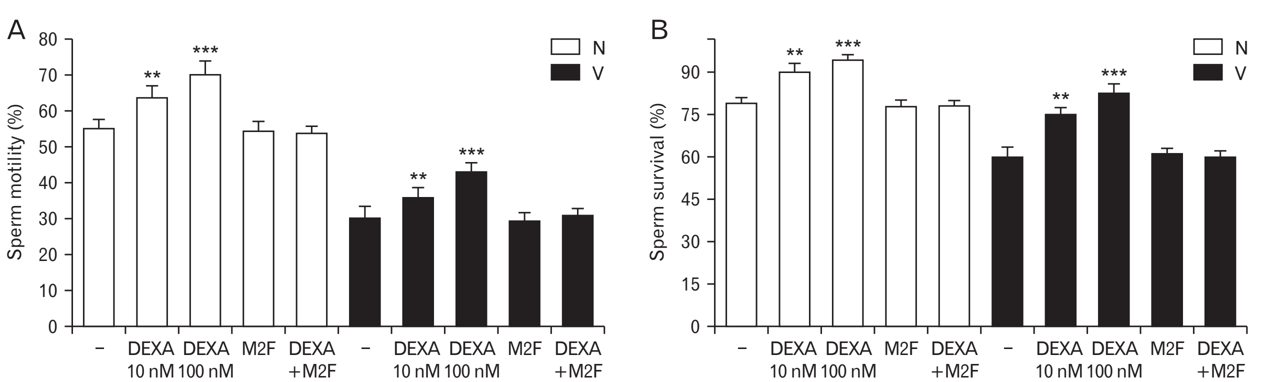

Fig. 3. DEXA induce sperm motility and survival. Sperm motility (A) and survival (B) expressed as percentage of total motile and survival sperm. Columns indicate the mean±SEM of six independent experiments performed in duplicate. N, normozoospermic; V, varicocele; DEXA, dexamethasone. $^{**}P<0.05$ and $^{***}P<0.01$ vs. untreated samples (-).

used for western blotting, evidenced a strong GR localization in the tail and in the head regions in the major part of the sperm (Fig. 2). We found a similar GR-D3 localization in varicocele sperm, but, as expected, the immunoreaction was significantly reduced (Fig. 2), confirming the decrease of GR-DR3 expression.

Glucocorticoids effect the human sperm motility and survival

Sperm motility is a distinctive parameter to measure of semen quality, describing the ability of sperm to move properly towards an oocyte. Therefore, we next investigated the effect of 10 and 100 nM DEXA treatment on sperm motility. As shown in Fig. 3, sperm motility of normozoospermic and varicocele samples was significantly enhanced at both the two DEXA concentrations, whereas the co-treatment with the inhibitor M2F reduced these effects, although were lower in varicocele sperm with respect to normozoospermic sperm (Fig. 3A). Another important hallmark of human sperm performance consists in its capacity to survive as much as

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possible to have the chance to find and fertilize the oocyte. Similar to motility, the DEXA treatment in both normozoospermic and varicocele samples increased sperm survival, which was rescued in presence of M2F (Fig. 3B).

Sperm capacitation and acrosome reaction are modulated by DEXA

The human sperm during its life passes through two stages of development: the first occurs in the male genital tract, where it acquires the morpho-anatomical maturation; the second in the female genital tract, where it gains the functional maturation during the capacitation process which prepares the gamete to the acrosome reaction. Therefore, we studied the effect of DEXA on these two important features of sperm. Particularly, upon treatment with DEXA in the incubation media of spermatozoa, we observed an increased cholesterol efflux, a gold marker of the capacitation process (Fig. 4A). Similarly, DEXA stimulated an augmented acrosome reaction in both normozoospermic and varicocele samples (Fig. 4B). These effects were abrogated using the inhibitor M2F (Fig. 4A, B). Capacitated sperms were used as positive control (Fig. 4B).

Cortisol in the sperm culture media is induced by DEXA

Really interesting was to investigate if sperm can secrete cortisol. The local concentration of cortisol in any cell or tissue is not only dependent on the concentration reaching that tissue from the circulation. Most tissues regulate local cortisol concentrations to suit their particular needs [33, 34]. Our results indicated that sperm secretes cortisol, which was higher in normozoospermic sperm with respect to the varicocele patients (Fig. 5). Furthermore, a positive effect of DEXA, both in normozoospermic and varicocele sperm, although in a lesser extent, has been noted (Fig. 5).

DEXA affects AKT, MAPK and JNK phosphorylation

To better define the GR-induced effects on sperm parameters, we analysed the molecular mechanism involved in these actions, exploring the main signalling that may be involved, *i.e.* the PI3K/Akt and MAPK 42/44 phosphorylations pathways, as previously showed by Aquila et colleagues [35, 36]. Our data showed that pAKT and prevalently the p44 band of pMAPK were significantly increased by DEXA treatments in

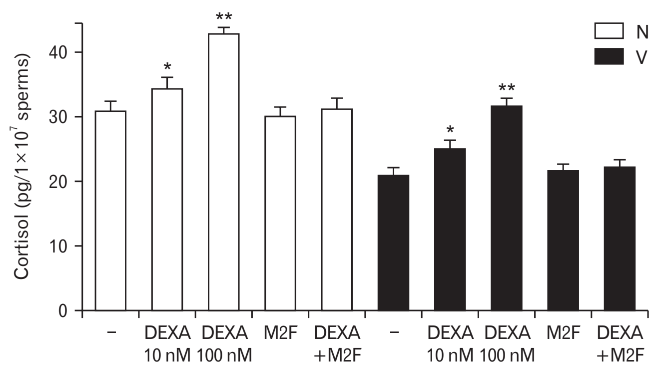

Fig. 5. Cortisol secretion is induced by dexamethasone (DEXA) in sperm. Cortisol amount in culture medium of purified spermatozoa from normal and varicocele samples was measured in the presence or not of the treatments, as indicated. Results are expressed as pg/1×10^7 sperm. *P<0.05 and **P<0.01 vs. untreated sperm (–).

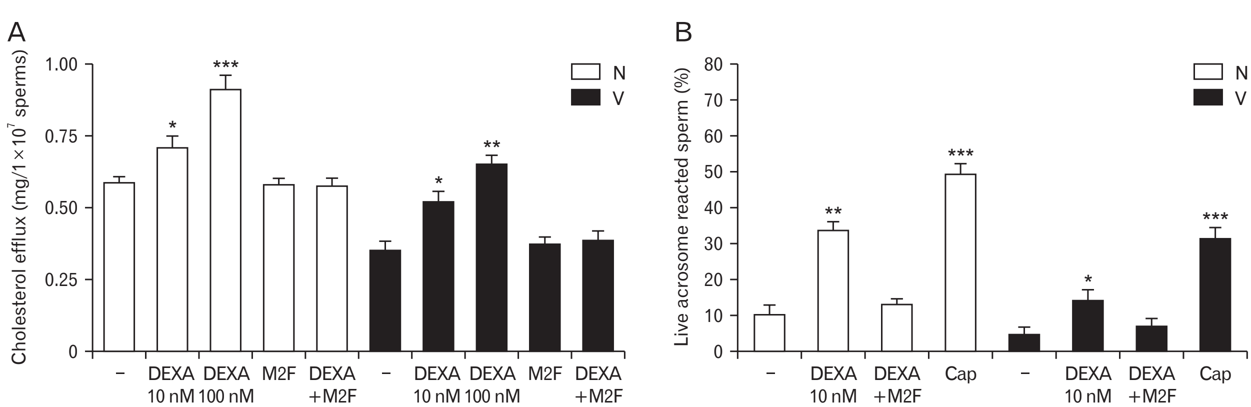

Fig. 4. Dexamethasone (DEXA) increases cholesterol efflux and induces acrosome reaction in sperm. (A) Cholesterol content in culture medium of purified spermatozoa from normal and varicocele samples was measured in the absence (–) or in the presence of the treatments, as indicated. Data are expressed as mg/1×10^7 sperm. Columns represent means±SEM of six independent experiments carried out in duplicate. *P<0.05, **P<0.02, and ***P<0.0001 vs. untreated sample. (B) Acrosome reaction was analyzed in sperm as indicated. Capacitated sperm (Cap) were used as positive control. Data are expressed as percentage of acrosome reacted sperm. *P<0.01, **P<0.005, and ***P<0.0001 vs. untreated samples (–).

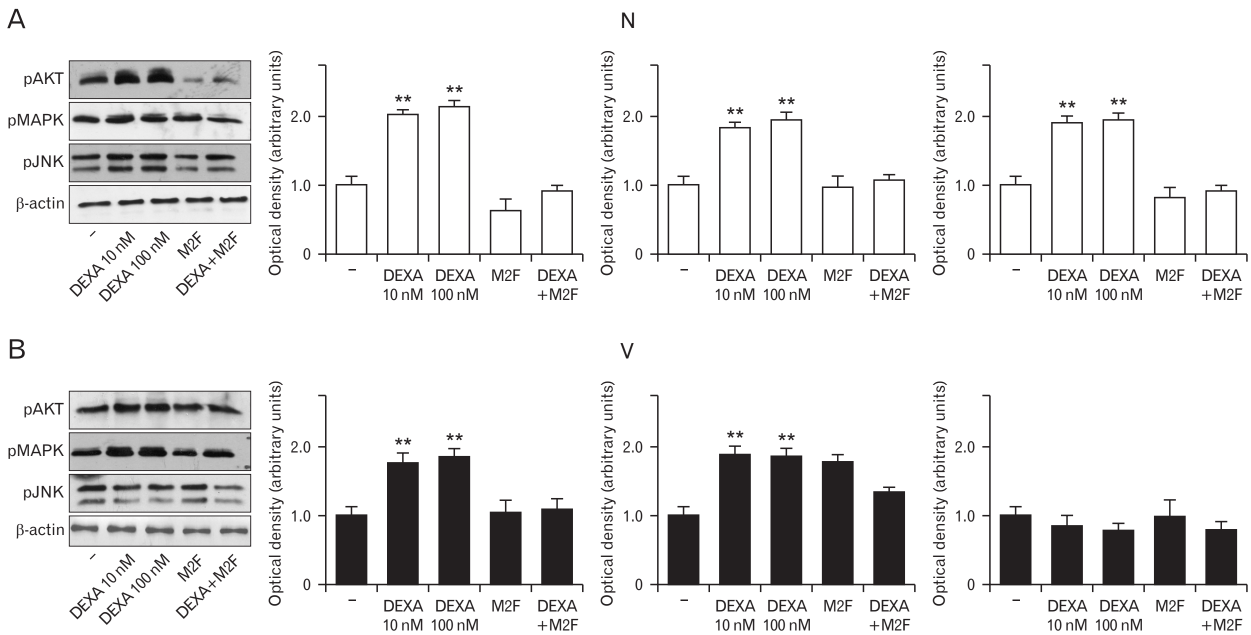

Fig. 6. Effect of DEXA on PI3k/AKT and SAPK/JNK pathways. Western blotting analyses were performed in normozoospermic (A) and varicocele sperm (B). Blots show the results of one representative experiment repeated at least four times. β-actin was used as loading control. The optical density is reported on the right. DEXA, dexamethasone; PI3K, PI3-kinase; JNK, c-jun N-terminal kinase; M2F, mifepristone. **P<0.005 vs. untreated sperm (–).

normozoospermic (Fig. 6A) and varicocele sperm (Fig. 6B). These effects were reversed by M2F. In normal samples, similar effects were obtained for pSAPK/JNK (Fig. 6A), members of the MAPK family, potently and preferentially activated by a variety of stresses, including inflammatory cytokines [37]. On the contrary in the varicocele patients DEXA treatments were not able to induce pSAPK/JNK modulation (Fig. 6B).

Discussion

Glucocorticoids are steroid hormones essential for adaptation to stress, behaviour, and reproduction. Moreover, they are also involved in preservation of energy homoeostasis, as well as in enhancement of vigilance, alertness, and attention [38].

In the present study, we investigated the presence of the GR in sperm and explored a possible role for the synthetic agonistic glucocorticoid DEXA, both in normal and in varicocele samples.

First, we showed, by western blotting, that sperm express GR and particularly the GR-D3 isoform which makes cells capable of undergoing apoptosis, even though not sensitive to glucocorticoid killing. The immunofluorescence assay evidenced the compartmentalization of this steroid receptor at the tail and head region level.

Furthermore, a reduced presence of GR-D3 in the sperm from varicocele subjects was evidenced. This may suggest that the varicocele sperm is less resistant to the cell-killing effects of glucocorticoids in female reproductive tracts.

Procreative events are associated with inflammatory, immunological, and transcriptional responses in the women reproductive system. After the deposition of semen in the uterus, a post-mating inflammation occurs and semen plasma as well as sperm are considered to be the cause, although the mechanism of action is unknown and never studied up to date. Uterus is a site of hostile inflammatory environment and a very small portion of spermatozoa migrate successfully to the fertilization site. The pathological sperm such as that derived by varicocele patients is weaker with respect to the normozoospermic one, showing more difficulty to accomplish its mission to fertilize an oocyte.

By treating sperm with DEXA we found that the main microscopic parameters, as survival and motility, were increased in both healthy and pathologic sperm.

Sperm to achieve fertilization need to switch into the capacitation status, which includes different aspects of sperm biology. It is logical to assume that during capacitation sperm is under stress conditions, *i.e.*, hyperactivate motility,

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energy consumption etc. Different steroids can help sperm to proceed towards capacitation, however we hypothesize that gametes also need to defense itself, and glucocorticoids/GR are the main candidate to help a fight and flight response. Thereafter, in our study DEXA/GR binding have been tested on the capacitation and acrosome reaction, unique feature of sperm cells, discovering that both processes are induced, particularly in the healthy sperm.

Emerging evidence suggests that glucocorticoids, like other steroid receptors, can also exert their actions in a more rapid manner (within minutes) [9]. This non-genomic mechanism, could be mediated by the activation of signal transduction pathways, such as PI3K/AKT pathways and mitogen-activated protein kinases, as demonstrated for other steroid receptors [35]. In this manuscript DEXA/GR-D3 increased both pAKT and pMAPK levels. Furthermore, in normal semen, but not in varicocele patients, similar effects were obtained for stress-activated protein kinases SAPK/JNK, members of the MAPK family, potently and preferentially activated by a variety of stresses, including inflammatory cytokines.

Cortisol is a steroid hormone released in response to stress. Therefore, cortisol can weaken the activity of the immune system. We hypothesize that sperm has also to fight against the immune system attach. Stress increase the cortisol levels to help the fight-or-flight mechanism function properly also in sperm cell. Nonetheless, findings regarding the possibility of sperm autonomous cortisol secretion is not available, although the ability of intact sperm for steroid interconversion has been proved [39].

Herein, from our data we discovered that sperm secrete cortisol and in a higher extent during capacitation. It needs to escape to the immune system attach, maintaining the immune privilege as in the testis, both resisting to apoptosis killing by immune system and splashing cortisol to fight and flight. Worthy, we demonstrated that sperm secrete different amounts of cortisol based on physiological/pathological status.

Glucocorticoids are believed to regulate both pro- and anti-inflammatory actions of the innate and adaptive immune systems during the inflammatory response. Although apoptosis of proinflammatory T cells contributes to the anti-inflammatory actions of glucocorticoids, killing of immune cells may increase the susceptibility to infection. The ability of the GR-D3 isoform to dissociate cytokine-suppressing and proapoptotic functions of DEXA, thus, may be beneficial in inhibiting inflammation and preventing immunosuppression.

We can assert that glucocorticoids ameliorate the sperm performance, particularly in pathologic sperm fortifying its defensive system. The sperm cortisol production may be considered a new feature of this cell, other than capacitation and acrosome reaction. Sperm-specific GR-D3/cortisol expression broadens glucocorticoids action.

Although further investigations are needed to deep the data obtained in our work, this novelty broads the role of glucocorticoids that could be considered towards innovative approaches in male infertility, opening new windows in the artificial insemination.

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Author Contributions

Conceptualization: VR, SA. Methodology: VR, AV. Validation: VR, AV, SA. Investigation: VR, AV. Drafting of the manuscript: SA,VR, AV. Critical revision of the manuscript: SA. Funding acquisition: SA. Approval of the final version of the manuscript: all authors.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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