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### **Reviewer A**

**Comment 1:** This manuscript by Atmoko and colleagues provides an excellent discussion of isolated teratozoospermia within the context of male infertility.

Although I understand the authors have framed the manuscript as a narrative review, it reads more like a book chapter rather than a review in an academic journal. I think this review would be significantly strengthened by:

1) Incorporating more details regarding how many studies were reviewed (screened, excluded at each branch point, and ultimately considered for this review). This can be further contextualized by highlighting how many of these articles were published over the last 5-10 years.

**Reply 1: Thank you very much for the acknowledgement and for pointing out the recommendation to strengthen our articles. We have added more details on the method and the abstract to better reflect our manuscript as a review for an academic journal. Additional details are outlined in table 1 and figure 1.**

#### **Changes in the text 1:**

*A broad and comprehensive literature search on PubMed was performed by two independent authors (WD, MS) using all iterations of the relevant search terms to identify articles from database inception to April 2023. From the initial articles retrieved during our search using PubMed, the title and abstract were screened, followed by a full-text review of selected abstracts. A subsequent manual search was conducted for additional articles. All the studies that discussed isolated teratozoospermia in the context of male infertility were considered. From a total of 145 articles reviewed, we included all the studies in English that provide strong evidence or were most relevant to the topic of interest. A complete search strategy for article selection can be found in Table 1. No prior registration protocol or ethical approval is required for the present review.*

*A total of 88 publications were included in this analysis, of which 55 of them were published in the last ten years. The included articles cover seven systematic reviews/reviews/meta-analyses and 81 original articles. Figure 1 summarizes the selection process for the present review. Information contained in these publications was extracted and synthesized to create a comprehensive narrative review of isolated teratozoospermia and its implication for male infertility.*

Comment 2: Incorporating language or data with respect to study quality. For example, it is hard to understand, particularly in the Genetic Causes and Molecular Pathways sections, if the studies included are robust and high quality, or if they likely represent spurious one-off examinations. Of note, the Management section does this well.

Overall, it is well written and was interesting to read, particularly with respect to certain historical aspects that were discussed. Also, integration of the SWOT analysis is excellent and provides a succinct snapshot of critical points of the manuscript.

**Reply 2: Thank you very much again for the suggestion. We agree and have added the additional information in term of study quality when mentioning a data particularly in the genetic causes and molecular pathways of teratozoospermia section.**

#### **Changes in the text 2:**

*A cohort study in Italy of 18 unrelated men found several genes to be responsible for globozoospermia*

including mutations or deletions of *DPY19L2*, *PICK1*, and *SPATA16* (47,48). A multicenter study in China also found *DPY19L2* variants in more than 55% of Chinese globozoospermic men (49). Additionally, a study in China on a young man with globozoospermic primary infertility found that a loss-of-function variant of the *SSFA2* gene contributed to infertility due to globozoospermia and failure of oocyte activation (46). Other genetic causes of sperm morphological abnormalities include mutations of the *SUN5*, *SPATA20*, *ACTL7A*, and *CCIN* genes which result in pinhead spermatozoa and acephalic spermatozoa syndrome, bubble-shaped acrosome and severe head malformations, respectively (38, 50, 51, 52). However, the majority of the findings come from case reports or case series.

Aside from the defect mentioned above, sperm tail defects were also found to correlate with several genetic defects. Tail defects are critical and can result in asthenozoospermia or abnormal motility. Several observational studies reported that mutations on *DNAH1*, *SPEF2*, and *SEPT12* were correlated with sperm tail anomalies (53-55). Patients who harbor a pathogenic variant of *ZMYND15* are also found to have abnormalities in sperm morphology based on sequencing analysis of 227 infertile men in China (56).

### **Reviewer B**

Comment 3: The manuscript offers an overview of a condition that has been insufficiently reported. Although the clinical significance of isolated teratozoospermia remains a subject of controversy, providing information on its causes and treatment options would be valuable to the field. Overall, the manuscript is well-written, with only a few minor errors that are outlined below. After addressing these revisions, I would recommend accepting the manuscript.

Regarding Line 256, could you please clarify whether the term "protaminatin" refers to protamine?

**Reply 3: Thank you for pointing out the importance of the manuscript. In regards to line 256, thank you we have corrected the word into protamination.**

#### **Changes in the text 3:**

*Oxidative stress results in lower protamination and disulfide bond formation*

Comment 4: In Line 326, the sentence "one study all sperm parameters, SDF rate and a higher rate of..." appears to be unclear in meaning. Please consider revising this sentence to enhance its clarity and coherence.

**Reply 4: Thank you, we agree that the sentence need to be clarified. We have reworded the sentence for better understanding and clarity.**

#### **Changes in text 4:**

*A study on men with isolated teratozoospermia and clinically palpable varicocele reported an increase in all sperm parameters (concentration, motility, and morphology), lower SDF, and a higher rate of natural pregnancy at 30.5% in the varicocelectomy groups compared to 16.7% pregnancy rate in the control group that only received an antioxidant (L-carnitine, vitamin C, and vitamin E) (99).*

### **Reviewer C**

Comment 5: This is a well written, though somewhat longer piece on the role of morphology in fertility and the minimal therapeutic options.

1. I appreciate that the authors recognize up front that there is high intra rater reliability
2. in the discussion of the WHO evolution of morphology %, I suggest that the authors add the historical point of the years of each publication. while readers can look up each WHO publication

date, it would be simpler for each mention of the WHO edition to also have the year of publication.

**Reply 5: Thank you for the suggestion. We agree and have added the year of each WHO publication date**

**Change in text 5:**

*In the first two editions (1990 and 1987), all spermatozoa without obvious defects...*

*The third edition of the WHO Manual in 1992 was the first to apply strict criteria for sperm morphology...*

*The fourth (1999) and fifth (2010) editions of the WHO manual adopted 14% and 4% as the cut-off of normal sperm morphology, respectively*

Comment 6: 3. consider reviewing for brevity. There are multiple paragraphs that can be shortened while still maintaining the same level of impact. consider:

section 3.1. paragraphs 3-4

3.1. page 9, line 162

3.2.1. the ROS section

4. the SWOT analysis is well considered and adds a valuable pictorial view

**Reply 6: Thank you for the suggestion and acknowledgement – we have revised and shortened the paragraph for section 3.1 and 3.2.1 as suggested by the reviewer.**

**Changes in text 6:**

Section 3.1

*Menkveld et al., in later years, proposed a Tygerberg strict criteria based on detailed observation of spermatozoa, particularly from the postcoital cervical mucus (29). This approach was based on the observation that the morphology of spermatozoa in the cervical mucus was better than those from the semen sample and similar to the morphology required for binding to zona pellucida (30, 31). These criteria consider a slight or borderline abnormality as abnormal, which results in a significantly lower threshold of normally shaped sperm compared to the liberal approach (27). It also emphasized the importance of optimal preparation and evaluation procedure of sperm morphology (29).*

*The third edition of the WHO Manual in 1992 was the first to apply strict criteria for sperm morphology using a cut-off of 30% for normal morphology, which was continually decreased in the later editions (10). The fourth (1999) and fifth (2010) editions of the WHO manual adopted 14% and 4% as the cut-off of normal sperm morphology, respectively (6,11). Notably, in the fourth edition, the cut-off was used with a statement that multicenter investigations were ongoing and that the data suggested a decrease in in-vitro fertilization (IVF) when the proportion of normal morphology was under 15% (11).*

Section 3.1 page 9

*Classification of sperm morphology characterizes sperm with a better fertilization potential. Specific structural defects such as globozoospermia, pin head defects, tapered sperm head, macro- or microcephalic sperm, multiple tail defects, or large residual cytoplasmic sperm droplets should be correctly reported as they significantly affect the prognosis. Nevertheless, a systematic detailing of abnormalities is unnecessary and not recommended in the WHO manual (11).*

*A detailed evaluation based on multiple sperm defect indices has also been proposed to give a better evaluation of sperm damage; these include teratozoospermia index (TZI), multiple anomalies index (MAI), and sperm deformity index (SDI). Even though these indices have been correlated to both in vivo and IVF outcomes, there is still an overlap between fertile and sub-fertile men, and they cannot be used as a strict assessment to define men's sperm potential (5). There needs to be more evidence of their clinical value (27). Moreover, even sperm with normal morphology can still exhibit other problems in function (22).*

### Section 3.2.1 ROS Section

*Causes for increased oxidative stress in teratozoospermic men include the increase of MDA (malondialdehyde), lipid peroxidation, iron, and NADPH (nicotinamide adenine dinucleotide phosphate), which is positively correlated with the damage to sperm DNA (76,77). Oxidative stress results in lower protamination and disulfide bond formation, leading to DNA breaks and increased free radicals (13). ROS production is also found to be higher among immature spermatozoa with abnormal morphology which may indicate abnormal regulation of spermatogenesis (78). During sperm migration to the epididymis, the ROS from immature spermatozoa will cause oxidative and DNA damage to the mature spermatozoa (79). A real-world study also showed that infertile men with isolated teratozoospermia have a higher sperm DNA fragmentation rate than those without abnormalities (20). However, other studies reported no correlation, or a very weak correlation, between abnormal sperm morphology and sperm DNA fragmentation (80-82).*

Comment 7: MINOR: section 3.4: line 314: major induced should likely be indices.

**Reply 7: Thank you for pointed out the typo in the manuscript. We have corrected it accordingly.**

#### **Changes in text 7:**

*Similar findings were reported by Fan et al. who showed that the outcomes of IVF were not influenced by isolated teratozoospermia (97).*

#### **Reviewer D**

**Comment 8:** This is a very interesting review on teratozoospermia and its relationship with infertility. Just comment that regarding ureaplasma infection, there is an article, which, although old, has not been reproduced subsequently. In this work, semen samples are experimentally contaminated with ureaplasma, and colonies of the same are observed attached to the head and the intermediate segment.

Núñez-Calonge R, Caballero P, Redondo C, Baquero F, Martínez-Ferrer M, Meseguer MA. Ureaplasma urealyticum reduces motility and induces membrane alterations in human spermatozoa. Hum Reprod. 1998 Oct;13(10):2756-61. doi: 10.1093/humrep/13.10.2756. PMID: 9804226.

**Reply 8: Thank you for the suggestion. We have incorporated the suggested study in the article.**

#### **Change in text 8:**

*An in-vitro study of semen contaminated with Ureaplasma urealityicum found that colonies of bacteria were attached to the head and mid-piece of sperm with abnormal morphology (66).*

#### **Reviewer E**

Comment 9: Overall excellent review highlights some of the clinical conundrums we have in the area of isolated teratospermia with some clinical guidance of clinical management  
- minor typos and grammar issues need to be corrected

**Reply 9: Thank you very much for the reviewer. We have reviewed and rechecked the manuscript thoroughly and made the necessary revision of any grammatical or typographical mistakes.**

#### **Change in text 9:**

Correction in grammar and typos were made throughout the manuscripts.

Comment 10: Can you provide summary of the clinical recommendations on the management of isolated teratospermia. I believe this could be valuable to readers

**Reply 10: We agree with the reviewer. We have added the summary for clinical recommendations to the isolated teratozoospermia**

**Change in text 10:**

*The clinical significance of isolated teratozoospermia is still unclear and treatment for this condition should be done cautiously due to the lack of recommendations from professional societies and controversies in contemporary literature. Finding specific sperm abnormalities, such as complete globozoospermia, can direct the patient to a specific treatment. However, it is yet to be seen if empiric therapy with antioxidants can improve the outcome in isolated teratozoospermia. In the case of isolated teratozoospermia with clinically palpable varicocele, although some studies showed some benefit after varicocelectomy, the evidence is still inadequate to make routine recommendations. The need for, and role of, ART for isolated teratozoospermia is also controversial, and other factors - female fertility status, comorbidities, duration of infertility, etc. - should be considered when deciding whether IUI or ART (IVF/ICSI) is required in a couple with isolated teratozoospermia as the only male factor.*

Comment 11: Can you provide prevalence of this issues in the overall male infertility spectrum? how prevalent? and is there any other sperm abnormalities that could be related

**Reply 11: Thank you for the important suggestion. We have added the prevalence of this isolated teratozoospermia. The discussion on other sperm abnormalities that can be related were mainly high SDF rate which was discussed under the molecular pathway section.**

**Change in text 11:**

*Isolated teratozoospermia contributed to 20% of all isolated sperm abnormality cases, accounting for more than 5% of infertile men cases based on a study of almost five thousand infertile men (85). In further analysis of 1084 men with isolated sperm defects the authors found that sperm DNA damage was more correlated with isolated asthenozoospermia as compared to isolated teratozoospermia (85).*

Comment 12: You could consider adding this reference showing relationship between marijuana use and poor sperm morphology

Evaluation of the impact of marijuana use on semen quality: a prospective analysis.

Hehemann MC, Raheem OA, Rajanahally S, Holt S, Chen T, Fustok JN, Song K, Rylander H, Chow E, Ostrowski KA, Muller CH, Walsh TJ.

Ther Adv Urol. 2021 Jul 20;13:17562872211032484. doi: 10.1177/17562872211032484. eCollection 2021 Jan-Dec.

PMID: 34367341

**Reply 12: Thank you for the suggestion. We have added the reference into the manuscript**

**Change in text 12:**

*Another survey of 409 infertile men found that the use of marijuana resulted in abnormal sperm parameters, especially semen volume and sperm morphology (61).*

**Reviewer F**

Comment 13: The article is scientifically very interesting; however, it has some minor aspects that need to be corrected.

Abstract.

Line 45. Assisted reproductive technology (ART)  
47. Intrauterine insemination (IUI)

**Reply 13: Thank you for the review and we have made the necessary changes into the abstract**

**Change in text 13:**

*However, the clinical correlation between teratozoospermia and ART (assisted reproductive technology) outcome shows conflicting data with recent meta-analyses suggesting that isolated teratozoospermia was not associated with poor fertility outcomes from ART and that IUI (intrauterine insemination) can be an effective option even in the presence of teratozoospermia. There is very limited data on effective therapeutic options to treat idiopathic isolated teratozoospermia. The opportunity for future research is huge to fill the gap in the medical literature on this topic.*

Comment 14: Introduction. The introduction described the well state of the art of study problem.  
117. In the first two editions (of the WHO manual, remove), all spermatozoa

**Reply 14: We have revised the line as suggested**

**Change in text 14:**

*In the first two editions (1990 and 1987), all spermatozoa without obvious defects were....*

Comment 15: Methods. The experiment of this study is well-designed. All the studies about isolated teratozoospermia were included.

Evidence Synthesis. In this section, the authors describe the findings of the literature review, and the section sing up-to-date literature which closely related to the study topic.

**Reply 15: Thank you for the acknowledgement. We have added more details to the method of the manuscript**

**Change in text 15:**

*A broad and comprehensive literature search on PubMed was performed by two independent authors (WD, MS) using all iterations of the relevant search terms to identify articles from database inception to April 2023. From the initial articles retrieved during our search using PubMed, the title and abstract were screened, followed by a full-text review of selected abstracts. A subsequent manual search was conducted for additional articles. All the studies that discussed isolated teratozoospermia in the context of male infertility were considered. From a total of 145 articles reviewed, we included all the studies in English that provide strong evidence or were most relevant to the topic of interest. A complete search strategy for article selection can be found in Table 1. No prior registration protocol or ethical approval is required for the present review.*

*A total of 88 publications were included in this analysis, of which 55 of them were published in the last ten years. The included articles cover seven systematic reviews/reviews/meta-analyses and 81 original articles. Figure 1 summarizes the selection process for the present review. Information contained in these publications was extracted and synthesized to create a comprehensive narrative review of isolated teratozoospermia and its implication for male infertility.*

Comment 16:

223. Pacey et al.

230. Mehta et al.

233. Ureaplasma Urealityicum : urealityicum

260. sperm morphology (74), The high generation of ROS in (immature) immature spermatozoa and may indicate

300. Knez et al. (88),

307, However, (once again, remove)

308. that morphology, that morphology, need a space.

309. Keegan et al.

313 Fan et al.

**Reply 16: We have made the necessary changes to the typos and re-review the whole manuscript for its grammar and typos**

**Change in text 16:**

*.... a multicentre study by Pacey et al. in 2014...*

*.... results. Mehta et al. found that the rate of....*

*... samples with Ureaplasma urealyticum also showed a lower...*

*.... among immature spermatozoa with abnormal morphology that may indicate abnormal regulation of spermatogenesis (74, 75).. During sperm migration to the epididymis, the ROS from immature spermatozoa will cause oxidative and DNA damage to the mature spermatozoa (75)....*

*Further, a study by Knez et al. (88) reported...*

*However, other studies...*

*Demir et al. who found that morphology did not ...*

*However, Keegan et al. in a study of 495 couples assessed ...*

*reported by Fan et al. which showed that the major indices of IVF ....*