



# Developing glycoproteomics reveals the role of post-translational glycosylation in the physiological and pathological processes of male reproduction

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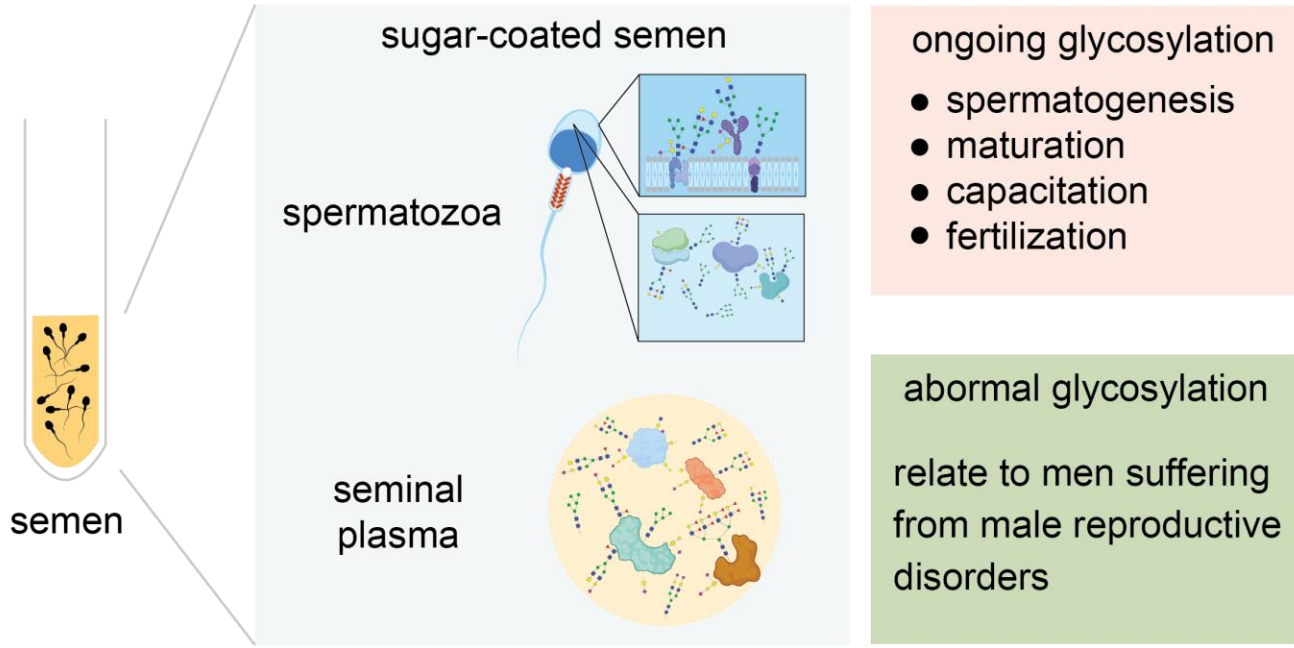


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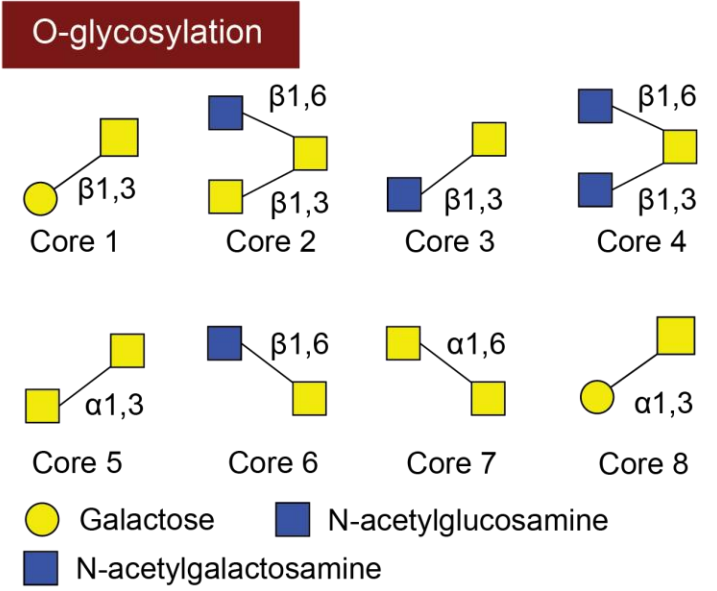
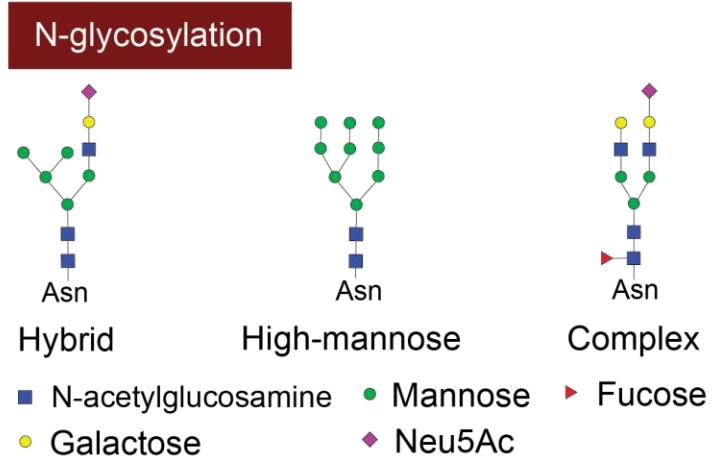


# Introduction

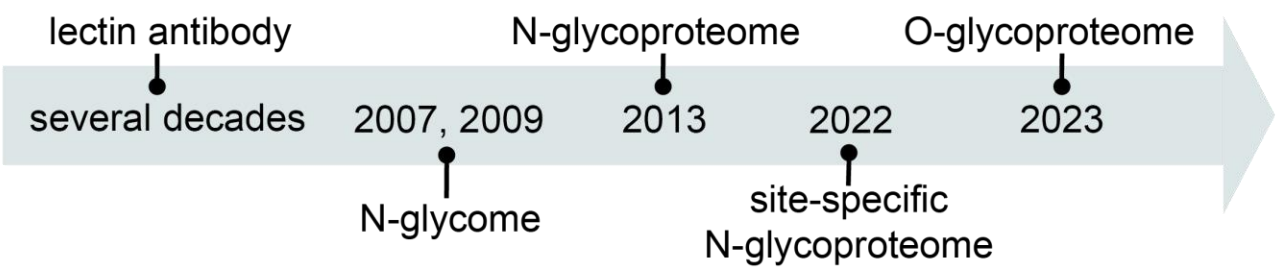
## The essential role of glycosylation in male reproduction



## Types of glycosylation



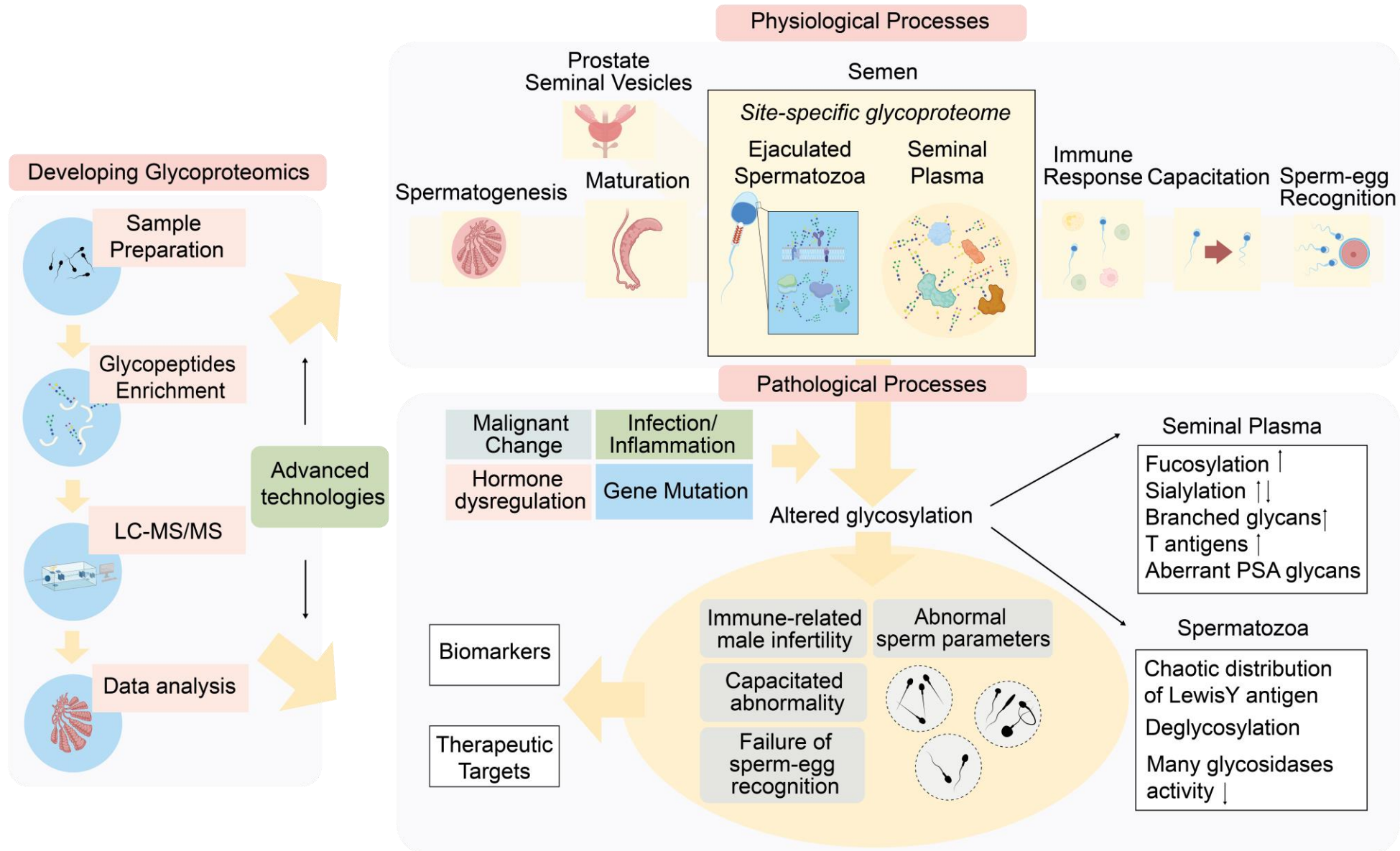
## Development of semen glycome and glycoproteome





# Introduction

## Review Framework





# Highlights

- ❑ The understanding of glycosylation in male reproduction is limited due to technical challenges in glycoproteomics.
- ❑ This review summarizes the current knowledge of glycosylation in the male reproductive system and potential applications of advanced glycoproteomics in male reproduction.
- ❑ This review presents a comprehensive overview of glycoproteomics in male reproductive diseases.

# Results

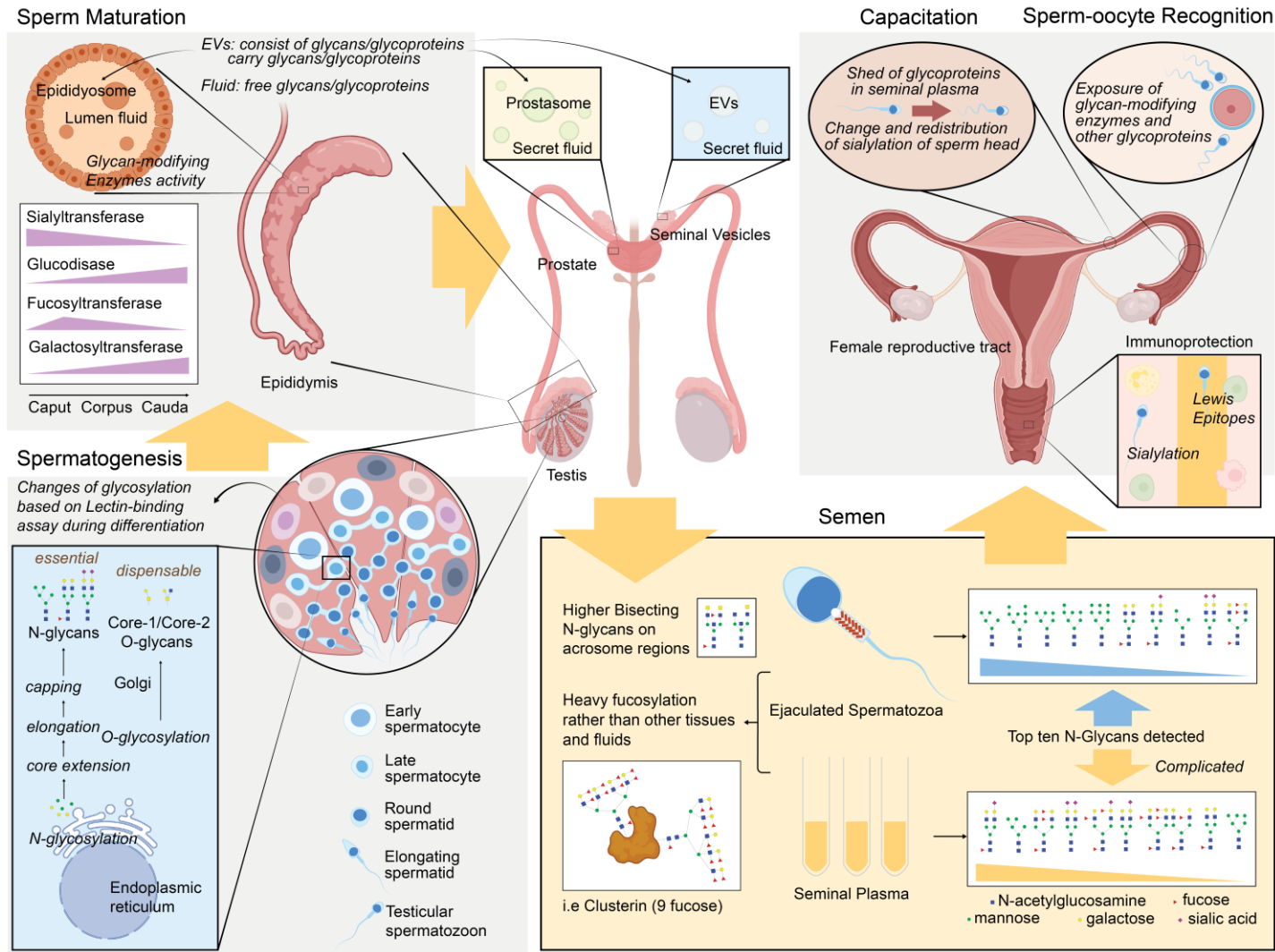
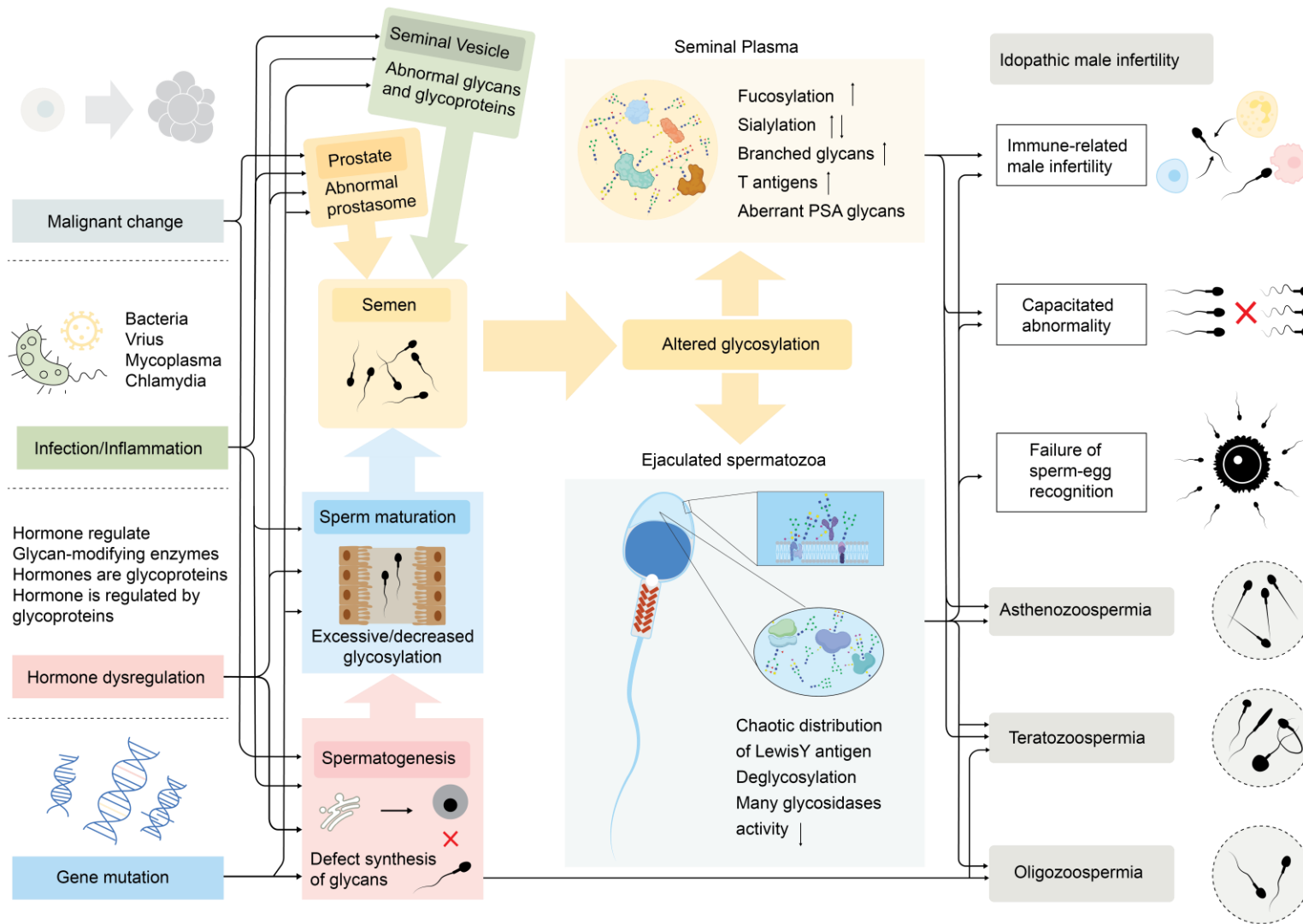


Figure 1. An overview of highly active, complex, spatiotemporal, and regulated glycosylation throughout the entire lifespan of human spermatozoa.

- Testis: N-glycans and O-glycans are actively synthesized
- Epididymis: orchestrated by various glycan-modifying enzymes in the luminal fluid; acquired glycoproteins delivered by the epididymosomes.
- Ejaculate sperm: capture from seminal plasma, containing glycoproteins, secreted by the prostate and the seminal vesicle.
- Glycoproteins protect spermatozoa from elimination of immune cells and premature capacitation. a series of changes occur in the glycosylation of spermatozoa to getting ready for sperm-egg recognition and fertilization.



# Results



- gene mutation, hormone dysregulation, infection or inflammation and carcinogenesis
- redistribution of glyicans, deglycosylation and decreased glycan-modifying
- impair the glycosylation during the spermatogenesis, maturation, and the function of accessory sexual glands.
- cumulatively result in the asthenozoospermia, oligozoospermia, and sexual gland diseases such as the prostate cancer

Figure 2. The pathogenesis of altered male reproductive glycosylation associated with male reproductive disorders.

# Results

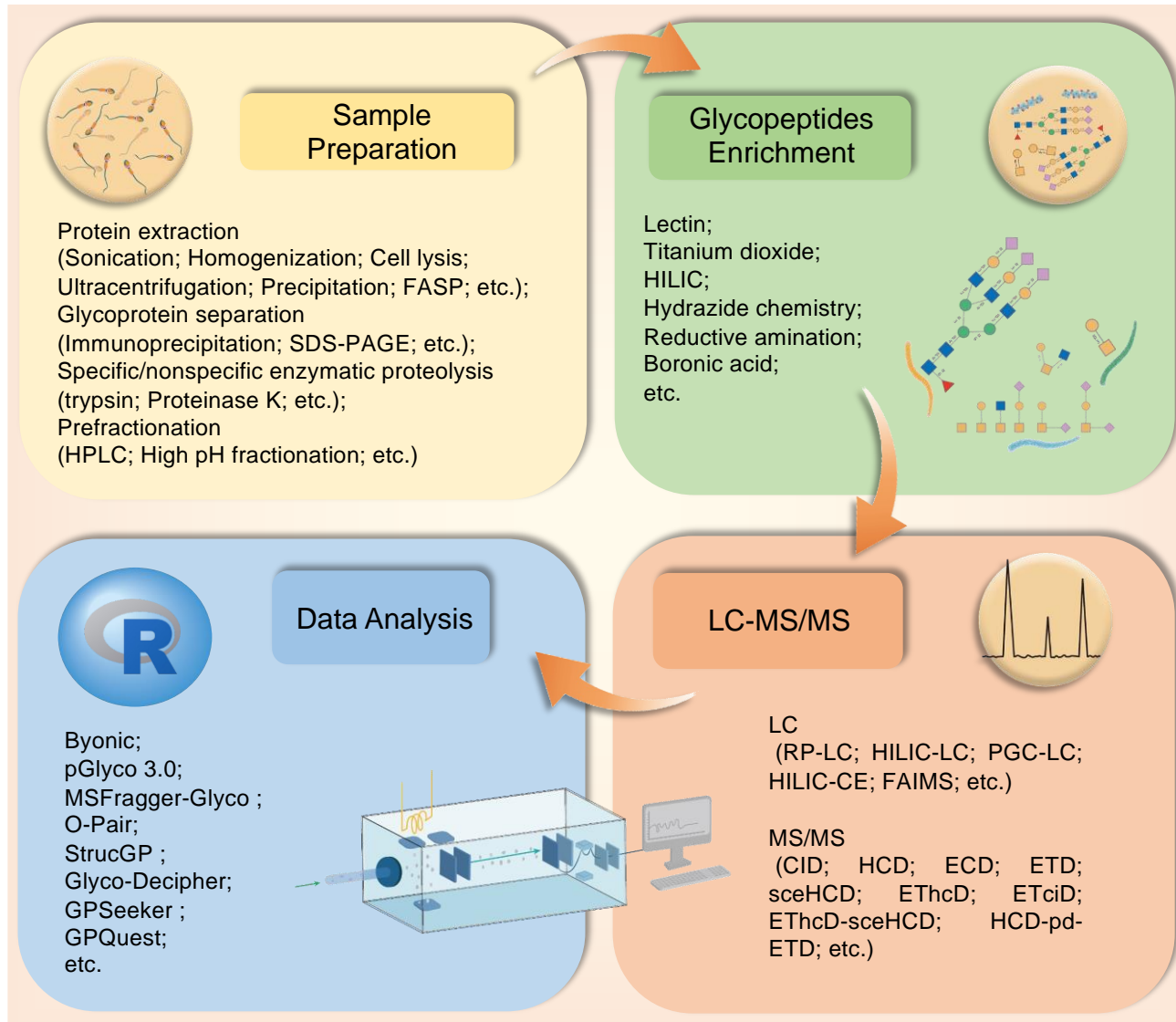


Figure 3. Overview of the methods and approaches of glycoproteomics and applications of human semen .

- purification of spermatozoa and seminal plasma from the ejaculate
- total proteins are extracted, and glycoproteins are separated for digestion to obtain the glycopeptides
- enrichment strategies included lectin, Hydrophilic interaction liquid chromatography (HILIC), chemical derivatization
- LC-MS/MS are performed to characterize the glycopeptide structures, combined with different fragment methods such as HCD, ECD and EThcD
- data analysis depends on different bioinformatic tools



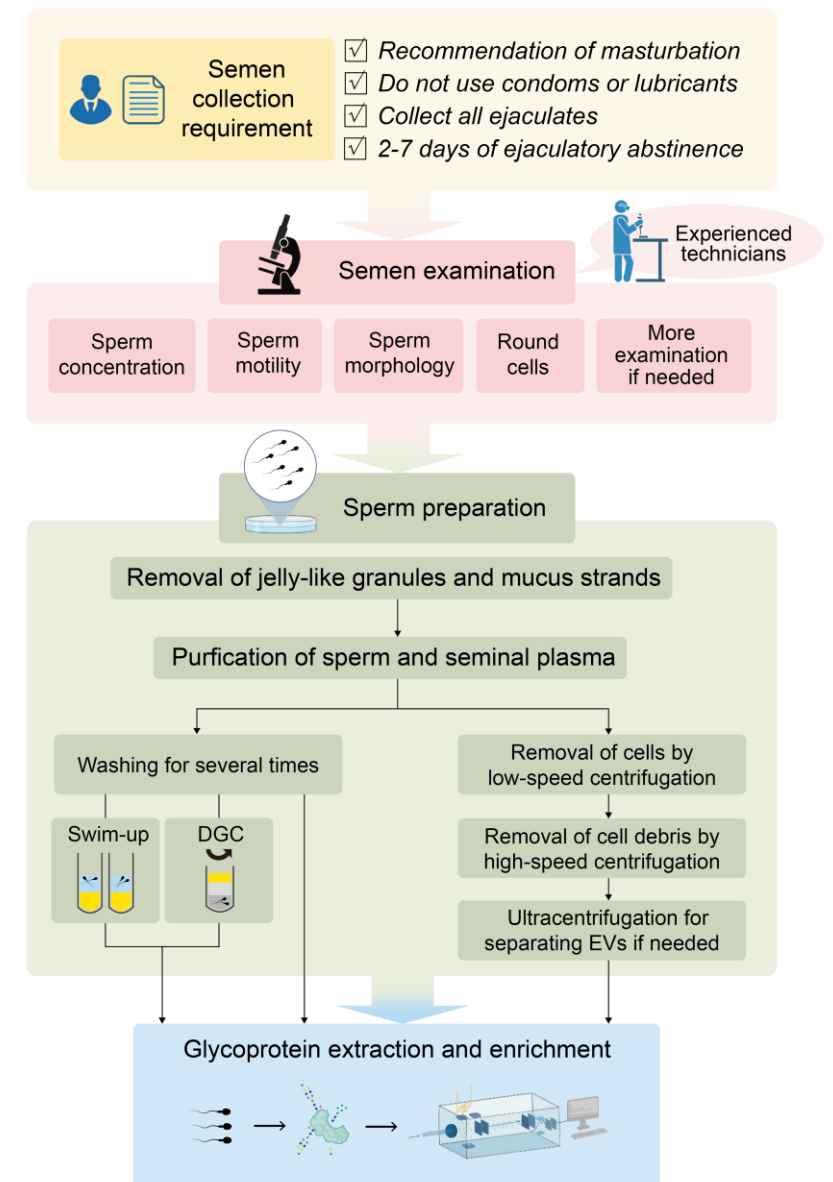
# Results

Table 3. Bioinformatic tools for identifying and/or quantifying intact glycopeptides

Name (cite)	N/O-glycans	Glycan ID method	Compatible fragmentation mode	False positive rate	Free?	Identification or/and quantification
Byonic	N/O	Mass only	Many modes	Peptide	No	Identification
pGlyco2.0	N	Y-type ions	sceHCD	Peptide + glycan	Yes	Identification
pGlyco3.0	N/O	Y-type ions	sceHCD; EThcD; ETciD	Peptide + glycan	Yes	Identification and quantification
StrucGP	N	Y+B-type ions	HCD	Peptide + glycan	Yes	Identification
MSFragger-Glyco	N/O	Y+B-type ions	HCD; sceHCD; HCD-pd-ETD	Peptide + glycan	Yes	Identification and quantification
PANDA	-	-	-	-	Yes	Quantification
pGlycoQuant	-	-	-	-	Yes	Quantification

Abbreviations: sceHCD, stepped collision energy high-energy collision dissociation; EThcD: electron-transfer/higher-energy collision dissociation; ETciD: electron-transfer collision-induced collision dissociation; HCD, high energy collision dissociation; HCD-pd-ETD: higher-energy collisional dissociation-product-dependent electron-transfer dissociation.

Figure 4. Recommended sample processing



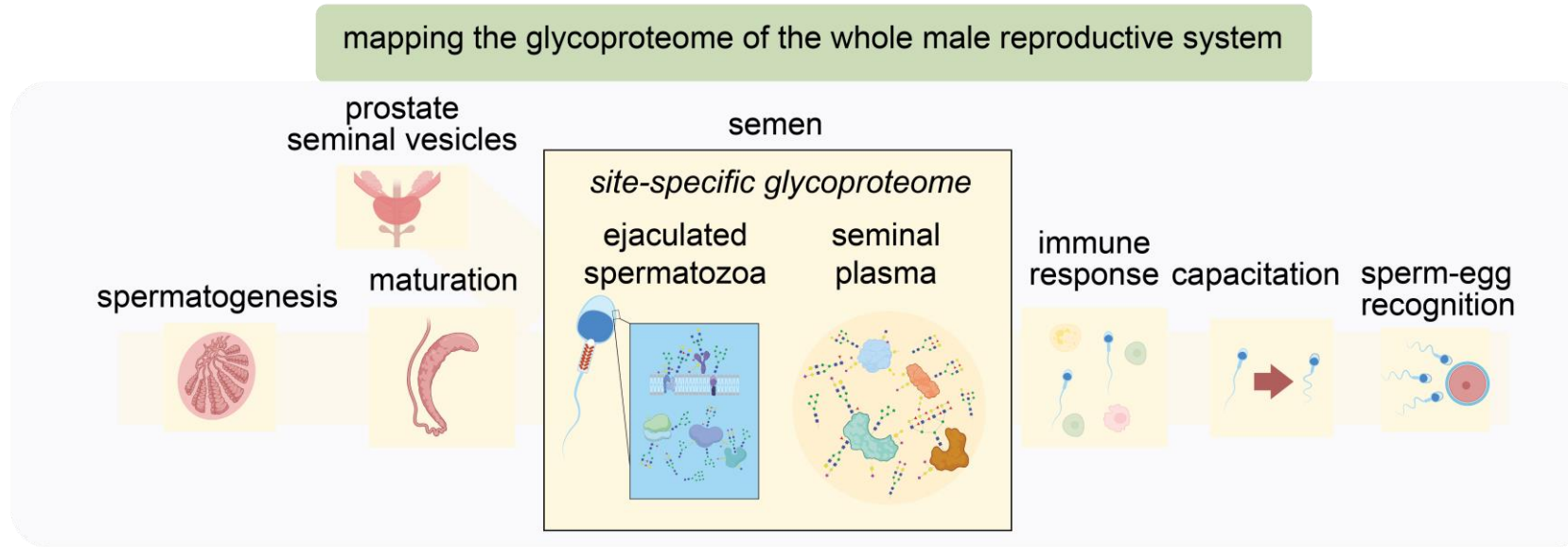


# Future Perspective

1

the precise *N*-glycoproteome of semen was mapped.

the *O*-glycoproteome of semen is continuously updated

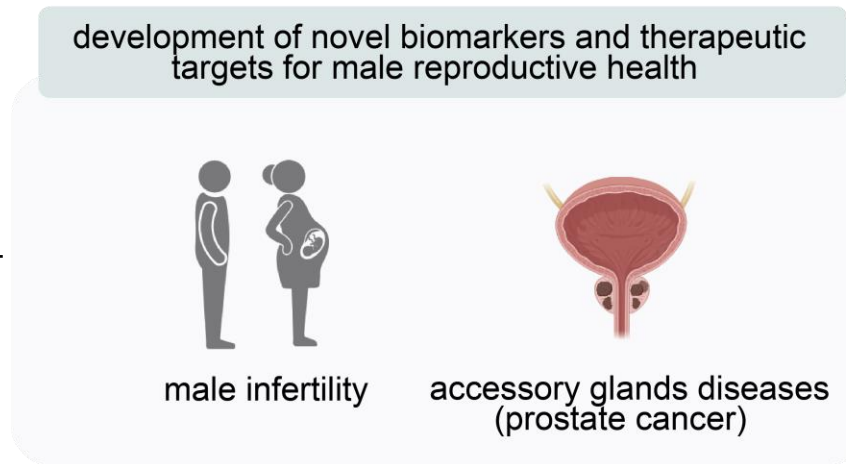


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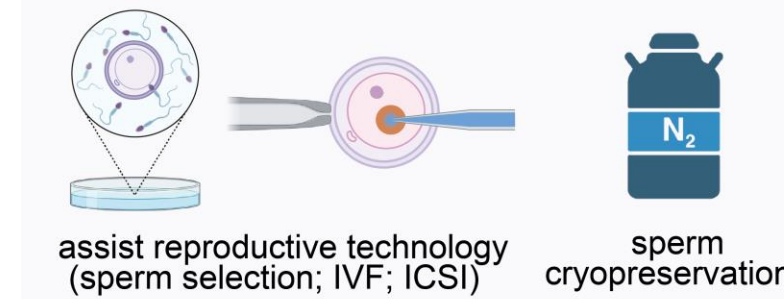
a precise glycoproteome of spermatogenesis, maturation, capacitation and fertilization is needed

3

glycan-modifying enzymes, glycans and glycoproteins are considered biomarkers of capacitation, sperm-egg binding, immune-related infertility and sexual glands.



help improve assisted reproductive technology



4

glycoproteome provides valuable information for the study of assisted reproductive technology such as sperm selection and freezability



# Conclusion

- ❑ While the investigation of glycosylation in male reproduction has a long history, recent studies have elaborated on the precise site-specific glycosylation in human ejaculated spermatozoa and seminal plasma.
- ❑ Alterations in the glycosylation profile of testicular, epididymal, and capacitated sperm may be closely linked to glycan-modifying enzymes and extracellular vesicles.
- ❑ Male infertility is associated with glycosylation abnormalities, which may result from genetic, endocrine, oxidative stress, or infection, and may explain some cases of idiopathic male infertility.
- ❑ This paper also discusses advanced glycoproteomic techniques that scientists can use to address current challenges in male reproduction such as mapping O-glycoproteome, detecting glyco-biomarkers and therapeutic targets for male infertility


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