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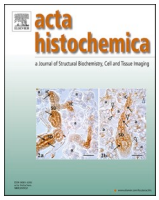

Sialylation status and its relationship with morphofunctional changes in human adult testis during sexually mature life and aging: A narrative review

Mirko Manetti [a], Mirca Marini [a], Angelica Perna [b], Alessia Tani [a], Eleonora Sgambati [c,*]

[a] Department of Experimental and Clinical Medicine, Section of Anatomy and Histology, Imaging Platform, University of Florence, Largo Brambilla 3, Florence 50134, Italy
[b] Department of Medicine and Health Sciences "Vincenzo Tiberio", University of Molise, Campobasso, Italy
[c] Department of Biosciences and Territory, University of Molise, Contrada Fonte Lappone, Pesche, Isernia 86090, Italy

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ABSTRACT

Sialic acids (Sias) are a family of electronegatively charged nine-carbon monosaccharides containing a carboxylic acid, mostly found as terminal residues in glycans of glycoproteins and glycolipids. They are bound to galactose or N-acetylgalactosamine via α2,3 or α2,6 linkage, or to other Sias especially via α2,8 linkage, which results in monomeric, oligomeric, and polymeric forms. Sias play determinant roles in a multitude of biological processes in human tissues from development to adult life until aging. In this review, we summarized the current knowledge on the sialylation status in the human testis with a main focus on sexually mature life and aging, when this organ shows significant morphofunctional changes resulting into variations of hormonal levels, as well as changes in molecules involved in mitochondrial function, receptors, and signaling proteins. Evidence suggests that Sias may have crucial morphofunctional roles in the different testicular components during the sexually mature age. With advancing age, significant loss of Sias and/or changes in sialylation status occur in all the testicular components, which seems to contribute to morphofunctional changes characteristic of the aging testis. Based on the current knowledge, further in-depth investigations will be necessary to better understand the mechanistic role of Sias in the biological processes of human testicular tissue and the significance of their changes during the aging process. Future investigations might also contribute to the development of novel prophylactic and/or therapeutic approaches that, by maintaining/restoring the correct sialylation status, could help in slowing down the testis aging process, thus preserving the testicular structure and functionality and preventing age-related pathologies.

1. Introduction

Aging is a natural process consisting in irreversible changes due to a myriad of endogenous and environmental factors found in all eukaryotic organisms at the molecular, cellular, tissue, organ, and system levels, comprising the human female and male reproductive systems (Perheentupa and Huhtaniemi, 2009; Basaria, 2013; Gunes et al., 2016). In general, the elderly population is currently defined as people aged 65 years or above, but the increase in life expectancy could push this threshold upwards in the next few years (Padilla Colón et al., 2018; Dumic et al., 2019; Gómez-Gómez and Zapico, 2019). As far as the female and male reproductive systems are concerned, a different aging trend occurs. In fact, while reproductive activity in women stops with menopause, a complete cessation of the reproductive potential does not occur in men. Anyway, there is clear evidence of decreased fecundity with advancing male age (Perheentupa and Huhtaniemi, 2009; Basaria, 2013; Gunes et al., 2016; Santiago et al., 2019; Bhasin et al., 2022). Indeed, aging can affect male reproductive function at multiple levels, from sperm production and quality to the morphology and histology of the male reproductive system. The morphofunctional changes occurring in the testis result in variations of hormonal levels, as well as changes in molecules involved in mitochondrial function, receptors, and signaling proteins (Paniagua et al., 1991; Sampson et al., 2007; Perheentupa and Huhtaniemi, 2009; Basaria, 2013; Paul and Robaire, 2013; Sibert et al., 2014; Zirkin and Papadopoulos, 2018; Gunes et al., 2016; Santiago et al., 2019). However, these age-related alterations show great individual

* Corresponding author.
E-mail address: eleonora.sgambati@unimol.it (E. Sgambati).

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variability, and their real impact on male fertility and reproductive health are still far from being fully understood (Zirkin and Papadopoulos, 2018; Santiago et al., 2019). In addition, epidemiological investigations point toward an association of low hormone levels, especially testosterone, with changes in sexual function, body composition, physical function and mobility, increased risk of diabetes, late life persistent depressive disorder (dysthymia), unexplained anemia of aging, osteoporosis, and bone fractures (Sampson et al., 2007; Bhasin et al., 2022). Another interesting aspect is the increased risk of specific genetic disorders related to paternal age among the offspring of older men (Paul and Robaire, 2013; Gunes et al., 2016; Santiago et al., 2019; Bhasin et al., 2022). Thus, reproductive aging of men is emerging as an important public health problem whose serious social consequences go far beyond the quality of life issues related to decreased fertility (Bhasin et al., 2022).

Among the numerous molecules involved in the structure and functionality of the human testis tissue, sialic acids (Sias) seem to play a crucial role (Arenas et al., 1998; Gheri et al., 2003, 2004a, 2004b, 2009; Marini et al., 2020). It is well established that Sias are molecules playing determinant roles in a multitude of biological processes in human tissues and organs, ranging from development and growth to adult life until aging, both in physiological and pathological conditions (Gheri et al., 2004c, 2007, 2009; Gnanapragassam et al., 2014; Marini et al., 2014, 2017, 2020, 2021; Schauer, 2016; Whited et al., 2018; Schauer and Kamerling, 2018; Li and Ding, 2019). They are a large family of electronegatively charged nine-carbon monosaccharides containing a carboxylic acid, mostly common in higher animals and some microorganisms (Varki, 2007; Schauer, 2009; Whited et al., 2018). Sias are typically found attached at the terminal position of glycan structures in glycoproteins and glycolipids (gangliosides) and of free oligosaccharides, located on the cell surface and in the cellular secretions (Wang and Brand-Miller, 2003; Wang et al., 2003; Schauer, 2009, 2016). They are usually bound to galactose (Gal) or N-acetylgalactosamine (GalNAc) via α2,3 or α2,6 linkage, or to other Sias via α2,8 or more rarely α2,9 linkage, which results in monomeric, oligomeric, and polymeric forms (Gnanapragassam et al., 2014; Varki et al., 2017; Whited et al., 2018). N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc) are the major Sias. Nevertheless, it appears that human healthy tissues are able to synthesize Neu5Ac, while Neu5Gc synthesis occurs only in some cancer types (Varki and Schauer, 2009; Gnanapragassam et al., 2014; Varki et al., 2017; Whited et al., 2018). In addition, 2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (KDN), a deaminated neuraminic acid, has been found in humans and other mammals (Whited et al., 2018). Modifications of 4-, 5-, 7-, 8-, and 9-hydroxyls of Sias can produce more than 80 sialoderivatives (Schauer and Kamerling, 2018), the most common of which are O-acetylated (Schauer, 2009; Varki et al., 2017; Schauer and Kamerling, 2018). Over the years, a number of useful tools have been developed for the recognition of Sias and to understand the functionality of either Sias or their derivatives and underlying linkage, such as lectins, antibodies, biochemical methods, imaging technology, and nanotechnology (Gheri et al., 2007, 2009; Marini et al., 2014, 2017, 2020; Schauer and Kamerling, 2018; Whited et al., 2018; Guo et al., 2019; Yang et al., 2021). Given their variability, Sias determine an extensive structural diversity of glycoconjugates, glycans and oligosaccharides (Wang and Brand-Miller, 2003; Wang et al., 2003; Whited et al., 2018). This variability, together with their electronegative charge and terminal location, accounts for Sias being involved in fundamental biological processes at the cellular level, such as cell−cell interactions, cell activation, differentiation, transformation, and migration (Whited et al., 2018). Sias can act as important biological recognition sites, interacting with specific proteins (lectins or other receptors) of vertebrate cells, protozoa, bacteria, mycoplasma, viruses, as well as with hormones, toxins, and antibodies (Schauer, 2009; Varki et al., 2017). Besides, Sias can even act as a biological mask, that is an antirecognition agent by shielding recognition sites such as penultimate monosaccharides of glycan chains or proteins and other macromolecules of cell membranes. This may be due to their electronegative nature together with their bulky, hydrophilic chemical structure (Schauer, 2009; Varki et al., 2017). In this regard, particular attention is given to the polymeric form, polysialic acid (PolySia), which is mostly attached to the neuronal cell-adhesion molecule (NCAM). PolySia mainly plays this masking role due to its remarkably steric hindrance, high hydration and slipperiness. It appears to be mainly expressed in developing and neuronal tissues, as well as in numerous types of cancer cells (Schauer, 2009; Varki et al., 2017; Marini et al., 2020, 2021; Sato and Kitajima, 2021). In such a context, a combination of several enzymes is involved in Sia synthesis (synthases), their transfer on carbohydrate chain (sialyltransferases), modification of Sia hydroxyls, such as the addition of acetylic groups (acetyltransferase) and their removal (acetylesterase), and Sia degradation (sialidases/neuraminidase), in order to maintain the normal and functional sialylation status in different types of tissues during development and adult life (Varki and Schauer, 2009; Schauer and Kamerling, 2018).

Herein, we aimed to overview the sialylation status in the human testis during the various stages of life, mainly focusing on the adult life until aging (Arenas et al., 1998; Gheri et al., 2003, 2004a, 2009; Marini et al., 2020), when several morphofunctional changes occur (Paniagua et al., 1991; Sampson et al., 2007; Perheentupa and Huhtaniemi, 2009; Basaria, 2013; Paul and Robaire, 2013; Sibert et al., 2014; Gunes et al., 2016; Zirkin and Papadopoulos, 2018; Santiago et al., 2019; Bhasin et al., 2022). In the different studies covered by this review, Sia detection was performed using lectin histochemistry and immunohistochemistry methods as reported in Table 1. First, we concisely reviewed the expression of Sias in the different components of testis and their possible role during testis organogenesis until reaching the correct structure and functionality in the adult life. Then, after an overview of the principal morphofunctional changes occurring in the aging testis, we focused on how during adult life, in sexually mature men, Sias can contribute to a variety of biological processes that are fundamental to maintain the structure and functions of the different testis components, and how changes in Sia content and/or distribution may be related to alterations of such processes in older age. Overall, the present narrative review can contribute to achieve a clearer outline of the role of sialylation amongst the mechanisms underlying the many morphofunctional changes affecting the human testis during sexually mature life and aging.

2. Methods

We performed this review by conducting a multiple database search (PubMed, Scopus, and Web of Science) for English language articles published between 1980 and 2023 using the following keywords: “aging”, “aging testis”, “sialic acids and testis”, “sialic acids and aging”. Among the identified articles, we mainly focused on those dealing with human testis. Some reference lists of the identified articles were further articles that had been searched. In particular, for the selection process of the articles we proceeded as follows: 1) identification of 115 titles concerning aging in general, morphofunctionality of human testis, Sias in aging, and Sias in testis; 2) exclusion of 48 titles as duplicate titles and/or abstracts, editorials, and commentaries; and 3) inclusion of 67 original articles, reviews or book chapters of which 30 concerning aging in general and/or morphofunctionality of human testis, and 37 concerning Sias in aging and/or Sias in testis.

3. Sialic acids in the developing human testis

In human testis, Sias are differentially expressed and seem to play crucial roles in the different testicular components from fetal development to the adult age (Arenas et al., 1998; Gheri et al., 2003, 2004a, 2004b, 2009; Marini et al., 2020). In fact, although the few studies available in literature employed lectin histochemistry that could not discriminate the different types of Sias (i.e., WGA lectin and PNA lectin

Table 1
Lectin histochemistry and immunohistochemistry for detection of sialic acids (Sias).

Lectins and Antibodies	Abbreviation	Sugar residues binding specificity	Treatments	References
Maackia amurensis agglutinin	MAA	Neu5Ac(α2,3)Gal		Arenas et al. (1998) Gheri et al. (2009) Marini et al. (2020)
Sambucus nigra agglutinin	SNA	Neu5Ac(α2,6)Gal/GalNAc		Arenas et al. (1998) Gheri et al. (2009) Marini et al. (2020)
Peanut agglutinin	PNA	D-Gal(β1,3)-D-GalNAc	Neuraminidase* KOH/Neuraminidase** Oxidation/neuraminidase° Oxidation/KOH/neuraminidase§	Arenas et al. (1998) Gheri et al. (2003, 2004a, 2009)
Soybean agglutinin	SBA	α/β-D-GalNAc > D-Gal	Neuraminidase*	Arenas et al. (1998)
Dolichos biflorus agglutinin	DBA	α-D-GalNAc	Neuraminidase*	Arenas et al. (1998)
Helix pomatia agglutinin	HPA	D-GalNAc(α1,3)-D-GalNAc	Neuraminidase*	Arenas et al. (1998)
anti-Polysialic Acid antibody	PolySia-specific mAb	Sia(α2,8)(Sia)n		Marini et al. (2020)

*Treatment for detection of Sias linked α−2,3, α−2,6 and α−2,8, before staining with PNA, SBA, DBA and HPA lectins (Arenas et al., 1998; Gheri et al., 2003, 2004a, 2009); **Treatments for detection of Sias containing acetylic groups on C4 of the pyranose ring before staining with PNA (Gheri et al., 2009); °Treatments for detection of Sias contain O-acetyl groups in the side chain before staining with PNA (Gheri et al., 2009); §Treatments for detection of Sias contain O-acetyl groups in the side chain and acetylic groups on C4 of the pyranose ring before staining with PNA (Gheri et al., 2009).

with neuraminidase treatment), both prenatal and postnatal developing testes already display a peculiar sialylation status.

3.1. Sias in the prenatal developing testis

In fetal testis, pre-Sertoli cells, pre-gonocytes, Leydig cells and vessels are all characterized by the presence of Sias since the early development stages (8–12 weeks of gestation) (Gheri et al., 2003).

It has been suggested that in both pre-Sertoli cells and pre-gonocytes Sias could not only act as structural molecules, but also play a role in male sexual differentiation, particularly by establishing junctions between the pre-Sertoli cells and the pre-gonocytes (Gheri et al., 2003). In addition, in a recent study (Marini et al., 2020), it has been hypothesized that likewise PolySia could be present and behave as either a masking factor in the seminiferous tubules favoring the proliferation of the pre-gonocytes without interferences, or a reservoir of some important factors and/or hormones, helping to release them in a coordinated manner in order to favor a correct and complete testicular histogenesis likely by blocking early germ cell maturation. This hypothesis arises mainly from the evidence of high levels of PolySia in testicular seminoma, which is thought to result from the proliferation of immature spermatogonia due to a failure in the normal maturation of germ cells arising from primordial germ cells during fetal life or postnatal development (Fukawa and Kanayama, 2018; Rajpert-De Meyts et al., 2018; Batool et al., 2019; Marini et al., 2020). This results in a severe impairment of the spermatogenesis process, which requires complex interactions between germinal cells and the other testicular components.

Regarding the endocrine Leydig cells, the presence of cells exhibiting morphological features of steroidogenic activity has been observed from approximately 7 to 8 weeks of gestation (Makabe et al., 1995; Svechnikov et al., 2010). Hence, a possible role played by Sias in inducing and maintaining the functionality of the Leydig cells has been hypothesized since early fetal development (Gheri et al., 2003). Moreover, there is substantial evidence that during fetal age the Leydig cells produce high levels of androgen (i.e., testosterone or androstenedione, depending upon the species) for differentiation of male genitalia and brain masculinization (Zirkin and Papadopoulos, 2018).

Sias present in endothelial cells of the fetal testicular capillary vessels could have a role in the uptake and transport of substances between the blood flow and the interstitial tissue in the developing testis (Gheri et al., 2003).

3.2. Sias in the postnatal developing testis

In newborn testis Sias were not present in Sertoli cells and endothelial cells (Gheri et al., 2004a). On the contrary, in the prepubertal testicular tissue Sias were detected in Sertoli cells, spermatogonia and endothelial cells, but not in Leydig cells (Gheri at al., 2004b). On the other hand, it is to keep in mind that gonadotrophin levels, which are high during fetal age, especially in the 17th-18th week stage, decrease in circulation towards the end of gestation, probably due to the negative feedback exerted by placental estrogens. Therefore, at birth, gonadotrophin levels are low and then increase after the first week, which greatly influences the morphofunctionality of testis in the postnatal period (Rey, 2014).

4. Sialic acids in human adult testis during sexually mature life and aging

In adult life, a wide characterization of the different types of Sias, especially regarding their linkages and O-acetylation modification, has been performed in the various testicular components including seminiferous tubule cells, Leydig cells, intertubular stroma and vessels (Arenas et al., 1998; Gheri et al., 2009; Marini et al., 2020). Collectively, these studies highlighted interesting changes in testicular expression of Sias between sexually mature men and aged men that were related to morphofunctional alterations.

4.1. Overview of the morphofunctional changes in the human testis during aging

Morphofunctional changes occur in the aging testis and result in variations of hormonal levels, as well as changes in molecules involved in mitochondrial function, receptors, and signaling proteins (Paniagua et al., 1991; Sampson et al., 2007; Perheentupa and Huhtaniemi, 2009; Basaria, 2013; Paul and Robaire, 2013; Sibert et al., 2014; Gunes et al., 2016; Zirkin and Papadopoulos, 2018; Santiago et al., 2019; Bhasin et al., 2022). However, such age-related alterations show individual variability (Paniagua et al., 1991; Sampson et al., 2007; Perheentupa and Huhtaniemi, 2009; Sibert et al., 2014; Zirkin and Papadopoulos, 2018; Santiago et al., 2019) (Fig. 1).

4.1.1. Testicular morphology in aging

Histomorphometric and ultrastructural studies on aging human testes detected various alterations such as narrowing of tubular diameter, tubular sclerosis, reduction in the number of Leydig cells, Sertoli cells and spermatogenic cells, cellular vacuolization and

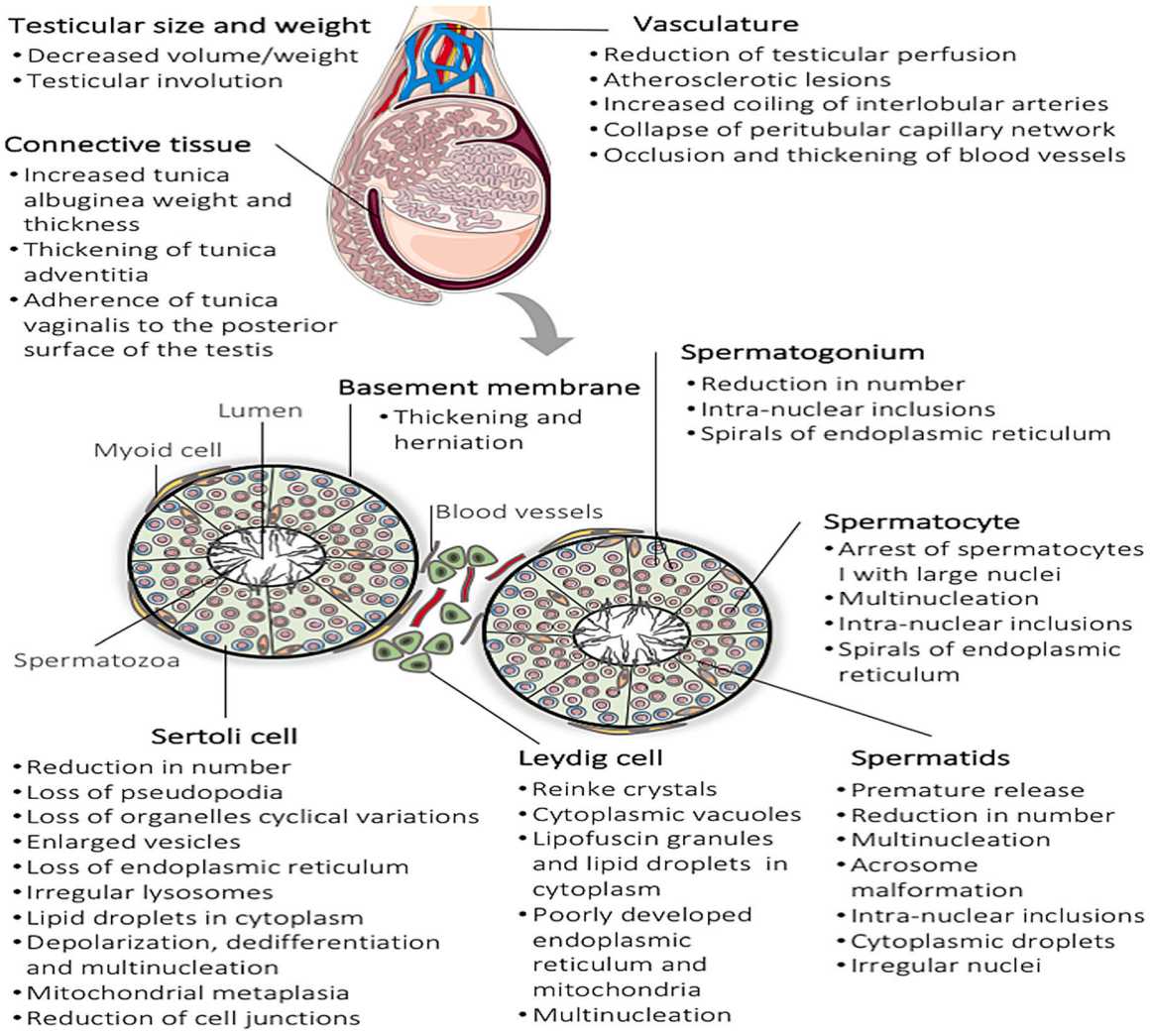

Fig. 1. Testicular structure. Upper part - Cross-section showing the location of the seminiferous tubules, the vas deferens and the epididymis as well as the tunica albuginea. Lower part - Schematic cross-section of testicular tubules illustrating the germ cells at different stages of maturation within a somatic Sertoli cell. Leydig cells and vasculature are present in the interstitium. Major morphological, cellular, and ultrastructural alterations associated with age in testis are indicated. (Reproduced with permission from Santiago et al., 2019).

multinucleation, thickening of seminiferous tubule basement membrane, modifications in the vasculature and stromal tissue fibrosis (Paniagua et al., 1991; Sampson et al., 2007; Perheentupa and Huhtaniemi, 2009; Sibert et al., 2014; Gunes et al., 2016; Santiago et al., 2019).

4.1.1.1. Leydig cells and seminiferous tubule cells. The majority of studies reported a reduction in the number of Leydig cells along with relevant ultrastructural changes with age such as intranuclear Reinke crystals or paracrystalin inclusions, multiple vacuoles, lipofuscin granules and lipid droplets in the cytoplasm. These cells also show signs of dedifferentiation and involution with poorly developed endoplasmic reticulum and mitochondria, as well as multinucleation (Paniagua et al., 1991; Sampson et al., 2007; Perheentupa and Huhtaniemi, 2009; Sibert et al., 2014; Gunes et al., 2016; Santiago et al., 2019). With increasing age, a reduction in testicular perfusion and an increase in arteriosclerotic lesions of testicular arterioles may partially explain the reduction in the number and function of Leydig cells. In fact, a decrease in blood supply and, therefore, in oxygen supply and luteinizing hormone (LH) levels may also be responsible for the reduction in testosterone production (Perheentupa and Huhtaniemi, 2009; Santiago et al., 2019).

Sertoli cells support the developing germ cells during their maturation, providing nutrients and protection from immune attack. Multiple alterations associated with aging have been observed in the Sertoli cell population such as a decrease in the cell number, linked to a decrease in seminiferous epithelial volume, odd-shaped nuclei, vesiculated endoplasmic reticulum, and irregular lysosomes (Paniagua et al., 1991; Sampson et al., 2007; Perheentupa and Huhtaniemi, 2009; Sibert et al., 2014; Gunes et al., 2016; Santiago et al., 2019). Of note, some of the lysosomes of Sertoli cells appeared large, oddly shaped, containing lipidic inclusions, and scattered all over the cytoplasm. Moreover, they comprised large, empty intercellular spaces, presumably previously occupied by developing germ cells and/or resulting from the loss of ability of aged Sertoli cells to break down waste products. An increased phagocytosis of degenerating germ cells results in an excessive accumulation of lipid droplets in the cytoplasm of Sertoli cells (Paniagua et al., 1991; Sampson et al., 2007; Perheentupa and Huhtaniemi, 2009; Sibert et al., 2014; Gunes et al., 2016; Santiago et al., 2019). Additional Sertoli cell abnormalities reported in aging include loss of polarity, dedifferentiation, multinucleation, and mitochondrial metaplasia (Paniagua et al., 1991; Sampson et al., 2007; Perheentupa and Huhtaniemi, 2009; Sibert et al., 2014; Gunes et al., 2016; Santiago et al., 2019). Sertoli cell intercellular tight junctions, which are essential to maintain the blood–testis barrier, are rarely detectable in aging and frequently replaced by focal contact points, thus compromising the specific microenvironment in which spermatogenesis occurs (Gunes et al., 2016;

Santiago et al., 2019).

Impaired spermatogenesis with loss of germ cells has also been observed in aging testes (Paniagua et al., 1991; Sampson et al., 2007; Perheentupa and Huhtaniemi, 2009; Sibert et al., 2014; Gunes et al., 2016; Santiago et al., 2019). This phenomenon was characterized by the disarrangement of spermatogenic cells and the release of premature spermatids from the seminiferous epithelium into the tubular lumen. Age-related changes in germinal cells seem to start with the spermatids and progressively affect less mature cell types. Therefore, the number of germinal cells in the seminiferous tubule wall usually decreases as age advances, resulting in a reduced diameter of the seminiferous tubules along with consistent epithelium vacuolization (Paniagua et al., 1991; Perheentupa and Huhtaniemi, 2009; Santiago et al., 2019). It has been observed that older men have reduced volume of seminiferous epithelia associated with decreased daily sperm production (Sampson et al., 2007; Perheentupa and Huhtaniemi, 2009; Sibert et al., 2014; Gunes et al., 2016; Santiago et al., 2019). Morphologically, aged human testes were found to exhibit multinucleated spermatocytes and spermatids, which possibly result from the fusion of cell membranes of neighboring spermatocytes and spermatids followed by cell degeneration (Paniagua et al., 1991; Sampson et al., 2007; Santiago et al., 2019). Other ultrastructural alterations in germ cells comprise acrosome malformation, redundant nuclear membranes, intra-nuclear inclusions, irregular nuclei, condensation of the chromatin and nuclear fragmentation, excessive droplet accumulation in the cytoplasm and spirals of endoplasmic reticulum in the cytoplasm (Santiago et al., 2019).

4.1.1.2. Basement membrane, vasculature and connective tissue. Thickening and herniation of basement membrane of the seminiferous tubules have been described in aged testes (Sampson et al., 2007; Perheentupa and Huhtaniemi, 2009; Santiago et al., 2019).

The interstitium surrounding the seminiferous tubules is highly vascularized. It has been shown that the pattern of testicular involution observed in aged men was similar to that observed after induction of tissue ischemia (Paniagua et al., 1991; Santiago et al., 2019). Indeed, such a pattern is influenced by alterations in testicular perfusion caused by vascular changes, mainly in the most distal regions of artery supply. The decrease of testicular perfusion is also caused by atherosclerotic alterations in testicular arterioles. In addition, the testicular microvasculature revealed increased coiling of interlobular arteries with age, which was accompanied by the degradation of the peritubular capillary network ultimately resulting in a notable reduction of blood flow. The decreased blood supply related to vascular changes might explain the involution of the seminiferous tubules (Santiago et al., 2019).

Moreover, a thickening of the boundary tissue surrounding the seminiferous epithelium, composed of myoid cells separated by collagen fibers, occurs with aging (Perheentupa and Huhtaniemi, 2009).

The tunica propria and tunica albuginea of the aging testis also become thickened due to increasing fibrosis (Paniagua et al., 1991; Perheentupa and Huhtaniemi, 2009; Sibert et al., 2014; 2019). These changes may be part of a vicious cycle, where oxygen deprivation due to vascular changes may have a leading role (Santiago et al., 2019).

4.1.2. Testicular dysfunction in aging

Aging has an effect on every level of the hypothalamic–pituitary–testicular axis, which is responsible for the regulation of the testicular functionality (Sampson et al., 2007; Basaria, 2013; Sibert et al., 2014; Santiago et al., 2019; Bhasin et al., 2022). The main functions of the testis are spermatogenesis and steroidogenesis, two processes that are not completely independent. These functions are affected by age resulting in alterations in hormonal levels during senescence, especially lower circulating levels of androgens. However, decreased androgen levels in aging men do not result in a complete cessation of reproductive capacity (Sampson et al., 2007; Basaria, 2013; Sibert et al., 2014; Gunes et al., 2016; Zirkin and Papadopoulos, 2018; Santiago et al., 2019; Bhasin et al., 2022).

4.1.2.1. Spermatogenesis. It is known that androgen stimulation of Sertoli cells is fundamental for the initial induction of spermatogenesis (Santiago et al., 2019). The reduction in testicular testosterone production with age can strongly impact in spermatogenesis by affecting the function of Sertoli cells (Santiago et al., 2019). The plasma levels of follicle-stimulating hormone (FSH), the second major stimulus for spermatogenesis, increase with age in older men, which is associated with a decrease in testicular size and spermatogenic efficacy (Basaria, 2013; Gunes et al., 2016; Santiago et al., 2019). In fact, increasing levels of FSH in elderly men has been linked to germ cell degeneration during meiosis, affecting sperm concentration (Santiago et al., 2019). In addition, circulating inhibin levels decline with aging, with older individuals presenting significantly lower concentrations of this glycoprotein already at the age of 40 (Santiago et al., 2019). Such a decrease in the inhibin secretion suggests that the testicular endocrine functions decline as soon as the fourth decade of life, and that Sertoli cell function declines earlier than that of other somatic testicular cells (Santiago et al., 2019).

4.1.2.2. Steroidogenesis. One of the most relevant age-related changes is the decline in testosterone levels, particularly plasma levels (Sampson et al., 2007; Basaria, 2013; Sibert et al., 2014; Gunes et al., 2016; Zirkin and Papadopoulos, 2018; Santiago et al., 2019; Bhasin et al., 2022). However, some studies failed to detect alterations in testosterone levels in aged healthy men (Basaria, 2013; Santiago et al., 2019). There is no consensus about the mechanism that causes age-related testosterone decline, but it may be associated with either alterations at the three levels of the hypothalamic–pituitary–testicular axis or a decrease in the testicular function (Sampson et al., 2007; Basaria, 2013; Santiago et al., 2019; Bhasin et al., 2022). The reduced number of Leydig cells or their aging and impaired testicular perfusion due to atherosclerosis, as well as a reduction in the release of testosterone upon human chorionic gonadotropin stimulation, support a primarily testicular cause for low testosterone levels (Sampson et al., 2007; Basaria, 2013; Sibert et al., 2014; Gunes et al., 2016; Zirkin and Papadopoulos, 2018; Santiago et al., 2019). Moreover, it was demonstrated that serum LH levels normally tend to rise with aging, possibly as a response to the decline in testosterone (Sampson et al., 2007; Basaria, 2013; Santiago et al., 2019).

4.1.3. Molecular changes in the aging testis

Several molecular mechanisms have been suggested to contribute to testicular aging, such as damage by reactive oxygen species (ROS) produced during aerobic metabolism, which directly implicates mitochondria in the aging process (Paul and Robaire, 2013; Gunes et al., 2016; Santiago et al., 2019). In fact, mitochondrial dysfunction has been reported in testicular aging (Gunes et al., 2016; Santiago et al., 2019). In addition, aging was associated with an increase in oxidative stress and free radical production, due to the alterations in the enzymatic activity of antioxidant enzymes (Paul and Robaire, 2013; Gunes et al., 2016; Santiago et al., 2019). Numerous age-related testicular alterations, including the decrease in steroidogenic capacity, have also been associated with an increase in tissue inflammation (Santiago et al., 2019). With aging, the testicular capsule begins thicker, as above mentioned, and the response to norepinephrine and prostaglandin becomes progressively higher, suggesting that neuro-humoral agents may have an important role in the maintenance of testicular capsular contractions (Santiago et al., 2019).

4.1.3.1. Leydig cells. Several molecular changes have been identified in the steroidogenic pathway of aged Leydig cells in humans that collectively result in the reduction of testosterone production (Santiago et al., 2019). Alterations in the expression profiles of several genes and a decrease in LH receptor levels have been reported (Santiago et al., 2019). Moreover, the response of Leydig cells to LH depends not only on

the number of receptors and the circulating concentration of glucocorticoids, but also on 11β-hydroxysteroid dehydrogenase enzyme activity that, with increasing age, decreases in expression with consequent reduction in testosterone production (Santiago et al., 2019). The impact of aging on the number and volume of Leydig cells and the above-mentioned molecular alterations may partly explain the reduction in testosterone levels often observed in older men (Santiago et al., 2019). However, such alterations may result from a combination of various elements, including environmental factors (Santiago et al., 2019).

4.1.3.2. Sertoli cells. Sertoli cell secretions and metabolites are crucial for the normal occurrence of spermatogenesis. The expression of some of these products is dependent on an intricate network of signaling molecules and hormones (Santiago et al., 2019). Aging is able to alter the production of some important secretions by the Sertoli cells and even their molecular components, such as transcripts, cytoskeleton proteins, and secretory proteins (Santiago et al., 2019). Additionally, age-related accumulation of intracellular amyloid fibrils in Sertoli cells has been reported. This accumulation is concurrent with the buildup of lipofuscin-loaded lysosomes and presence of damaged mitochondria, in Sertoli cells. Furthermore, dysfunctional mitochondria produce more ROS and lipofuscin accumulation sensitizes cells to ROS-induced damage in a kind of vicious cycle amplifying Sertoli cell damage and dysfunction (Santiago et al., 2019).

4.1.3.3. Germ cells. The decreased number of germinal cells in aged testes is accompanied by genomic instability and several changes in gene expression profile, particularly in the expression of spermatogonia markers (Paul and Robaire, 2013; Santiago et al., 2019). In addition, changes in protein amount, especially those involved in oxidative stress, were detected (Paul and Robaire, 2013; Gunes et al., 2016; Santiago et al., 2019). Of note, the balance between proliferation of spermatogonia and apoptosis of different germ cell types appears to be disturbed with aging. Increased ROS production, decreased ATP production, and apoptosis are three features of dysfunctional mitochondria related to aging. Indeed, high oxidative stress causes an increase in lipid peroxidation, DNA damage and apoptosis, which ultimately culminates in loss of sperm motility and vitality (Gunes et al., 2016).

4.2. Sialic acids in the sexually mature human testis

Data concerning the expression of Sias in the testis of sexually mature men are not always in agreement. In fact, by using a direct lectin histochemistry method, the study of Arenas et al. (1998) demonstrated the presence of the monomeric form Sia α2,3 linked to galactose (MAA lectin), but not monomeric form Sia α2,6 linked to galactose or galactosamine (SNA lectin), in all cell types of the human seminiferous epithelium (i.e., spermatogonia, spermatocytes, spermatids, and Sertoli cells), in Leydig cells and in the peritubular stroma (i.e., lamina propria). In contrast with the findings of Arenas et al. (1998), Gheri et al. (2009) showed that Sia α2,3 linked to galactose was absent in all seminiferous tubular components, Leydig cells, lamina propria and interstitial tissue, while Sia α2,6 linked to galactose or galactosamine was present in the interstitial tissue and lamina propria in some testicular samples. Of note, it has been hypothesized that these discrepancies could be due to the different fixatives employed (Gheri et al., 2009). It should also be noted that the testis samples analyzed in these studies arose from a small number of men with different age ranges (number of cases: 17, age range: 25–70 years, Arenas et al., 1998; number of cases: 10, age range: 18–30 years, Gheri et al., 2009). However, another more recent investigation performed on testicular tissue specimens from men with age range of 18–39 years (number of cases: 6, Marini et al., 2020) revealed some different findings and provided additional interesting observations with respect to those reported in the study of Gheri et al. (2009). Indeed, a variability in the content and distribution of both α2,3- and α2,6-linked Sias was observed. In particular, α2,3-linked Sia was present in some germinal cells, especially spermatides/spermatozoa and in microvessels. On the contrary, α2,6-linked Sia was detected in the intertubular stroma and microvessels from almost all samples (Fig. 2 A, B). Of note, it was found that only the testicular tissue collected from an 18 year-aged man almost lacked both Sia types with the exception of microvessels. Overall, as far as Sias in monomeric form are concerned, data arising from the

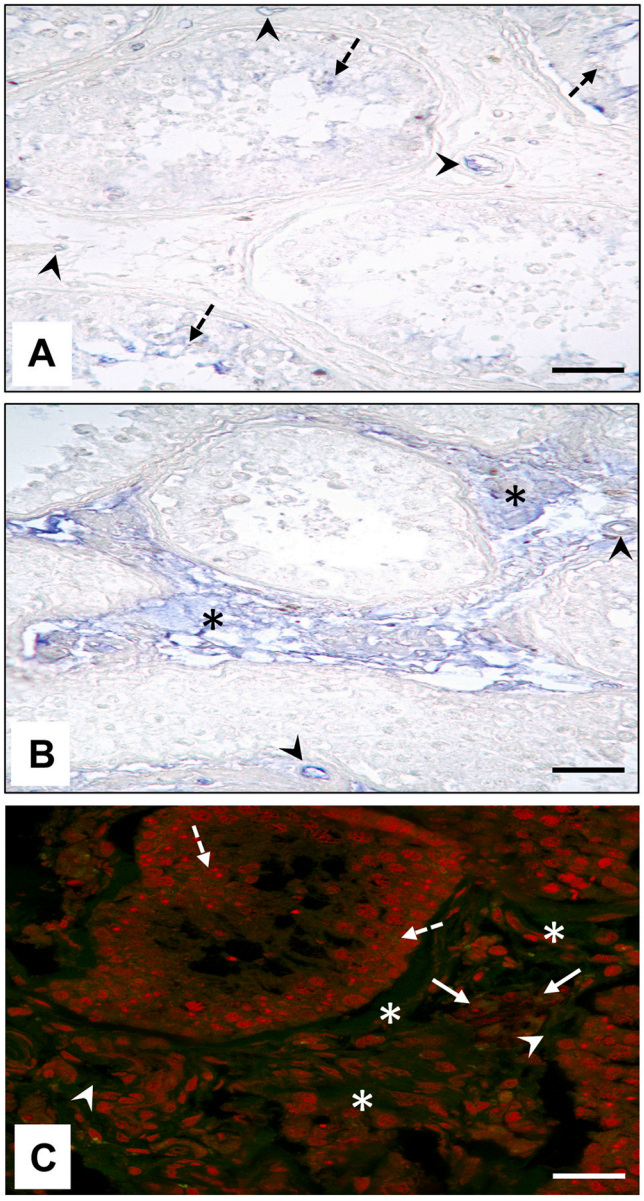

Fig. 2. MAA and SNA lectin reactivity (digoxigenin-labeled) showing α2,3 and α2,6 galactose (Gal)- or N-acetyl-D-galactosamine (GalNAc)-linked Sias, and PolySia-specific monoclonal antibody immunoreactivity (Alexa Fluor 488-conjugated IgG-labeled) in testicular samples from a man aged 25 years (A-C). (A) Weak MAA lectin reactivity is present in some germinal cells of the seminiferous tubules (dashed arrows) and in the endothelial cells of microvessels (arrowheads). No reactivity is seen in peritubular and interstitial stromal tissue, as well as in Leydig cells. (B) Moderate SNA lectin reactivity is detected in the intertubular stroma (asterisks) and microvessels (arrowheads). Seminiferous tubules, peritubular stroma and Leydig cells are negative. (C) Weak PolySia immunoreactivity (green) is present in the seminiferous tubules (dashed arrows), peritubular and intertubular stromal tissue (asterisks), Leydig cells (arrows), and microvessels (arrowheads) of normal testis. Nuclei are stained red with propidium iodide. Scale bar = 40 μm (A, B), 25 μm (C). Sias, sialic acids; PolySia, polysialic acid.

aforementioned studies seem to reveal a certain variability in their content and distribution in the different testicular components among healthy individuals. This could be related to interindividual differences in testicular functionality, probably due to different external stimuli represented by hormones and/or a variety of other factors (Marini et al., 2020).

Another interesting finding was observed using indirect lectin histochemistry methods. In fact, Arenas et al. (1998) reported that treatment with neuraminidase increased reactivity to PNA, SBA and HPA lectins in all the seminiferous tubule cells, as well as in Leydig cells and in lamina propria, demonstrating the presence of Sia linked to D-galactose-β(1–3)-N-acetyl-D-galactosamine and N-acetyl-D-galactosamine. Some discrepancies were subsequently observed in the work by Gheri et al. (2009), in which using PNA lectin with neuraminidase treatment the authors detected the presence of Sia linked to D-galactose-β(1–3)-N-acetyl-D-galactosamine in seminiferous tubule cells, interstitial tissue, lamina propria and endothelium of microvessels. In addition, in all the samples deacetylation treatments demonstrated the presence of Sia containing acetylic groups in both Sertoli cells and germ cells, and further revealed that steroidogenic Leydig cells exhibited only acetylated Sia. Interestingly, in this study the direct and indirect lectin histochemistry methods did not always gave similar results. Hence, Gheri et al. (2009) hypothesized the presence of PolySia long chains interfering with binding by SNA and MAA, which recognize monomeric Sia and its α2,3 and α2,6 linkages to the penultimate sugar residues galactose and/or galactosamine. Indeed, by using immunohistochemistry Marini et al. (2020) have subsequently demonstrated the presence of the PolySia in all tissue components of all testicular specimens (Fig. 2 C). Interestingly, acetylated Sias and PolySia seem to be more evenly distributed in the testicular components with respect to non-acetylated Sias in monomeric form.

Crucial morphofunctional roles have been ascribed to Sias in the different testicular components during the sexually mature age.

Concerning the seminiferous tubules, Sias and other carbohydrates have been demonstrated to play a crucial role during fertilization, particularly as constituents of the human sperm (1994; Fliniaux et al., 2022). In addition, different carbohydrates including Sias present in the germ cell plasma membranes seem also to be needed for Sertoli cell-germ cell interactions during spermatogenesis and, later on, for interactions with the male excurrent duct epithelia, as well as cell surfaces in the female genital tract (Ertl and Wrobel, 1992; Akama et al., 2002; Fliniaux et al., 2022). Moreover, the presence of different carbohydrates including Sias in the Golgi complex and cytosol of Sertoli cells might be related to either secreted or structural glycoproteins (Arenas et al., 1998). Indeed, it is well known that Sertoli cells secrete various paracrine factors that are involved in the control of germ cells, peritubular cells, and Leydig cells (Skinner, 1993; Santiago et al., 2019). The expression of some of these factors are dependent on an intricate network of signaling molecules and hormones (Santiago et al., 2019). With regard to PolySia, it is known that it plays not only a role as anti-adhesive molecule involved in tissue plasticity, but it is also able to bind various growth factors (Rollenhagen et al., 2013; Hänsch et al., 2014; Strubl et al., 2018). In a study on roe deer testis, variability in the level of PolySia has been observed during key steps of the "on/off mechanisms" of spermatogenesis, which led to propose that PolySia may influence the interaction and communication of Sertoli cells with germ cell precursors (Hänsch et al., 2014). In particular, PolySia could influence cell-cell interactions and support spermatogenesis, the self-renewing of undifferentiated spermatogonia and survival of germinal cells through a mechanism of retention/release of growth factors (Hänsch et al., 2014). Therefore, it has been proposed that PolySia might play such a dual role also in human testis, where it could be involved in the regulation of normal spermatogenesis in the seminiferous tubules (Marini et al., 2020). Interestingly, Simon et al. (2013a),(2013b) demonstrated that PolySia is partially integrated into the sperm membrane of the postacrosomal region during the epididymal transit. It is proposed that PolySia in semen represents a cytoprotective element to increase the amount of vital sperm, counteracting histone as well as neutrophil extracellular trap-mediated cytotoxicity against host cells, which plays a role after insemination.

As far as Leydig cells are concerned, Sias in general seem to play a role in maintaining the normal cell structure (Arenas et al., 1998; Gheri et al., 2009; Marini et al., 2020). In addition, as in the tubule components, PolySia could mostly act as a reservoir of growth factors also in the Leydig cells (Marini et al., 2020).

Interestingly, the presence of both monomeric Sias and PolySia in microvessels is also noteworthy (Gheri et al., 2009; Marini et al., 2020). Besides the repulsive action of Sias between the luminal surface of the vascular endothelium and erythrocytes, it is known their role in the formation of a lumen in developing blood vessels aa well as their involvement in the angiogenic process (Chiodelli et al., 2018; Strubl et al., 2018; Li and Ding, 2019). In particular, it has been demonstrated that the association of Sias with several proangiogenic molecules in endothelial cells is involved in angiogenesis through the regulation of various molecular interactions (Chiodelli et al., 2018). Moreover, PolySia in the endothelium might exert a role of protection against inflammation and atherogenesis owing to its repulsive properties, as well as binding of various growth factors to concentrate and protect them from proteolytic cleavage (Strubl et al., 2018). PolySia associated with other pro-angiogenic molecules, in particular neuropilin-2, seems also involved in angiogenesis (Rollenhagen et al., 2013; Chiodelli et al., 2018).

Regarding the testicular stroma, the sialylation status seems to be important to maintain the normal connective tissue structure as support for testicular tubules together the vascular component (Ayisi et al., 1982; Paniagua et al., 1991; Gheri et al., 2009).

Finally, concerning Sia acetylation in testis tubule cells and Leydig cells, it has been hypothesized that it could play a crucial role in the maintenance of the structure and functionality of these cells, including the intercellular interactions between Sertoli cells and germinal cells during their maturation and the production of androgens by Leydig cells (Gheri et al., 2009). Interestingly, it is known that Sia acetylation may also control the apoptotic activity of sialylated glycoconjugates, especially gangliosides (Malisan et al., 2002; Mandal et al., 2015).

4.3. *Sialic acids in the human testis during aging*

To our knowledge, at present only one study has investigated the expression of Sias in the human aging testis (Gheri et al., 2009). However, the reported data were sufficiently exhaustive to provide a first relationship of the sialylation status with morphofunctional changes typical of the aging testis. The findings arising from this investigation showed that in testis from 15 older men, from 70 to 93 years, drastic changes occur in the expressions of the different types of Sias in the various testicular components. In fact, both Sias α2,3 linked to galactose and Sias α2,6 linked to galactose or galactosamine were observed in the testicular interstitial tissue and in the lamina propria but not in tubules, Leydig cells and vascular endothelium of all testicular samples examined (Gheri et al., 2009). After the necessary enzymatic and chemical treatments, PNA lectin revealed structural changes and/or the gradual disappearance of Sia linked to D-galactose-β(1–3)-N-acetyl-D-galactosamine in the different testicular components. In particular, the presence of unacetylated Sia linked to D-galactose-β(1–3)-N-acetyl-D-galactosamine was detected in all the seminiferous tubule cells and the lamina propria. Instead, Leydig cells, interstitial tissue and endothelium did not show the presence of Sia linked to D-galactose-β(1–3)-N-acetyl-D-galactosamine (Fig. 3 A–D). Therefore, loss of Sias and/or important changes in sialylation status seem to occur with aging. In particular, most noteworthy was the lack of acetylation and of likely PolySia chains in the aging testis. In addition, the interindividual variability in Sia distribution found in mature adult testis was no longer observed in the aging testis.

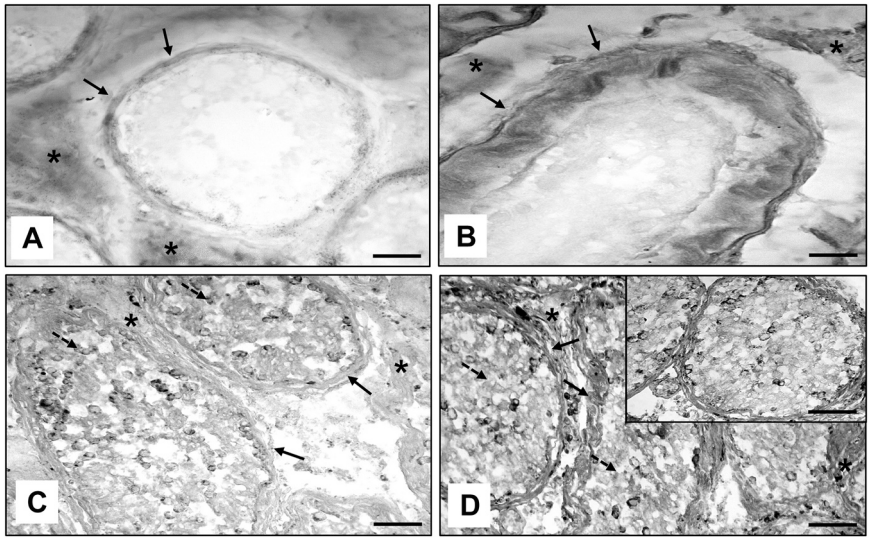

Fig. 3. MAA and SNA lectin reactivity (digoxigenin-labeled) showing α2,3 and α2,6 galactose (Gal)- or N-acetyl-D-galactosamine (GalNAc)-linked Sias and PNA, Neuraminidase-PNA, KOH-Neuraminidase-PNA (digoxigenin-labeled) staining for detection of acetylated or unacetylated Sias linked to D-galactose-β(1,3)-N-acetyl-D-galactosamine (D-Gal(β1,3)-D-GalNAc) in testicular samples from a man aged 90 years (A-D). MAA (A) and SNA (B) reactivity is observed in the interstitial tissue (asterisks) and in the lamina propria (arrows). PNA reactivity (C) is detectable in the seminiferous tubules (dashed arrows), interstitial tissue (asterisks) and lamina propria (arrows). After neuraminidase (D) and KOH-Neuraminidase (inset in D) treatments, PNA reactivity in the seminiferous tubules (dashed arrows) and in the lamina propria (arrows) is increased with respect to PNA without treatments. No differences in reactivity intensity is observed between the two treatments. Scale bar = 50 μm (A, C, D, inset in D), 25 μm (B). Sias, sialic acids.

Concerning the expression of Sias in seminiferous tubules, besides an evident decrease, important changes in the acetylation of Sias were found in the Sertoli cells and in the developing germinal cells in the oldest men, which could be implicated in the structural alterations of these cells, as well as in the impairment of intercellular interactions between Sertoli cells and germinal cells (Gheri et al., 2009). Indeed, it is known that aging is related to multiple structural, molecular and functional alterations of Sertoli cells and germ cells, arrests germ cell division and decreases the number of both cell types of (Schulze and Schulze, 1981; Holstein, 1986; Paniagua et al., 1987, 1991; de Miguel et al., 1997; Baird et al., 2005; Dakouane et al., 2005; Miething, 2005; Sampson et al., 2007; Perheentupa and Huhtaniemi, 2009; Sibert et al., 2014; Gunes et al., 2016; Santiago et al., 2019). In addition, the balance between proliferation of spermatogonia and apoptosis of different germ cell types appears to be disturbed with aging (Gunes et al., 2016). Of note, as above reported, Sia acetylation critically seems also to play a role in control of the apoptotic activity of sialylated glycoconjugates (Malisan et al., 2002; Mandal et al., 2015). These alterations, together with the significant reduction in the number of normally structured seminiferous tubules (Paniagua et al., 1991; Perheentupa and Huhtaniemi, 2009; Santiago et al., 2019), could result in the reduced, although not abolished, fertility of aged men.

Even the lack of Sias in Leydig cells in the testes from the oldest men has been hypothesized to be related to structural alterations of these cells (Gheri et al., 2009). In this regard, it has been demonstrated that during aging Leydig cells show various structural changes (Paniagua et al., 1991; Sampson et al., 2007; Perheentupa and Huhtaniemi, 2009; Sibert et al., 2014; Gunes et al., 2016; Santiago et al., 2019), as well as a decrease in their number, molecular changes and dysfunction, which results in the decline in testosterone levels (Paniagua et al., 1991; Sampson et al., 2007; Perheentupa and Huhtaniemi, 2009; Sibert et al., 2014; Gunes et al., 2016; Santiago et al., 2019; Basaria, 2013; Zirkin and Papadopoulos, 2018; Bhasin et al., 2022).

Of note, the consistent Sia absence in the endothelial cells of vessels in the aging testis may also have important consequences (Gheri et al., 2009). In particular, it has been hypothesized that aging endothelial cells no longer produce Sias, and this could contribute to changes in plasma membrane integrity and intercellular adhesion, contributing to the development of vascular lesions (Gheri et al., 2009). Furthermore, it has been demonstrated that the development of tubular involution with age is similar to that observed after experimental ischemia, suggesting that vascular alterations may play an important role in age-related testicular atrophy (Paniagua et al., 1991; Santiago et al., 2019).

Concerning the testicular stroma, the Sia changes occurring with aging seem to be related to structure alterations as well (Gheri, 2009). In this regard, thickening and herniation of basement membrane of the seminiferous tubules, as well as thickening of the boundary tissue surrounding the seminiferous epithelium and of tunica propria and tunica albuginea with increasing fibrosis have been noticed in aged testes (Sampson et al., 2007; Perheentupa and Huhtaniemi, 2009; Santiago et al., 2019; Paniagua et al., 1991; Perheentupa and Huhtaniemi, 2009; Sibert et al., 2014; Gunes et al., 2016). Of note, the fibrotic process affecting the tunica propria and tunica albuginea may be part of a vicious cycle, where oxygen deprivation due to vascular changes may have an important role (Santiago et al., 2019). Both changes in vascular and stromal Sias may work together to determine testicular atrophy (Gheri et al., 2009).

5. Conclusions and perspectives

Sias play a multitude of roles in physiological and pathological human tissues and organs, ranging from development to adult life until aging. In this review, we focused on the sialylation status in the human testis tissue during the sexually mature life and during aging, when this organ shows significant morphofunctional changes resulting in variations of hormonal levels, changes in molecules involved in mitochondrial function, receptors, and signaling proteins. Although the reproductive potential is not abolished, there is clear evidence of decreased fecundity with advancing male age. In addition, it has been demonstrated a relationship of variations in hormone levels not only with changes in sexual function, but also with alterations of body composition, physical function and mobility, as well as increased risk of various metabolic disorders. Therefore, the aging of testicular tissue seems to affect the healthy status of the whole man body. Overall, age-related alterations in sialylation status showed great interindividual variability. Nevertheless, it appears that Sias may have relevant

morphofunctional roles in the different testicular components during the sexually mature age. Moreover, with advancing age, significant loss of Sias and/or changes in sialylation status, such as lack of acetylation, occur in all the testicular components, which can contribute to a variety of structural and functional changes characteristic of the aging testis.

The main limitation of the present overview is that to date only one study investigated the expression of Sias in the human aging testis. However, currently available data are sufficiently exhaustive to highlight a relationship of the sialylation status with morphofunctional changes typical of the aging testis. In addition, various discrepancies are present in literature regarding the sialylation status in sexually mature testis. Therefore, having comprehensively resumed the current state-of-the-art, we are confident that this review will provide the basis for further in-depth investigations that will be necessary to fill data gaps and resolve discrepancies found in the literature, ultimately helping to better understand the role of sialylation status in the biological processes of human testicular tissue and the importance of Sia changes during the aging process. In particular, it will be needful to investigate Sia expression on a major number of cases and age groups, possibly covering the entire postnatal life period up to the advanced age. In-depth histochemical analyses by using a wider panel of lectins and various chemical and enzymatic treatments, as well as immunohistochemical investigations are also required. In addition, it could be also interesting to investigate possible changes in the enzymatic apparatus involved in Sia metabolism. In perspective, such a deeper knowledge might lead to the development of novel prophylactic strategies and therapeutic approaches that, by maintaining and/or restoring the correct sialylation status, could contribute to the preservation of the morphofunctionality of testis, ultimately slowing down the testicular aging process and helping to prevent age-related pathologies.

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Angelica Perna: Investigation, Writing – review & editing. **Mirca Marini:** Supervision, Visualization, Writing – original draft, Writing – review & editing. **Mirko Manetti:** Conceptualization, Investigation, Supervision, Visualization, Writing – original draft, Writing – review & editing. **Eleonora Sgambati:** Conceptualization, Investigation, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing. **Alessia Tani:** Investigation, Writing – review & editing.

Conflict of interest

The authors declare no conflict of interests.

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