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Inflammatory Pathologies in Reproductive Tract: Role of NETs and METs on Fertilization Disorders

Patologías Inflamatorias del Tracto Reproductivo: Papel de NETs y METs en los Trastornos de la Fecundación

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SUMMARY: The role of neutrophil extracellular traps (NETs) and monocyte extracellular traps (METs) is well established in fertilization disorders by adverse effects on sperm motility, acrosome integrity, membrane lipoperoxidation, and disturbing oocyte sperm binding. Moreover, NETs/METs remove efficiently excessive spermatozoa within the female reproductive tract (FRT) after natural coitus or artificial insemination (AI). Depending on their location in reproductive organs, NETs/METs may be beneficial to the host by their capability of either limiting the dissemination of infective pathogens, promoting wound healing, and exerting anti-inflammatory properties through the degradation of pro-inflammatory components. Conversely, excessive or imbalanced NETs/METs release within FRT or urogenital tract (UGT) might be associated with worse fertility due to deleterious effects on sperm functions, oocyte fertilization, endometrium microenvironment, and embryo implantation. In the UGT, patients with epididymitis or urogenital bacterial infections and with leukocytospermia spontaneous release of NETs/METs in seminal fluids. Moreover, in infertile male/female patients without infectious etiologies but with autoimmune (i. e. systemic lupus erythematosus, rheumatoid arthritis)-, metabolic (diabetes mellitus, obesity)- and vascular (vasculitis, hypertension, and preeclampsia)- diseases uncontrolled NETs/METs might negatively influence their fertility. Thus, this review aims to provide novel insights into the beneficial as well as adverse effects of NETs/METs on male gametes, oocyte fertilization, and endometrium by highlighting broad implications on early diagnosis of extracellular traps (ETs) in seminal fluid samples of infertility patients with inflammatory etiologies. As well as, for early diagnosis of spontaneous formation of NETs/METs in infertile couples with one or both being affected with chronic inflammatory-, metabolic-, vascular- and/or autoimmune diseases to increase the chance of conception.

KEY WORDS: Neutrophil extracellular traps; Monocyte extracellular traps; Fertility; Reproduction; Sperm; Infertility.

INTRODUCTION

Infertility is a significant cause of morbidity affecting more than 48.5 millions of couples worldwide (Inhorn & Patrizio, 2015), appearing in one of 7 couples in Western industrialized countries and one of four couples in developing countries, meaning that 8 to 12% of couples trying to conceive within reproductive ages are affected this reproductive disorder (Szkodziak *et al*., 2016; Vander Borght & Wyns, 2018).

The male factor is responsible for approximately 50% of these infertility cases and between 6 and 15% of these cases being associated to secondary urogenital infections, mainly prostatitis and epididymitis, in addition to chronic diseases (e. g. diabetes, autoimmune diseases, vasculitis) (Inhorn & Patrizio, 2015; Punab *et al*., 2017) resulting in pro-inflammatory microenvironments of male urogenital tract (UGT) accompanied with enhanced

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oxidative stress (OS). When compared to other cells, spermatozoa are extremely vulnerable to OS due to their limited cytoplasmic space and their lower concentrations of enzymatic antioxidants (e. g. superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic antioxidants (e. g. vitamin C, vitamin E, selenium, hypotaurine). Therefore, OS is until now main cause of impaired sperm function and linked to male infertility (Uribe *et al*., 2022). In this context, the persistence of potent pro-inflammatory professional phagocytes in semen, leukocytospermia, can result in detrimental effects on sperm cells due to increased production of reactive oxygen species (ROS) by activated leukocytes thereby increasing OS in seminal fluids. In patients with leukocytospermia, the principal cells are polymorphonuclear neutrophils (PMN) and monocytes. Seminal PMN/monocytes to secrete pro-inflammatory cytokines/chemokines (interleukin-8), to release ROS, conduct degranulation and to extrude neutrophil extracellular traps (NETs) (Brinkmann *et al*., 2004; Hagan *et al*., 2015; Zambrano *et al*., 2016, 2021) as well as monocyte extracellular traps METs (Schulz *et al*., 2019a; Zambrano *et al*., 2020). ROS and OS are produced under physiological conditions secondary to cellular metabolism, however, their excess formation can be detrimental in UGT as well as FRT (Aziz *et al*., 2004).

Consistently, presence of NETs/METs and particularly large aggregated NETs (aggNETs) can result in deleterious effects in sperm functional parameters, including lower sperm numbers, reduction of sperm motility and alteration of sperm morphologies (Schulz *et al*., 2019b; Zambrano *et al*., 2020). All above mentioned sperm alterations of are induced by ROS and OS resulting in sperm membrane damage due to polyunsaturated fatty acids, which are extremely susceptible to ROS/OS and suffering more significant lipid peroxidation (Agarwal *et al*., 2008). At sperm DNA level, ROS production has been associated with DNA fragmentation (Tremellen, 2008) that is strong association to male infertility disorders (Shekarriz *et al*., 1995; Tremellen, 2008; Uribe *et al*., 2022), thereby confirming ROS-mediated adverse effects against male gametes as demonstrated elsewhere (Zambrano *et al*., 2016; Uribe *et al*., 2022).

Infiltration of PMN/monocytes into seminal fluid and thereafter presence of NETs/METs in freshly ejaculated semen of patients with leukocytospermia or with epididymitis, resulted in altered sperm function and massive sperm entrapment through *agg*NETs/METs *ex vivo*. For instance, *agg*NETs resulted in reduced sperm motility of 26.8% in the first h of incubation determined by CASA computerized system (Zambrano *et al*., 2016). In addition, the presence of *agg*NETs/METs affect integrity of sperm membrane thereby decreasing their viability in the first two h by 30 % using SYBR/PI fluorophores with variable data depending on the concentration of leukocytes exposed to sperm (Zambrano *et al*., 2016). Also the evolution of acrosome reaction was significantly affected by decreasing the percentage of spermatozoa with their acrosome intact by 26.4 % using PNA/PI fluorescent probes in flow cytometry. Likewise, human spermatozoa exposed to PMN and NETs reduced oocyte binding capacities as demonstrated by standard hemizona assay (Zambrano *et al*., 2021). A 49 % reduction of sperm-oocyte binding capacities were detected after contact to extruded NETs thereby directly impacting probability of achieving successful oocyte fertilization (Zambrano *et al*., 2016, 2021).

There is currently little information on possible role of human NETs/METs in physiopathology of reproductive disorders of female and male patients with chronic diseases and their impact on infertility due to excess presence of this cells. Therefore, the aim of this review is to present available evidence on the role of the pro-inflammatory responses, with particular emphasis on beneficial as well as adverse effect of NETs/METs on sperm function or fertilization potential, focusing on possible adverse effects on fertility when non-infectious diseases and chronic pathologies are present.

Neutrophil Extracellular Traps

PMN are the most abundant leukocytes to fight pathogens within the human innate immune system but also having further roles on fertility alteration (Carestia *et al*., 2016). PMN make up to 70 % of leukocytes in human blood, representing the first line of defense and being the first ones to be recruited to site of infections (Kumar & Sharma, 2010; Hermosilla *et al*., 2014). PMN have a cytoplasm rich in antimicrobial granules peptides and having abilities for synthesis of leukotrienes and thromboxanes, pro-inflammatory cytokines (among others of TNF family, IFN-γ) and chemokines such as CXCL8 (IL-8) (Mantovani *et al*., 2011). Additionally, PMN have the NADPH oxidase (NOX) enzyme complex responsible for ROS production (Nathan, 2006). Brinkmann *et al*. (2004) visualized for the first formation of neutrophil extracellular structures (NETs) by human PMN stimulated with either IL-8 or lipopolysaccharides (LPS). NETs are composed of linear elements of 15-17 nm in diameter and the main architecture or backbone corresponding to DNA, whereas 70 % of the proteins are nuclear histones (i. e. H1, H2A/H2B, H3, H4), covered by granular elements such as myeloid cell nuclear differentiation antigen (MNDA),

myeloperoxidase (MPO), neutrophil elastase (NE), proteinase 3 (PR3), cathepsin G (Cat-G), lactoferrin, lysozyme C, cathelicidin LL37 (LL-37), pentraxin (PTX) and bactericidal permeability-increasing protein (BPI) among others (Wartha *et al*., 2007; Papayannopoulos *et al*., 2010; Hermosilla *et al*., 2014).

NETs are considered as pivotal ancient and well-conserved host defense mechanism of innate immune system within animal kingdom, including terrestrial and marine mammals (Villagra-Blanco *et al*., 2019) as well as invertebrates (Lange *et al*., 2017; Neumann *et al*., 2020; Adrover *et al*., 2022). During NETotic process there will be extrusion of DNA, covered with global histones and antimicrobial proteins/peptides (e. g. NE, MPO, Cat-G, lactoferrin, PTX, gelatinase, PR3, LL-37, peptidoglycan-binding proteins and other components with bactericidal activity able to destroy pathogens as well as virulence factors to the extracellular space which in some cases can lead to the cell death of PMN (Brinkmann *et al*., 2004). Nowadays, suicidal- and vital NETosis have been reported and differing not only on signaling pathways but also on their induction times (Jorch & Kubes, 2017; Zhou *et al*., 2019). During vital NETosis activated PMN release mitochondrial DNA to form NETs but thereby keeping their plasma membrane integrity, crawling- and paghocytosis activities and releasing small NETs in vesicle forms (Jorch & Kubes, 2017). Irrespective of suicidal or vital NETosis two intracellular events of PMN are required for the formation of adequate NETs. Firstly, the generation of ROS through the NOX system, which will trigger significant intracellular ROS release, mainly superoxide anion (O2-) and later on the generation of hydrogen peroxide (H_2O_2), hypochlorite (ClO-) and of singlet oxygen ($^1O_2$), inducing oxidation of carbohydrates, lipids, lipoproteins and proteins as well as granule membranes (Fuchs *et al*., 2007). The second relevant process is the unfolding of chromatin through the activation of peptidylarginine deaminase type 4 (PAD4), a calcium-dependent enzyme present in the granules of PMN that enables citrullination of histones (Wang *et al*., 2009). By changing the residues of arginine to citrulline, PAD4 makes possible decondensation of chromatin, aided by NE released after ROS-dependent lysis of azurophilic granules (Amulic & Hayes, 2011). This enzyme, together with MPO, are translocated to nucleus after PMN activation and degrade histones, mainly H4, allowing decondensation of chromatin (Papayannopoulos *et al*., 2010). Later on, nuclear membrane is ruptured and cytoplasmic granules are released, leading to the binding of nuclear and cytoplasmic components to extruded DNA. Finally, the change in permeability of the membrane in combination with PMN cytoskeleton permit release of NETotic content into extracellular space. NETs-associated cytoxicity for pathogens results mainly through their exposure to MPO, NE, histones, PTX and ROS, thereby altering the membrane integrity in bacteria , fungi (Urban *et al*., 2009) and protozoan (Silva *et al*., 2014) and metazoan parasites Villagra-Blanco *et al*., 2019). Another proposed mechanism is the cytotoxic function of histones, mainly H2A, altering the mitochondrial membrane potential and therefore reducing ATP synthesis (Guthrie *et al*., 2008) as well as inducing mitochondrial edema, a change in membrane permeability with the release of cytochrome c and apoptosis-inducing factor (AIP) thereby activating caspase-dependent or -independent cell death (Lobascio *et al*., 2015).

Effect on Sperm Function and Fertilization Potential of Nets/Mets Induced by Infectious Diseases. Although NETs/METs initially constitute an important host defense mechanism in humans, the excessive formation of NETs/METs could cause significant tissue damage (Czaikoski *et al*., 2016). Their increased presence in UGT and FRT, for instance in patients with chronic disease, unbalanced clearance of these fibers could negatively influence fertility (Zambrano *et al*., 2016; Condorelli *et al*., 2017). The excess of NETs and the presence of MPO, NE and cathepsin G induce mitochondrial damage in exposed macrophages and dendritic cells (DCs) (Zambrano *et al*., 2016; Condorelli *et al*., 2017). Similarly to macrophages and DCs, human sperm exposed to NETs result in significant changes of permeability, including loss of sperm membrane integrity (Zambrano *et al*., 2016, 2021), added to the damage induced by OS due to presence of elevated ROS concentrations causing peroxidation of long-chain polyunsaturated fatty acids (PUFAs), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) (Waterhouse *et al*., 2006). Subsequent to loss of sperm plasma membrane integrity, there is a change at acrosomal level, leading to a decrease in the parameters of viability, motility and binding to the oocyte (Maldjian *et al*., 2005). Alghamdi *et al*. (2009) published first reports indicating that equine spermatozoa can induce formation of NETs. Later on, *in vitro* studies demonstrated for the first time that human spermatozoa can as well induce formation NETs (Zambrano *et al*., 2016). Different subtypes of NETs have been identified after exposure to motile human sperm resulting in aggregated NETs (*agg*NET), diffuse NETs (*diff*NET) and spread NETs (*spr*NET) (Zambrano *et al*., 2016). Similarly to PMN, human monocytes in seminal fluid of patients with marked leukocytospermia also extruded *agg*METs *ex vivo* thereby entrapping efficiently sperm and impeding proper movement (Schulz *et al*., 2019).

Mammalian leukocytes, including PMN, monocytes and macrophages, can sense size of pathogens through

(Branzk) and more importantly motility through so-called mecanosensitive (MS) ion channels such as Piezo 1 and 2, MscL and TREK-2 (Ridone *et al*., 2019; Canales Coutiño & Mayor, 2021). MS ion channels are integral membrane proteins which play a crucial role in fast signaling during mechanosensory transduction processes in various cell types, including PMN. MS ion channels are not only activated by mechanical forces but also by chronic inflammation. The majority of MS ion channels have high specificity for Ca^{++} and often referred in as stretch-activated Ca^{++}-channels (Canales Coutiño & Mayor, 2021). Whether motility of human spermatozoa are capable to induce MS ion channel activation during NETs/METs needs further clarification.

There is evidence that spermatozoa exposed to increasing concentrations of NETs significantly reduce their progressive motility (Zambrano *et al*., 2016). This has been seen in bacterial infections of the male reproductive tract, with the most frequent cause being acute bacterial epididymitis produced by *Chlamydia trachomatis* or *Escherichia coli* (Pilatz *et al*., 2015), which induce greater infiltration of leukocytes into seminal fluid, releasing NETs/METs, and explaining decrease in sperm motility as well as quality (Zambrano *et al*., 2020). The generation of NETs/METs is not exclusive to PMN and monocytes as they can also be released by activated mast cells (von Köckritz-Blickwede *et al*., 2008), eosinophils (Yousefi *et al*., 2008; Muñoz-Caro *et al*., 2015), and macrophages (Chow *et al*., 2010; Wei *et al*., 2018).

Seminal macrophages and monocytes play an prominent role in infections such as chronic epididymitis and prostatitis; hence, they can be an important substrate for formation of METs in seminal fluid of these patients. The exposure of human monocytes to spermatozoa resulted in a greater formation of METs, mainly *diff*METs. Similarly to *agg*NETs, that immobilized numerous sperm mainly through their binding to sperm mid-piece and flagellum (Schulz *et al*. 2019a). Subsequently, a greater production of *agg*METs has been determined in the presence of *E. coli*, mainly resulting in diffuse phenotypes (Zambrano *et al*., 2020). As well as, has been demonstrated of NETs/METs in semen samples of men with epididymitis associated to *Ureaplasma urealyticum*-, *Chlamydia trachomatis*- and *E coli*-infections (Zambrano *et al*., 2020). In the same way, a strong correlation was found between elastase levels and the percentage of positive peroxidase cells, both considered indicative markers of a seminal inflammatory state (Rusz *et al*., 2012). Induction of NETs (which contain neutrophil elastase) in the seminal fluid of patients with epididymitis due to *E. coli*, *Ureaplasma urealyticum*, and *C. trachomatis* has also been reported by other groups (Rajeeve *et al*., 2018; Yu *et al*., 2019).

However, presence of NETs/METs in seminal fluid originating from healthy patients has also been documented suggesting that there might be physiological concentrations in normospermic patients as well but digested by DNases present in semen without producing alterations of seminal parameters (Schulz *et al*., 2019b). Therefore, presence of vital spermatozoa and motility constitute biological stimuli for NETs/METs extrusion which would not be harmful at basal levels due to compensation mechanisms. Nevertheless, the triggering molecular mechanisms of human sperm-triggered NETs/METs are still unknown.

Presence of NETs/METs has been associated with human reproductive pathologies which result in infertility in cases where there is no infection and where the release of spontaneous ETs exceeding physiological threshold resulting in damage to male gametes (Schulz *et al*., 2019b). The presence of NETs is also associated with a reduction of sperm motility in co-culture of spermatozoa with PMN. Evaluated progressive motility in 4 groups with different PMN concentrations for 0, 1, 2 and 3 h of incubation for each group a significant reduction of progressive motility was observed in all experimental group. This was associated with the increased formation of NETs in the time (Zambrano *et al*., 2016). In the other hand, the PMN co-cultures isolated from healthy women's peripheral blood with spermatozoa of seminal fluid from healthy patients, after 180 min, NETs were generated and spermatozoa were trapped, demonstrating an increased concentration of extracellular DNA in co-cultures of sperm with PMN (Fig. 1). In addition, a significant reduction was detected in plasma membrane and acrosomal membrane integrity mediated by NETs (Zambrano *et al*., 2021). Interestingly, the spermatozoa co-cultured with PMN also presented a significantly lower ability to bind to the zona pellucida than the spermatozoa in the control group. A mechanism that could be involved in the generation of sperm-mediated ETs is the entry of extracellular calcium regulated by intracellular deposits through SOC channels (SOCE). Other studies have also proposed that calcium transport is involved in the formation of NETs through ROS generation and histone citrullination (Gupta *et al*., 2014). Its relevance in sperm-triggered NETosis was evaluated in this study through treatment with 2-ABP, a functional SOCE inhibitor. Pre-treatment of PMN with 2-ABP and then co-culture with spermatozoa showed a significant reduction in NETs compared to the control group (PMN + spermatozoa). Furthermore, it increased the number of spermatozoa bound to the zona pellucida, demonstrating the relevance of SOCE in the physiopathology of NET generation.

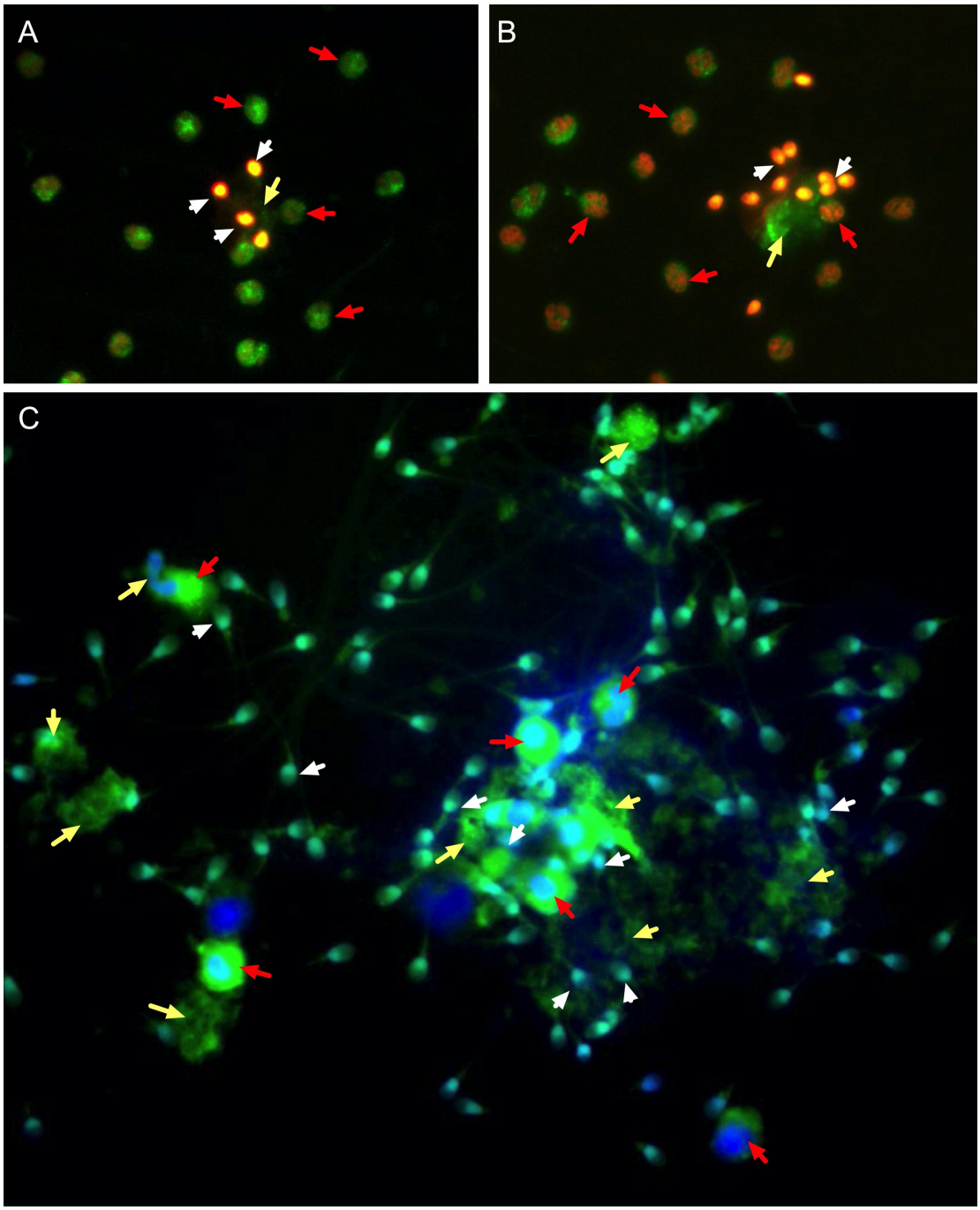

Fig. 1. Representative inmunofluoresce photographs showing ET/sperm interaction in the human model. A and B show neutrophil elastase staining in green and DNA in red. C, shows staining with global histones in green and DNA co-localized with green and blue staining. The sperm are trapped after 180 min of interaction. Red arrows indicate neutrophils, yellow arrows indicate ET, white arrows indicate sperm.

Effect of Extracellular Traps on Fertility in Chronic Diseases. The presence of the three ET phenotypes has also been described in seminal fluid with no infectious cause but with chronic inflammatory states or immunological pathologies, leading to greater activation of neutrophils and seminal macrophages (Zambrano *et al*., 2016; Schulz *et al*., 2019b). ETs could be of importance in the physiopathology of non-infectious diseases (Fig. 2) such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), obesity (OB), diabetes mellitus (DM), vasculitis and

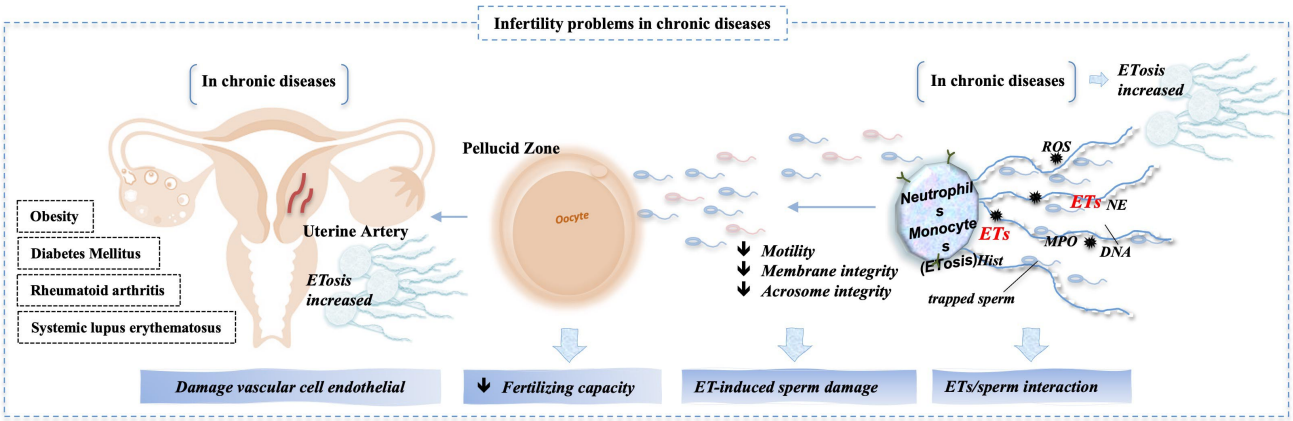

Fig. 2. Model of the ETs/sperm interaction with the consequences on fertility in cronic diseases. Abbreviations: MPO: myeloperoxidase. ROS: reactive oxygen species. NETs: neutrophil extracellular traps. Hist: histones. NE: neutrophil elastase. PMN: polymorphonuclear neutrophils.

neoplasias(Jorch & Kubes, 2017). Autoimmune disorders are hyper-responsive to autoantigens and NETs /or their products could induce a greater formation of antibodies (Jorch & Kubes, 2017). Nevertheless, the role of constituent NETosis in patients with autoimmune or chronic diseases has not been fully studied and, therefore, the real consequences during the reproductive process are unknown. Accordingly, we consider it relevant to study the presence of NETs in the reproductive tract of male and female patients, since it would enable their quantification, as well as the generation of information to aid in understanding and answering such questions as: What direct effect would this have on sperm function and motility or capacity to bind to the oocyte?, or to demonstrate what the real impact would be of the largest number of NETs in the vaginal fluid of these patients on the activation of the oocyte, migration or implantation?. Other autoimmune disorders or chronic pathologies such as diabetes mellitus could produce a larger number of ETs in seminal fluid. In these diseases having a proinflammatory state, the neutrophils may have a role in the alteration of patients' fertility (Fig. 2) (Carestia *et al*., 2016). In diabetic patients there is an increased presence of NETs in the blood (Menegazzo *et al*., 2015) because they have a chronic inflammatory and metabolic disorder that may be associated with a greater activation of neutrophils related to an increase in PAD4 (Wong *et al*., 2015). In addition, they present increased levels of nucleosomes, elastase-DNA complex (Carestia *et al*., 2016), IL-6 and TNF alpha in plasma, responsible for neutrophil activation (Joshi *et al*., 2013). In *in vitro* studies, neutrophils exposed to high glucose concentrations experience NETosis more frequently than those with lower concentrations (Menegazzo *et al*., 2015). High glucose in the blood of diabetic patients could at least partly be responsible for the increase in the susceptibility of neutrophils at the beginning of the NETosis process.

The obesity-induced proinflammatory state can also affect the function of the epididymal epithelium, altering the environment with increasing the influx of neutrophils and macrophages into the epididymal lumen, resulting in increased cytokine expression and epithelial apoptosis, thus impairing sperm maturation and fertilization capacity. Consequently, it can induce changes in sperm parameters such as sperm motility and increased abnormal morphology (Shukla *et al*. 2014, Guo *et al*. 2017). In addition, both the spontaneous acrosomal reaction and the progesterone-induced acrosomal reaction are altered in obese men, possibly associated with oxidative stress and altered membrane lipids (Samavat *et al*. 2014). Obesity-related changes in spermatogenesis, such as altered sperm RNA levels, DNA methylation, protamination, and histone acetylation, can affect offspring. This suggests that many factors in obese men may impair sperm quality, including sex hormone imbalance, oxidative stress, and chronic inflammation (McPherson & Lane, 2015). In particular, weight loss, exercise, lifestyle changes, or bariatric surgery increase serum testosterone levels and sperm count (Häkonsen *et al*., 2011). This increase in sperm count may be associated with decreased inflammatory activation and reduced NET production with consequent less sperm entrapment.

In autoimmune diseases like systemic lupus erythematosus (SLE), where 90 % of patients are diagnosed during reproductive age (Wang *et al*., 2015), a greater production of NETs has been noted, in addition to an altered ability to degrade them, thereby increasing their average lifespan (Heidari *et al*., 2016). This disease is characterized by the presence of antibodies, mainly antiDNA, related to the degree of activity of the disease, and it has been suggested that these antibodies could be induced by NETs (Gupta & Kaplan, 2016). Additionally, a higher

concentration of NETs has been suspected in pregnant women with SLE and placental insufficiency because in murine models, a greater formation of extracellular traps was demonstrated, which is why the greater presence of NETs could contribute to the immunological disorder of the placenta (Jiang *et al*., 2021). It has been suggested that they may be involved in perpetuating vascular damage in the placenta of patients with SLE, as well as fetal loss, limited intrauterine growth and pre-eclampsia (Gupta *et al*., 2006; Marder, 2016).

In patients with RA, other chronic diseases of autoimmune nature has also been described that neutrophils produce more NETs and higher amounts of ROS than healthy patients. Khandpur *et al*. (2013) demonstrated enhanced NETosis in circulating and synovial fluid RA neutrophils, compared to neutrophils from healthy controls.

The immune response to oxidative stress in diseases such as chronic prostatitis could persist in the seminal fluid for a time after the eradication of the infectious agent, inducing a more prolonged harmful effect on sperm function (Moretti *et al*., 2005; Calogero *et al*., 2017). This is supported in a review published by Condorelli *et al*. (2017), which comprised 27 studies, in which 3241 participants were included, including 1670 patients with chronic prostatitis (51.5 %). In this group of patients, a reduction in seminal volume, sperm concentration and motility was observed. One of the proposed mechanisms was increased oxidative stress generated by the residual inflammatory response, inducing greater synthesis of ROS and proinflammatory cytokines, mainly IL-8, a primary leukocyte mediator. As well as, the presence of *ex vivo* NETs in semen could be an element to evaluate inflammatory activation in the genital tract (Sardi *et al*., 2024).

DISCUSSION

ETs are an important defense mechanism through which neutrophils can trap and destroy germs; however, they might be detrimental to the increase in the inflammatory response and alteration of male and female fertility parameters. The different phenotypes of ETs are associated with different leukocyte sub-populations; however, neutrophils can release the three characteristic morphologies, whereas macrophages release mainly *diff*ETs (Schulz *et al*., 2019a). The presence and activation of inflammatory cells and the subsequent generation of ETs has a negative effect on sperm quality, altering their functional parameters and oocyte-binding capacity, reducing their fertilization potential. According to this review, we can state that the formation of ETs causes a loss of plasma membrane integrity, motility, viability and reduction in oocyte-binding capacity (Zambrano *et al*., 2016; Zambrano *et al*., 2021). The activation of leukocytes and formation of ETs can induce greater ROS synthesis and generate oxidative stress and secretion of proinflammatory cytokines such as IL-6, IL-8 and TNF (Allam *et al*., 2008), affecting the sperm membrane due to increased lipid peroxidation, altering mainly DPA and DHA. An important mechanism involved in this formation is SOCE-mediated calcium influx (Bréchard & Tschirhart, 2008). It has been shown that this NET-induced damage to the spermatozoa decreased significantly with the functional inhibition of SOCE with 2 – ABP (Zambrano *et al*., 2021). Therefore, in patients with a symptomatic or asymptomatic infection of the reproductive tract, the functional parameters of the spermatozoa could be affected not only by the microorganisms, but also by the permanent activation of PMN and macrophages (Fraczek & Kurpisz, 2007). The presence of chronic diseases or autoimmune pathologies shows an exacerbated inflammatory response and greater production of free radicals, with a constant generation of ETs, which also seem to be a factor during pregnancy, increasing the risk of placental vascular damage, intrauterine growth restriction and pre-eclampsia (Gupta *et al*., 2006; Marder, 2016). Nevertheless, studies are still lacking that provide more evidence about the real impact of this inflammatory response at the reproductive. However, these findings are relevant since the identification of increased ROS production or high NETs concentration can help to elucidate idiopathic infertility causes, in addition to providing tools that allow establishment specific therapeutic alternatives that directly reduce ROS production or exaggerated activation of neutrophils. Recently, it has been suggested that the presence of *ex vivo* NETs in semen could be an element to evaluate inflammatory activation in the genital tract by demonstrating that all patients with systemic Covid infection had NETs compared to healthy controls who were negative (Sardi *et al*., 2024).

CONCLUSION

The presence of infections in the reproductive tract and leukocyte activation with the formation of ETs in concentrations that exceed the compensatory mechanisms is associated with a negative effect on sperm quality and function. The increase of NETs in non-infectious etiologies such as autoimmune chronic diseases or pathologies, may play a silent role in cases of idiopathic infertility. However, additional studies are needed to confirm these theories, that can explain their possible adverse effects on the reproductive tract and that assess the direct impact on male or female fertility. Future research in this area would contribute with relevant information to design therapeutic strategies that can help reduce infertility rates in patients with chronic pathologies.

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RESUMEN: El papel de las trampas extracelulares de neutrófilos (NETs) y las trampas extracelulares de monocitos (METs) está bien establecido en los trastornos de la fertilización por efectos adversos sobre la motilidad de los espermatozoides, la integridad del acrosoma, la lipoperoxidación de la membrana y la alteración de la unión de los espermatozoides al ovocito. Además, las NETs/METs eliminan de manera eficiente el exceso de espermatozoides dentro del tracto reproductivo femenino (FRT) después del coito natural o la inseminación artificial (IA). Dependiendo de su ubicación en los órganos reproductivos, las NET/MET pueden ser beneficiosas para el huésped por su capacidad de limitar la diseminación de patógenos infecciosos, promover la cicatrización de heridas y ejercer propiedades antiinflamatorias a través de la degradación de componentes proinflamatorios. Por el contrario, la liberación excesiva o desequilibrada de NETs/METs dentro del FRT o el tracto urogenital masculino (UGT) podría estar asociada con una baja fertilidad debido a efectos nocivos sobre las funciones de los espermatozoides, la fertilización de los ovocitos, el microambiente del endometrio y la implantación del embrión. En UGT, los pacientes con epididimitis o infecciones bacterianas urogenitales y con leucocitospermia presentan liberación espontánea de NETs/METs en los fluidos seminales. Además, en pacientes masculinos/femeninos infértiles sin etiologías infecciosas pero con enfermedades autoinmunes (es decir, lupus eritematoso sistémico, artritis reumatoide), metabólicas (diabetes mellitus, obesidad) y vasculares (vasculitis, hipertensión y preeclampsia), los NETs/METs no controlados pueden influir negativamente en su fertilidad. Por lo tanto, esta revisión tiene como objetivo proporcionar nuevos conocimientos sobre los efectos beneficiosos y adversos de los NETs/METs en los gametos masculinos, la fertilización de ovocitos y el endometrio, destacando las amplias implicaciones en el diagnóstico temprano de trampas extracelulares (TE) en muestras de fluido seminal de pacientes infértiles con etiologías inflamatorias. Así como para el diagnóstico precoz de la formación espontánea de NETs/METs en parejas infértiles en las que uno o ambos padecen enfermedades inflamatorias, metabólicas, vasculares y/o autoinmunes crónicas, con el fin de aumentar las posibilidades de concepción.

PALABRAS CLAVE: Trampas extracelulares de neutrófilos; Trampas extracelulares de monocitos; Fertilidad; Reproducción; Espermatozoides; Infertilidad.

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