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RESPONSIVENESS TO PROGESTERONE AND POTASSIUM CHANNEL BLOCKERS 4-AMINOPYRIDINE, TETRAETHYLAMMONIUM AND FREE Ca^{2+} CONTENTRATION IN SPERMATOZOA OF PATIENTS WITH OLIGOZOOSPERMIA/ LEUCOCYTOSPERMIA

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The present study was undertaken to evaluate $[Ca^{2+}]_i$ signals that occur in human sperm cells exposed in vitro to three diverse compounds; progesterone, 4-aminopyridine (a highly effective inducer of hyperactivation in human sperm) and tetraethylammonium. The $[Ca^{2+}]_i$ reached after the extracellular calcium treatment was always higher in normozoospermic samples pretreated with progesterone than in pathozoospermic samples pretreated with progesterone. There were no changes in calcium signal in spermatozoa pretreated with progesterone from patients with oligozoospermia and leucocytospermia compared to control samples (without progesterone). $[Ca^{2+}]_i$. was always higher in pathozoospermic samples without 4-aminopyridine and always lower in pathozoospermic samples with 4-aminopyridine compared to these values in normozoospermic men. The 2 mM extracellular calcium administration to spermatozoa pretreated with tetraethylammonium did not result in a detectable increase in $[Ca^{2+}]_i$ in normo- and pathozoospermic samples. The mechanisms of progesterone-dependent activation of the Ca^{2+}-entry and the functioning of the voltage gated Ca^{2+}-channels of plasmalemma are disturbed in pathologies – there was no increase in the Ca^{2+} level in the conditions of K^+-depolarization (in the presence of inhibitors of K^+-channels).

K e y w o r d s: calcium, spermatozoa, progesterone, inhibitors of K^+ channels.

Reproductive problems occur in 15% of couples. Male infertility is up to 50% of all infertility cases. Oligozoospermia and leukocytospermia are the most common causes of male infertility. Oligozoospermia is low spermatozoa concentration in ejaculate (< $20 \cdot 10^6$ cells/ml). Leukocytospermia (the presence of leucocytes ≥ $1 \cdot 10^6$ cells/ml) is a well-known indicator of infectious process in the genitourinary tract which affects male reproductive function and fertility [1]. Up to now the data regarding ionic aspects of male infertility remain poorly understood. Calcium is the major second messenger which plays an important role in sperm physiology. Fluctuations in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) mediate transmission of information from receptors on plasmatic membrane to nucleus or other internal response systems [2]. The ability of $[Ca^{2+}]_i$ to encode and mediate rapid transmission of information depends on the ability of the cell to generate calcium signals of sufficient precision and complexity. In particular, regulation of sperm motility, hyperactivation and chemotaxis depends on intracellular calcium level [3]. Hyperactivation facilitates progressive movement of spermatozoa and enables them to penetrate oocyte. It is also associated with capacitation leading to acrosome reaction. Disturbances in Ca^{2+} signalling in sperm are associated with male subfertility [4]. In fact, abnormal motility might be explained by abnormally low cytoplasmic calcium [5]. Sperm cells are not able to perform either of these crucial functions in the absence of extracellular Ca^{2+} [6]. However, mo-

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lecular mechanisms that trigger the increase in intracellular Ca^{2+} to initiate sperm hyperactivation are still not very clear.

In sperm cells the structural complexity that is characteristic of somatic cells (particularly the network of intra-membranous organelles) is greatly reduced during spermiogenesis. The "standard" components and Ca^{2+}-signaling capabilities are retained, though possibly in a modified form [3]. There are at least two mechanisms of Ca^{2+} entrance that contribute to hyperactivation in sperm cells: entry of Ca^{2+} via Ca^{2+}-channels (CatSper) in the plasma membrane and Ca^{2+} mobilization of intracellular calcium stored. The plasma membrane of spermatozoa is the primary component in Ca^{2+} regulation since sperm intracellular Ca^{2+} is maintained at a very low level (< 0.1 μM) in a medium containing millimolar Ca^{2+} (sperm plasma). Several types of CatSper were detected: voltage-operated Ca^{2+}-channels, ligand-activated channels and cyclic nucleotide-regulated channels in mammalian and invertebrate sperm cells [7]. Sperm from CatSper-null male mice are motile but do not hyperactivate. It is unable to make them to migrate within the oviduct and unable to fertilize oocytes [8]. Plasma membrane Ca^{2+}, Mg^{2+}-ATPase is expressed at high levels in spermatozoa and has the greatest contribution to Ca^{2+} extrusion in mammalian sperm. Inhibition of this pump by pharmacological agents disrupts control of $[Ca^{2+}]_i$ in the spermatozoa and causes the loss of sperm motility [9]. Na^+/Ca^{2+} exchangers have also been identified in spermatozoa and contribute significantly to $[Ca^{2+}]_i$ regulation [10]. There is considerable evidence that calcium stores exist in spermatozoa and play important roles in $[Ca^{2+}]_i$ signaling [11]. The presence of endoplasmic reticulum Ca^{2+}, Mg^{2+}-ATPase in spermatozoa is controversial [12]. It was shown that both human and sea urchin sperm express the nonendoplasmic reticulum Ca^{2+}-store pump secretory pathway and that this pump plays a significant role in sperm Ca^{2+} storage [13, 14]. The acrosome is another calcium store which exists in spermatozoa. It has IP_3 receptors on its external membrane and plays an important role in Ca^{2+} regulation [15]. Sperm mitochondria may also serve as intracellular Ca^{2+} stores, although their role in signaling is still unclear [16]. Regulation of $[Ca^{2+}]_i$ is achieved by a combination of channels, pumps and exchanges on both the plasma and intracellular membranes. The present study was undertaken to evaluate $[Ca^{2+}]_i$ signals that occur in human sperm cells exposed to three diverse compounds; progesterone, 4-aminopyridine (a highly effective inducer of hyperactivation in human sperm) and tetraethylammonium. The last two are known as non-specific inhibitors of the voltage gated K^+ channels of the plasma membrane.

Materials and Methods

Donors and semen sample preparation. Human semen was obtained from 10 healthy volunteers and 12 pathozoospermic men undergoing routine semen analysis for couple infertility at Lviv Regional Clinical Hospital (Ukraine). The approval for the study was taken from the ethics committe of Danylo Halytsky Lviv National Medical University (Ethical Committee Approval, protocol No 6 of March 29, 2017). All patients and healthy donors were matched by age and gave a written informed consent to participate in research. Exclusion criteria: subjects currently on any medication or antioxidant supplementation were not included. In addition, subjects with infertility over 10 years, azoospermia, testicular varicocele, genital infection, chronic illness and serious systemic diseases, smokers and alcoholic men were excluded from the study because of their well-known high seminal reactive oxygen species levels and decreased antioxidant activity which may affect calcium level. Samples were obtained by masturbation after 3-4 days sexual abstinence and processed immediately upon liquefaction. The classical semen parameters of spermatozoa concentration, motility, and morphology were examined according to World Health Organization criteria (2010). Sperm cells were washed from semen plasma by 3 times centrifugation at 3000 g for 10 min in media which contained (mM): 120 NaCl, 30 KCl, 30 Hepes (pH 7.4).

Calcium measurements. Changes of ionized Ca content in spermatozoa were identified with 2 μM Fluo-4 AM (λ_{ex} = 495 nm, λ_{em} = 520 nm). The probe was mixed with Pluronic F-127 (0.02%) to facilitate loading [17]. All experimental procedures were conducted in physiological Henks's solution (mM): NaCl – 136.9; KCl – 5.36; KH_2PO_4 – 0.44; $NaHCO_3$ – 0.26; Na_2HPO_4 – 0.26; $CaCl_2$ – 0.03; $MgCl_2$ – 0.4; $MgSO_4$ – 0.4; glucose – 5.5; Hepes (pH 7.4, 37 °C) – 10. The sperm cells were incubated for 30 min at 37 °C. In order to compare the fluorescence intensities of different studied groups the same number of cells was used in spectrofluorimetric measurements. The levels of Ca^{2+} in sperm cells were measured using a spectrofluorimeter (Quanta Master 40 PTI, Canada) with a software FelixGX 4.1.0.3096. Extra-

cellular calcium and effectors were administrated into cuvvete. The following reagents were used in the study: glucose, Pluronic F-27, $CaCl_2$ (Sigma, USA), Fluo-4 AM (Invitrogen, USA). Progesterone obtained from injection form (Biopharma, Ukraine) was added directly to the incubation medium. Other reagents were of local manufacture and analysis-grade purity.

Statistical analysis. Data are expressed as means ± SE of the numbers of determinations. Differences between paired sets of fluorimetric experiments were analysed using paired Student's *t*-tests in Microsoft Excel. Differences were considered significant at $P < 0.05$ as the minimum significance level.

Results and Discussion

In physiological conditions, calcium ions are intracellular second messengers which play an important role in sperm physiology. However, the role of calcium in hyperactivation, capacitation of spermatozoa and acrosome reaction was always regarded as controversial. There is a strong relationship between calcium homeostasis and male infertility (sperm count and motility). A statistically significant difference between intracellular Ca^{2+} level ($[Ca^{2+}]_i$) in infertile versus fertile males was found, while no differences were observed for seminal plasma Ca^{2+} concentrations [18]. Then, $[Ca^{2+}]_i$ seems to be a marker of sperm fertility, since males with unexplained infertility have lower intracellular concentrations of this divalent cation. We examined the influence of various physiological (progesterone) and pharmacological (4-aminopyridine and tetraethylammonium) agents on the resting $[Ca^{2+}]_i$ of human sperm cells loaded with Fluo-4.

As a physiological agent we used progesterone, a calcium-mobilizing classical agonist which is able to deplete the intracellular calcium stores. It is present throughout the entire length of female genital tract with the greatest concentration up to 10^{-6} M in cumulus cells that surround the oocyte [19]. It has been shown that progesterone is a chemoattractant for human spermatozoa which indicates at least one of the membrane progesterone receptors might act as a chemotaxis receptor [20]. Progesterone is the best known Ca^{2+} mobilizing classical agonist (effector) for which detailed study has been carried out on human sperm cells. The mean fluorimetric responses from a series of parallel experiments are represented on Fig. 1. In the absence of extracellular calcium (calcium-free medium), the basal intracellular Ca^{2+} level tended to be lower in spermatozoa from oligozoospermic and leucocytospermic men than in men with preserved fertility (normozoospermia), although these changes were not significant. Also there was no difference in $[Ca^{2+}]_i$ in sperm cell preatreated with progesterone compared to control in both normo- and pathozoospermic men.

As can be seen from Fig. 1 the exposure to extracellular calcium at milimolar dose (at 5 min of incubation) causes an immediate (within seconds)

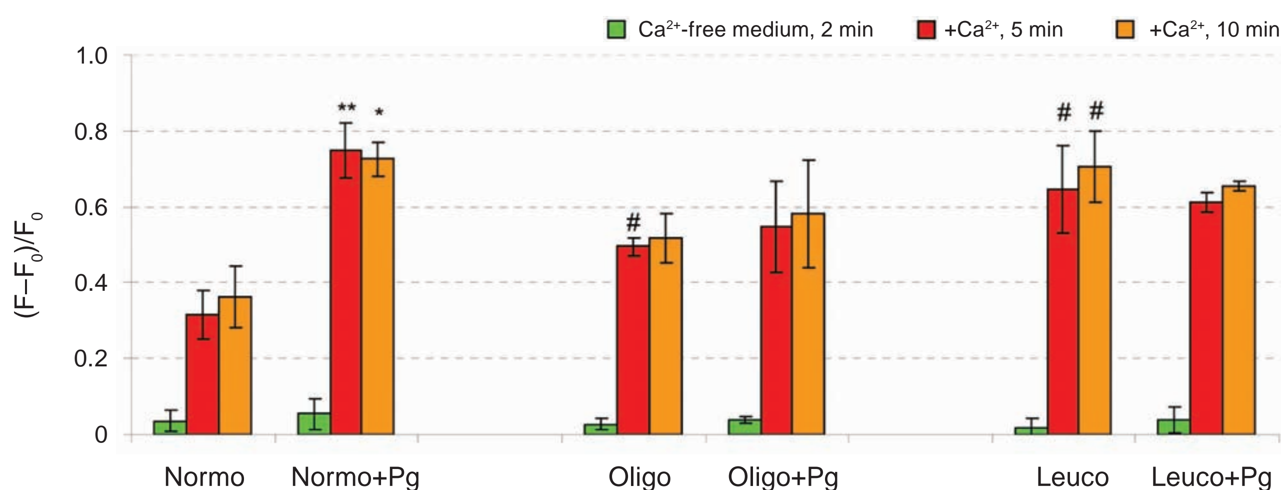

*Fig. 1. Ca^{2+}-induced increase in $[Ca^{2+}]_i$ in sperm cells pretreated with 1 nM progesterone (Pg) within 45 min. Bars are mean fluorimetric responses from a series of parallel experiments; administration of $CaCl_2$ (2 mM) at 5 min, n = 4-5. *P < 0.05; **P < 0.01 significantly different from samples without progesterone treatment; #P < 0.05 significantly different relative to the control group (normozoospermia)*

increase in $[Ca^{2+}]_i$ in both normozoospermic and pathozoospermic human sperm cells pretreated with progesterone and without progesterone indicating of calcium entry or the release of calcium from intracellular stores. The calcium-induced increase in intracellular free Ca^{2+} was observed after long periods of time (to 15 min of incubation, not represented on Fig. 1). However, the $[Ca^{2+}]_i$ reached after the extracellular calcium treatment was always higher in normozoospermic samples pretreated with progesterone than in pathozoospermic samples pretreated with progesterone. There were no changes in calcium signal in spermatozoa pretreated with progesterone from patients with oligozoospermia and leucocytospermia compared to control samples (without progesterone). The $[Ca^{2+}]_i$ was higher in pathozoospermic samples without progesterone than in normozoospermic samples.

These results are in perfect agreement with those obtained in human spermatozoa obtained from infertile men with another form of pathospermia – asthenozoospermia. It was shown that in asthenozoospermic men the progesterone-induced calcium transient was undetectable and subsequent calcium entry was much smaller compared to normospermic patients. These results may be explained either by a failure to localise a calcium signal to its site of action or by reduced or absent expression of progesterone receptors.

It is known than progesterone induces a biphasic Ca^{2+} influx and consequent acrosome reaction in human spermatozoa. It was shown that the amplitude (but not the kinetics) of the transient $[Ca^{2+}]_i$ response to progesterone shows strong dose sensitivity over the range of 0.3 nM – 3 μM, saturating at ≈ 300 nM (ED_{50} ≈ 30-50 nM) [21]. It has been suggested that both a high- and a low-affinity binding site for progesterone are present in sperm, corresponding to a biphasic dose–response curve for calcium increase stimulated by progesterone [22].

It was shown that $[Ca^{2+}]_i$ transient is apparently mediated by Ca^{2+} influx since it was greatly inhibited by a non selective Ca^{2+} channel blocker La^{3+} and was abolished when the extracellular medium contained the Ca^{2+} chelator EGTA. It has been concluded that the transient response of human spermatozoa to progesterone is a combination of Ca^{2+}-influx and mobilization of a labile, EGTA-sensitive store [23].

In our study measurements of $[Ca^{2+}]_i$ showed that responsiveness to progesterone in sperm from oligozoospermic and leucocytospermic subjects in the presence of extracellular calcium is lower than in healthy men with normozoospermia. Decreased responsiveness to progesterone was shown earlier for infertile men with oligozoospermia and asthenozoospermia [4]. It was suggested that reduced responsiveness to progesterone in sperm from asthenozoospermic subjects could be mainly due to decreased levels on membrane progesterone receptors, which could be translated in abnormal calcium signalling, and probably not to a direct effect on calcium release process. Also it was shown a significant decrease in the percentage of progesterone receptors in asthenozoospermic men [24]. We believe that decreased responsiveness to progesterone in sperm from oligozoospermic and leucocytospermic subjects is also due to decreased levels on membrane progesterone receptors.

4-aminopyridine is known as effective K^+-channel blocker used as a therapeutic agent in many neurological and neuromuscular disorders [25]. Since human spermatozoa express different types of K^+-channels the blocking of these channels can cause depolarization induced calcium influx. Fig. 2 demonstrates the increase of $[Ca^{2+}]_i$ in sperm cells pretreated with 1 mM 4-aminopyridine in the presence of extracellular calcium. The 2 mM extracellular calcium administration to spermatozoa pretreated with this pharmacological agent resulted in a detectable increase in $[Ca^{2+}]_i$ only in normozoospermic samples. In spermatozoa from both oligozoospermic and leucocytospermic men treatment with 4-aminopyridine did not result in significant changes in $[Ca^{2+}]_i$. It should be noted that $[Ca^{2+}]_i$. was always higher in pathozoospermic samples without 4-aminopyridine and always lower in pathozoospermic samples with 4-aminopyridine compared to these values in normozoospermic men.

It was shown that the application of 2 mM 4-aminopyridine caused a dose dependent, tonic increase in $[Ca^{2+}]_i$ in human sperm and at least part of increase in $[Ca^{2+}]_i$ reflects the mobilisation of stored Ca^{2+}, though the prolonged effect is probably dependent upon Ca^{2+}-influx [26]. The alternative explanation of 4-aminopyridine induced increase in $[Ca^{2+}]_i$ is that 4-aminopyridine induces Ca^{2+}-influx in the sperm tail (and sustained hyperactivation) by activation of pH-sensitive sperm K^+ current [26, 27]. However, Xia and Ren have demonstrated that recorded $[Ca^{2+}]_i$ influx in mouse sperm was not dependent on membrane potential and activation of voltage gated Ca channels [28].

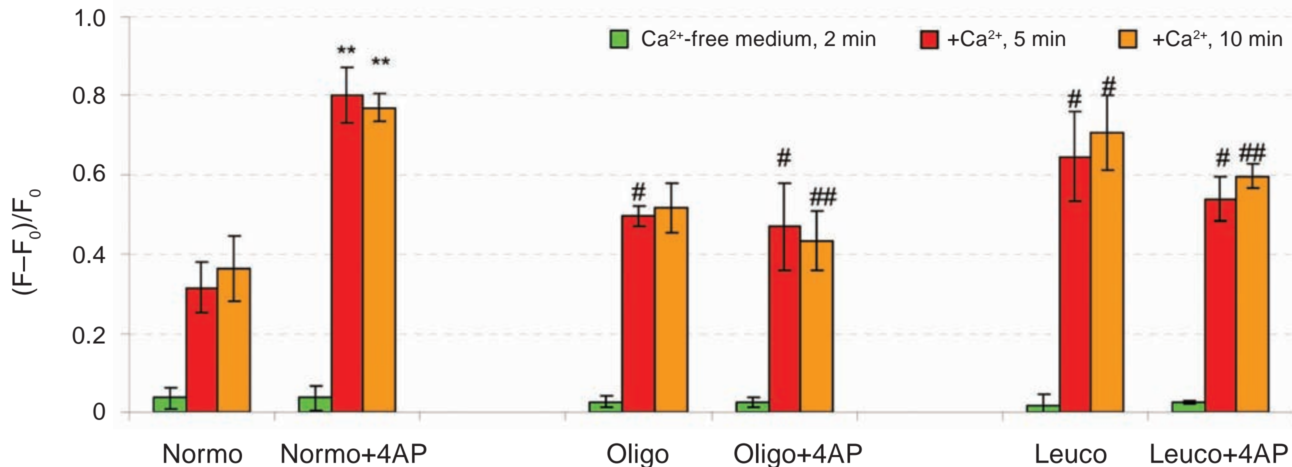

*Fig 2. Ca^{2+}-induced increase in $[Ca^{2+}]_i$ in sperm cells pretreated with 1 mM 4-aminopyridine (4AP). Bars are mean fluorimetric responses from a series of parallel experiments; administration of $CaCl_2$ (2 mM) at 5 min, n = 4-5. *P < 0.05; **P < 0.01 significantly different from samples without 4-aminopyridine treatment; $^{\#}P < 0.05$; $^{\#\#}P<0.01$ significantly different relative to the control group (normozoospermia)*

4-aminopyridine is also one of the most potent inducers of sperm hyperactivation. It was suggested that increase in $[Ca^{2+}]_i$ in the head piece and/or mid-piece of the flagellum is apparently key to initiation and maintenance of hyperactivation [5]. Using valinomycin (K^+ ionophore) it was shown that 4-aminopyridine induced hyperactivation was not significantly affected by valinomycin. Hovewer, it caused a significant sustained increase in $[Ca^{2+}]_i$ in single cell responses which may be due to modulation of sperm mitochondria as Ca stores [29].

Tetraethylammonium is known as a pharmacological research agent that blocks selective potassium channels. The 2 mM extracellular calcium administration to spermatozoa pretreated with tetraethylammonium did not result in a detectable increase in $[Ca^{2+}]_i$ in normo- and pathozoospermic samples (Fig. 3). However, calcium signal tended to be a bit smaller in spermatozoa from leucocytospermic samples pretreated with tetraethylammonium compared to calcium signal obtained in control (without tetraethylammonium), although this decrease was not sig-

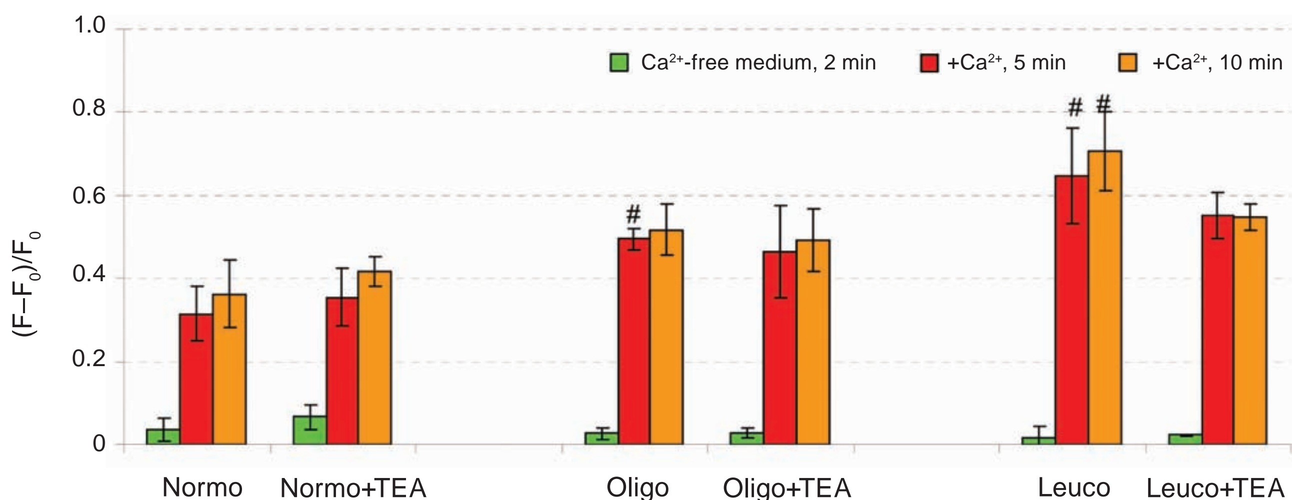

Fig 3. Ca^{2+}-induced increase in $[Ca^{2+}]_i$ in sperm cells pretreated with 1 mM tetraethylammonium (TEA). Bars are mean fluorimetric responses from a series of parallel experiments; administration of $CaCl_2$ (2 mM) at 5 min, n = 4-5. $^{\#}P < 0.05$; $^{\#\#}P < 0.01$ significantly different relative to the control group (normozoospermia)

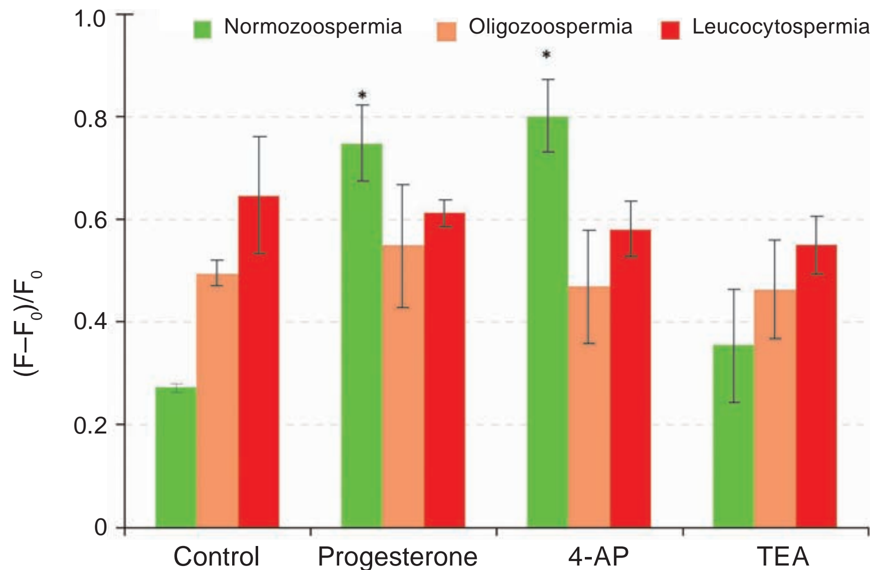

*Fig. 4. The peak increase in $[Ca^{2+}]_i$ in response to extracellular calcium at milimolar dose in sperm cells pretreated with physiological and pharmacological agents; the bars represent means ± SE of different experiments, n = 4-5. *P < 0.01 significantly different compared to peak increase in $[Ca^{2+}]_i$ in control (samples without effectors treatment)*

nificant. Tetraethylammonium-sensitive potassium channel are present in sperm cells. It was shown that application of the venom of the Chilean Latrodectus mactans blocks these K^+ currents and then alters the passive properties of the plasma membrane of spermatozoa. This leads to the entrance of Ca^{2+} from the extracellular medium, reflected by an increase in basal fluorescent units [30].

The peak increase in $[Ca^{2+}]_i$ in response to extracellular calcium at millimolar dose was lower in both oligozoospermic and leucocytospermic sperm cells pretreated with progesterone and 4-aminopyridine (Fig. 4). The peak increase in $[Ca^{2+}]_i$ in response to extracellular calcium was at the same level in spermatozoa pretreated with tetraethylammonium in normozoospermic men and both pathozoospermic types. These data clearly demonstrate that significant changes in the mechanisms involved in Ca^{2+} regulation occur in pathozoospermic patients.

Despite the fact that both 4-aminopyridine and tetraethylammonium are the potassium channel blockers, there were some differences in the sperm responsiveness to these pharmacological agents. A detectable increase in $[Ca^{2+}]_i$ after 2 mM extracellular calcium administration was observed only in spermatozoa pretreated with 4-aminopyridine, and changes in $[Ca^{2+}]_i$ in sperm cells pretreated with tetraethylammonium were not significant. This might be explained by the fact that 4-aminopyridine combines the properties of depolarizing agent with the ability to sequester intracellular Ca^{2+} [31]. It was shown that the increase in $[Ca^{2+}]_i$ stimulated by 4-aminopyridine in Ca^{2+}-free conditions had resulted from Ca^{2+} efflux from endoplasmic reticulum and were abolished by intracellular free Ca^{2+} chelator BAPTA.

Most importantly, we have found that sperm from oligozoospermic and leucocytospermic men reduces responsiveness to physiological (progesterone) and nonphysiological (potassium channel blockers 4-aminopyridine and tetraethylammonium) stimuli. These findings suggest a functional alteration in the ability of sperm cells from oligozoospermic and leucocytospermic men to initiate hyperactivation and the acrosome reaction in response to physiological and nonphysiological stimuli.

The mechanisms of progesterone-dependent activation of the Ca^{2+}-entry and the functioning of the voltage gated Ca^{2+}-channels of plasmalemma are disturbed in pathologies – there was no increase in the Ca^{2+} level in conditions of K^+-depolarization (in the presence of inhibitors of K^+-channels). In comparison with the physiological norm the increase in $[Ca^{2+}]_i$ in pathozoosermic samples can be provided by the activation of the mechanisms of Ca^{2+} release from the internal subcellular structures.

Since $[Ca^{2+}]_i$ is associated with sperm hyperactivation, capacitation and acrosome reaction, the decreased responsiveness to physiological and nonphysiological stimuli as a calcium-mobilizing agonist in pathozoospermic men contributes to the explanation of reduced fertility potential. Although obtained data need to be confirmed in a higher number of subjects, they are highly indicative of a link between responsiveness to progesterone and potassium channel blockers and the spermatozoa concentration (the presence of leucocytes in semen samples > $1.0 \cdot 10^6$ ml^{-1}) and suggest an involvement of these responsivenesses in the pathogenesis of male infertility.

It was shown that evaluation of human sperm to respond to progesterone by increasing $[Ca^{2+}]_i$ may be used as a fast and objective tool for the diagnosis of the human sperm quality, especially in cases of unexplained sterility [32]. It would be interesting, in future studies, to evaluate intracellular calcium transients and responsiveness to nitric oxide in sperm cells of pathozoospermic men and its relation to levels of reproductive hormones, in the intent of demonstrating how calcium homeostasis is regulated. Therefore, these studies indicate that intracellular calcium signals appear to be important potential targets for pharmacological interventions of spermatozoa function for improving the sperm fertility potential (for infertile men) or for reduction of sperm fertilising capacity (for male contraception).

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ЧУТЛИВІСТЬ ДО ПРОГЕСТЕРОНУ ТА БЛОКАТОРІВ КАЛІЄВИХ КАНАЛІВ 4-АМІНОПІРИДИНУ, ТЕТРАЕТИЛАМОНІЮ ТА КОНЦЕНТРАЦІЯ Ca^{2+} В СПЕРМАТОЗОЇДАХ ПАЦІЄНТІВ З ОЛІГОЗООСПЕРМІЄЮ/ ЛЕЙКОЦИТОСПЕРМІЄЮ

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Метою роботи було оцінити Ca^{2+}-сигнали в сперматозоїдах фертильних та інфертильних чоловіків за інкубації сперматозоїдів *in vitro* із прогестероном, 4-амінопіримідином (високоефективні індуктори гіперактивації сперматозоїдів) та тетраетиламонієм. $[Ca^{2+}]_i$ у разі додавання 2 мМ зовнішньоклітинного Ca^{2+} була вищою в сперматозоїдах нормозооспермічних чоловіків за інкубації із прогестероном, ніж у патозооспермічних зразках. Водночас не відзначалось вірогідних змін Ca^{2+}-сигналу в сперматозоїдах інфертильних чоловіків із олігозооспермією та лейкоцитоспермією під час інкубації із прогестероном. $[Ca^{2+}]_i$ була вищою в сперматозоїдах патозооспермічних чоловіків (без інкубації з 4-амінопіримідином) та нижчою в сперматозоїдах патозооспермічних чоловіків (за інкубації з 4-амінопіримідином) порівняно з величинами у фертильних чоловіків (нормозооспермія). Додавання зовнішньоклітинного 2 мМ Ca^{2+} до сперматозоїдів, преінкубованих із тетраетиламонієм, не спричинювало зростан-

ня $[Ca^{2+}]_i$ в нормозоо- та патозооспермічних зразках. Показано, що за патології порушуються механізми прогестеронзалежної активації Ca^{2+} входу та функціонування потенціалкерованих Са-каналів плазмалеми, також не спостерігається зростання $[Ca^{2+}]_i$ у разі K^+-деполяризації (в присутності інгібіторів). Підвищення $[Ca^{2+}]_i$ порівняно з нормою може забезпечуватись активацією механізмів виходу Ca^{2+} із внутрішніх субклітинних структур.

К л ю ч о в і   с л о в а: кальцій, сперматозоїди, прогестерон, інгібітори K^+-каналів.

ЧУВСТВИТЕЛЬНОСТЬ К ПРОГЕСТЕРОНУ И БЛОКАТОРАМ КАЛИЕВЫХ КАНАЛОВ 4-АМИНОПИРИДИНА, ТЕТРАЭТИЛАММОНИЯ И КОНЦЕНТРАЦИЯ Ca^{2+} В СПЕРМАТОЗОИДОВ ПАЦИЕНТОВ С ОЛИГОЗООСПЕРМИЕЙ/ ЛЕЙКОЦИТОСПЕРМИЕЙ

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Целью работы было оценить Ca^{2+}-сигналы в сперматозоидах фертильных и нефертильных мужчин, при инкубации сперматозоидов *in vitro* с прогестероном, 4-аминопиримидином (высокоэффективные индукторы гиперактивации сперматозоидов) и тетраэтиламмонием. $[Ca^{2+}]_i$ при добавлении 2 мМ внеклеточного Ca^{2+} была выше в сперматозоидах нормозооспермических мужчин при инкубации сперматозоидов с прогестероном, чем в патозооспермических образцах. Вместе с тем, не было достоверных изменений Ca^{2+}-сигнала в сперматозоидах нефертильных мужчин с олигозооспермией и лейкоцитоспермией инкубированных с прогестероном. $[Ca^{2+}]_i$ была выше в сперматозоидах патозооспермических мужчин (без инкубации с 4-аминопиримидином) и ниже в сперматозоидах патозооспермических мужчин (инкубированных с 4-аминопиримидином) по сравнению с величинами у фертильных мужчин (нормозооспермия). Добавление внеклеточного 2 мМ Ca^{2+} к сперматозоидам, преинкубированным с тетраэтиламмонием, не изменяло уровень $[Ca^{2+}]_i$ в нормозоо- и патозооспермических образцах. Показано, что при патологии нарушаются механизмы прогестеронзависимой активации входа Ca^{2+} и функционирования потенциалуправляемых Са-каналов плазмалеммы, также не наблюдается изменения концентрации Ca^{2+} при K^+-деполяризации (в присутствии ингибиторов). Повышение $[Ca^{2+}]_i$ по сравнению с нормой может обеспечиваться активацией механизмов выхода Ca^{2+} из внутренних субклеточных структур.

К л ю ч е в ы е   с л о в а: кальций, сперматозоиды, прогестерон, ингибиторы K^+-каналов.

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