Original Article

Effects of Day 3 Versus Day 5 PGT-A Embryo Biopsies on IVF/ICSI Cycles Outcome : A Systematic Review and Meta-Analysis

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ABSTRACT

Background: The effectiveness of pre-implantation genetic testing for aneuploidy (PGT-A) remains a topic of intense debate.
Objectives: Is to determine the optimal time for PGT-A biopsies.
Methods: Up until May 2024, MEDLINE/PubMed, Embase, and the Cochrane Central Library were searched thoroughly for relevant literature. PGT-A with comprehensive chromosomal screening (CCS) on Days 3 and 5 was used in 11 randomized controlled studies.
Results: In the overall population, PGT-A did not increase live-birth rates (LBR) per patient (RR:1.11; 95%CI:0.87-1.42; n=1513; I2=75%). Nonetheless, PGT-A reduced the whole population's miscarriage rate (RR:0.45; 95%CI:0.25-0.80; n=912; I2=49%). Remarkably, PGT-A increased cumulative LBR per patient (RR:1.36; 95%CI:1.13-1.64; n=580; I2=12%). Only the day-5 biopsy procedure showed enhanced LBR per ET in terms of optimal scheduling (RR: 1.37; 95% CI: 1.03-1.82; I2=72%).
Conclusion: PGT-A only increased live-birth rates when applied to embryos at the blastocyst phase; it had no effect on clinical results for the overall population.

Key Words: Blastocyst, day 3, IVF/ICSI, live birth rate, PGT-A.

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INTRODUCTION

The gold standard for embryo choice in accordance to euploidy is preimplantation genetic testing for aneuploidy (PGT-A)[1]. It is primarily advised for the extremely specific group of patients who have planned single ET, profound male infertility, recurring failure of implantation, repeated loss of pregnancy, or old maternal age[2,3].

There are two, if not more, lines of thought that are beginning to emerge over "when" and "how" PGT-A should be used. Getting an agreement on the best course of action is made more difficult by the fact that PGT-A is a factually complex condition that involves the difficult processes of biopsy and consequent genetic analysis, in addition to a variety of alternatives and combinations[1].

Although trophectoderm biopsies is the newest fashion, D3 biopsy remains evidently useful in clinical settings[4,5]. The danger of fewer embryos achieving the blastocyst phase may be mitigated by doing biopsy during the cleavage phase. However, it has been documented in the scientific publications that embryos that were identified as mosaic in a biopsy at the cleavage phase were later assessed as euploid in a biopsy at the blastocyst phase[6]. Trophectoderm biopsy may have great potential since it permits a large number of cells to be biopsied, whereas cleavage phase biopsy carries the risk of an incorrect diagnosis based on single-cell analysis[7].

Mosaicism may be the cause of misdiagnosis after trophectoderm biopsy. Inconsistency between trophectoderm and inner cell mass is uncommon, but in the few instances that it is seen, it should not be disregarded[8,9]. There are clear disagreements around the biopsy procedure[7,10,11]. Since newer research supports doing a biopsy at the blastocyst phase in conjunction with comprehensive chromosomal screening (CCS), the debate between day 3 (D3) and day 5 (D5) biopsy has been in the news for years[12].

When using the FISH technique, PGT-A was associated with reduced ongoing pregnancy and live-birth rates relative to traditional cycles, according to two out of three meta-analyses that examined the benefits of PGT-A[3,13,14]. On the other hand, PGT-A founded on CCS has been shown to increase live birth and clinical pregnancy rates[15,16]. Because PGT-A has recently been offered and sold on a more horizontal basis, knowledgeable patients now see it as a risky "add-on" to enhance the clinical results of IVF[17,18].

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This work attempts to add to the literature on PGT-A regarding the effectiveness of conducting biopsies at the cleavage or blastocyst phases.

METHODS

Search strategy

Only publications released by peer-reviewed journals up until June 2023 were included in the systematic literature research conducted in the databases of PubMed/ Medline, Embase, and the Cochrane Central Library."In Vitro Fertilization," "IVF," "Intracytoplasmic Sperm Injection," "ICSI," "Preimplantation Genetic Screening," "aneuploidies," "disorders," "Day 3," "cleavage stage embryo," "Day 5," "Day 6," and "blastocyst," were among the key phrases and their corresponding mixtures that were part of the search approach.

The three databases produced 1819 papers in the first search. 215 studies were eliminated as duplication from the total results. After a preliminary review of all records' headings and abstracts, 1504 were honed to produce pertinent articles. After that, complete texts were carefully vetted, and citation mining of a few chosen, pertinent articles was done for ultimate inclusion. Eleven studies in all[4,5,19–27] have been incorporated in our meta-analysis after this extensive screening.

Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) chart (Figure 1) was created. Three separate writers conducted the screening and study selection. The senior author convened an arbitration to settle any disputes between the two writers.

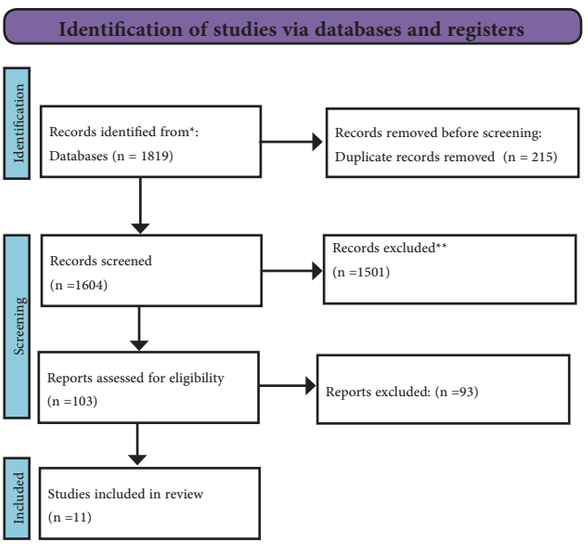

Fig. 1: PRISMA flow chart

Study selection

In an effort to eliminate insignificant researches, We firstly evaluated the study headings and abstracts. Full-text publications of the remainder studies were acquired and carefully examined after the initially made selection.

Inclusion criteria

1. Women who underwent IVF/ICSI cycles with or without PGT-A before embryo transfer (ET) were included in the population; the study group was represented by the former, while the control group was represented by the latter. Before ET, only morphological evaluation of D3 or D5 embryos was done for the control group,

2. Trials that included both fresh and frozen PGT-A cycles,

3. Only studies that performed 24 chromosome aneuploidy screening (PCR, aCGH, and NGS), were enrolled in the study.

Exclusion criteria

1. Polar body biopsy or PGT for structural chromosomal defects, translocations, or monogenic diseases,

2. Studies involving patients who were not randomized with respect to the impact of PGT-A effectiveness on pregnancy results,

3. Studies that performed the analysis on a specific number of chromosomes (FISH), were excluded from this meta-analysis.

Data extraction

In accordance with the selection standards, information extraction was carried out separately. Personal interaction was tried with the authors of papers that did not include age-subgroup analysis.

Outcome measures

The live-birth rate per patient and the miscarriage rate per clinical pregnancy serve as the main outcome metrics for this meta-analysis. The following are the secondary outcome measures: cumulative live-birth rate per patient, live-birth rate per ET, continuing pregnancy rate per ET, clinical pregnancy rate per ET, cumulative live-birth rate per ET, and ongoing pregnancy rate per patient.

Clinical pregnancy is regarded as the appearance of a gestational sack at 4–5 weeks of gestation, whereas

ongoing pregnancy is recognized as a viable pregnancy at 20 weeks. The number of live births after several ETs is known as the cumulative live-birth rate.

Assessment of risk of bias

Using the "Cochrane Risk of Bias Tool for Randomized Controlled Trials," evaluation of bias was carried out separately by 2 investigators. An additional writer resolved any disputes between the writers (see Figures 2,3).

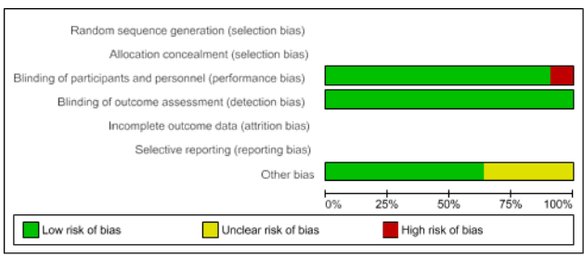

Fig. 2: Evaluation of the studies' risk of bias that were part of the meta-analysis

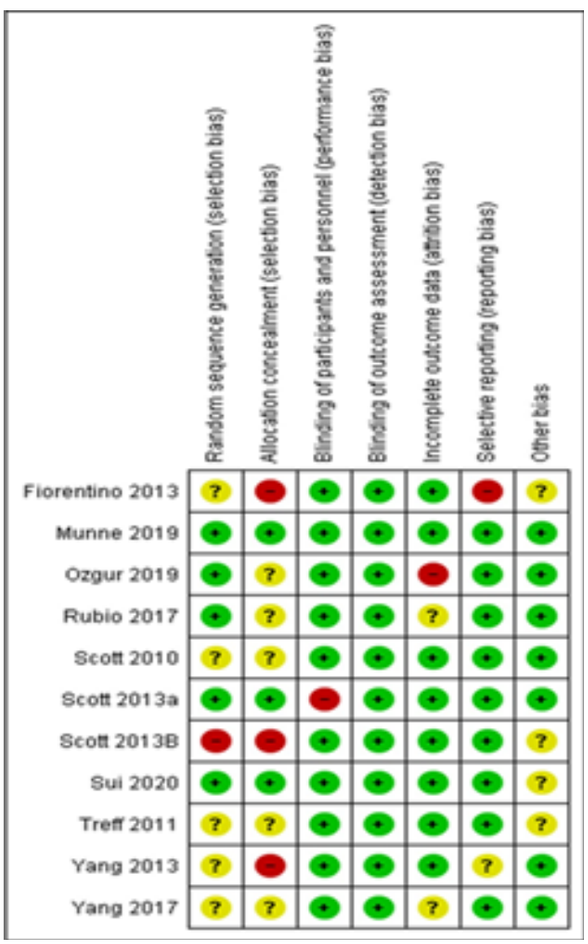

Fig. 3: Synopsis of the risk of bias evaluation for every item for every study that was part of the meta-analysis

Statistical analysis

Using the RevMan (v.5.3), a meta-analysis was conducted with respect to the age groups. For statistical reasons, the network was constructed using the R computer language's package. Direct and indirect effects are compared in network meta-analysis. While the indirect effect is calculated through contrasting the two groups to another "reference" group, the direct effect is calculated by directly contrasting the two groups. For the analysis of the included studies, a risk ratio with 95% CIs was used. For results pooling based on heterogeneity, either the fixed effect or the random effects model was used. The I2 statistic was used to assess the exposure effect's heterogeneity. The meta-analysis was not conducted since high heterogeneity was indicated by an I2 value of 80% or above. In accordance to the Cochrane Handbook's sixth edition, the model with random effects was used if the I2 value was higher than 0 and there was a discernible difference in sample size across the trials. This meta-analysis's study sizes varied greatly, hence the fixed effects model was only used when I2=0%.

RESULTS

Analysis according to day of PGT-A biopsy

Rates of live births per patient

Data on live-birth rates per patient have been released in 7 trials. Two distinct biopsy days were contrasted across three distinct study designs (Day 5 biopsy vs. Day 3 biopsy; Day 5 biopsy vs. control; and Day 3 biopsy vs. control). The term "control group" describes the selection of embryos centered only on morphology assessment and without biopsy. 1629 individuals in all were assessed. We assessed seven pairwise contrasts. The model with random effects was used since the studies' acknowledged heterogeneity was very high (I2=72.4%). The network estimate for both of the biopsy days (Day 3 vs. Day 5) did not show any statistically significant differences (RR: 0.90; 95% CI: 0.59–1.38). Concerning Day 3 biopsy, there was no statistically significant distinction between D3 and control (RR: 1.07; 95% CI: 0.71–1.60). When compared to control (Day 5 vs. control), PGT-A using Day 5 biopsy did not show statistically significant greater live-birth rates (RR: 1.18; 95% CI: 0.93–1.51) (see Figure 4A).

Live-birth rates per ET

Data on live birth rates per ET cycle have been published by 7 studies. Two distinct biopsy days were contrasted across three distinct research designs (Day 5 biopsy vs. Day 3 biopsy; Day 5 biopsy vs. control; and Day 3 biopsy vs. control). The term "control group" describes the selection of embryos based only on morphology assessment and without biopsy. In all, 1450 ET cycles were assessed. We assessed seven pairwise contrasts. The

random effects model was used since the studies' stated heterogeneity was very high (I2=71.5%). The network estimate for the two biopsy days (Day 3 vs. Day 5) showed insignificant differences (RR: 0.92; 95% CI: 0.58–1.48). When comparing the Day 3 biopsy to the control, there was no statistically significant difference (RR: 1.26; 95% CI: 0.81–1.97). Comparing the D5 biopsy to the control, the live-birth rates were statistically significantly higher (RR: 1.37; 95% CI: 1.03–1.82) (Figure 4B, Table 1).

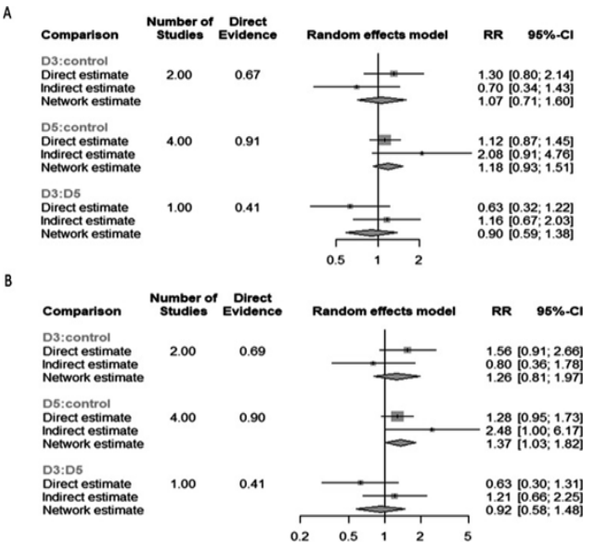

Fig. 4: (A) Forest plot of the network contrasting PGT-A with day 5 biopsy to PGT-A with day 3 biopsy to morphology analysis (control) in the general population with respect to the live-birth rate per individual. **(B)** Forest plot contrasting PGT-A with day 5 biopsy to PGT-A with day 3 biopsy to morphology analysis (control) in the overall population with respect to live-birth rate per ET result

Clinical pregnancy, miscarriage, and cumulative live-birth rates

After Day 3 biopsy PGT-A, merely one study revealed cumulative live-birth rates, ongoing pregnancy, clinical pregnancy, and miscarriage, and no research showed the Day 3 vs. Day 5 configuration. For one of the two designs, a network meta-analysis conducted on a single trial and utilizing only indirect estimates will produce extremely poor quality data that is prone to bias. Therefore, a network meta-analysis of these results was not conducted.

Fresh vs frozen ET

Only women 35 years of age or beyond were included in the contrast between the fresh and frozen ET approach after PGT-A in order to remove any potential confounders and extra biases. The selection requirements were met by four studies. PGT-A only increased the live-birth rate (RR: 1.39; 95% CI: 1.09–1.78; n=384) when frozen ET was used. However, when contrasting PGT-A subsequently receiving a new ET with the control group, there was no statistically significant distinction was seen (RR: 1.56; 95% CI: 0.73–3.34; n=228).

DISCUSSION

One of the very rare meta-analyses contrasting D3 and D5 biopsies for PGT-A is the one being conducted here. In accordance to the headline and protocol, the upcoming study is intended to assess various biopsy days for pre-implantation genetic testing for monogenic illnesses (PGT-M), even though a Cochrane guideline on the day of biopsy has been released.

Our study's findings only pertain to the outcome of a live birth when discussing the biopsy day. There was no discernible difference between the Day 3 and Day 5 biopsies. Yet, the live-birth rate per ET was only statistically substantially higher in the Day 5 biopsy group. It is important to remember that just one study in this collection directly compared Day 3 and Day 5 biopsies[21] (Table 1).

Table 1: lists the attributes of every study that was part of this meta-analysis

Author	Study type	no of patients	Fresh/ frozen cycles	Biopsy day/ embryo stage	Biopsy technique	Outcome measures
Fiorentino et al., 2013[4]	Cohort study	65	Fresh	Day 3	aCGH	OPR, CLB
Munné *et al*., 2019[19]	RCT	661	Frozen	Day 5	NGS	CPR, OPR, MR
Ozgur *et al*., 2019[20]	RCT	220	Frozen	Day 5	NGS	CPR, OPR, MR
Rubio *et al*., 2017[5]	RCT	205	Fresh	Day 3	aCGH	CPR, OPR, MR, CLB
Scott *et al*., 2010[22]	RCT	28	Fresh	Day 5	qPCR	CPR
Scott *et al*., 2013a[23]	RCT	155	Fresh	Day 5	qPCR	CPR, OPR, MR
Scott *et al*., 2013b[11]	RCT	226	Fresh	Day 3 & day 5	Microarray analysis and SNP	OPR
Sui *et al*., 2020[27]	RCT	207	Frozen	Day 5	SNP	IR, CPR, CMR, OPR, and LBR
Treff *et al*., 2011[24]	Cohort study	76	Fresh	Day 5	qPCR	IR, CPR, CMR, and LBR
Yang *et al*., 2013[26]	RCT	103	Frozen	Day 5	aCGH	CPR, CMR, and LBR
Yang *et al*, 2017[25]	Cohort study	169	Frozen	Day 5	NGS	CPR, OPR, MR

In contrast to individuals assigned to D5 biopsy, those subjected to D3 biopsy had a significantly lower live-birth rate, according to this study[21]. There was merely one study contrasting D3 biopsy and PGT-A vs. control, which referred to the choice of embryos centered only on morphology changes criteria without conducting biopsy, and no studies juxtaposing Day 3 vs. Day 5 biopsies, so outcomes on ongoing pregnancy, clinical pregnancy, miscarriage, and cumulative live-birth rates could not be offered. This could be explained by the fact that the majority of research use FISH rather than CCS and undergo cleavage stage biopsies.

The embryo's ability to implant appears to be unaffected by trophectoderm biopsy. For the first time, Tiegs and associates[29] tried to highlight the actual effect of trophectoderm biopsy solely on live-birth rates. Trophectoderm biopsy was performed as part of the study's design, and a frozen ET was conducted using just morphology criteria, not the PGT-A outcomes, which were unknown and revealed at the moment of ET.

According to the authors, the live-birth rates in the biopsy and no-biopsy groups were comparable. Additionally, the previously mentioned research found that when PGT-A was used in conjunction with a day 5 biopsy, the negative predictive value was 100%. Even while it is commonly accepted that RCTS may be trusted to agree on safety and effectiveness, not all RCTs are created equal. Because of this, the findings from RCT studies might be of varying quality.

Considering non-selection investigations and their role in inference, they should be taken into account even though, as a study method, they are typically regarded as having less weight than RCT data. To further explain this, RCTs and non-selection studies yield distinct kinds of results. RCTs evaluate the use of PGT-A in terms of pregnancy and live birth rates, but they don't tell us how accurate PGT-A is. Since sensitivity, specificity, and positive and negative predictive value cannot be gleaned from RCTs, only non-selection investigations should be examined in order to gauge the accuracy of diagnostic tests.

Without any question, accounting for the aspect of embryo shape would provide more light on the link between result and embryo quality at the time of ET. However, most research offers a broad categorization of embryo quality. Based on morphological evaluation, all of the investigations that made up the current meta-analysis used what are referred to as "the higher quality embryos" that were accessible for transfer. It should be noted that in the studies that offer data on embryo quality, embryos that were deemed to be of "good quality" were not initially exposed to biopsy. Every study that used the frozen embryo transfer method also used luteal stage support.

Just one age group was included in the analysis of the fresh and frozen ET strategies in an effort to reduce potential confounders and extra bias. The > 35 year age group was chosen since only this patient group showed a statistically significant distinction, allowing for comparability. Investigating the best course of action for individuals older than 35 should be given priority because they are a more time-sensitive demographic. Although both fresh & frozen ET studies report on Day 5 ET, the contrast between them is prone to bias a priori because frozen ET studies report on Day 5 ET, whereas fresh ET studies conducted a Day 3 biopsy. It is impossible to ignore this significant disparity.

When compared to the frozen ET group, Scott's 2013 trial on Day 5 biopsy and a fresh Day 6 ET would be the only study strategy that would not be affected by the confounding factor of the discrepant biopsy day. The different ET days, specifically Day 5 vs. Day 6, present another confounding factor in this instance, though. However, the study could not be included since the investigators did not include age-subgroup analysis. It would be excellent to confirm the same biopsy and ET days in order to accurately compare fresh ET to frozen ET after PGT-A. However, this would not be consistent with standard clinical practice.

Notwithstanding the differences, the contrast between fresh and frozen food is legitimate because it details the various approaches used; yet, there are two reasons why the frozen ET technique is superior. First, as evidenced by the findings cited here, blastocyst biopsy is generally accepted as the preferred biopsy day; second, the cryopreservation method of vitrification allows for good results, a claim that has been backed up by substantial data[28].

The efficiency of the thawed embryo and endometrial receptivity should be taken into consideration while examining variables related to either the PGT-A technique using a fresh or frozen embryo transfer. There are fewer embryos accessible for ET in contrast to the fresh transfer method, even if the vitrification procedure is preferable than slow freezing because the survival rate of embryos after thawing has not yet surpassed 100%[30]. However, the frozen ET method enables greater synchrony and excellent endometrial receptivity, even if it may appear to impede the embryo's implantation dynamics[31].

Unlike CCS, FISH did not increase the rates of pregnancy or live births. This can be explained by FISH producing false-positive results and not evaluating every chromosome[2,32]. Studies that used FISH were therefore disqualified. Because a high degree of agreement has been observed in the data obtained by PCR, aCGH, or NGS, the authors chose to include studies using these methods[4,5,19–26]. Because a lesser percentage of cells are eliminated, it's possible that just trophectoderm biopsy produced a statistically meaningful increase in live birth rates. Trophectoderm biopsy's improved outcomes are consistent with a number of studies[33,34].

PGT-A's improved cumulative live-birth rate statistics could show how successful it is in cycles with a high embryo richness. When the evaluation was conducted per ET, it was shown that PGT-A resulted in noticeably better clinical outcomes; this finding was not supported by the per patient assessment.

We have noticed that several research have randomised at the embryo's biopsy phase rather than at the oocyte retrieval step. This could imply that the study eliminated patients who failed to get to the necessary stage or the threshold for the number of embryos that do. This could lead to the assumption that when evaluating the efficacy of PGT-A, the quantity of embryos accessible for biopsy may be very important. Yet, before seeking to make a comment regarding the significance and the function that the number of embryos performs in determining whether PGT-A is useful, more research with a different design must be conducted.

Regarding the present study's shortcomings, there are still relatively few papers that meet the inclusion parameters for this meta-analysis. Nevertheless, it is one of the very first meta-analyses to compare the days of biopsy and only include RCTs using full chromosomal screening. Another drawback is that there was just one study that directly compared cleavage and trophectoderm biopsies.

In order to get an adequate number of embryos, two of the studies that were part of the current meta-analysis[5,27] provided with multiple oocyte retrievals. The PGT-A & reference groups did not vary in the quantity of oocytes harvested or the number of oocyte retrievals carried out. Since there was no statistically significant distinction between the two groups, the many retrievals technique did not produce any differences, which could have been a confounding factor given PGT-A's effectiveness in terms of clinical outcomes. Therefore, research with numerous retrievals were considered appropriate for inclusion by the meta-analysis's investigators.

Given that multiple investigations have documented reduced live-birth rates after day 6 ET[4], the data from Scott's 2013 study may pose a confounding factor for the current meta-analysis. Nonetheless, we have chosen to incorporate this research. When it comes to fresh ETs, delay in ET on day 6 may have a detrimental effect on PGT-A clinical results by exhibiting a decreased PGT-A effectiveness. However, as there are a few explanations to postpone ET to day 6 in the absence of PGT-A, which has been known to increase the chance of skipping the implantation window, executing a delayed day 6 ET after PGT-A might better represent clinical practice.

CONCLUSION

The current study's findings indicate that PGT-A on Day 5, using CCS and frozen Day 5 ET, increases live birth rates. Yet, the quantity and caliber of embryos accessible for biopsy, along with the age of the mother, can be a decisive factor in determining whether PGT-A is advantageous from the standpoint of the IVF cycle's success.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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