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DDX3Y and *USP9Y* in Male Infertility: A Critical Review of Genetic Evidence and Clinical Implications

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ABSTRACT

Y-chromosomal abnormalities are established causes of severe spermatogenic impairment, but regional deletions do not establish the contribution of each affected gene. This critical narrative review examines human genetic observations, expression studies, experimental models, and clinical guidance concerning DDX3Y, an RNA helicase, and USP9Y, a ubiquitin-specific protease, within the azoospermia factor a (AZFa) region. Evidence is interpreted by lesion type, reproductive phenotype, developmental stage, and experimental endpoint, without meta-analysis. Rare *DDX3Y* loss-of-function variants associated with non-obstructive azoospermia, protein localization in premeiotic germ cells, and experimental complementation converge on a major role in early human germ-cell development. However, the small, clinically selected carrier series do not establish penetrance across variant classes. In contrast, isolated *USP9Y* deletions have been naturally transmitted, and complete gene loss has been documented with preserved sperm production. A reported *USP9Y*-deleting, *DDX3Y*-retaining partial AZFa deletion coexisted with congenital bilateral absence of the vas deferens and intact spermatogenesis, illustrating the need to distinguish obstruction from production failure. Mouse fertility and somatic-cell paralog buffering cannot establish functional substitution in human germ cells. Overall, the gene-specific evidence is stronger for *DDX3Y* than for *USP9Y*. Clinical interpretation should integrate deletion boundaries, variant-level evidence, and phenotypic assessment. Selection, language, and publication bias remain possible in this non-systematic synthesis. Experimental rescue of early germ-cell development does not establish a clinically available fertility treatment.

Keywords: *DDX3Y, USP9Y,* Male Infertility, Y Chromosome, AZFa, Non-Obstructive Azoospermia, Spermatogenesis

INTRODUCTION

Male infertility is defined as abnormalities in sperm production, transport and function. Identification of a genetic cause in men with azoospermia or severe oligozoospermia can guide prognosis, reproductive management and counseling. Idiopathic male infertility is a male reproductive disorder in which no underlying cause is determined after evaluation. It differs from unexplained infertility in couples. Pelzman and Hwang (2021) point out that a single estimate cannot be implemented in different clinical settings, since the percentage of cases classified as idiopathic depends on the population and the diagnostic work-up.

Azoospermia is a clinical presentation, not a mechanism. Obstructive azoospermia may be suggested by impaired sperm transport, while non-obstructive azoospermia (NOA) may be suggested by impaired sperm production. Spermatogenesis occurs in the seminiferous tubules and involves maintenance and differentiation of spermatogonia, meiosis and spermiogenesis; epididymal maturation is a separate process. Therefore, germ-cell stage and testicular histology are important for the interpretation of genetic results (Neto et al., 2016; Pelzman & Hwang, 2021).

Deletions of Y-chromosomal azoospermia factor (AZF) regions are well established genetic causes of severe spermatogenic impairment. The Sertoli-cell-only (SCO) pattern, characterized by the presence of Sertoli cells within the seminiferous tubules without germ cells, is strongly related to azoospermia and complete AZFa deletion. This regional evidence alone does not pinpoint the deleted gene driving the phenotype (Krausz et al., 2024). Two major AZFa genes have distinct molecular functions. DDX3Y, previously called DBY, encodes a DEAD-box RNA helicase, while USP9Y, previously called DFFRY, encodes a deubiquitinating enzyme. Their close genomic proximity has historically made gene-specific interpretation difficult.

Now human evidence differentiates these loci. Conversely, isolated USP9Y deletions have been inherited through natural conception and can be present with significant sperm production, while DDX3Y loss-of-function variants have been associated with severe NOA (Dicke et al., 2023; Krausz et al., 2006; Luddi et al., 2009). But the link between molecular activity, developmental timing and clinical outcome is not yet fully understood. These results demand a gene-specific synthesis, distinguishing intragenic defects from effects of regional deletions and experimental developmental rescue from restoration of fertility.

This review aims to critically assess the genetic and functional evidence for the role of DDX3Y and USP9Y in male infertility and to define its implications for diagnosis, counseling, reproductive management and future studies.

MATERIALS AND METHODS

Review Design and Literature Identification

This study is a focused critical narrative review, not a systematic review. It integrates heterogeneous human genetic reports, developmental expression studies, experimental investigations, and clinical guidance to address gene-specific questions. No prospectively registered protocol,

comprehensive reproducible search, PRISMA study-selection accounting, or formal pooled effect estimate was available. The purpose was to evaluate convergent and conflicting evidence rather than to estimate the prevalence of every *DDX3Y* or *USP9Y* alteration.

Literature identification used targeted searches of PubMed, consultation of the PubMed Central full-text repository, publisher websites, and professional guideline sources. The original source-checking period was April–June 2026; targeted literature updating and bibliographic verification for this revision were completed on November 11, 2026. Search concepts combined *DDX3Y* or *DBY* with azoospermia, spermatogenesis, expression, rescue, complementation, or knockout; *USP9Y* or *DFFRY* with mutation, deletion, fertility, or natural transmission; and AZFa microdeletions with diagnosis, deletion boundaries, or clinical guidelines. Reference lists and linked records were used to identify relevant original publications. The assessed sources were English-language publications or accessible English-language records; a comprehensive multilingual search was not undertaken.

Inclusion and Exclusion Criteria

Eligible sources were referenced along with authoritative diagnostic or variant-interpretation guidance, transcriptomic or developmental datasets, functional experiments into the gene or its paralogue, or directly relevant peer-reviewed human genotype-phenotype observations. Studies useful for the current analysis were heeded. Most recent reports were added to a growing body of evidence where it was either gene specific or it clarified clinical differences. Animal and cultured-cell studies were offered to provide mechanistic context, with species, cellular setting and endpoints explicitly specified in respect to human reproductive outcomes.

Duplicate reports, publications without a material connection to the review question, unsupported placeholder identifiers, and unresolved bibliographic entries were excluded. In reports lacking sufficient clinical or deletion-boundary information, definitive gene-specific reproductive phenotypes could not be assigned. Secondary reviews were used to help establish context and source of primary studies but were not used as independent confirmation of the same patients or experiments. Primary studies were identified by their original participants, experimental methods, or newly generated observations. Professional guidelines were interpreted as guidelines and not as new groups of patients.

Evidence Extraction and Synthesis

The selection of sources was initially based on a targeted assessment of the relevance of titles and abstracts and then on an assessment of accessible full texts or source records. This was a qualitative selection without independent duplicate screening or systematic recorded counts of inclusions. Important studies were analyzed in regard to: study design; gene or interval involved; retention of flanking genes; obstructive vs. non-obstructive phenotype; semen findings; histology; inheritance; functional endpoints; study-specific limitations. The bibliographic information was checked against indexed records and publisher metadata, but not reconstructed if unavailable from accessible primary sources. The weight of the conflicting evidence was judged by the question to which it could speak. The gene and phenotype information that most accurately related to clinical outcomes was the presence of human loss-of-function observations and mapped deletions phenotyped for reproductive impact. The use of naturally transmitted deletions and semen-assessed carriers made claims of obligate gene requirement particularly meaningful. Biological plausibility was suggested but not confirmed to be human fertility outcomes, in the form of expression, complementation, mouse and somatic cell studies. The tables 1 and 2 evidence strength descriptors are qualitative interpretive terms, not formal risk of bias— or gene validity scores.

No patients were recruited, biological samples collected or new individual-level dataset generated. Published experiments were not treated as observations from a new clinical cohort. Meta-analysis, regression modeling and diagnostic-accuracy calculations were not undertaken because the selected evidence combined heterogeneous case reports, pedigrees, expression studies and experimental models without a common estimand. Conclusions therefore reflect qualitative convergence and contradiction across evidence types, with uncertainty stated alongside the affected interpretation.

RESULTS AND DISCUSSION

AZFa Architecture and the Meaning of Deletion Boundaries

The male-specific region of the human Y chromosome contains X-degenerate and ampliconic sequence classes. *DDX3Y* and *USP9Y* are single-copy X-degenerate genes with X-linked counterparts, *DDX3X* and *USP9X*, respectively; they are not members of the multicopy gene families' characteristic of much of AZFb and AZFc (Skaletsky et al., 2003). Complete AZFa deletion removes two focal genes, whereas partial deletions may affect one gene, nearby regulatory sequence. Thus, a severe phenotype after regional deletion provides strong regional evidence but does not assign equal causal importance to every affected locus (Krausz & Abrardo, 2025; Krausz et al., 2024).

The European Academy of Andrology/European Molecular Genetics Quality Network (EAA/EMQN) guidance recommends validated multiplex polymerase chain reaction (PCR) testing followed by deletion-extension analysis. The routinely tested AZFa sequence-tagged sites (STSs), sY84 and sY86, are anonymous upstream markers rather than assays of the *DDX3Y* or *USP9Y* coding sequence. Extension analysis and exclusion of technical amplification failure are therefore necessary (Krausz et al., 2024).

A partial deletion cannot automatically inherit the prognosis of a confirmed complete AZFa deletion. Interpretation requires the affected interval, the integrity of both genes, and the relevant clinical phenotype. Table 1 summarizes the unequal gene-specific evidence and its main interpretive boundaries.

Table 1: Comparison of *DDX3Y* and *USP9Y* Evidence Relevant to Human Infertility

Domain	*DDX3Y*	*USP9Y*	Evidence strength and principal limit
Molecular function	DEAD-box RNA helicase involved in RNA metabolism.	Ubiquitin-specific protease involved in deubiquitination.	Biological plausibility; relevant human germ-cell targets and substrates remain incompletely defined.
Expression	Protein predominantly detected in spermatogonia despite broader transcript detection (Ditton et al., 2004); developmental localization supports an early role (Gueler et al., 2012).	Expression and enzyme identity support a potential role but not an obligatory reproductive requirement (C. Sun et al., 1999).	Localization supports developmental relevance, not causal necessity or proven paralog substitution.
Human genetics	Three MERGE and one GEMINI loss-of-function carriers had NOA; the three MERGE cases had SCO histology and unsuccessful TESE (Dicke et al., 2023).	Initial azoospermic case contrasts with naturally transmitted partial deletions and preserved sperm production after complete gene loss (Krausz et al., 2006; Luddi et al., 2009; C. Sun et al., 1999).	Direct human observations; small selected series and family-specific phenotype assessment limit penetrance and prevalence inference.
Functional and reproductive endpoints	Complementation increased early germ-cell-like formation; mature human sperm or live birth was not demonstrated (Ramathal et al., 2015).	Deletion carriers demonstrate compatibility with sperm production and transmission, without identifying the compensatory mechanism (Krausz et al., 2006; Luddi et al., 2009).	Developmental rescue and human reproduction are different endpoints; no established gene-based fertility treatment.
Clinical interpretation	Stronger evidence for a major role in severe spermatogenic impairment; variant-specific assessment remains necessary.	Isolated loss does not establish inevitable or irreversible spermatogenic failure.	Interpretation requires mapped lesions, gene integrity, and distinction between obstruction and production failure (Brannigan et al., 2024; Krausz et al., 2024).

Note. AZFa = azoospermia factor a; NOA = non-obstructive azoospermia; SCO = Sertoli-cell-only pattern; TESE = testicular sperm extraction. MERGE and GEMINI are independent infertility research cohorts.

DDX3Y Expression and Developmental Regulation

DDX3Y is a DEAD-box RNA helicase implicated in ATP-dependent remodeling of RNA-containing complexes. This function provides a plausible link between gene disruption and altered developmental translation, but helicase-family functions should not automatically be assigned to *DDX3Y* in human spermatogonia. Its physiological germ-cell RNA targets and downstream pathways remain incompletely characterized.

Ditton et al. (2004) reported widespread DDX3Y transcription in investigated tissues; however, the protein was only found in male germ line and predominantly localized to spermatogonia. The studied testis sections observed DDX3X protein in spermatids after meiosis. These observations is related to translational regulation and development rather than only testis DDX3X transcription or DDX3X compensation for DDX3Y in earlier human germ cells.

Gueler et al. (2012) detected DDX3Y protein in fetal prospermatogonia and postnatal spermatogonial populations, which supports a role during pre-meiotic period and could indicate that some AZFa diseases are caused prior to adulthood. The two are, however, distinct from development: the study focuses on the populations that are to be affected, rather than when a pathogenic allele initiated germ-cell loss.

Vogt et al. (2022) reported the existence of two primate DDX3Y transcript variants associated with proximal 3′ UTR polyadenylation and germ-cell-specific translation. We cannot assume that the way it works in humans is like in rodents, because there is species-specific transcript processing. While a single, shortened transcript may also be needed for ongoing mating. Humans are better limited in their expression studies to an early phase of germ-cell development, centering on a stage-specific phenotype than on a generic stress-triggered way of seeing why humans are infertile..

Human *DDX3Y* Variants and Spermatogenic Failure

Dicke et al. (2023) investigated exomes of 1,655 men with unexplained cryptozoospermia or azoospermia across the MERGE cohort. Three unrelated men were heterozygous for putative DDX3Y loss-of-function variants, and they identified a carrier in the independent GEMINI cohort. The variants were a splice site deletion, a stop-generating duplication, and two frameshift indels. Experimental testing via minigene assay demonstrated that c.1609+1del created an abnormal splice, though not all alleles have been experimentally tested this way.

Azoospermia was noted in all of the four carriers of these variants, who also mentioned testicular volume reductions and higher levels of follicle-stimulating hormone (FSH). The three MENDE carriers underwent testicular sperm extraction (TESE), had severe impairment of sperm production, and no sperm were recovered. No biopsy was done on the GEMINI carrier. These gene-specific speculations are especially noteworthy as they implicate DDX3Y (with little to no effect on the rest of the AZFa region). The discussion, however, does not include population prevalence, penetrance, or identical consequences for missense, regulatory, or mosaic variants (Dicke et al., 2023).

A subsequent case report by Abou et al. (2026) described a man with NOA, negative routine AZF STS testing, and a *DDX3Y* stop-gain variant, c.1501C>T, p.Arg501*. Microdissection TESE retrieved no sperm, and sampled tissue showed an SCO pattern. This additional observation supports the distinction between gene-level sequence defects and regional microdeletions. It remains a single case; segregation was unavailable, and a de novo origin was not established.

Experimental Complementation of *DDX3Y* Deficiency

Ramathal et al. (2015) used two induced pluripotent stem-cell lines derived from one man with an AZFa deletion. *DDX3Y*-complemented and control clones were xenotransplanted into mouse seminiferous tubules. Complementation increased the formation of donor-derived early germ-cell-like populations and shifted transcriptional programs toward germ-cell and prospermatogonial development, without a significant improvement in engraftment.

This experiment provides functional evidence beyond localization or clinical association. Restoration of a defined molecular activity within an AZFa-deleted background is consistent with *DDX3Y* contributing to early germ-cell competence. The endpoint was early germ-cell-like formation and molecular differentiation, not complete human spermatogenesis (Ramathal et al., 2015).

USP9Y: Initial Pathogenic Observations and Subsequent Contradictions

USP9Y encodes a deubiquitinating enzyme, which has biological plausibility to be involved in spermatogenesis through its effect on protein stability. C. Sun et al. (1999) reported a de novo deletion of four nucleotides in a splice-donor site of USP9Y in an azoospermic man. Aberrant transcript processing and predicted truncation established early gene-specific association with spermatogenic failure. The finding did not prove USP9Y to be necessary in all genetic backgrounds or carriers.

Later, Krausz et al. (2006) reported two unrelated families with partial deletions of USP9Y and independent mapping of retention of DDX3Y. The deletions were of different sizes and affected different parts of USP9Y (31,925 bp and 389,404 bp, respectively). Both probands exhibited moderate oligo-astheno-teratozoospermia compared to the normal semen profiles. Natural transmission was seen in both pedigrees, but the reproductive outcomes were markedly different, with one proband requiring in vitro fertilization and the other conceiving naturally. Therefore, preserved transmission implies compatibility with reproduction, rather than uniform normal semen quality.

Luddi et al. (2009) reported a deletion of 513,594 bp that eliminated the entire USP9Y gene but left the known DDX3Y coding and regulatory sequence intact. The proband was able to produce sperm at a high concentration (54–66 million/mL) and had mildly reduced progressive motility, despite the complete loss of USP9Y. His brother had the same deletion and the father had a documented reproductive history, but neither relative donated semen. Therefore, the evaluation of the proband cannot be used to infer the individual semen phenotypes of the individuals.

Y. Sun et al. (2025) reported a 384.9-kb partial AZFa deletion that deleted USP9Y but not DDX3Y in a man with congenital bilateral absence of the vas deferens and a homozygous CFTR TG12-5T variant. Histology demonstrated normal spermatogenesis and testicular sperm were retrieved. Both his brother and his father also had the same deletion on the Y-chromosome. The proband was obstructive azoospermic, so this cannot be considered as a failure to produce sperm due to USP9Y. The reported outcome of assisted reproduction was an ongoing pregnancy and not a documented live birth.

Such observations in humans establish variable clinical consequences and do not support a universally obligatory standalone requirement of USP9Y. A role in spermatogenic efficiency, genetic-background-dependent effects or functional buffering is still plausible but not yet resolved. Key studies and limitations of their inferences are shown in Table 2.

Table 2: Selected Human Genetic and Functional Studies: Findings and Inference Limits

Study	Design and Principal Finding	Evidence Strength and Principal Limitation
C. Sun et al. (1999)	Human genetic investigation: de novo four-nucleotide *USP9Y* splice-donor deletion in an azoospermic man; aberrant transcript and predicted truncation (C. Sun et al., 1999).	Gene-specific case evidence; one severe phenotype cannot establish an obligatory requirement or penetrance.
Krausz et al. (2006)	Two pedigrees with partial *USP9Y* deletions (31,925 and 389,404 bp) retaining *DDX3Y*; probands had oligoasthenoteratozoospermia and natural transmission occurred in both families (Krausz et al., 2006).	Direct transmission evidence; natural conception is not equivalent to uniformly normal semen or spontaneous conception by every proband.
Luddi et al. (2009)	One family with a 513,594-bp complete *USP9Y* deletion sparing known *DDX3Y* sequence; the proband had 54–66 million sperm/mL with mild asthenozoospermia (Luddi et al., 2009).	Direct sperm-production evidence in the proband; father and brother had deletion testing but no semen assessment.
Ramathal et al. (2015)	Two AZFa-deleted iPSC lines from one donor: *DDX3Y* complementation increased early germ-cell-like formation and altered developmental transcriptional programs (Ramathal et al., 2015).	Functional developmental evidence; one donor and mouse xenografts, with no mature human sperm or fertility endpoint.
Dicke et al. (2023)	Exome analysis: three *DDX3Y* loss-of-function carriers in MERGE (n = 1,655) and one in GEMINI; three MERGE cases had SCO histology and no TESE sperm (Dicke et al., 2023).	Convergent gene-level evidence; small infertility-selected series, and no biopsy in the GEMINI carrier.
Y. Sun et al. (2025)	Single case: 384.9-kb *USP9Y*-deleting, *DDX3Y*-retaining partial AZFa loss with *CFTR* TG12-5T and CBAVD; intact spermatogenesis and retrieved sperm (Y. Sun et al., 2025).	Direct obstruction/production distinction; one family, and reported pregnancy was ongoing rather than a documented live birth.
Abou et al. (2026)	Single NOA case with *DDX3Y* c.1501C>T, p.Arg501*, negative routine AZF STS testing, SCO histology, and unsuccessful micro-TESE (Abou et al., 2026).	Additional gene-level concordance; segregation unavailable and no population or allele-wide prognostic estimate.

Note. AZF = azoospermia factor; AZFa = azoospermia factor a; CBAVD = congenital bilateral absence of the vas deferens; *CFTR* = cystic fibrosis transmembrane conductance regulator; iPSC = induced pluripotent stem cell; NOA = non-obstructive azoospermia; SCO = Sertoli-cell-only pattern; STS = sequence-tagged site; TESE = testicular sperm extraction; micro-TESE = microdissection TESE. MERGE and GEMINI are independent infertility research cohorts. Qualitative strengths describe the question addressed, not a validated scoring system.

Species Differences and Paralog Buffering

Matsumura et al. (2019) reported normal spermatogenesis, morphologically normal sperm, and offspring production in Ddx3y-deficient mice. Their evidence for combined Ddx3x/Ddx3y loss came from mutant germ cells in embryonic-stem-cell chimeras, not viable whole-animal double-knockout males. The additional murine autosomal paralog D1pas1 is a possible explanation for divergent requirements, but its compensation for Ddx3y was not directly demonstrated. Consequently, it is not appropriate to

assume that the continuous disappearance of DDRX3 gene, particularly the DDX3Y gene, after loss of functional evidence for human dispensability.

Rengarajan et al. (2025) demonstrated negative post-transcriptional cross-regulation of *DDX3X* and *DDX3Y* in cultured human cells, including primary fibroblasts and lymphoblastoid lines. These findings support buffering of combined helicase dosage in the studied settings. They did not demonstrate rescue of *DDX3Y*-deficient human spermatogonia or fertility. Likewise, a natural AZFa deletion comparison in that study removed both *DDX3Y* and *USP9Y* and could not identify a separate *USP9Y* effect.

Discussion

Interpreting the Unequal Evidence for the Two Genes

The subclinical nature of the developmental evidence for DDX3Y and USP9Y. While DDX3Y is supported by evidence along 3 in human loss-of-function observations, localisation in the early germ cells and experimental complementation These evidence types are designed to address complementary questions: localization identifies relevant cell populations, genetic disruption links a locus to phenotype, and complementation supports restoration of a developmental function. Their convergence is more convincing than biological plausibility alone seems, as for evidence of penetrance across all alleles or complete fertility restoration (Dicke et al., 2023; Ditton et al., 2004; Ramathal et al., 2015).

The other 2026 stop-gain case is consistent with this gene-level interpretation, but does not consider population-based prognostic estimates (Abou et al., 2026). The USP9Y studies consider a separate causal proposition. The severe initial case suggests an association in one person, whereas naturally transmitted deletions and the semen-assessed complete-deletion proband, are underwhelming for a constantly essential requirement. These findings are consistent with context-dependent effects, not with mutually exclusive conclusions, while no one has quantified the efficiency-modifier model nor whether USP9X can compensate enough to forecast an individual carrier's reproductive success (Krausz et al., 2006; Wu et al., 1999; C. Sun et al., 1999). Krausz and Abrardo (2025) likewise separate complete AZFa deletion, DDX3Y loss of function and isolated USP9Y loss, instead of classifying all the findings of AZFa as belonging to a single clinical entity.

Mechanistic comparisons further illustrate why shared sequence does not guarantee shared developmental function. In a human HCT116 cell line, Venkataramanan et al.(2021) showed overlapping translation functions of DDX3X/DDX3Y. In contrast, Shen et al. (2022) identified differences in the tendency to phase separate, ATPase activity, and translation repression with recombinant proteins and cellular assays. The results reflect distinct endpoints, need not be inconsistent: a single translational setting can exist across, with different tissue regulation and differential condensate behavior In either case, none demonstrated DDX3X rescuing DDX3Y-deficient human germ cells (Shen et al., 2022; Venkataramanan et al., 2021). Quite similar to this, recent synthesis suggests the use of paralog requirements is more about the variation in tissue and developmental context as opposed to applying redundant RNA-regulatory activity and equating it with reproductive outcomes (Chen et al., 2026).

Houston et al. (2022) provide a broader framework to evaluate male-infertility gene–disease relationships from clinical and functional evidence. EC109's assessment predates the RRID: DDX3Y carrier series 2023 and does not represent gene-by-gene validation of the information uncovered, i.e. both DDX3Y and USP9Y were listed as unable to classify in the supplementary assessment. When it comes to comparing successive evidence syntheses, this chronology is of importance. General NOA exome studies and multiple site analyses of sperm retrieval outcomes suggest that expanding genetic diagnosis is better, but gene-specific evidence precedes general enough to transfer their results to these two loci. (Kherraf et al., 2022; Riera-Escamilla et al., 2025).

Diagnostic Testing and Variant Interpretation

Genetic testing should be used to supplement clinical phenotyping. Semen analysis, reproductive history, examination, endocrine studies, and vasal anatomy are helpful in distinguishing production failure from obstruction. The 2024 American Urological Association/American Society for Reproductive Medicine (AUA/ASRM) amendment recommends testing for Y-chromosome microdeletions in men with primary infertility and azoospermia or a sperm concentration ≤1 million/mL, supported by elevated FSH, testicular atrophy, or a diagnosis of impaired sperm production. The concentration threshold alone is not an indication. CFTR testing, including for the 5T allele, should be offered to patients with vasal agenesis or idiopathic obstructive azoospermia (Brannigan et al., 2024). The USP9Y-deletion/CFTR case demonstrates that the identification of a Y-chromosomal lesion does not exclude the need for an obstruction investigation (Y. Sun et al., 2025). Classification of deletions requires validated markers, adequate controls and extension analysis. The complete-AZFa extension pattern expected according to EAA/EMQN guidance is characterized by the presence of sY82 and absence of sY1064 proximally, and absence of sY1065 or sY1182 and presence of sY88 distally. It prefers the use of sY1064 versus sY83 in defining the proximal border. Complete AZFa loss is not recommended to be reported in case of assay failure or isolated loss of STS or incompletely mapped partial deletion (Krausz et al., 2024). Do not present the AUA/ASRM threshold as a universal international threshold as the indications for testing differ according to professional guidance and clinical settings.

Deletion testing based on STS uses complements and not duplicates. The DDX3Y sequence variants described by Dicke et al. (2023) and Abou et al. (2026) are examples of defects that are missed by routine regional markers (Abou et al., 2026; Dicke et al., 2023). The structure complexity and haplotype variation of complete and population Y-chromosome assemblies are important for the design and interpretation of assays (Hallast et al., 2023; Rhie et al., 2023). Sequence-variant and copy-number detection can be incorporated into validated exome workflows, but locus coverage, paralog discrimination and structural-variant performance need to be established for the specific platform (Oud et al., 2025). A negative exome result does not rule out inaccessible, regulatory, or poorly covered variation (Pelzman & Hwang, 2021).

Variant interpretation should follow the established classification principles including rarity, predicted molecular consequence, functional evidence, phenotype and segregation (Richards et al., 2015). Rarity or an adverse computational prediction alone do not establish causality. For isolated USP9Y variants, it is important to consider the known compatibility of USP9Y loss with sperm formation and natural propagation. A variant of uncertain significance should not be the sole basis for a permanent reproductive decision.

Prognosis, Counseling, and Reproductive Management

Confirmed in the literature that men with a complete AZFa deletion have an extremely poor sperm-retrieval prognosis, many professional opinions say not using TESE in men with complete AZFa and/or AZFb deletions for assisted reproduction should again be made on the evidence of a complete deletion. It is often not beneficial to automatically extrapolate the clinical recommendation to isolated USP9Y loss or an uncharacterized marker abnormality. AZFc and smaller partial AZFa/AZFb deletions have different implications and require lesion-specific counseling (Brannigan et al., 2024; Krausz et al., 2024).

The lack of any retrieved sperm and the ensuing explanted cases demonstrating classical SCO have important clinical implications in all three examined MERGE DDX3Y carriers, and also apply to the subsequent stop-gain case. We almost certainly cannot speak to a probable range of sperm procurement rates for all genotypes of DDX3Y, and we cannot say that the mere existence of a variant in the gene constitutes an absolute contraindication to surgery. The strength of the evidence for severe dysfunction in these loss of function cases must be shared within the limits of the details of the specific clinical cases available (Abou et al., 2026; Dicke et al., 2023). It is necessary to differentiate between genotype transmission and the certainty of a particular reproductive phenotype, especially for USP9Y deletions with sporadic semen findings and families that transmit it spontaneously (Krausz et al., 2006; Luddi et al., 2009). Reproductive options should be tailored to the demonstrated lesion and clinical phenotype; resolving the diagnosis at the molecular level is not a substitute for sperm (Brannigan et al., 2024.

Limitations of the Evidence and this Review

Most of the literature with human evidence pertains to rare variants, small pedigrees, or clinically selected populations that have been examined for infertility - the inheritance patterns among probands and affected family members are not representative populations for comparison. Using this design one can extrapolate gene-specific observations, but not unbiased prevalence, penetrance, or allele-wide population sperm retrieval. Some studies only perform direct semen or histological assessment for the proband so the phenotype cannot be confidently attributed to relatives.

The link between expression and function is one-way; the exploration of heterologous complementation is not an argument for reproducing the endogenous regulation and substitution of germ cells by somatic cells has yet to be proven to ever exist. Species-specific paralogs and developmental regulation may limit the generalizability of findings derived from mouse models. Furthermore, our narrative review is also limited by selection, language and publication bias, given that our source identification was intentionally targeted, most of the reviews were formed in the English language and the screening process was not independently replicated (no standardized risk-of-bias score or pooled estimate was generated). These are the limits within which the synthesis should be interpreted without contradicting the consistent human observations on DDX3Y.

Future Research and Therapeutic Boundaries

Human relevant models are needed to correlate specific sequence changes with developmental function. Zhen et al. (2024) generated an engineered human embryonic-stem-cell line that retained pluripotency, marker-based trilineage differentiation, and a ∼1.2-Mb AZFa deletion. It is a resource for further experimentation, not a proof of germ-cell differentiation or restoration of fertility. Endogenous editing used together with matched controls could be used to compare DDX3Y alleles and test if physiological re-expression can reverse molecular defects. Direct targets should be identifiable through RNA-binding and translational studies and the proposed compensation in appropriate developmental contexts can be tested by studying USP9Y substrates and by carefully perturbing USP9X.

Clinical research should be based mainly on multi-center recruitment and harmonized phenotyping. Repeated semen assessments, endocrine findings, deletion boundaries mapped, sequence variants, histology when clinically indicated, and reproductive outcomes should be consistently documented. Fertile carriers and relatives are useful comparators, but reproductive history should not be used as a substitute for semen assessment. These designs could differentiate technical misclassification from ascertainment and biological modification, providing the groundwork for future variant-specific risk estimates.

The DDX3Y complementation remains a proof-of-concept for the development of early germ cells. Functional sperm; the right testicular environment; completion of meiosis; the right target cells; and regulated expression would be necessary to translate into a fertility treatment. Severe depletion of germ cells in complete-AZFa-associated SCO testes may limit the availability of autologous cells for correction. The published xenograft experiment (Ramathal et al., 2015) does not completely satisfy these clinical demands. Editing of spermatogonial cells for reproductive purposes, even if done in an adult, can result in heritable changes and should not be considered a non-heritable somatic therapy. For gene delivery that does not involve editing, persistence, integration, off-target exposure, and potential transmission must also be assessed. Governance and oversight are at the heart of the World Health Organization's recommendations for human genome-editing research (World Health Organization, 2021).

CONCLUSION

Existing genetic, developmental and functional evidence indicates that DDX3Y rather than USP9Y is the more likely responsible for the severe spermatogenic impairment linked to the AZFa disruption. These DDX3Y loss-of-function observations line up with the early germ-cell localization and experiments demonstrating complementation. This seems in contrast to some cases of isolated USP9Y loss which have been reported to allow is almost indefinite sperm production and natural transmission. These findings cannot be extrapolated to all variant classes of penetrance given that current clinical data derives from a handful of series of limited sample size and this review was made from non-representative source selection. Clinical interpretations should distinguish full regional deletions vs gene-specific changes, assess the integrity of DDX3Y and the limits of the deleted region, and also differentiate between obstruction vs impaired sperm production. To better understand the prognostic framework and mechanisms of such changes will require larger, consistent outcomes in phenotyped human cohort with appropriately models for developmental contexts. Though germ cell complementation has helped unravel more about the biology, it is not a gene-based fertility treatment that is yet applicable in clinic.

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