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Review

A search for molecular mechanisms underlying male idiopathic infertility

An Bracke [a], *Kris Peeters* [b], *Usha Punjabi* [b], *David Hoogewijs* [c], *Sylvia Dewilde* [a,*]

[a] *Department of Biomedical Sciences, University of Antwerp, Universiteitsplein 1, Antwerp 2610, Belgium*
[b] *Center for Reproductive Medicine, University Hospital Antwerp, Wilrijkstraat 10, Edegem 2650, Belgium*
[c] *Department of Medicine/Physiology, University of Fribourg, Chemin du Musée 5, CH 1700 Fribourg, Switzerland*

An Bracke is a PhD student in the Protein Science, Proteomics and Epigenetic Signalling laboratory at the Department of Biomedical Sciences, University of Antwerp. Her doctoral thesis focuses on the role of androglobin in male infertility.

KEY MESSAGE
Multiple studies have been undertaken to unravel the genetic and molecular background underlying idiopathic male infertility. This review summarizes and discusses the results of these studies and thus supports the development of improved genetic screenings and relevant biomarkers necessary for an adequate diagnosis and more personalized treatment of male infertility.

A B S T R A C T

Infertility affects approximately 15% of the couples wanting to conceive. In 30 – 40% of the cases the aetiology of male infertility remains unknown and is called idiopathic male infertility. When assisted reproductive technologies are used to obtain pregnancy, an adequate (epi)genetic diagnosis of male infertility is of major importance to evaluate if a genetic abnormality will be transmitted to the offspring. In addition, there is need for better diagnostic seminal biomarkers to assess the success rates of these assisted reproductive technologies. This review investigated the possible causes and molecular mechanisms underlying male idiopathic infertility by extensive literature searches of: (i) causal gene mutations; (ii) proteome studies of spermatozoa from idiopathic infertile men;(iii) the role of epigenetics; (iv) post-translational modifications; and (v) sperm DNA fragmentation in infertile men. In conclusion, male infertility is a complex, multi-factorial disorder and the underlying causes often remain unknown. Further research on the (epi)genetic and molecular defects in spermatogenesis and sperm function is necessary to improve the diagnosis and to develop more personalized treatments of men with idiopathic infertility.

Introduction

According to the World Health Organization (WHO), the definition of infertility is 'the inability of a sexually active, non-contracepting couple to achieve spontaneous pregnancy in one year' (WHO, 2010). About 15% of all couples are infertile and seek medical treatment for fertility and in 50% a male-infertility-associated factor is found together with abnormal semen parameters (Nieschlag et al., 2010). Known causes of male infertility are: congenital or acquired urogenital

* Corresponding author.
E-mail address: sylvia.dewilde@uantwerpen.be (S Dewilde).
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abnormalities, malignancies, urogenital tract infections, increased scrotal temperatures, endocrine disturbances, immunological factors and some characterized genetic abnormalities. However, in 30 to 40% of the cases, the aetiology of male infertility remains unknown and it is called idiopathic male infertility (Nieschlag et al., 2010). Despite the fact that these men have no history of diseases affecting fertility and show normal findings of physical examinations and endocrine, genetic and biochemical laboratory testing, their semen analysis often reveals abnormal semen parameters. The most common abnormalities in a routine semen analysis are: absence of spermatozoa (azoospermia); very low sperm count in the ejaculate (oligozoospermia); abnormal sperm morphology (teratozoospermia) and/or abnormal sperm motility (asthenozoospermia). These idiopathic sperm abnormalities are assumed to be caused by several factors, including reactive oxygen species (ROS), unknown genetic and epigenetic abnormalities and endocrine disruption by environmental pollution (European Association of Urology, 2015). According to the human protein atlas, 74% of all human proteins (*n* = 19692) are expressed in the testis and 1980 of these genes display an elevated expression in testis compared with other tissue types. An analysis of the genes with elevated expression in the testis shows that most of the corresponding proteins are involved in spermatogenesis (Djureinovic et al., 2014; Fagerberg et al., 2014; Uhlen et al., 2015; Yu et al., 2015). Spermatogenesis covers a complex network of processes that occur in the seminiferous tubules of the testis. The subsequent processes of spermatogenesis can be described as: (i) proliferation of spermatogonia; (ii) spermatogonial differentiation into spermatocytes; (iii) meiotic division of round spermatids; and (iv) the release of highly specialized mature spermatozoa into the testicular tubule lumen (Neto et al., 2016). Mutations in any of these genes may directly or indirectly lead to abnormalities in one of the spermatogenic processes and may subsequently result in male infertility. These genetic defects are mostly not genetically transferable (because of the infertile phenotype) and are a result of *de novo* mutations during the gametogenesis. This renders appropriate characterization of the genetic aetiology of male infertility difficult. Notwithstanding these difficulties, many studies were undertaken to unravel the genetic and molecular background underlying the different male infertility phenotypes (azoospermia, oligozoospermia, teratozoospermia and asthenozoospermia).

The condition with an absence of spermatozoa in the ejaculate is referred to as azoospermia and is identified in 11% of infertile men. In 40% of the cases azoospermia is caused by a physical blockage to the male excurrent ductal system (obstructive azoospermia, OA) and here the aetiology is mostly known. The remaining cases are called non-obstructive azoospermia (NOA) and are frequently associated with testicular failure, characterized by a smaller testis volume and elevated LH and FSH concentrations (Wosnitzer et al., 2014). However, often the cause of NOA remains unknown and might be due to genetic abnormalities. These genetic defects may result in a total absence of spermatogenesis [also named 'Sertoli Cell Only (SCO) syndrome'] or in a maturation arrest (MA) of spermatogenesis.

An ejaculate spermatozoa count of less than 15 million/ml is termed oligozoospermia (WHO, 2010). A recently published study on 1737 patients with reduced total sperm counts revealed that in 60% of the cases the primary causal factor could not be assigned and that almost 75% of all oligozoospermic cases remained idiopathic (Punab et al., 2016).

Teratozoospermia is a condition where more than 96% of the spermatozoa in the sperm sample have an abnormal morphology (WHO, 2010). Two severe and rare phenotypes of teratozoospermia are recognized: macrozoospermia and globozoospermia. Macrozoospermia is characterized by the presence of a very high percentage of spermatozoa with enlarged and irregular shaped heads and multiple flagella. These spermatozoa display a high rate of polyploidy, many being tetraploid. Globozoospermia is characterized by the presence of a large majority of round spermatozoa lacking the acrosome in the ejaculate. These sperm cells are unable to adhere and penetrate the zona pellucida. These phenotypes are monomorphic since all the spermatozoa display the same abnormality. A third, heterogeneous phenotype of teratozoospermia has been described by Ben Khelifa *et al.* (Ben Khelifa et al., 2014) and is called 'multiple morphological anomalies of the flagella (MMAF). This phenotype includes spermatozoa with absent, short, bent and coiled flagella and flagella of irregular width. These morphological abnormalities of the sperm flagella also lead to impaired sperm motility (asthenozoospermia).

WHO criteria delineate the normal percentage of motile spermatozoa to be 40%. A lower percentage of motile spermatozoa is referred to as asthenozoospermia (WHO, 2010). Asthenozoospermia represents a common cause of male infertility and is observed in up to 81% of all abnormal semen analyses, frequently combined with oligo- and/or teratozoospermia. Only 18% of the cases are isolated asthenozoospermic (immotile spermatozoa with a normal morphology and sperm count) (Curi et al., 2003). Possible causes of decreased sperm motility include prolonged duration of sexual abstinence, unhealthy lifestyle, varicocele and/or infection. However, mostly the aetiology of asthenozoospermia remains unknown and may be caused by genetic factors.

In some male infertility cases no abnormalities are detected after conventional semen analysis (sperm count, motility and morphology) and the aetiology of infertility remains unclear. These cases are referred to as normozoospermic infertility. Mature mammalian spermatozoa require capacitation in the female reproductive tract before binding to and crossing the zona pellucida and finally fusing with the oocyte plasma membrane. Defects in these processes are not detectable during sperm analysis and may represent a possible cause of idiopathic normozoospermic male infertility. At the cell biology level, capacitation induces changes in the sperm motility pattern known as hyperactivated movement and prepares the sperm to undergo an exocytotic process known as the acrosome reaction. At the molecular level, capacitation is associated with cholesterol loss from the sperm plasma membrane, increased membrane fluidity, changes in intracellular ion concentrations, hyperpolarization of the sperm plasma membrane, increased activity of the protein kinase A (PKA) and protein tyrosine phosphorylation (Stival et al., 2016). Defects in any of these molecular mechanisms may result in male infertility.

This review investigates the possible causes of male idiopathic infertility by extensive literature searches of: (i) causal gene mutations linked to male infertility; (ii) proteome studies of sperm from idiopathic infertile men; (iii) the role of epigenetics; (iv) post-translational modifications; and (v) sperm DNA fragmentation in infertile men. In this way, an extensive overview of the knowledge obtained during the last decades about idiopathic infertility and its underlying molecular mechanisms was created. We believe that this information is important to properly understand the aetiology of male infertility and to develop adjusted genetic screenings and biomarkers towards a better diagnosis and more personalized treatment of male infertility.

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