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INVITED REVIEW

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Germ cell apoptosis and survival in testicular inflammation

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Abstract

Male infertility is due to genetics, hormonal or environmental causes, or is idiopathic. Azoospermia is linked to local testicular microenvironment deregulation, with inflammatory cells present in the 15% of testicular biopsies of infertile patients. As widely reported, spermatogenesis and steroidogenesis are controlled by local immunoregulatory agents produced by immune and nonimmune cells. Moreover IL-6R, TNFR1, Fas and IL-1R are expressed on germ cells, indicating a direct action of pro-inflammatory agents on these cells. Beyond the known function of cytokines and nitric oxide on testicular function at the stable levels present in the normal testis, this review focalises on the effect of pro-inflammatory factors on germ cell survival and death when inflammatory conditions are established in the testis. As no cure for male infertility has been found up to the present, intracytoplasmic sperm injection is the therapeutic option for azoospermic patients who wish to achieve genetic parenthood. Therapies with antioxidant and anti-inflammatory agents in experimental models of testicular damage have been successful. However, clinical implementation is uncertain in cases with a prolonged inflammatory state of the testis. Therapies offering multiple approaches to treat infertility by restoring the spermatogonial stem cell niche and protecting germ cells from apoptosis should be considered.

KEYWORDS
germ cells, immune cells, nitric oxide, pro-inflammatory cytokines, testis

1 | INTRODUCTION

Inflammatory factors present in the normal testis, such as interleukin-6 (IL-6), TNFα, IL-1, FasL and NO, participate in the regulation of spermatogenesis, including proliferation of premeiotic germ cells (GCs), spermatocyte apoptosis, sperm release from seminiferous tubules (STs) and changes at the blood–testis barrier (BTB) to allow the passage of preleptotene spermatocytes into the luminal compartment (Loveland et al., 2017).

Spermatogenesis and steroidogenesis are affected by inflammation. In fact, inflammation is widely accepted as an important aetiological factor of subfertility and infertility. Many factors have been described as partially responsible for the disruption of the dynamic equilibrium among inflammatory/anti-inflammatory agents during spermatogenesis (O'Bryan & Hedger, 2008). These stressors alter local cytokine production and/or the number of effector and regulatory immune cells leading to infertility. In this review, we will describe the mechanisms by which different inflammatory mediators such as cytokines or oxidative stress are involved in germ cell apoptosis (GCA). Experimental models of acute and chronic testis inflammation are analysed, focusing on results obtained in an experimental model of autoimmune orchitis.

2 | GENERAL MECHANISMS OF APOPTOSIS

Apoptotic cell death can be triggered from outside the cell by the activation of death receptors TNFR1 and Fas, which upon binding with their own ligands, TNF-α and FasL, respectively, activate initiator caspases 8 and 10, which cleave and activate downstream

effector procaspases (such as caspase 3, 6 and 7) to kill the cell. Apoptosis may also be triggered by signals that activate the mitochondrial pathway regulating the release of cytochrome *c*. Release of cytochrome *c* from mitochondria depends on mitochondria outer membrane permeabilisation (MOMP). Once released, cytochrome *c* promotes the activation of procaspase 9 within the apoptosome complex, with the subsequent proteolytic activation of effector caspases (Elmore, 2007). The mitochondrial pathway is modulated by members of the Bcl-2 protein family; pro-survival proteins such as Bcl-2 and Bcl-xL inhibit mitochondrial pathway, whereas pro-apoptotic members including Bax, Bak and the BH3-only proteins (such as Bid, Bim, Puma, Bad and Noxa) stimulate it. BH3-only proteins are upstream regulators of Bax and Bak activation. Activated Bax and Bak are able to induce MOMP and cytochrome *c* release from mitochondria to cytosol (van Delft & Huang, 2006). Endoplasmic reticulum (ER) can also initiate apoptosis through the release of Ca^{2+}, which sensitises mitochondria to extrinsic and intrinsic death stimulus or directly activating caspases. Bcl-2, Bax and Bak modulate ER Ca^{2+} homeostasis and ER-mediated apoptosis (Yuan et al., 2016).

Inhibitors of apoptosis (IAPs) are multifunctional molecules that protect cells from accidental caspase activation. The IAP protein family regulates apoptosis by direct inhibition/degradation of caspases and modulation of the transcription factor nuclear κB (NF-κB; Elmore, 2007).

3 | APOPTOSIS IN ACUTE INFLAMMATION: LPS MODEL

Bacterial lipopolysaccharide (LPS) is a component of the cell wall of Gram-negative bacteria that has been widely used to study the impact of acute inflammation on testis physiology. LPS exerts its effects through the Toll-like receptor 4 (TLR4) complex. TLR4 is expressed by rat macrophages, Sertoli and Leydig cells. LPS administered to rats generates an inflammatory–oxidative microenvironment that induces temporal apoptosis of spermatocytes and spermatids turning on the apoptotic mitochondrial pathway (Liew, Meachem, & Hedger, 2007; Metukuri, Reddy, Reddy, & Reddanna, 2010; Reddy et al., 2006). Testicular macrophages and Sertoli cells respond to LPS by secreting pro-inflammatory cytokines IL-6 and IL-1α, while Leydig cells respond by secreting TNF-α, IL-1β and TGF-β1 (Hu et al., 2017; O'Bryan & Hedger, 2008); these cytokines may favour GCA activating TNFR1 and IL-6R, thereby inducing BTB disruption as IL-1α and TGF-β1 and generating more local inflammation as is the case of IL-1β (Theas, Jacobo, Pérez, Guazzone, & Lustig, 2018).

Pro-inflammatory testicular macrophages increase transiently 12 hr after LPS challenge decreasing to normal values by Day 3, and GCA starts at this time and proceed for 7 days more (Gerdprasert et al., 2002). This temporal asynchrony and the lack of markers of activation, as is the steady levels of inducible NOS (iNOS) expression in testicular macrophages, suggest that macrophages are not involved in the apoptotic response to LPS. On the contrary, in regions of testicular microhaemorrhage, neutrophils show an upregulation of iNOS expression, which points to them as inducers of GCA (Gerdprasert et al., 2002).

Acute LPS administration in mice induces apoptosis of spermatocytes, spermatids and also spermatogonia followed by a temporary reduction in sperm concentrations in the epididymis. GCA is mediated by Fas-FasL system in an autocrine–paracrine way, as Fas is upregulated in GCs and FasL in GCs and in Sertoli cells (Kajihara, Okagaki, & Ishihara, 2006). IL-18 seems to be a central cytokine in the induction of GC apoptosis in response to LPS in mice. IL-18 activates death and mitochondrial pathways by upregulating Fas-FasL, TNFα-TNFR1 systems and iNOS; IL-18 is expressed by activated testicular macrophages, Sertoli and Leydig cells, and however, the contribution of each population to the testicular increase in IL-18 levels was not evaluated. Together with IL-18, other pro-inflammatory cytokines with a known pro-apoptotic effect on GCs such as IL-6 and TNFα are produced in the testis after acute LPS administration. However, the overall apoptotic effect might depend only on IL-18, as depletion of IL-18 completely prevents GCA (Inoue et al., 2015). The effect of LPS on spermatocyte and spermatid apoptosis may certainly depend on TNFR1, Fas and IL-6R expressed by these cells; however, regarding spermatogonia whose Fas death receptors expression has not been clearly documented, the apoptotic effect of LPS may be indirect and probably mediated by Sertoli cells; in this regard, LPS is able to reduce spermatogonia self-renewal through inhibition of glial-derived neurotrophic factor secretion by Sertoli cells (Zhang, Shi, Li, Zhang, & Hao, 2014).

Most of the normal functions of the seminiferous epithelium depend upon androgen support. LPS decreases testicular testosterone, but not below 30% of normal levels in rats and mice (Kajihara et al., 2006; O'Bryan, Schlatt, Phillips, de Kretser, & Hedger, 2000); up to 20%–30% are sufficient to support full spermatogenesis (Sharpe, Donachie, & Cooper, 1988). Overall, the data point to a pro-apoptotic effect of LPS on GCs mediated by inflammatory factors such as NO, IL-1, IL-6, TNFα, FasL and IL-18 produced by macrophages, Sertoli cells and Leydig cells. Moreover, somatic cells are also able to respond to pro-inflammatory cytokines by releasing more inflammatory mediators; a positive feedback may be established which collaborates with the disruption of spermatogenesis during endotoxemia.

4 | APOPTOSIS IN CHRONIC INFLAMMATION: EXPERIMENTAL AUTOIMMUNE ORCHITIS (EAO) MODEL

In EAO induced in adult rats by active immunisation with testis homogenate in complete Freund's adjuvant using Bordetella pertussis, as co-adjuvant, focal orchitis develops 50 days after the first immunisation and a severe disease develops by 80 days. Testicular histopathology is characterised by an interstitial lymphomononuclear cell infiltrate associated with damage of STs. At last, aspermatogenesis

and atrophy of the STs and fibrosis are observed (Lustig, Rival, & Tung, 2013).

Apoptosis of postmeiotic GCs, spermatocytes and spermatids, and sloughing from the STs to epididymis occur progressively in orchitis. The number of apoptotic GCs in the severe phase of orchitis is approximately 10 times higher than in the focal phase. Caspases initiating external and internal pathways, caspase 8 and caspase 9, respectively, are activated and both responsible for the activation of the executioner caspase 3. Caspase 8 activity is highest in severe than in focal orchitis; concordantly, the percentage of apoptotic GC expressing TNFR1 and Fas increases in the damaged STs (Suescun, Rival, Theas, Calandra, & Lustig, 2003; Theas, Rival, & Lustig, 2003). Activation of caspase 9 occurs together with Bax/Bcl-2 imbalance, MOMP and cytochrome *c* release to cytosol (Figure 1; Theas, Rival, Jarazo, Guazzone, & Lustig, 2006).

Two isoforms of Bax protein, Baxβ (24 kDa) and Baxα (21 kDa), exist and are products of alternative mRNA splicing. Both isoforms are expressed in the normal testis. In orchitis, Baxα translocates from cytosol to mitochondria while pro-survival Bcl-2 content decreases, and the imbalance of these proteins in favour of pro-apoptotic Bax may induce MOMP and mitochondrial pathway activation. Baxβ, highly expressed in EAO, may sequester Bcl-2 and prevent its translocation to mitochondria. Baxα also translocates to the ER, suggesting that it may also regulate the ER apoptosis pathway (Theas et al., 2006).

Overexpression of Bcl-2 increases cell viability and prevents apoptosis in adverse tissue circumstances (Toruner et al., 2006).

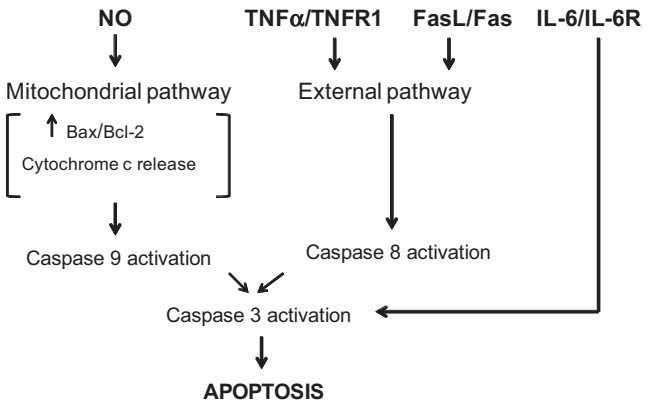

FIGURE 1 Apoptotic pathways and mediators involved in germ cell apoptosis (GCA) in experimental autoimmune orchitis (EAO). In EAO, spermatocytes and spermatids up regulate death receptors TNFR1 and Fas and also IL-6R. TNFα produced by interstitial macrophages activates caspase 8, which in turn activates caspase 3. FasL shed from interstitial lymphocytes (soluble Fas) or expressed by neighbouring GCs trigger Fas activation in a paracrine or autocrine pathway, inducing apoptosis. NO released by interstitial macrophages activates both external and mitochondrial pathway by promoting release of cytochrome *c* from mitochondria and caspase 9 activation followed by caspase 3 execution of apoptosis. Release of cytochrome *c* from mitochondria is facilitated by increased Bax/Bcl-2 ratio in the mitochondria. IL-6 contributes with GCA through the activation of caspase 3

The rise in Bcl-2 protein levels in basal GCs of the STs in EAO supports a protective role of Bcl-2 to counteract apoptotic stimuli in spermatogonia. Spermatogonia that remain attached to the STs during severe orchitis (Méndez et al., 2017) may guarantee the damaged testis responds to a normalised situation. Cytokines IL-1β, TNFα and IFNγ are able to regulate Bcl-2 family protein expression (Diaz-Ganete et al., 2015). In EAO, a specific GC pro-apoptotic/anti-apoptotic Bcl-2 protein family balance may occur in response to the pro-inflammatory cytokines, in order to prompt apoptosis of postmeiotic GCs and to preserve premeiotic GCs.

4.1 | Inflammatory Factors Involved in Germ Cell Apoptosis in EAO

4.1.1 | Fas-FasL system

Fas is a Type I membrane protein belonging to the TNF/nerve growth factor receptor family. Its ligand, FasL, is a Type II transmembrane protein belonging to the TNF family that may be processed into soluble form by metalloproteinases. Activation of Fas induces the binding of procaspase 8 to Fas-bound FADD leading to the activation of caspase 8 and apoptosis (Elmore, 2007).

In orchitis, Fas and membrane FasL (mFasL) are upregulated, being expressed by spermatocytes and spermatids in the STs, Leydig cells and CD4+ and CD8+ T cells in the interstitium, and testicular macrophages do not express membrane and intracellular FasL (Jacobo et al., 2012; Theas et al., 2003). The number of GCs expressing Fas and FasL or both molecules increased between focal and severe orchitis (Theas et al., 2003), for instance Fas expressing GCs, might die by the activation of Fas induced by FasL in an autocrine and/or paracrine way. Pro-inflammatory cytokines released by lymphomononuclear cells in EAO could modulate Fas expression on GCs. TNFα and IFNγ have been shown to increase Fas expression in the normal testis (Riccioli et al., 2000).

In focal EAO, testicular content of soluble FasL (sFas) is similar to that detected in normal rats; however, we cannot rule out sFasL involvement in GC death during this phase of the disease as the higher number of GCs expressing Fas may sensitise the testis to sFasL action. Intratesticular content of sFasL reaches its highest level in the severe phase. During severe orchitis, when paracrine interactions between Fas and mFasL bearing GCs are maximally disturbed, apoptosis could be mediated primarily by sFasL instead of mFasL. FasL, intratesticularly injected, enters the STs and induces GCA providing that sFasL participates in the control of GCA by binding to Fas expressed by these cells (Jacobo et al., 2012).

4.1.2 | TNFα-TNFR1 system

TNFα exerts its effects by binding to either cell membrane TNF-α receptor (TNFR) TNFR1 or TNFR2. TNFR1 contains a cytoplasmatic death domain (DD) and belongs to the family of death receptors; survival apoptotic and pro-inflammatory signals may also be initiated by stimulation of TNFR1. Which type of signal predominates depends on the balance of intracellular adaptor proteins interacting

with TNFR1. As the intracellular domain of TNFR2 lacks DD, it efficiently activates NF-κB pathway and JNK and is generally unable to elicit apoptosis (Vandenabeele, Declercq, Herreweghe, & Vanden Berghe, 2010).

In EAO, TNFα, produced by pro-inflammatory infiltrating monocytes and resident immunosuppresor macrophages, increases in the testis between the end of the immunisation and severe orchitis and induces GCA (Theas et al., 2008). Lymphocytes and Leydig cells are also a source of TNFα in orchitis (Jacobo, Pérez, Theas, Guazzone, & Lustig, 2011; Theas et al., 2008). Upregulation of TNFRI occurs in orchitis in STs, mainly in spermatocytes, most of the TNFR1+ GCs being apoptotic (Suescun et al., 2003). Apart from a direct effect of TNFα on GCA, indirect actions are also possible through TNFα disruption of the Sertoli cell tight junction barrier and the induction of other pro-apoptotic factors released from somatic cells. TNFα stimulates IL-6 release from Sertoli cells and peritubular cells and in combination with other cytokines induces NO production by Sertoli cells (Riccioli et al., 1995; Schell et al., 2008). In orchitis, a pro-survival role of TNFα is possible through the modulation of Bcl-2 family protein, and Fas-FasL system, as a compensatory mechanism to curb excessive GC death; TNFα is able to induce anti-apoptotic Bcl-xL expression in GCs and the release of soluble Fas (sFas) from Sertoli cells (Riccioli et al., 2000; Suominen, Wang, Kaipia, & Toppari, 2004). We propose that sFas might prevent FasL to interact with Fas expressed by GCs. TNFα is undetectable in the serum of EAO rats supporting the concept that TNFα acts locally. Aside from its role in GCA, TNFα is able to increase endothelial cell permeability, facilitating lymphomonocyte extravasation and activation in the testis (Bauché, Stéphan, Touzalin, & Jégou, 1998; Riccioli et al., 1995; Schell et al., 2008). In fact, Yule and Tung (1993) defined TNFα as an important cytokine in the pathogenesis of autoimmune orchitis developed in mice as i.p. neutralisation of the cytokine reduced the incidence and severity of the disease.

4.1.3 | IL-6-IL-6R system

IL-6 has pro-inflammatory and anti-inflammatory actions and induces a broad range of effects including cell proliferation and differentiation, cell cycle arrest and apoptosis (Hunter & Jones, 2015).

In orchitis, a significant increase in IL-6 secreted by inflammatory infiltrating monocytes occurs as well as an upregulation of IL-6R. Peritubular and Leydig also contribute to the rise in the testicular content of IL-6 in the inflammatory microenvironment of orchitis. On the contrary, Sertoli cells down regulate IL-6 expression in both focal and severe EAO; this behaviour may reflect a Sertoli cell function impairment or an attempt to cope with the deleterious effect of IL-6 in the STs (Rival, Theas, Guazzone, & Lustig, 2006). In orchitis, the number of spermatocytes and spermatids IL-6R+ in the STs rises simultaneously with the number of apoptotic GCs, indicating that IL-6 triggers GC death in a direct way (Pérez et al., 2012; Rival et al., 2006). Exogenous IL-6 induces GCA through caspase 3 activation and GC sloughing from STs. IL-6 upon binding to IL-6R activates JAK/STAT signalling which finally acts on the nucleus to change gene expression, through this pathway IL-6 may prompt Bcl-2/Bax imbalance and GCA (Ju, Byun, Mok, & Joo, 2016). An indirect mechanism of death mediated by IL-6 is possible through an increase in the number of leucocytes (CD45+ cells) able to secrete inflammatory mediators with a pro-apoptotic effect on postmeiotic GC. Exogenously IL-6 disrupts BTB and also impacts on premeiotic GCs in the ST (Pérez et al., 2012). IL-6 inhibits DNA synthesis in spermatogonia, and in spermatogonia B GC-1 cell line, IL-6 promotes the maintenance of the stem germ status (Hakovirta, Syed, Jegou, & Parvinen, 1995; Huang et al., 2016); in this way, beyond inducing GCA of postmeiotic GCs, IL-6 may promote premeiotic germ cell cycle arrest in orchitis (Méndez et al., 2017).

4.1.4 | NO-NOS system

Nitric oxide (NO) is a pro-oxidative molecule with multiple biological actions synthesised by enzymatic conversion of L-arginine to L-citrulline catalysed by NOS. Three isoforms of NOS exist, inducible (iNOS), endothelial (eNOS) and neuronal (nNOS). In general, low concentrations of NO promote cell survival, proliferation and homeostasis, whereas higher levels such as those occurring during inflammatory processes generate oxidative stress favouring cell cycle arrest, apoptosis and senescence (Thomas et al., 2008).

In the inflamed testis, NO is produced at higher levels than in normal testis, and an oxidative condition is established in this organ (Jarazo Dietrich et al., 2015; Jarazo-Dietrich et al., 2012). Upregulation of the NOSs isoform expression in the STs and in the interstitial cells occurs together with NOS-increased activity, being iNOS the isoform selectively upregulated. Pro-inflammatory cytokines such as TNFα, IFNγ and IL-6 produced in large amounts by interstitial lymphocytes and macrophages during EAO development might be involved in iNOS upregulation (Jacobo et al., 2011; Rival et al., 2008; Theas et al., 2008). In fact, in vitro stimulation of Sertoli and peritubular cells with combined cytokines (TNFα, IFNγ and IL-1) and LPS induce iNOS expression (Bauché et al., 1998).

Many studies performed on experimental models of testicular damage have shown the upregulation of NOS associated with pathological alterations of the testis (Turner & Lysiak, 2008). Our group has demonstrated for the first time the involvement of NO-NOS system in the induction of pathological alterations occurring during chronic inflammation (Jarazo Dietrich et al., 2015).

Pro-inflammatory macrophages are the main producers of NO in EAO testis. NO released by EAO macrophages disrupt testicular function, as it induces GCA and increased testosterone production by Leydig cells. NO activates the mitochondrial pathway of apoptosis and provokes a rise in Bax/Bcl-2 ratio by targeting Bax to the mitochondria, thereby triggering cytochrome *c* release and caspase 9 activation (Jarazo Dietrich et al., 2015).

During EAO development, spermatocytes and spermatids are the main targets of pro-inflammatory microenvironment. However, diethylenetriamine DETA-(NO), a slow-releasing of NO, and NO released by EAO macrophages induce apoptosis of basal GCs of normal testis (spermatogonia and/or preleptotene spermatocytes) (Jarazo

Dietrich et al., 2015). Pro- and anti-apoptotic protein expression may diverge in GCs in normal and inflamed testicular microenvironment, resulting in cells with different sensitivities to apoptotic stimuli. Premeiotic GCs of the basal compartment of STs express low levels of the anti-apoptotic Bcl-2 protein, and spermatocytes expressing death receptors are scarce, in the normal but not in the EAO testis (Rival et al., 2006; Suescun et al., 2003; Theas et al., 2003, 2006).

4.1.5 | IL-17-IL-17R system

IL-17A plays an essential role in inflammatory and autoimmune disease (Isailovic, Daigo, Mantovani, & Selmi, 2015). In EAO, the content of IL-17 (prototypical Th17 cytokine) increases in the testis, reaching its maximum level during the severe stage (Jacobo et al., 2011). IL-17 intratesticular injection induces BTB permeability, apoptosis and sloughing of spermatocytes through activation of executioner caspase 3 (Pérez et al., 2014). No receptors for IL-17 have been identified in GCs, and no apoptotic actions of this cytokine were reported. IL-17 stimulates a host of inflammatory cytokines and chemokines along with other inflammatory mediators such as NO, IL-6 and TNFα mRNA (Isailovic et al., 2015). An indirect effect of IL-17A on GCA is possible, mediated by pro-apoptotic agents released by somatic cells and/or recruited lymphocytes and macrophages, such as NO, IL-6 and TNFα, and the impairment of the BTB.

5 | AGEING

Testicular ageing is associated with decreased steroidogenesis and impaired spermatogenesis, events that are also prompted by local microenvironment alterations and deregulation of the hypothalamic–pituitary–gonadal axis (Lacombe et al., 2006). iNOS and TNFα upregulation, failure of the spermatogonia stem cell (SSC) niche to support an appropriate balance between SC self-renewal and differentiation, are associated with GCA during ageing in mice (Banerjee, Anjum, Verma, & Krishna, 2012; Hikim et al., 2005; Ryu, Orwig, Oatley, Avarbock, & Brinster, 2006). Matzkin et al., (2016) by comparing mice with delayed (GHRH-KO) to those with accelerated longevity, (GH-transgenic mice), demonstrated that the testis of the aged mice exhibits testicular inflammation, oxidative and apoptotic processes. This topic is reviewed by Frungieri et al., in this supplement of Andrologia.

6 | OBESITY

Obesity is a global health problem associated with dietary, genetic and hormonal factors, a high-fat diet being the most common cause of obesity**.** There is substantive evidence supporting that obesity causes peripheral inflammation based on the presence of cytokines (TNFα and IL-6) and the activation of pro-inflammatory signalling pathways; however, until recently, it was not clear that inflammation associated to obesity damages spermatogenesis . In the testis of obese mice, upregulation of TNFα mRNA, IL-6 mRNA, IL-1β protein, NOD-like receptor family pyrin domain-containing-3 protein (NLRP3), active caspase 1 and increased number of macrophages are associated with GCA (Fan et al., 2018; Wellen & Hotamisligil, 2005; Zhang et al, 2017). Intracellular inflammasome is a multiprotein complex, sensor of damage, infection and pathogen-associated signatures, comprised of several proteins (NLRP3, procaspase 1 and an adaptor molecule) that function by activating pro-IL-1β and IL-18 by caspase 1-dependent proteolytic cleavage. Activation of inflammasome present in macrophages and Sertoli cells may explain the increased content of IL-1β in the testis of obese mice (Hayrabedyan et al., 2016; Jo, Kim, Shin, & Sasakawa, 2016).

The mechanism involved in spermatogenesis disruption in obesity needs further clarification to unveil the role of inflammatory agents in GCA.

7 | CLINICAL IMPLICATIONS

Recent reports have estimated that almost 15% of couples are infertile worldwide, with over half of these cases being attributable to factors in the male partner (Inhorn & Patrizio, 2015). Testicular biopsies from infertile patients revealed asymptomatic focal inflammatory lesions in approximately 30% of the cases (Schuppe et al., 2008). GCs undergo a unique series of genetic and epigenetic modifications to finally become functional quality gametes, events that require an optimal testicular microenvironment to succeed (Hajj & Haaf, 2013). No cure for male infertility has yet been found until today. Intracytoplasmic sperm injection (ICSI) is the therapeutic option, for patients who wish to achieve genetic parenthood. ICSI performed with gametes of bad quality poses risks in terms of miscarriage, health and well-being of the offspring and perpetuation of the infertile phenotype (Aitken, 2018). In vitro gametogenesis provides an alternative source of gametes with the potential to cure infertility but is presently premature (Nagamatsu & Hayashi, 2017). Therefore, reconstitution of the testicular milieu may guarantee the production of quality gametes. In accordance, antioxidant and anti-inflammatory treatments have been employed experimentally to ameliorate testicular function; however, no specific treatment exists for infertility associated with a testicular inflammatory process (Arena et al., 2017; Theas et al., 2018).

8 | FUTURE PERSPECTIVES

Targeting apoptosis may represent an alternative therapeutic strategy in the treatment of infertility. This approach might be achieved by blocking activated apoptotic pathways or by inducing repressors of apoptosis. Caspase inhibitors have been employed in testicular torsion and varicocele to reduce GCA (Lysiak, Zheng, Woodson, & Turner, 2007). The mitochondria-derived cytoprotective peptide humanin, protects GCs from apoptosis induced by hormonal deprivation by preventing pro-apoptotic Bax to enter the mitcohondria

(Jia et al., 2013). Nortriptyline, a well-tolerated second-generation antidepressant inhibits MOMP, a central event for mitochondrial pathway induction, reduces GCA after testis de-torsion (Yazdani et al., 2017). Polydeoxyribonucleotide, an agonist of adenosine A2A receptor, stimulates the expression of IAPs, modulates Bcl-2 family protein and restores spermatogenic function in experimental varicocele (Minutoli et al., 2015).

A combination of antioxidant/anti-inflammatory therapies together with IAPs may be considered for full recovery of normal testicular function. This strategy may facilitate the reconstitution of a suitable microenvironment to re-start spermatogenesis from the remaining spermatogonia within STs and to prevent apoptosis of postmeiotic GCs which are the most sensitive cells to death insult in pathological conditions. Improvement of the spermatogonia niche to favour autorenovation, differentiation and proliferation of SSC might also be considered together with anti-inflammatory therapies, by transplantation of healthy nurse cells. Experimental Sertoli cell and mesenchymal SC transplantation have been demonstrated to stimulate partial recovery of endogenous spermatogenesis in the damaged testis (Hsiao, 2015; Malolina, Kulibin, & Kushch, 2016).

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