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The Effect of Recombinant LH Addition to Recombinant FSH on Assisted Reproductive Technologies Outcomes in Overweight and Obese Patients without Polycystic Ovary Syndrome

Merve Demir[1]*, Emre Sertel[2], Ozge Senem Yucel Cicek[1], Leylim Yalcınkaya[1], Sare Dogan[1], Osman Onur Daloglu[3], Emek Doger[1], Birol Vural[4]

[1]Department of Obstetrics and Gynecology, Kocaeli University Faculty of Medicine, Kocaeli, Turkey
[2]Department of Obstetrics and Gynecology, Kiziltepe State Hospital, Mardin, Turkey
[3]Department of Internal Medicine, Kocaeli State Hospital, Kocaeli, Turkey
[4]Department of Obstetrics and Gynecology, Kolan International Hospital, Istanbul, Turkey
Email: *mervedemir_yeditepe@hotmail.com

Abstract

Background: Obesity and overweight negatively affect fertility. Protocols need to be developed in ART treatments for obese and overweight women. **Objectives:** To investigate the effect of recombinant luteinizing hormone (rLH) addition to recombinant follicle stimulating hormone (rFSH) on treatment outcomes in assisted reproductive technologies (ART) cycles in obese and overweight women without polycystic ovary syndrome. **Methods:** This retrospective cohort study was carried out in Kocaeli University Faculty of Medicine, Assisted Reproductive Techniques Clinic between January 2016 and March 2019. To analyze the impact of rLH addition to rFSH in GnRH antagonist cycles in overweight and obese, the patients were divided into four groups according to body mass index (BMI) and gonadotropin type; Group 1: patients with BMI ≥ 25 stimulated with rFSH alone (n: 37), Group 2: patients with BMI ≥ 25 stimulated with rFSH + rLH (n: 37), Group 3: patients with BMI between 18.5 - 24.99 stimulated with rFSH alone (n: 33), Group 4: patients with BMI between 18.5 and 24.99 stimulated with rFSH + rLH (n: 30). Patients with polycystic ovary syndrome were excluded. **Results:** Basal LH levels were found to be significantly lower in obese and overweight patients compared to normoweight patients (p = 0.01). Grade 1 embryo ratio in obese and overweight patients was higher in rLH added obese group than in group LH not included (64.9%, p = 0.005). Ongoing pregnancy rates (OPR) in obese and

overweight patients were significantly higher in rLH added group compared to rFSH only group (43.2% vs 18.9% respectively, p = 0.044). However, OPR did not differ significantly in rLH added and rFSH only groups in normoweight patients (p = 0.588). **Conclusion:** This study has shown that obese and overweight non-PCOS patients have lower endogenous LH levels. It has also shown that rLH supplementation in GnRH antagonist cycles in obese and overweight women improves embryo quality and ongoing pregnancy rates. However rLH addition to rFSH doesn't seem to have a value in normoweight patients.

Keywords

Recombinant LH, *In Vitro* Fertilization, Obesity, GnRH Antagonist Protocol

1. Introduction

According to the World Health Organization, a person with a body mass index (BMI) of 25 or more is considered overweight and a BMI of 30 or more is considered obese [1]. In addition to being a risk factor for a number of chronic diseases, obesity also contributes to infertility and poor assisted reproductive technologies (ART) outcomes [2]. Overweight and obese women have a lower chance of pregnancy compared with normoweight women following ART treatment [3]. In addition, total gonadotropin dose used in ART cycles in obese and overweight women is higher and the number of oocytes obtained is lower [4].

Leptin, known as the satiety factor, establishes an important link between obesity and fertility. Body fat content and serum leptin levels are directly proportional leading to increased leptin levels in obese and overweight individuals. Leptin affects the reproductive functions centrally by stimulating GnRH and subsequent luteinizing hormone (LH) secretion and regulating the neuropeptides called kisspeptins. Kisspeptins also increase gonadotrophin-releasing hormone (GnRH) release by stimulating the arcuate nucleus of the hypothalamus [5]. A severe leptin resistance has been suggested in overweight and obese individuals leading to defects in leptin actions. Consequently, irregularity and reduction in GnRH release and a subsequent decrease in LH pulse amplitude have been observed in obese women [6] [7].

Generally, follicle-stimulating hormone (FSH) only regimens are administered in controlled ovarian stimulation (COS) protocols for ART cycles as they can achieve adequate follicular development. Although there is a clear need for LH for optimal follicular response and maturation in a natural cycle, the impact of LH addition to FSH during a COS protocol remains controversial. LH addition has been proven to be beneficial in specific populations, especially in women of advanced age, poor responders and women with low LH levels [8]. In obese and overweight patients without polycystic ovary syndrome (PCOS), subclinical deficiency of luteinizing hormone (LH) has been shown in several studies [9]

[10]. However, it is not clear whether recombinant FSH (rFSH) + recombinant LH (rLH) treatment in this group of patients contributes to pregnancy rates.

The aim of this study was to investigate the effect of adding rLH to rFSH on GnRH antagonist cycle outcomes in obese and overweight infertile patients without PCOS.

2. Materials and Methods

Study design and participants

This was a retrospective cohort study conducted in Kocaeli University Faculty of Medicine, Assisted Reproductive Techniques Clinic between January 2016 and March 2019. The study was approved by the ethical committee of Kocaeli University, Kocaeli, Turkey.

Couples with a diagnosis of unexplained infertility, diminished ovarian reserve, tubal factor or male factor were included. Patients who had no fresh embryo transfer, a BMI less than 18 kg/m^2, basal FSH concentrations more than 12 mIU/mL were excluded from the study, as well as patients with PCOS and a visible endometrioma on ultrasound examination. Patients with a BMI between 18.5 and 24.99 kg/m^2 were considered normoweight, a BMI between 25 and 29.99 kg/m^2 were considered overweight and a BMI $\geq$ 30 kg/m^2 were considered obese.

Outcome measures

Primary outcome of the study was ongoing pregnancy rate (OPR). Secondary end points were clinical pregnancy rate (CPR), biochemical pregnancy rate, duration of COS, total dose of gonadotropins used, estradiol (E2) and progesterone levels, endometrial thickness, follicle number greater than 12 mm and 17 mm on hCG day, number of oocytes retrieved, number of metaphase II (MII) oocytes, fertilization rate, number of good quality embryos.

Protocol

A total of 137 patients undergoing COS with GnRH antagonist protocol and fresh embryo transfer between January 2016 and March 2019 were enrolled in this retrospective study. Of 137 patients, 37 had a BMI $\geq$ 25 and received rFSH alone (Group 1), 37 had a BMI $\geq$ 25 and received rFSH + rLH (Group 2), 33 had a BMI between 18.5 - 24.99 and received rFSH alone (Group 3) and 30 had BMI between 18.5 and 24.99 and received rFSH + rLH (Group 4).

All patients received GnRH antagonist protocol for COS. On cycle day 3, vaginal ultrasonography was performed and patients with endometrial thickness < 5 mm and no follicle > 10 mm were started on daily injections of rFSH (Gonal-F®, Merck Serono, Aubonne Switzerland, subcutaneous and Puregon®, Organon, The Netherlands, subcutaneous). Initial rFSH dosage was determined based on age, BMI, anti-Mullerian hormone (AMH) level and previous response. Repeat examination was performed on Day 5 of stimulation then every 1 to 3 days as indicated. Follicular development was monitored by transvaginal ultrasonography, and when the leading follicle diameter reached 12 mm, a daily dose of 250 μg cetrorelix acetate (Cetrotide flakon® 0.25 mg, Baxter Oncology GmbH,

Frankfurt, Germany, subcutaneous) was iniated. In LH group, rLH (Luveris, Serono, Switzerland, subcutaneous) was added on days 5 - 9. In this group, rFSH dose remained constant and 75 U rLH was added. When the dominant follicles reached 17 - 19 mm, 250 μg recombinant hCG (Ovitrelle®, Merck-Serono, Modugno, Italy, subcutaneous) was administered and oocyte collection was performed 34 - 36 hours later. Oocytes were fertilized using either conventional IVF or ICSI. Normal fertilization was defined as zygotes with two pronuclei (2PN). Before transfer, embryos were graded on the basis of morphological condition. Embryos were evaluated on the inverted microscope according to blastomer number and fragmentation rates. Grading was determined according to this evaluation. For transfer, embryos with less than 50% fragments and cleavage were preferred. One or two embryos will be transferred on day 3 or 5, taking into consideration the patient's age, the number of embryos suitable for transfer, and the medical indication. Luteal phase support was started with progesterone as 200 mg vaginal tablet or 90 mg vaginal gel (Progestan, Kocak Farma, Tekirdag, Turkey, vajinal tablet or Crinanoe, M.Y. Healthcare Packaging, Bedford, England, vaginal jel) on the day of oocyte retrieval and was continued until the documentation of fetal heart activity by ultrasound.

Baseline evaluations including serum FSH, LH, estradiol, AMH and antral follicle counts at the beginning of the menstrual cycle (induced or spontaneous) were examined. The type, duration and total dose of gonadotropins used were evaluated. Estradiol level, progesterone level and endometrial thickness, follicle number greater than 12 mm and 17 mm were recorded and compared between groups. The embryology records of the patients were examined and total oocyte count, M2 oocyte percentage, fertilization rate, number of transferred embryos, and quality of transferred embryos were evaluated.

Pregnancy results of all patients were examined and evaluations were made according to the groups. Serum b-hcg level above 20 was considered as chemical pregnancy. Intrauterine gestational sac with fetal heart beat on TV USG after 6 weeks after embryo transfer was accepted as clinical pregnancy. Presence of at least one live fetus at the end of the 12th week following embryo transfer was considered to be ongoing pregnancy.

Statistical analysis

Statistical analysis was performed with IBM SPSS 20.0 (IBM Corp., Armonk, NY, USA). The normal distribution suitability test was evaluated by Kolmogorov-Smirnov Test. Numerical variables with normal distribution were given as mean ± standard deviation, the numerical variables without normal distribution were given as median (25th - 75th percentile), and categorical variables were given as frequency (percentage). Differences between the groups were determined by one-way analysis of variance for numerical variables having normal distribution and by Kruskal-Wallis and Mann Whitney U Test for numerical variables without normal distribution. Tukey and Dunn tests were used for multiple comparisons. The relationships between categorical variables were evaluated by Chi-square analysis. To compare proportion for two samples, Fisher's

exact test was used. For the test of two-way hypotheses, p < 0.05 was considered sufficient for statistical significance.

3. Results

In the study, 137 IVF cycles were analysed. The sociodemographic characteristics of all patients included in the study are shown in Table 1. The basal clinical and laboratory characteristics of the patients were compared in Table 2 according to their BMI. There was no significant difference between basal FSH, basal E2, AMH levels and antral follicle counts in both groups. However, basal LH levels were significantly lower in obese and overweight patients without PCOS (**p = 0.01**).

Groups formed according to BMI and gonadotropin type were compared in Table 3 according to stimulation time, total FSH and LH dose used, ultrasonographic and laboratory findings on HCG day, the embryological results after oocyte collection. The duration of stimulation and the total dose of rFSH used were significantly lower in Group 3 than in Groups 1 and 2. There was no significant difference between Group 2 and Group 4 in terms of total rLH dose. There was no significant difference between the groups in terms of HCG day E2 value, hCG day progesterone value, follicle number greater than 17 mm and 12 mm on HCG day (p > 0.05). And also there was no difference between the groups in terms of total oocyte count, M2 oocyte count, M2 oocyte percentage and fertilization rate.

Table 1. The socio-demographic characteristics of the patients.

PARAMETER	VALUE
Age (years)	33.37 (±4.88)
Infertility period (years)	6.33 (±3.66)
Body mass index (kg/m^2)	26.53 (±5.87)
IVF indication, n (%)	
Unexplained	58 (42.3%)
Low ovarian reserve	33 (24.1%)
Tubal factor	8 (5.8%)
Male factor	21 (15.3%)
Female + Male factor	17 (12.4%)

Variables are shown as mean ± SD or n (%).

Table 2. Comparison of basal clinical and laboratory characteristics of patients according to body mass index.

PARAMETER	BMI ≥ 25 (n = 74)	BMI < 25 (n = 63)	p
Basal FSH, (IU/L)	7.82 (6.54 - 9.01)	8.13 (6.75 - 9.55)	0.344
Basal LH, (mIU/mL)	4.74 (3.25 - 6.04)	5.92 (4.48 - 7.1)	**0.001**
Basal E2, (pg/mL)	40 (31.25 - 53)	40 (32 - 49.5)	0.609
Basal antral follicle count, (n)	8.5 (4.75 - 12)	8 (4 - 10)	0.305
AMH	2 (0.9 - 3.17)	1.91 (0.83 - 3.56)	0.820

Variables are given as median (25 - 75 percentile values). Mann Whitney U test.

Table 3. Comparison of the groups based on body mass indexes and gonadotropin type in terms of stimulation time, total FSH and LH dose used, ultrasonographic and laboratory findings on HCG day, the embryological results after oocyte collection.

	GROUP1 (BMI ≥ 25, rFSH) (n = 37)	GROUP2 (BMI ≥ 25, rFSH + rLH) (n = 37)	GROUP3 (BMI < 25, rFSH) (n = 33)	GROUP4 (BMI < 25, rFSH + rLH) (n: 30)	P
Stimulation duration (days)	9 (8 - 10)[a]	9 (8 - 11)[b]	8 (7 - 8)[ab]	8.5 (7.75 - 10)	**<0.001**
Dose of rFSH used (IU)	2325[c] (1837.5 - 2775)	2400[d] (2062.5 - 2925)	1800[cd] (1300 - 2100)	2062.5 (1781.25 - 2587.5)	**<0.001**
Dose of rLH used (IU)		375 (225 - 600)		375 (281.25 - 543.75)	1.0
E2 value on HCG day (pg/ml)	1033 (722 - 1485)	754 (525 - 1180.5)	1062 (597.5 - 1446.5)	976.5 (690.7 - 1967.2)	0.130
progesterone value on HCG day (pg/ml)	0.87 (0.65 - 1.1)	0.69 (0.39 - 0.95)	0.89 (0.54 - 1.14)	0.73 (0.58 - 1.15)	0.86
≥12 mm follicle on HCG day (n)	8 (5 - 10.5)	6 (4 - 7.5)	7 (4.5 - 8.5)	6 (3.75 - 9)	0.212
≥17 mm follicle on HCG day (n)	3 (2 - 3.5)	3 (2 - 3.5)	2 (1 - 3)	2.5 (1 - 3.25)	0.409
Number of oocytes retrieved (n)	8 (5 - 10)	6 (4 - 8)	7 (3.5 - 8.5)	6 (3 - 10.25)	0.065
Number of M2 oocytes (n)	5 (3.5 - 8.5)	4 (3 - 7)	5 (2 - 6.5)	4 (2.75 - 6.25)	0.403
M2 oocyte (%)	78 (58 - 93)	80 (66 - 100)	75 (52 - 94)	78 (63 - 92)	0.429
Fertilization rate (%)	75 (52 - 93)	71 (52 - 100)	66 (5 - 100)	75 (5 - 100)	0.710

Variables are given as median (25 - 75 percentile values) and mean ± SD. ** One Way Analysis of Variance. a: There is a significant difference between Group 1 and Group 3 (p = 0.012); b: There is a significant difference between Group 2 and Group 3 (p < 0.001); c: There is a significant difference between Group 1 and Group 3 (p = 0.004); d: There is a significant difference between Group 2 and Group 3 (p < 0.001).

A total of 195 embryos were transferred to 137 patients. Table 4 shows the distribution of embryos transferred to patients by groups and there was no significant difference between the groups in terms of the number of transferred embryos (p = 0.66). In addition, the comparison of the groups formed based on gonadotropin type and body mass indexes in terms of embryo quality is shown in Table 5. According to Table 5, a statistically significant difference was found between the groups with and without rLH in terms of embryo quality between obese and overweight patients. The number of Grade1 embryos in Group 2 (40 embryos (70.2%)), the group with rLH added and BMI ≥ 25; was observed more than Group 1 (27 embryos (49.1%)), the group without rLH and BMI ≥ 25 (**p = 0.34**). However, there was no significant difference in embryo quality between Group 3 and Group 4, *i.e.* with and without rLH from normal weight patients.

The groups based on gonadotropin type and body mass indexes were compared according to pregnancy rates after embryo transfer and shown in the **Table 6**. There was no significant difference between the groups in terms of chemical pregnancy and clinical pregnancy rates. Ongoing pregnancy rates were significantly higher in Group 2 than in Group 1 (**p = 0.044**), but there was no significant difference between Group 3 and Group 4 (p = 0.588).

Table 4. Distribution of the number of embryos transferred to patients by groups.

	GROUP 1 (BMI ≥ 25, rFSH) (n = 37)	GROUP 2 (BMI ≥ 25, rFSH + rLH) (n = 37)	GROUP 3 (BMI < 25, rFSH) (n = 33)	GROUP 4 (BMI < 25, rFSH + rLH) (n = 30)	Total	p
Single embryo transfer	19 (34.5%)	17 (29.8%)	25 (60.9%)	18 (43.9%)	79	
Double embryo transfer	18 (32.7%)	20 (35%)	8 (19.5%)	12 (28.6%)	58	0.66
Total transferred embryo	55	57	41	42	195	

Chi-square test.

Table 5. Comparison of the groups based on body mass indexes and gonadotropin type in terms of embryo quality.

	GROUP 1 (BMI ≥ 25, rFSH)	GROUP 2 (BMI ≥ 25, rFSH + rLH)	p (Group 1-2)	GROUP 3 (BMI < 25, rFSH)	GROUP 4 (BMI < 25, rFSH + rLH)	p (Group 3-4)
Grade 1	27 (49.1%)	40 (70.2%)	**0.034**	22 (53.7%)	21 (50%)	0.827
Grade 2	28 (50.1%)	13 (22.8%)	**0.002**	14 (34.1%)	15 (35.7%)	1
Grade 3	0	4 (7%)	0.119	5 (12.1%)	6 (14.2%)	1
Total transferred embryo	55	57		41	42	

Fisher's exact test.

Table 6. Comparison of the groups formed based on body mass indexes and gonadotropin type in terms of pregnancy rates after embryo transfer.

	GROUP 1 (BMI ≥ 25, rFSH) (n = 37)	GROUP 2 (BMI ≥ 25, rFSH + rLH) (n = 37)	p value (Group 1-2)	GROUP 3 (BMI < 25, rFSH) (n = 33)	GROUP 4 (BMI < 25, rFSH + rLH) (n = 30)	p value (Group 3-4)
Chemical pregnancy n (%)	15 (40.5%)	18 (48.6%)	0.640	12 (36.3%)	17(56.7%)	0.173
Clinical pregnancy n (%)	12 (32.4%)	17 (45.9%)	0.341	11(33.3%)	13 (43.3%)	0.578
Ongoing pregnancy n (%)	7 (18.9%)	16 (43.2%)	**0.044**	10 (30.3%)	12 (40%)	0.588

Variables are given as n (%). Chi-square test.

4. Discussion

In this retrospective study, we compared the effects of rLH addition to rFSH for COS in GnRH antagonist protocol in normoweight and obese patients. Our results showed that endogenous LH levels were lower in obese and overweight women. Few data are available in regard to the effect of gonadotropin type on ART outcomes in obese women. This study demonstrated that rLH supplementation did not provide any benefit on ART outcomes in normoweight patients. However rLH addition in GnRH antagonist cycles lead to better embryo quality and higher OPR in obese and overweight patients without PCOS.

Management of overweight and obese patients in ART is a challenging situation. Higher total gonadotropin dose and longer duration of stimulation are required in overweight and obese women [3] [4]. In addition, ART cycles in obese patients result in lower implantation and pregnancy rates compared to normoweight patients [11]. New approaches are needed in obese to improve ART outcomes.

A study conducted in patients with poor ovarian response has shown that basal LH levels were significantly lower in obese compared to normoweight. However, no significant difference was observed in basal FSH, AMH, E2 levels and AFC [9]. Another study examining hormonal status in obese gynecologically normal women found E2 and LH levels lower in obese group [12]. In line with these studies, we found LH levels lower in obese and overweight non PCOS patients than in normoweight patients. However, there was no significant difference in FSH, AMH, E2 levels and AFC. Similarly, De Pergola et al. found decreased LH levels and similar AFC in obese women and suggested a possible direct inhibitory effect of body mass on gonadotropin production and no effect of body mass on AFC [13]. Further studies suggested central leptin resistance as a potential mechanism for decreased LH levels and hypogonadotropic state in obese and overweight individuals [6] [7].

Evidence from IVF cycles in women with hypogonadotropic hypogonadism has clearly shown a direct primary role of LH in development and complete maturation of the follicles [14]. The effects of LH on folliculogenesis vary according to follicular developmental stages. During follicular phase, LH acts on production of androgens by affecting the theca cells that carry LH receptors. Androgens produced serve as a substrate for aromatization to estrogens and estrogens induce a series of events which are critical for follicle development [15]. Therefore LH plays a role in optimal follicular response and patients with low endogenous LH levels as in hypogonadotropic hypogonadism may benefit from rLH supplementation [16] [17].

A beneficial effect of rLH supplementation to rFSH for ovarian stimulation is controversial. A meta-analysis demonstrated that the addition of rLH to rFSH in ART cycles did not affect live birth rate and OHSS risk, but there was a moderate increase in OPR in the group that received rLH [18]. The meta-analysis showed no effect of rLH addition in women of advanced age. However there is also evi-

dence showing a potential benefit of rLH supplementation in specific populations including patients with advanced age (≥35 years), hypo-response to rFSH, and patients with poor ovarian reserve [8]. On the other hand, rLH addition to rFSH for ovarian stimulation in obese non-PCOS patients who have relatively low LH levels has not been studied previously.

In agreement with previous studies, we found higher total rFSH consumption and longer duration of stimulation in obese and overweight patients and rLH addition did not seem to have a positive effect on rFSH dose and stimulation duration in obese women [3] [4].

Several studies investigated the effect of rLH addition to rFSH on ovarian stimulation outcomes. In a randomized controlled study of 244 women with poor ovarian reserve, the addition of rLH to rFSH for ovarian stimulation did not result in a significant difference in number of oocytes retrieved, embryos and fertilization rate as well as embryo quality [19]. However a meta-analysis found a significant benefit on the number of oocytes retrieved in r-FSH + r-LH group compared to r-FSH alone in poor responders. In addition a number of good quality embryos were significantly higher in rLH added group both in poor and normal responders [20]. Another randomized controlled trial investigated rLH addition in GnRH antagonist cycles and found similar number of oocytes and fertilization rates in both rLH added and rFSH only groups however there were more top quality embryos in rLH added group [21]. Taken together, these data suggest that rLH supplementation may improve ovarian stimulation outcomes in certain groups. Our analysis showed that number of oocytes retrieved, number of MII oocytes and fertilization rates were similar in rLH added and rFSH only groups both in obese and normoweight patients. However number of grade 1 embryos was significantly higher in rLH added obese group although rLH addition did not have any effect on embryo quality in normoweight patients. These findings indicate that rLH addition in GnRH antagonist cycles does not improve ovarian stimulation outcomes in normoweight patients; on the other hand may improve embryo quality in obese non-PCOS patients. A possible explanation for improved embryo quality in rLH added obese patients is that rLH supplementation may restore optimal follicular environment in obese patients with relatively low endogenous LH levels and therefore lead to better oocyte and embryo quality.

For normoweight women, the results of the study did not show any improvement in clinical and ongoing pregnancy rates when rLH was added to rFSH for ovarian stimulation in ART cycles. For overweight and obese women, addition of rLH to rFSH for COS did not improve CPR however OPR were significantly higher with the addition of rLH compared to rFSH administration alone. In addition, extensive researches have shown that obesity is associated with unfavorable ART outcomes including lower pregnancy rates and higher preclinical and clinical miscarriage rates [22]. However, our results have shown that comparable OPR with normoweight patients were achieved in obese patients with the addition of rLH. A possible factor that might be contributing to better OPR in rLH

added obese group is the better embryo quality obtained in this group.

The strength of our study is that it is the first study investigating the effect of rLH addition to rFSH on ART outcomes in obese and overweight non-PCOS patients.

Limitations of our study were its retrospective nature and relatively small sample size consisting of 137 cases. Another limitation was that our primary end point was OPR as it would be preferable to use live birth rate. Randomised controlled trials are needed to further evaluate the role of rLH addition in GnRH antagonist cycles in obese women.

5. Conclusion

In conclusion, this study has shown that obese and overweight non-PCOS patients have lower endogenous LH levels. It has also shown that rLH supplementation in GnRH antagonist cycles in obese and overweight women improves embryo quality and ongoing pregnancy rates. However rLH addition to rFSH doesn't seem to have a value in normoweight patients.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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