The Effect of Methotrexate Systemic Treatment on Ovarian Reserve: A Systematic Review and Meta-analysis

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ABSTRACT

Objectives: To assess anti-Mullerian hormone (AMH) levels before and after Methotrexate (MTX) treatment to get an insight into ovarian function following therapy and compare the effect of the single and multi-dose therapy of MTX.

Methods: A thorough search was conducted for pertinent literature using PubMed, SCOPUS, Web of Science, Science Direct, and Wiley Library. Comprehensive meta-analysis software was used for all analyses and assessed statistical heterogeneity with the I^2 index.

Results: Our results included ten studies with a total of 354 women who received MTX therapy. The follow-up duration ranged from 1 month to 23 months. Our analysis showed a significant reduction in the AMH level following MTX therapy (SMD = -0.393, 95% CI 0.197, 0.032, p= 0.028). When comparing the single and multi-MTX dosages on AMH levels, both showed insignificant reduction; (SMD = -0.301, 95% CI 0.301, 0.091, p= 0.318) and (SMD = -0.516, 95% CI 0.276, 0.076, p= 0.061), respectively.

Conclusion: This analysis demonstrated that MTX significantly affects the AMH levels; however, there was no reported difference according to the treatment regimens. This topic is arising and there is a lack of data about it which requires more future prospective and RCTs to interpret.

Keywords: Methotrexate; Anti-mullerian hormone; Ovarian reserve; Meta-analysis; Systematic review.

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INTRODUCTION

Methotrexate (MTX) is used as a medicinal therapy for many medical conductions such as ectopic pregnancy and cancer. Its mean efficacy is claimed to be 89%, with rates ranging from 64 to 94% [1]. This treatment allows patients to avoid surgery, particularly when they have minor symptoms and a barely established ectopic pregnancy [2].

MTX is an anti-metabolite that rapidly inhibits cell proliferation, particularly in trophoblasts, and may also impair pre-antral, antral, and primordial follicles [3]. In the context of regulated ovarian stimulation, blood circulation to the ovaries is definitely augmented, and follicle metabolism may be accelerated; hence, MTX may have a direct apoptotic impact on granulosa cells such as oocytes [4].

The use of AMH as an ovarian reserve marker has piqued researchers' curiosity over the last two decades. Preantral and antral follicles, which continue in the growth phase for many weeks regardless of FSH or menstrual fluctuations, produce AMH [5]. AMH is more accurate than other traditional indices of ovarian activity, such as menstruation, estradiol, and FSH [6, 7].

In our meta-analysis, we assessed AMH levels before and after MTX treatment to get an insight into ovarian function following therapy. In addition, this analysis compared the effect of the single and multi-dose therapy of MTX.

METHODOLOGY

Study design and duration

This meta-analysis follows PRISMA criteria, which stand for Preferred Reporting Items for Systematic Reviews and Meta-Analyses [8]. We did this analysis in July 2024.

Literature search

To find relevant literature, an extensive and comprehensive search of PubMed, Web of Science, Scopus, Science Direct, and Clinical Key was performed. We tailored our search to the specific requirements of each database and confined it to English. To find relevant research papers, the following keywords were translated into Mesh terms in PubMed or topic terms in Scopus: "Ovarian reserve," "Ovarian function," "Anti-mullerian hormone," "AMH," " Methotrexate," and "MTX." Keywords representing Boolean operators such as "OR," "AND," and "NOT" were allocated.

Study selection and Data extraction

Rayyan (QCRI) was used to screen results and remove duplicates [9]. Before integrating the search results, the researchers assessed the relevance of the titles and abstracts using inclusion/exclusion criteria. Reviewers carefully assessed all papers that met the inclusion criteria. The authors discussed how to solve disagreements. The approved study was uploaded using a pre-designed data extraction form. Data on study titles, authors, study year, nation, sample size, mean age, underlying disease, follow-up duration, MTX dosage, and AMH level before and after MTX therapy. The risk of bias assessment was conducted on a different sheet.

Selection criteria

The inclusion criteria are as follows: (1) any study design that reported the mean AMH level before and after MTX therapy (2) single or multi-dose MTX regimen, and (3) Patients who received any camel dairy product.

We omitted case reports, case series (with fewer than 10 patients), narrative or systematic reviews, communications, and perspectives. We also overlooked studies conducted in languages other than English.

Risk of bias assessment

To assess the risk of bias in the identified randomized control trials (RCTs), the Cochrane Collaboration Risk of Bias (ROB) tool [10] was used. The results are presented as a table with a variety of color schemes. Red indicates a considerable risk of bias; green indicates a low risk; and yellow indicates an inability to estimate the risk due to insufficient information.

The ROBINS-I risk of bias evaluation method was used to evaluate the quality of the non-randomized study. The included papers were evaluated [11]. The seven issues covered included confounding, research participant selection, intervention classification, divergence from planned treatments, insufficient data, outcome evaluation, and the choice of the stated result.

Statistical analysis

The software that was used for data analysis was Comprehensive Meta-Analysis (Version 3.0) [12]. We chose the random effect model because it allows us to take study-to-study volatility into account when determining study weights. Statistical significance was defined as $P < 0.05$. Before estimating the pooled effect size and assessing the specific contributions of each study in the meta-analysis, a sensitivity analysis was performed on each study individually. Cochran's Q and I2 statistics were used to evaluate heterogeneity in effect sizes. A substantial Q value ($p < 0.05$) suggested heterogeneity across trials, but I2 statistics revealed the fraction of variance in observed effects that represented variance in genuine effects rather than sampling error [13, 14].

RESULTS

Search results

After a thorough search, 1453 study articles were found; 712 duplicates were eliminated. After 723 studies had their titles and abstracts screened, 611 were not included. Only 3 items out of the 112 reports that were requested were not found. After screening, 109 papers for full-text assessment, 65 were rejected due to incorrect study results, 26 were rejected due to incorrect population type, 7 abstracts, and 2 articles were editor's letters. This analysis contained nine acceptable research papers. An overview of the procedure used to choose studies is provided in **Figure 1.**

Characteristics of the included studies

Table 1 shows the sociodemographic features of the study articles that were considered. Our results included ten studies with a total of 354 women who received MTX therapy. Six studies were prospective cohorts [15, 16, 18, 20, 21, 22], two were prospective observational studies [17, 19], and one study was a prospective RCT [23]. Two studies were conducted in Iran [16, 23], two in Turkey [17, 18], two in Egypt [2021], one in Vietnam [15], one in Spain [19], and one in Indonesia [23]. Seven studies included women diagnosed with ectopic pregnancy [16-21, 23] and two included women diagnosed with gestational trophoblastic disease/ neoplasia [15, 22]. Five studies discussed multi-dose MTX regimens [16, 18, 19, 21, 23] and four discussed the single-dose MTX regimen [15, 17, 20, 22]. The follow-up duration ranged from 1 month [18, 22] to 23 months [19].

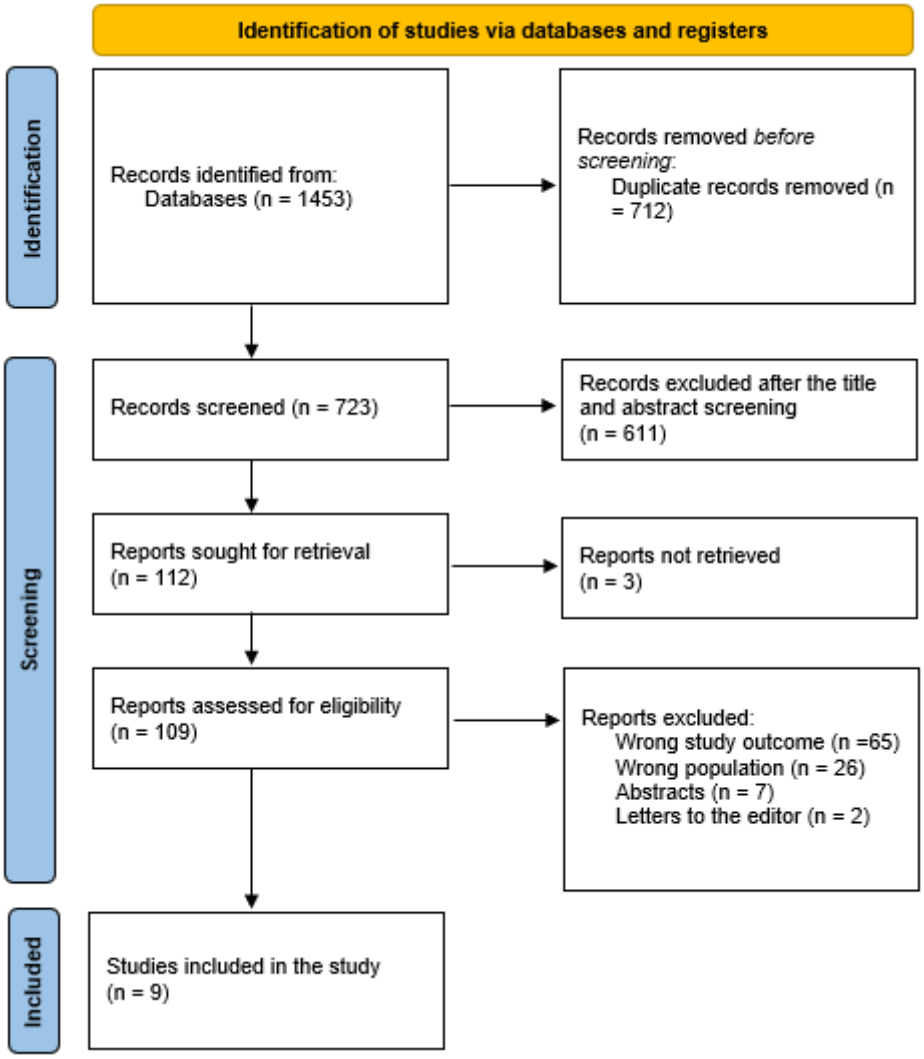


Figure 1. Study selection is summed up in a PRISMA flowchart

Table 1. Sociodemographic characteristics of the included participants

Study	Study design	Country	Age range/ mean	Underlying disease	Follow-up (months)	Single/ multi	Dosage	ROBIN-II
Dat et al., 2024 [15]	Prospective cohort	Vietnam	<40	Women with gestational trophoblastic neoplasia	12	Multi-dose	1 mg/kg IM	Moderate
Shirazi et al., 2020 [16]	Prospective cohort	Iran	21-43	Women with ectopic pregnancy	2	Single dose	50 mg/m2 IM	Moderate
Çetin et al., 2023 [17]	Prospective observational	Turkey	18-44	Women with ectopic pregnancy	3 - 6	Multi-dose	80 mg/day IM	Moderate
Sahin et al., 2016 [18]	Prospective cohort	Turkey	29.6 ± 4.04	Women with ectopic pregnancy	1	Single dose	50 mg/m2 IM	Moderate
Oriol et al., 2008 [19]	Prospective observational	Spain	33	Women with ectopic pregnancy	23	Single dose	1 mg/kg IM	Moderate
Dawood et al., 2022 [20]	Prospective cohort	Egypt	28.24 ± 5.48	Women with ectopic pregnancy	3	Multi-dose	50 mg/m2 IM	High
El-Wahed et al., 2023 [21]	Prospective cohort	Egypt	19-40	Women with ectopic pregnancy	3	Single dose	50 mg/m2 IM	Moderate
Madjid et al., 2024 [22]	Prospective cohort	Indonesia	20-40	Women with gestational trophoblastic disease	1	Multi-dose	1 mg/kg IM	Moderate
Tavana et al., 2018 [23]	Prospective RCT	Iran	23.9	Women with ectopic pregnancy	6	Single dose	NM**	NA*

*NA=Not-applicable
**NM=Not-mentioned

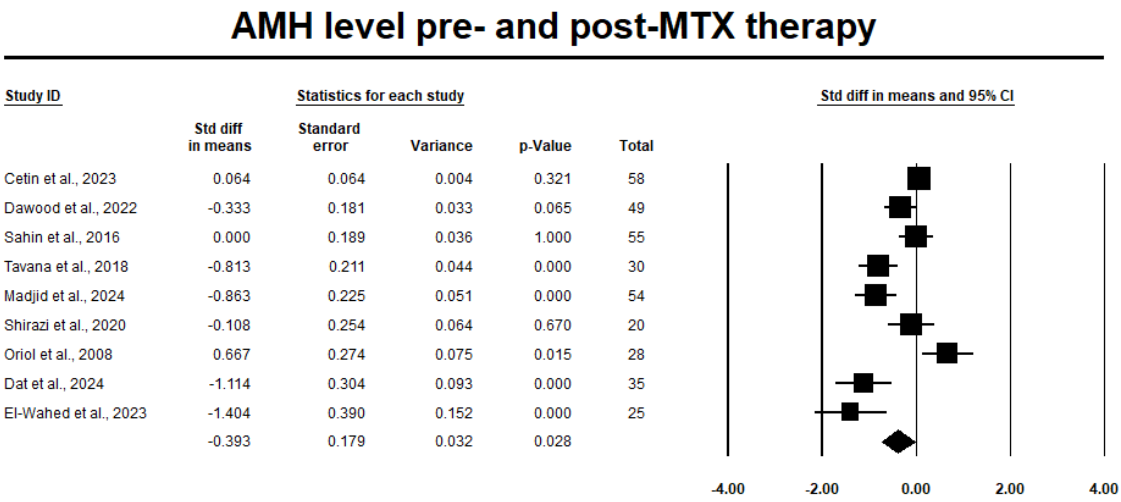
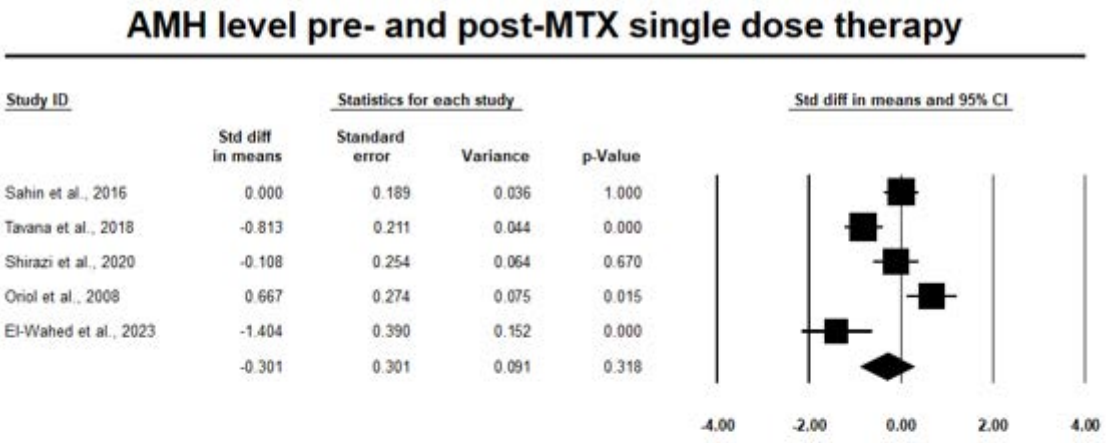
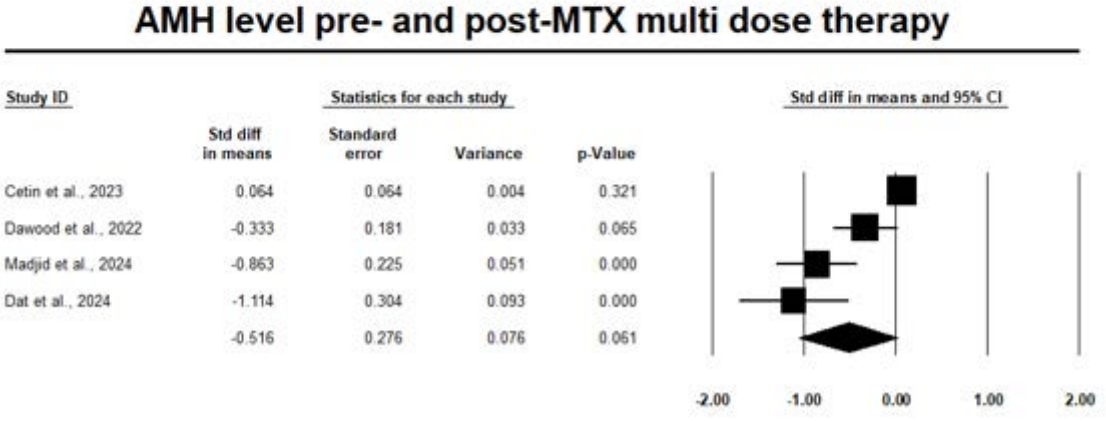


Figure 2. Forest plot of the effect of pre-and post MTX therapy on AMH level**.**



(A)



(B)

Figure 3. Forest plot comparing the efficacy of the single and multi-dose MTX regimens on AMH levels

Meta-analysis of primary effect size

Results of pre- and post-MTX therapy

A meta-analysis of 9 eligible comparative studies involving 354 women showed an overall significant reduction in the AMH level following MTX therapy (SMD=-0.393, 95% CI 0.197, 0.032, p= 0.028) **Figure (2).**

Comparison between single and multi-dose regimens of MTX

Five eligible comparative studies involving 158 women who received a single dose of MTX therapy showed an overall insignificant reduction in AMH level following treatment (SMD=-0.301, 95% CI 0.301, 0.091, p= 0.318) **Figure (3A).** Another 4 eligible comparative studies involving 196 women who received multi-dose of MTX therapy showed an overall insignificant reduction in AMH level following treatment (SMD=-0.516, 95% CI 0.276, 0.076, p= 0.061) **Figure (3B).**

Publication bias

The funnel plot demonstrated evident asymmetry, suggesting the presence of publication bias (I^2=86.9%, (P=0.00)). This shows that the study's findings were rather strong **Figure (4)**.

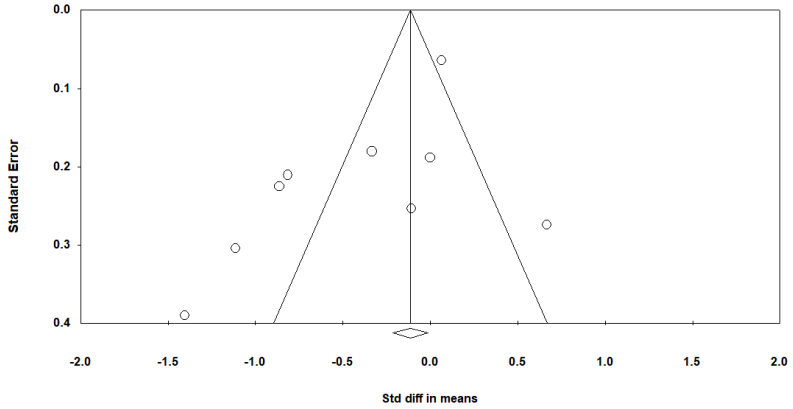


Figure 4. Funnel plot of the risk of bias assessment of the previously included studies

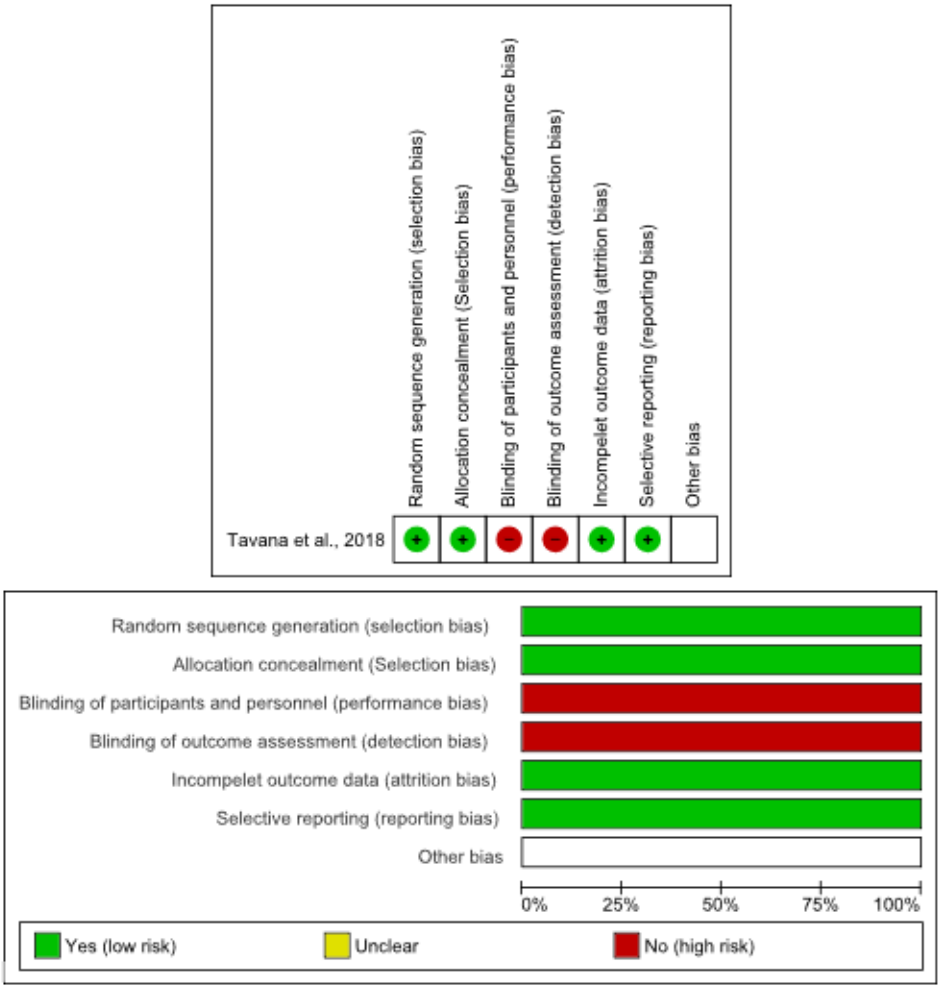


Figure 5. A) Risk of bias graph, and B) Risk of bias Summary

DISCUSSION

This meta-analysis reported a significant reduction in the AMH level following MTX therapy (SMD = -0.393, 95% CI 0.197, 0.032, p= 0.028). In a similar analysis, **Romito *et al.*** also reported a statistically significant reduction in AMH levels −1.97 (95% CI: −3.12, −0.82) among women with breast cancer after chemotherapy regimens that comprised MTX [24]. In the last 15 years, AMH has demonstrated a good connection with ovarian reserve, with more precision than other markers such as FSH [25]. Therefore, women who underwent MTX treatment for different medical conditions, after ovarian stimulation, will probably attain a low number of oocytes which consequently results in poor chances of pregnancy [26].

The considerable statistical heterogeneity seen in our meta-analysis could be attributed to the high clinical diversity, which includes varied ages, MTX regimens, AMH assay kits, follow-up durations, and different clinical conditions [49]. To reduce heterogeneity, we conduct a sub-group analysis for the different MTX regimens. We compared the single and multi-MTX dosages on AMH levels and both showed insignificant reduction; (SMD = -0.301, 95% CI 0.301, 0.091, p= 0.318) and (SMD = -0.516, 95% CI 0.276, 0.076, p= 0.061), respectively. This indicates that there is no difference in the single and multi dosages of MTX.

STRENGTHS

This systematic review and meta-analysis identified many gaps in current understanding, which may help guide future studies. Indeed, many sub-analyses that might be therapeutically beneficial and informative cannot be conducted with the data supplied. Many key questions remain unresolved: What is the potential role of women's age on the results? What is the difference between the underlying clinical conditions that required MTX treatment on AMH levels? These questions need further prospective studies with large sample sizes and adequate follow-up durations to answer.

This study has many strengths as this is the first systematic review and meta-analysis to investigate the direct effect of MTX treatment on ovarian reserve in light of AMH level. All of the included studies are prospective in nature which enhances the reliability of our findings. The study encompassed all relevant study designs, used independent evaluations in duplicate to assess the quality of the selected studies, and reported on all important study outcomes.

LIMITATIONS

The findings of this meta-analysis should be determined in light of some limitations, such as (1) publication bias, (2) the inclusion of only peer-reviewed and the English language documents, and (3) the low-to-medium quality of the methodology of the studies, which were unrepresentative and conducted on small sample sizes, with no stratified analysis carried out to account for potential confounding factors.

CONCLUSION

This analysis demonstrated that MTX significantly affects the AMH levels; however, there was no reported difference according to the treatment regimens. This topic is arising and there is a lack of data about it which requires more future prospective and RCTs to interpret.

Authorship Contribution: All authors share equal effort contribution towards (1) substantial contributions to conception and design, acquisition, analysis and interpretation of data; (2) drafting the article and revising it critically for important intellectual content; and (3) final approval of the manuscript version to be published. Yes.

Potential Conflict of Interest: None

Competing Interest: None

Acceptance Date: 11-11-2024

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