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The Effect of Male Sexual Abstinence Periods on the Clinical Outcomes of Fresh Embryo Transfer Cycles Following Assisted Reproductive Technology: A Meta-Analysis

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Abstract

A sexual abstinence period (SAP) lasting for 2–7 days is recommended before undertaking semen analyses. However, there is no consensus regarding the length of the SAP for couples using assisted reproductive technology (ART). Therefore, a meta-analysis was performed to compare the effect of short SAPs (less than 4 days) and long SAPs (4–7 days) on the clinical outcomes of fresh embryo transfer cycles after ART. A total of four studies were included in the meta-analysis. Although the fertilization rate in short SAP couples was higher than that in long SAP couples, a pooled analysis demonstrated that it was not statistically significant (p = .09). The implantation rate was, however, significantly higher in short SAP couples (p = .0001). The pooled analysis revealed that the pregnancy rate was significantly higher in short SAP couples than that in long SAP couples. The overall odds ratio (OR) for the pregnancy rate was 1.44 (p = .0006). No significant difference in miscarriage rates between the short and long SAP couples was found (p = .88). The meta-analysis indicates that a shorter abstinence period could result in higher implantation and pregnancy rates for patients undertaking ART treatments.

Keywords

Sexual abstinence period, ART, clinical outcomes, meta-analysis

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Semen analysis is a critical component of the evaluation of infertile men. Among numerous factors affecting semen quality, sexual abstinence period (SAP) is often overlooked. According to the guidelines published by the World Health Organization (WHO), 2–7 days of SAP is recommended; however, 3–4 days are advised by the European Society of Human Reproduction and Embryology (Barratt et al., 2011; ESHRE; WHO, 2010). In contrast to these recommendations, several studies have reported that even a less than 2 days of SAP can result in better semen quality and higher pregnancy rates following assisted reproductive technology (ART; Ayad et al., 2018; Hanson et al., 2018). No studies have verified whether 2–7 days or 3–4 days of SAP is the optimal period for achieving pregnancy after ART, and there is no consensus concerning whether there is a need to update SAP recommendations following ART.

In the present study, short SAP was defined as an SAP of less than 4 days, while long SAP was defined as an

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SAP from 4 to 7 days. This meta-analysis aimed to evaluate the influence of short and long SAPs on the clinical outcomes of fresh embryo transfers (ETs) following ART.

Methods

Design and Search Strategy

All studies related to the evaluation of clinical outcomes between short and long SAPs on fresh ETs during in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycles were identified for this meta-analysis. A literature search was performed in July 2019 and no language or geographic region restrictions were applied. The PubMed (1966 to July 2019) and EMBASE (1984 to July 2019) databases were searched. The search terms included *abstinence, sexual abstinence, SAP, ejaculatory abstinence, IVF, ICSI, in vitro fertilization, intracytoplasmic sperm injection, assisted reproductive technology*, and *ART*. Additional articles or unpublished data were manually retrieved after reviewing the reference lists from relevant publications.

Study Selection and Data Extraction

All potentially eligible studies were independently evaluated by two investigators and then cross-checked. The following criteria were used for study selection: (a) The outcome measures in the original studies included implantation rate, fertilization rate, pregnancy rate, miscarriage rate, or live birth rate, (b) the original studies compared the clinical outcomes undergoing ART between short and long SAPs, and (c) the type of ART treatment included fresh IVF cycles, fresh ICSI cycles, or a combination of the two. Studies using surgically retrieved, cryopreserved, and donor sperms as well as artificial insemination using the husband's semen were excluded. Studies reported only as an abstract were also excluded.

Relevant data from the included studies were independently extracted by two reviewers and then cross-checked. Any disagreements that could not be reconciled through discussions were decided by a third person. When short or long SAPs were divided into subgroups in the original studies, the pooled mean and standard deviations (*SD*s) were calculated using the formulas (Hozo et al., 2005):

$$(N1 * M1 + N2 * M2 + Nn * Mn) / (N1 + N2 + Nn)$$

and

$$\sqrt{SD1^2 * (N1\text{-}1) + SD2^2 * (N2\text{-}1) + SDn^2 * (Nn\text{-}1) / (N1 + N2 + Nn\text{-}2)}$$

The following information was extracted from the included studies: first author, study design, geographic region, SAP, type of ART, inclusion/exclusion criteria, and outcome measures.

Quality Assessment and Statistical Analysis

The quality of the included studies was assessed using the Newcastle–Ottawa quality assessment scale (NOS). Briefly, this scale includes three factors (selection, comparability, and exposure) and a total of 9 points (Stang, 2010). Two investigators independently evaluated the quality of original studies and a NOS score ≥7 indicated high quality.

Statistical analyses were performed using Review Manager, version 5.3.0 (Cochrane Collaboration, Oxford, UK). The Mantel–Haenszel chi-square test and the I^2 tests were used to assess the heterogeneity of the study results. If the I^2 value was less than 50% and the p value was more than .10, the heterogeneity was considered acceptable and a fixed effect model was applied for calculations. Otherwise, a random effect model was used. Mean differences (MDs) and odds ratios (ORs) were used to evaluate continuous and dichotomous data, respectively. All results are reported with 95% confidence intervals (CIs). A p value $<.05$ was considered statistically significant.

Results

Study Selection and Quality of the Included Studies

The initial database search yielded 210 reports, and two additional reports were identified through other sources (Figure 1). Of these, 188 were excluded based on titles or abstracts, and 17 were excluded after full-text reading. The remaining four studies (Borges et al., 2019; Periyasamy et al., 2017; Sánchez-Martín et al., 2013; Shen et al., 2019) met the inclusion criteria, and the data were subsequently extracted. Table 1 presents the characteristics of the four included studies. The overall quality of the four studies was high according to their NOS scores. Table 2 presents the specific scoring on selection, comparability, and exposure factors.

The fertilization rate was reported as mean +/− *SD* in two studies (Borges et al., 2019; Periyasamy et al., 2017) and data from these studies were extracted for meta-analysis. Although fertilization rates in short SAP couples were higher than those in long SAP couples, the pooled analysis was not statistically significant (MD = 5.18; 95% CI [−0.89, −11.26]; p = .09; Figure 2A). Three studies (Borges et al., 2019; Periyasamy et al., 2017; Shen et al., 2019) evaluated implantation rates and the pooled

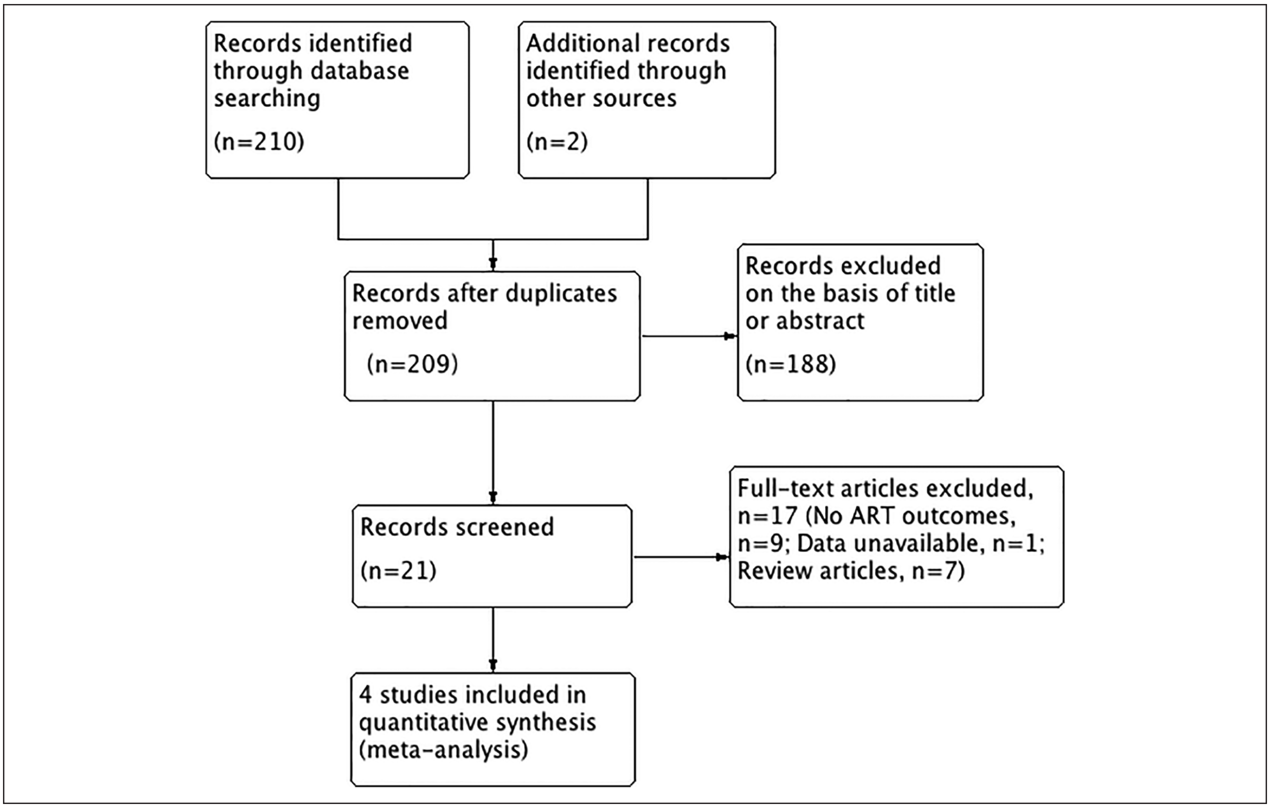

Figure 1. Flow diagram of the study selection process.

analysis revealed that this rate was significantly higher in short SAP couples than in long SAP couples (overall OR = 1.39; 95% CI [1.17, −1.65]; p = .0001; Figure 2B). The pooled analysis revealed that short SAP couples, in contrast to long SAP couples, had significantly higher pregnancy rates (Borges et al., 2019; Periyasamy et al., 2017; Sánchez-Martín et al., 2013; Shen et al., 2019). The overall OR for the pregnancy rate was 1.44 (95% CI [1.17, −1.78]; p = .0006; Figure 2C). Data on miscarriage rates were extracted from three studies for Forest plotting (Borges et al., 2019; Periyasamy et al., 2017; Shen et al., 2019). The results revealed that no significant difference existed for miscarriage rates between the short and long SAP couples (overall OR = 0.99; 95% CI [0.65, −1.52]; p = .88; Figure 2D).

Discussion

Although recommendations for SAPs are provided by WHO and ESHRE, the optimal period of sexual abstinence following ART is still under debate. Until now, there are no published meta-analyses that compare the clinical outcomes between short and long SAP during IVF/ICSI cycles. Therefore, this meta-analysis provides, for the first time, pooled evidence for the influence of SAP on fresh ETs following ART. With regard to semen parameters, a large amount of data indicated that longer abstinence periods were associated with increases in semen volume and total sperm count; however, a shorter abstinence period might improve the morphology, motility, and other semen parameters (e.g., Alipour et al., 2017; Deng et al., 2019; DNA fragmentation; Hanson et al., 2018; Keihani et al., 2017; Said & Reee, 2015). For couples undergoing intrauterine insemination (IUI) cycles, previous studies have reported that the highest observed pregnancy rates following the IUI cycle belonged to those whose abstinence interval was 3 days or less (Jurema et al., 2005; Marshburn et al., 2010). For IVF/ICSI cycles, clinical outcomes varied among different studies. In the present analysis, while there was no significant difference in the fertilization rate between groups, the short SAP couples showed higher implantation pregnancy rates than couples with long SAPs. One possible explanation for these results may be that the SAP does not appear to impact the fusion of an ovum and sperm. The meta-analysis also revealed that the miscarriage rate in short SAP couples showed a similar trend compared to long SAP couples.

Previous studies have evaluated the impact of SAP on IUI outcomes. In a retrospective analysis (Marshburn

Table 1. Characteristics of Included Studies.

Study	Design	Geographic region	Sexual abstinence period	Assisted reproductive technology	Inclusion criteria	Exclusion criteria	Outcome measures
Periyasamy et al. (2017)	Retrospective study	India	Group Ia: SAP 2–4 days; Group Ib: SAP 5–7 days; Group II: SAP >7 days	ICSI or IVF/ ICSI	Fresh ET	Women ≥40 years; surgically retrieved sperms or cryopreserved samples; IVF-only cycles; poor responders; all embryos were cryopreserved	Live birth rate; development of top-quality embryos; fertilization rate; implantation rate; clinical pregnancy rate; miscarriage rate
Borges 2018	Perspective cohort study	Brazil	SAP ≤4 days vs. SAP >4 days	ICSI	Isolated male infertility undergoing first ICSI cycle; fresh embryo transfer	Vitrified/thawed or donated oocytes; surgical sperm retrieval; vitrified/thawed embryo transfer; preimplantation genetic diagnosis or screening; female infertility	Fertilization rate; implantation rate; pregnancy rate; miscarriage rate; high-quality embryo rate on Day 3; blastocyst formation rate on Day 5
Sánchez-Martín et al. (2013)	Cohort study	Spain	SAP: 12 h vs. SAP: 4 days	ICSI	ICSI cycle with non-severe male factor; ovum donation with non-severe male factor	Azoospermic, oligospermic, and asthenospermic with less than 10% of a + b sperm motility	Pregnancy rate
Shen 2018	Perspective study	China	SAP: 1–3 h vs. SAP 3–7 days	IVF	First round of IVF; normal ovarian reserve; normal serum thyroid-stimulating hormone concentration; normal prolactin concentration	NA	Implantation rate; clinical pregnancy rate; miscarriage rate; live birth rate

Note. ICSI = intracytoplasmic injection; IVF = in vitro fertilization; NA = not available; SAP = sexual abstinence period.

Table 2. Newcastle–Ottawa Scale Scores of Included Studies.

	Selection				Comparability		Exposure			
Study	Definition of cases	Representativeness	Selection of controls	Definition of controls	Main risk factor	Other risk factors	Assessment of exposure	Same methods of ascertainment for cases and controls	Nonresponse rate	Total
Periyasamy et al. (2017)	★	★	★	★	★	★	★	★	☆	8
Borges 2018	★	★	★	★	★	★	★	★	☆	8
Sánchez-Martín et al. (2013)	★	★	★	★	☆	☆	★	★	☆	6
Shen 2018	★	★	★	★	★	☆	★	★	☆	7

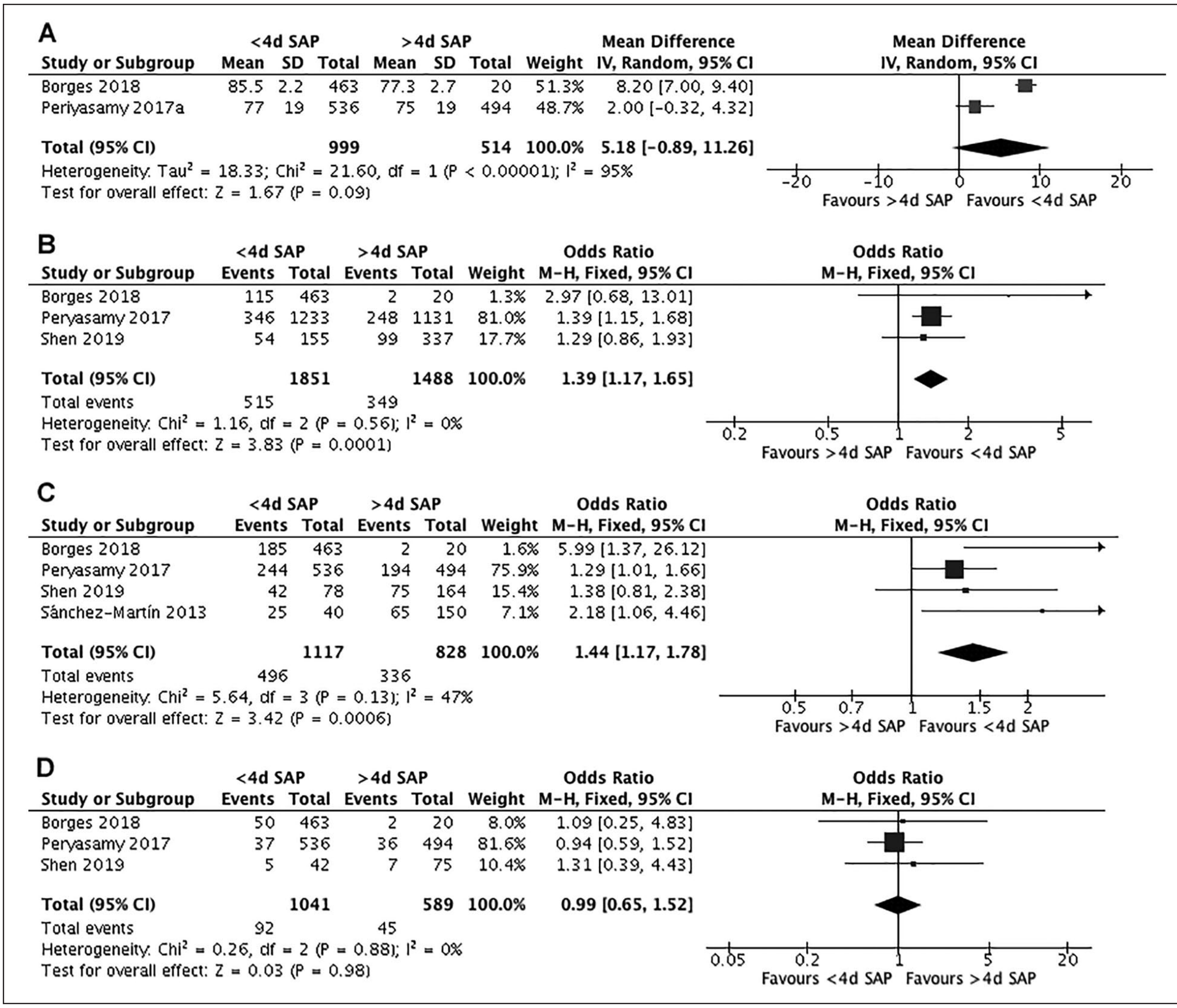

Figure 2. (A) Random effect model of the mean differences (MDs) with 95% confidence intervals (CIs) of fertilization rate: long sexual abstinence period S(AP) versus short SAP; (B) fixed effect model of the odds ratios (ORs) with 95% CIs of implantation rate: long SAP versus short SAP; (C) fixed effect model of the ORs with 95% CIs of pregnancy rate: long SAP versus short SAP; (D) fixed effect model of the ORs with 95% CIs of miscarriage rate: long SAP versus short SAP ; M-H: Mantel-Haenszel; IV: inverse variance.

et al., 2010), an ejaculatory abstinence period of no more than 3 days achieved the highest pregnancy rate (11.27%). In this study, while the pre/postwash total motile sperm (TMS) count was the highest when the abstinence time was 7 days, the pregnancy rate did not show a similar trend. While TMS was considered the variable most predictive of conception in IUI cycles, a higher pregnancy rate was observed despite a lower TMS in the insemination specimens. However, a retrospective, observational, and transversal study from Mexico showed that the pregnancy rate after 4–7 days of SAP appears to be somewhat higher than that after 1–4 days of SAP (Kably-Ambe et al., 2015). Therefore, in IUI cycles, the reported pregnancy rate in different SAPs is contradictory. Still, the present study can conclude that although TMS can be achieved with prolonged SAPs, the pregnancy rate in prolonged SAP couples is not necessarily higher than that in short SAP couples. In addition, published data regarding the clinical outcomes in IVF/ICSI cycles are also inconsistent. In a retrospective cohort study evaluating 131 ICSI cycles, the fertilization and clinical pregnancy rates were not significantly different between 5–7 days of SAP and 2–4 days of SAP groups ($p = .1$ and $p = .9$, respectively; Lee et al., 2015). In another retrospective study that included 445 ICSI cycles, the investigators evaluated the impact of 1, 2, 3, 4, 5, 6–10, and ≥11 days of SAP on clinical outcomes (Colturato et al., 2007). The highest and lowest pregnancy rates were reported in couples with

1 and 5 days of SAP (67.2% and 42.1%, respectively, $p = .007$). The study reported an inverse relationship between SAP and ICSI results, and a short period of abstinence was recommended during ICSI cycles. Despite the different conclusions arrived at by these studies, in neither study did short SAP couples exhibit lower pregnancy rates than high SAP couples.

Human spermatozoa are produced in the seminiferous tubules and then stored in the epididymis. As the storage time of spermatozoa in the reproductive ducts can be affected by ejaculatory frequency, short abstinence periods could eliminate the detrimental effects of possible oxidative stress associated with sperm storage (Marshburn et al., 2014). Other studies have also indicated that prolonged exposure to reactive oxygen species arising from dead spermatozoa and leukocytes may be one reason for the association between decreases in sperm quality and an increase in DNA fragmentation rates with low ejaculatory frequencies (Borges et al., 2019; Comar et al., 2017). However, data regarding abstinence periods and DNA fragmentation rates is also heterogeneous. In 2019, Shen et al. confirmed the possible molecular diversity of spermatozoa in ejaculates after 1–3 h of SAP and 3–7 days of SAP (Shen et al., 2019). A total of 322 differentially expressed proteins with four reproductive tract–related tissues (testis, epididymis, seminal vesicles, and prostate) were reported and the authors suggested that testis-related spermatogenesis may play an important role in the reproductive potential of spermatozoa after a shorter abstinence interval. To date, little is known about the role of these proteins in abstinence-related sperm function; however, the research suggests that short abstinence intervals can alter the expression of sperm proteins, which may be one of the reasons why short SAPs may improve clinical outcomes following ART.

There are some limitations with this meta-analysis. First, the present meta-analysis only compared the main clinical outcomes following ART—semen parameters were not analyzed. Previous studies have demonstrated that shorter abstinence times could improve semen parameters and is proposed as a method for reducing sperm DNA fragmentation (Borges et al., 2019; Deng et al., 2019; Hanson et al., 2018). Decreased sperm DNA fragmentation might be one of the reasons underlying improvements in pregnancy rates. Second, an analysis to clarify if spermatozoa preparatory procedures affect clinical outcomes was not performed. The most common preparatory techniques in clinical practice are density gradient centrifugation (DGC) and the swim-up method. Whether the effects of these two methods on sperm quality are different is still a matter of debate. Some researchers have suggested that both the DGC and swim-up procedures are not optimal semen-processing techniques and that emerging microfluidic sperm sorters might be a better choice for semen preparation (Shirota et al., 2016). Third, the present meta-analysis did not analyze whether the locations of the included studies impact the clinical outcomes following ET. Consales et al. (2014) reported that the sperm DNA methylation levels differ between distinct geographic locations, and previous studies also reported that sperm DNA methylation is important in maintaining proper sperm health and function (Du et al., 2016; Kläver et al., 2013). Although there is no direct evidence that location will affect the clinical outcomes following ART, it should be considered a promising new direction for future research. Further, cigarette smoking, caffeine intake, and lifestyle were not analyzed in this meta-analysis, although a significant relationship of the aforementioned factors with semen quality was concluded by previous studies (Ricci et al., 2017; Shi et al., 2018; Sharma et al., 2016). Finally, all the included studies evaluated various populations, including healthy normal men as well as oligozoospermic men. Meanwhile, in original studies, there was no clear discrimination between populations. Therefore, this meta-analysis failed to analyze whether male patients with severe oligozoospermia will benefit from short SAP.

Based on the available data, a shorter abstinence period could achieve higher implantation and pregnancy rates during ART procedures. While recommendations for SAP lengths have been provided by WHO and ESHRE, there is increasing evidence indicating that shorter SAPs could result in better semen parameters and better clinical outcomes following ART procedures. Therefore, a new criterion for abstinence periods should be considered. Further study is needed to investigate the effect of sperm preparation processing on clinical outcomes following ART procedures.

Author Contributions

All authors made substantial contributions to the following: (a) the research design and critical revision; (b) searching and selecting the studies; (c) analyzing, interpreting the data, and drafting the paper; and (d) final approval of the version to be submitted.

Declaration of Conflicting Interests

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