

Impact of COVID-19 Pandemic on Clinical and Embryological Outcomes of Assisted Reproductive Techniques

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ABSTRACT

Background: The emergence of the COVID pandemic unfolded a series of precautions and dilemmas and the complete suspension of health services. With the gradual emergence of data showing near minimal effects of the virus on pregnancy, Assisted Reproductive Techniques (ART) services were gradually resumed following guidelines and advisories. **Aim:** The purpose of this study was to detect the COVID positivity rate in women undergoing ART treatment during the COVID pandemic and compare clinical and embryological outcomes to the ART cycles performed in the pre-COVID era. **Study Setting and Design:** This was a retrospective cohort study of all women undergoing controlled ovarian stimulation, followed by a fresh or frozen embryo transfer (ET) between 1st October 2019 and 31st March 2020 (control group) and between 1st April 2020 and 31st September 2020 (study group) at Nova IVF Fertility Clinic, Ahmedabad. **Material and Methods:** The study group underwent ART during the first wave of COVID-19 pandemic in India and when gradual unlocking of facilities including ART was advised as per the national ART advisory by the ICMR in December 2020. The outcomes were compared with the control group (cycles in pre-covid time). **Statistical Analysis:** Statistical analysis was performed in SPSS (v25.0) and included Mann-Whitney U, Fisher's exact and Pearson Chi-square as appropriate. Values of $P < 0.05$ were considered statistically significant. **Results:** A total of 367 *in vitro* fertilisation (IVF) stimulations were initiated. A total of 342 retrievals and 606 ETs (171 fresh and 435 frozen) were completed during the study period with a COVID positivity rate of 6.8% (25/367) amongst fresh and 3.9% (18/453) amongst frozen ETs, respectively; the PR and IR in the study group was similar to the control group (47.6 vs. 55.1 $P = 0.4$ and 68.7 vs. 66.4; $P = 0.52$, respectively). The maternal complication rates were similar in both groups with a COVID positivity rate of 10.2% (23/225) and 1 maternal death in the study group. The live birth rates were similar. **Conclusions:** We did not find a noteworthy difference in the clinical and embryological outcomes in the IVF cycles conducted in the COVID era as compared to the pre-COVID time. Thus, with adequate precautions and safety measures, ART services conducted during the COVID pandemic have comparable birth outcomes and can be safely advocated.

KEYWORDS: Assisted reproductive techniques, COVID 19, *in vitro* fertilisation, lockdown, pandemic, pregnancy

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INTRODUCTION

The 2019 novel coronavirus (2019-nCoV) or COVID-19 is the third coronavirus that has emerged among the human population in the last two decades, after Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS).^[1] On 31st December 2019, cases of pneumonia of unknown aetiology detected in Wuhan, China, were reported to the World Health Organisation (WHO) China Country Office.^[2] The causative agent was identified as a coronavirus and was named 2019-nCoV or 2019-novel coronavirus.^[3] It was declared a public health emergency of international concern by the WHO.^[4] On 11th March 2020, the WHO declared COVID-19 as a pandemic.^[5]

The first case of COVID-19 in India was reported on 30th January and another two cases were reported on 2nd and 3rd February 2020.^[6,7] The first death due to COVID-19 in the country was reported on 12th March 2020.^[7] Initial demographic studies based on crowdsourced databases showed that while around two-thirds of the infected cases were in the age group between 21 and 50 years, majority of deaths were reported among people above 50 years of age.^[7] In the first 2000 deaths reported from India, more than 96% of patients had comorbidities, and 71% had multiple comorbidities.^[8] There was a nationwide lockdown implemented by the government in phases to curb the spread of the virus (Phase 1: March 25–April 14, Phase 2 (April 15–May 3, Phase 3 (May 4–May 17 and Phase 4 (May 18–May 31, 2020).^[9]

Based on previous literature on maternal complications in MERS and SARS, some authors had warned against possible adverse clinical outcomes including life-threatening maternal disease in pregnant women affected with COVID-19, in as early as February 2020.^[10] The same has also been reported in recent studies assessing the effect of COVID-19 on maternal outcomes.^[11] However, its exact effects on Assisted Reproductive Techniques (ART) outcomes are not yet known. Major concerns reported in the literature regarding ART treatment, other than the risk of spreading the infection to the patients and staff, included its impact on fertility, impact on success rates of infertility treatments, its potential teratogenic effect and handling of gametes in the clinical laboratories.^[12] As a result, the suspension of new *in vitro* fertilisation (IVF) programmes was recommended by many professional bodies,^[13] and the IVF facilities remained closed for some time during the pandemic. A joint statement was issued by the Indian Society of Assisted Reproduction (ISAR), Indian Fertility Society (IFS) and Academy of Clinical Embryologists (ACE) in India in March 2020 on

resumption/opening up ART services, in which it was observed that while information on effects of COVID-19 infection on fertility treatment and early pregnancy was limited, there was no evidence that infection increased the risk of or miscarriage or foetal malformations.^[14]

There are limited data on the possible effects of COVID-19 on ART outcomes, and the limited number of studies published till date have failed to find any significant influence of COVID-19 on ART outcomes. A study from Italy compared the ART outcomes from a COVID-19 risk period (866 fresh cycles, 628 embryo cycles and 26 oocyte warming cycles) with those from a non-COVID-19 risk period (883 fresh, 538 embryo cycles and 37 oocyte warming cycles), and did not find any significant difference in the rates of clinical pregnancy, biochemical pregnancy, ectopic pregnancy and miscarriage.^[15] Another study from Wuhan also did not find any significant effect of mild SARS-CoV-2 infection in females on ovarian reserves and responses as well as on laboratory parameters and clinical outcomes in ART treatments.^[16] Clinical data on the potential effects, if any, of COVID-19 on ART outcomes are required for both policy decisions as well as for prognostication of patients. Keeping this in view, the present study was conducted to compare the IVF outcomes in the COVID era with pre-COVID time. The purpose of this study was to compare the COVID positivity rate in women undergoing ART treatment during the COVID pandemic and comparison of clinical and embryological outcomes to ART cycles performed in the pre-COVID era.

MATERIALS AND METHODS

Design

This is a retrospective cohort study of all patients seeking treatment using Assisted Reproductive Technology at Nova IVF Fertility, Ahmedabad, a single tertiary fertility centre in Gujarat, India, India during the COVID-19 pandemic in the unlocking phase (April 2020 to September 2020). The control population included women undergoing similar ART treatment in pre-COVID time (1st October 2019, to 31st March 2020). The study has been registered in CTRI as CTRI/2021/11/037862. The study adhered to the principles of Helsinki Declaration (2013), is approved by the institutional Ethics committee. All participants have provided consent for use of anonymised data for research or educational purposes.

Subjects

All women undergoing controlled ovarian stimulation (COS), followed by a fresh or frozen embryo transfer (ET) performed between 1st October 2019 and 31st March 2020 (pre-COVID or control group) and

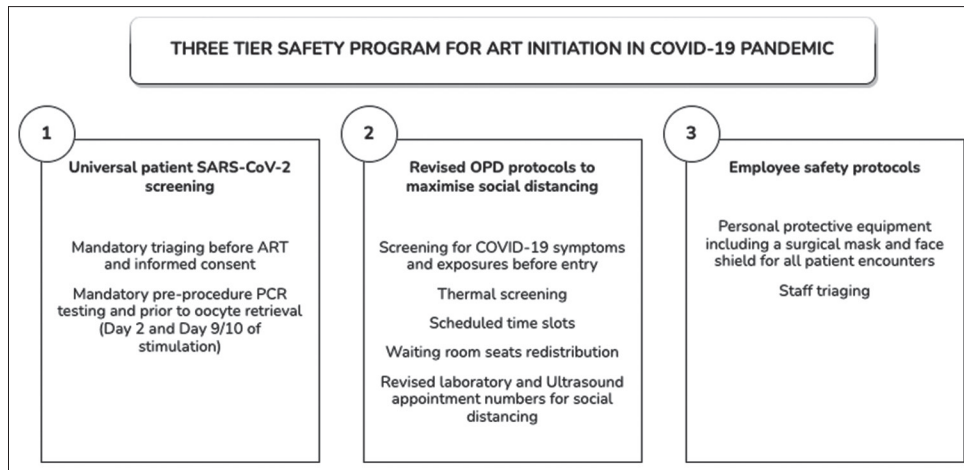


Figure 1: Three tier safety programme for ART initiation in COVID pandemic

between 1st April 2020 and 31st September 2020 (study or COVID group) were eligible for inclusion. The study group underwent ART during the first wave of COVID-19 pandemic in India and when gradual unlocking of facilities including ART was advised. We followed the national ART advisory regarding the resumption of ART services published by the ICMR in December 2020.^[17]

COVID precautions

Our centre followed a three-tier safety program after the careful resumption of patient services in the ongoing COVID-19 pandemic: (1) universal patient COVID-19 screening including mandatory pre-procedure PCR testing; (2) revised outpatient protocols to ensure social distancing and (3) employee safety protocols [Figure 1]. All patients and staff were triaged according to the ART risk assessment questionnaire issued by the Indian Council of Medical Research (ICMR).^[17]

All patients were offered testing at any time during their cycle if symptomatic and those testing positive were cancelled and referred for appropriate treatment. Patients with a prior positive COVID-19 RT-PCR test did not require repeat testing if the positive test was under 90 days and the patient remained asymptomatic and was advised to delay treatment for a total of 3 months. Treatment was initiated if CBC and CRP reports were normal.

Patients undergoing ET during the COVID pandemic were further stratified according to the type of transfer (fresh or frozen embryos). Patients with a positive pregnancy result had confirmation of an intrauterine pregnancy (presence of at least one gestational sac) and were followed up for pregnancy outcome till live birth. Obstetric care was provided at our clinic till the first trimester and thereafter at a maternity unit of patient's choice and regular follow-up

regarding maternal and foetal well-being was obtained telephonically. The control population comprised all women undergoing ART in the pre-COVID era (October 19–March 20) under the same ART treatment subtypes. Controls were identified from the electronic medical records during a 6 month period and were referred to as the 'pre-COVID-19 Fresh and Frozen ET' group.

Outcomes

The primary objective of this study was to evaluate both the prevalence of COVID-19 in patients undergoing IVF-ICSI and the impact of the COVID pandemic on clinical and embryological outcomes. Safety was measured using SARS-CoV-2 PCR positivity during ART cycles, COVID-related cycle cancellation rates and adverse materno-foetal outcomes during pregnancy.

Clinical and embryological outcomes were compared amongst the two groups of patients undergoing ART. Clinical outcomes included pregnancy rate, clinical and biochemical abortion rate and live birth rate. Secondary clinical outcomes included the comparison of prematurity rate, perinatal morbidity and maternal morbidity and mortality rate. Embryological outcomes studied were fertilisation rate, blastulation rate and implantation rate. Maternal morbidity and foetal morbidity data included the comparison of maternal deaths, prematurity rate and perinatal mortality rates amongst the two groups.

Sample size and statistical analysis

The study included all women fulfilling the inclusion criteria and undergoing COS followed by an ET during the first wave of between 1st April 2020 and 31st September 2020 (study or COVID group). The control or pre-COVID group identified a similar group between 1st Oct 2019 and 31st March 2020.

Statistical analysis was performed in SPSS (v25.0, IBM Corp. Released 2017, Armonk, NY) and included Mann–

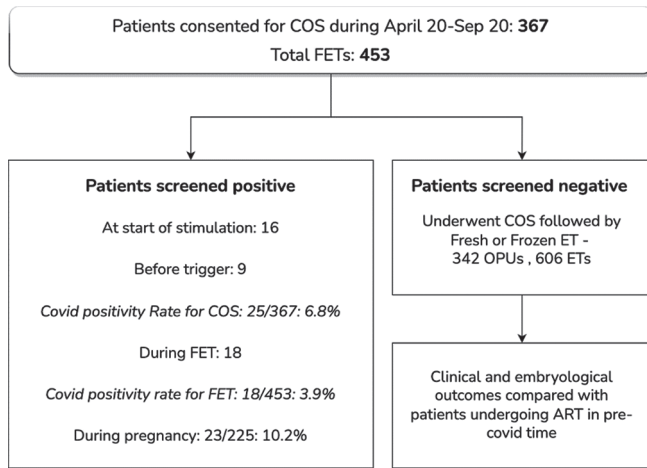


Figure 2: Case selection for the study

Whitney U, Fisher's exact and Pearson's Chi-square tests as appropriate. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Of the 367 IVF cycles initiated during the study period (between 1st April 2020 and 30th September 2020), 25 were cancelled due to COVID 19. 342 oocyte retrievals were completed with 171 fresh and 435 frozen ETs, resulting in 225 pregnancies. A total of 18 frozen ET cycles were cancelled due to COVID and 23 women were diagnosed to have COVID during ongoing pregnancy. The COVID positivity rate for IVF-ICSI cycles was 6.8% (25/367), 3.9% (18/453) for frozen ET and 10.2% in pregnancy (23/225) [Figure 2]. Majority of the COVID-affected women were symptomatic (35/45; 77.8%). The patients in both study and control groups had a similar demographic profile and the details are shown in Table 1:

A total of 342 oocyte retrieval procedures were performed and the embryology data were compared with the control group [Table 2]. The cycles cancelled due to COVID-19 were eliminated from statistical analysis for studying the outcomes. It was found that despite having a significantly higher fertilisation rate in the study group, the blastulation rate was similar among both groups [Table 2].

A total of 171 fresh and 435 frozen ETs were performed in the study group and 352 and 654, respectively, in control group. In the women who had a fresh transfer, IR and PR (31% and 47.6%, respectively) and abortion rates were similar to the control group. In women of the study group undergoing a frozen ET, despite having a significantly higher IR (53.3% vs. 42.8%; $P = 0.0007$), the PR was similar among both groups. The clinical abortion rate was significantly higher in the study

Table 1: Demographic details of the patients in both groups

	Study group (n=606)	Control group (n=1006)	P
Mean age (years)	30.7	31	0.8
BMI (kg/m ²)	26.5	26.2	0.3
Infertility (%)			
Primary	54.4	51.9	
Secondary	45.6	48.1	
Average embryos per ET	1.6	1.7	0.2

ET=Embryo transfer, BMI=Body mass index

Table 2: Comparison on embryology data after OPU among both groups

	Study group	Control group	P
Number of oocyte retrievals	342	526	
Fertilisation rate (%)	79.2	77.1	0.015
Blastocysts formed	1172	1954	
Blastulation rate (%)	41.7	41.4	0.798

OPU=Ovum Pick Up

group as compared to women undergoing thaw ET in the pre-COVID era (26.1 vs. 19.5; $P = 0.001$). The live birth rates were also found to be similar between both groups for fresh and frozen transfers [Table 3].

Common antenatal complications seen were gestational diabetes and pregnancy-induced hypertension (PIH). The incidence of PIH was significantly more in the control group in a frozen transfer cycle. The incidence of other complications was found to be similar [Table 4].

A total of 23 women contracted COVID-19 infection in the antenatal period (9 following fresh, 14 following frozen transfer with a positivity rate of 10.2% (23/225). A total of 20 women had mild infections and were managed conservatively, 3 women needed intensive care unit (ICU) admission. There was 1 maternal death (following a frozen transfer) after the patient succumbed to COVID and delivered a preterm stillborn child. None of the other children had COVID infection after birth.

DISCUSSION

The WHO declared COVID-19 a global pandemic on March 11, 2020, and its dramatic spread gradually unfolded its novel implications on multiple systems. Global and national lockdown of all services were announced causing disruption of the health-care system. All the elective health services were put on hold in view of the unknown effects of the virus. Fertility services were also not left untouched and whether to initiate ART services with restrictions due to the possible unknown effects of the virus on gametes and pregnancy was a

Table 3: Comparison of *in vitro* fertilisation outcomes in women undergoing a fresh and frozen embryo transfer among both groups

	Fresh ET			Frozen ET		
	Study group	Control group	P	Study group	Control group	P
Total ET	171	352		435	654	
Total sacs	53	97		232	280	
IR (%)	31.0	27.6	0.420	53.3	42.8	0.0007
PR (%)	47.6	55.1	0.210	68.7	66.4	0.525
BCM (%)	10	7.89	0.657	6.73	7.22	0.838
CAR (%)	24.4	12.4	0.067	26.1	19.5	<0.001
MPR (%)	20.0	25.7	0.45	29.4	35.4	0.189
LBR (%)	32.4	40.6	0.345	46.3	47.2	0.852
Pre-maturity rate (<37 weeks) (%)	47.00	48.80	0.859	42.30	43.10	0.035
IUD/still birth	2	2		3	4	
Neonatal deaths	1	1		4	5	

IR=Implantation rate, PR=Pregnancy rate, BCM=Biochemical miscarriage rate, CAR=Clinical abortion rate, MPR=Multiple pregnancy rate, LBR=Live birth rate, IUD=Intrauterine death, ET=Embryo transfer

Table 4: Antenatal complications in the study and control group*

	Fresh ET			Frozen ET		
	Study group	Control group	P	Study group	Control group	P
PIH (n)	1.2% (2)	4.1% (12)	0.068	2.4% (4)	9.5% (28)	0.003
IUGR (n)	0.6% (1)	1.4% (4)	1	2.4% (4)	1.4% (4)	0.375
Oligo (n)	1.2% (2)	2% (6)	0.419	0.6% (1)	1% (3)	1
GDM (n)	0.6% (1)	2.7% (8)	0.169	0.6% (1)	3.4% (10)	0.169
PROM (n)	1.8% (3)	1.7% (5)	1	1.8% (3)	0.7% (2)	0.375
PPH (n)	0.6% (1)	0.7% (2)	1	0.6% (1)	0% (0)	0.085
APH (n)	0% (0)	0.3% (1)	0	0.6% (1)	0.3% (1)	0.085
CPD (n)	0% (0)	0% (0)	0	1.8% (3)	1.4% (4)	0.375
Mortality	0%(0)	0%(0)		0.7%(1)	0%(0)	

*Calculated from ongoing pregnancies: Study group: 194, Control 296. IUGR=Intra uterine growth restriction, Oligo=Oligohydramnios, PROM=Pre-mature rupture of membranes, PPH=Post-partum haemorrhage, APH=Antepartum haemorrhage, CPD=Cephalopelvic disproportion, PIH=Pregnancy-induced hypertension, GDM=Gestational diabetes, ET=Embryo transfer

dilemma. Provision of safe ART services during the pandemic, to avoid the possible vertical transmission of infection through gametes and preimplantation embryos was a challenge.

Some evidence extrapolated from previous SARS infections on gametes and embryos triggered genuine concerns, but specific data from COVID affected women and men started coming up mitigating the fear. Evidence-based information regarding pregnancy outcomes, including miscarriage and live birth rates, in women affected with COVID-19, is a useful tool for creating guidelines and counselling women for fertility services, but this was initially unavailable for ART pregnancies due to the stoppage of services. It was assumed that the virus affected various organs through the ACE-2 receptors. Although the ACE-2 receptors are present in the testes, ovaries, uterus and vagina, studies did not find the presence of this COVID-19 virus in gametes.^[18] Reports suggested that the virus was not detected in the seminal fluid of

recovered men.^[19,20] Studies from Demirel *et al.* and Barragan *et al.* failed to demonstrate the presence of COVID viral RNA in the follicular fluid of infected women.^[21,22]

Evidence on the near minimal effect on eggs and sperms led to re-initiation of suspended ART services by following due care and precautions. Global fertility societies laid down consensus guidelines regarding controlled and limited initiation of ART services American Society of Reproductive Medicine, European Society of Human Reproduction and Embryology (ASRM, ESHRE).^[23] Indian guidelines were formulated and released jointly by the ISAR-IFS-ACE and then by ICMR in December 2021.^[14,17]

This is probably the first Indian study to evaluate the COVID positivity rate in ART cycles and compare the outcomes of ART cycles performed during the COVID pandemic with those performed in pre-COVID times. This study also evaluates both clinical and embryological

outcomes of ART cycles conducted in the unlocking phase right up to live birth. An Indian study by Jirge *et al.* provides practical insights for the resumption of ART services in India and found satisfactory Key Performance Indicators (KPIs) for cycles conducted in the COVID era; however, clinical indicators were not compared.^[24]

We followed the stepwise protocol of initiating ART services in the national unlocking phase of the global pandemic strictly following the guidelines and all necessary precautions in the clinic. We noted a COVID positivity rate of 6.8% amongst patients undergoing COS in the unlocking period. The majority of these (64%; 16/25) were detected at the start of stimulation, others before trigger (36%). COVID positivity was 3.9% (18/453) in those undergoing FET. The COVID positivity rate during pregnancy was 10% (23/225); majority (20/23) had the mild disease; 3 had severe infections needing ICU care and there was 1 maternal death.

Initial studies by Setti *et al.* compared the early pregnancy outcomes following ART in Lombardy County and compared it with pre-COVID time. They noted that clinical pregnancies per cycle (25.38% vs. 27.30%; $P = 0.237$), biochemical pregnancy rates (3.57% vs. 2.50%; $P = 0.089$), ectopic pregnancies, spontaneous miscarriages (22.70% vs. 24.82%; $P = 0.487$) and the intrauterine ongoing pregnancies (76.22% vs. 74.46%; $P = 0.569$) were similar. A multivariate analysis showed no differences in spontaneous miscarriage rate between the two timeframes suggesting no differences in outcomes of ART between the pre-COVID and COVID-19 periods.^[15] Our analysis also showed similar embryological and pregnancy outcomes, except showing higher rates of clinical miscarriages in frozen cycles.

A study from Italy compared the effect of the early phase of COVID-19 on natural and ART-mediated birth rates with the pre-COVID time. They noted a similar contribution of ART births (4.4% in 2019 and 4.5% in 2020), but a 16.7% reduction in overall birth rate, of which 76% was related to natural and 25% was related to ART conceptions. They suggested that such findings should be viewed in the context of stress-related outcomes triggered by this pandemic.^[25] We noted similar live birth rates in ART cycles compared to those conducted in the pre-COVID times.

Recent data have reported an increase in the incidence of adverse pregnancy events in COVID-positive women.^[26] In our study, we found similar incidences of antenatal complications in women during and before the pandemic, except a significant reduction in the incidence of PIH

during the pandemic. This, though seems odd, might be explained by the fact that during the pandemic, there was stringent screening before the start of IVF and only low-risk women without any comorbidities were offered treatment. Data from 78 centres from the Spanish registry documented the perinatal outcomes of ART pregnancies in COVID-infected women. They noted a higher incidence of preeclampsia and ICU admission in these women compared to naturally conceived women with other maternal and neonatal outcomes being similar.^[27] Recently, the ESHRE working group collected data from 80 cases from 32 countries from May 20 to June 21 and noted that medically assisted pregnancies are not differentially affected by COVID-19 infection compared to spontaneous pregnancies and that performing ART with due precautions and triaging can be advocated.^[18]

An Indian study published on materno-foetal outcomes in IVF pregnancies noted no maternal mortality and a neonatal mortality rate of 21/1000 births in IVF cycles using self-oocytes.^[28] We observed one maternal death (COVID related) and five neonatal deaths in the COVID period (three due to prematurity and two due to meconium ileus in twins with suspected cystic fibrosis; all in COVID unaffected mothers); neonatal mortality rate of 30/1000 (similar to the above study; $P = 0.5$). The numbers are low to arrive at definite conclusions regarding the possible unknown effect of the virus. Thus, with adequate precautions and safety measures, ART services conducted during the COVID pandemic have comparable birth outcomes and can be safely advocated.

Data regarding the embryology and pregnancy outcomes for IVF cycles initiated during the COVID-19 pandemic are scarce in India. Although our findings are preliminary, further studies including a wider database from multiple centres across the country and accounting for births in 2021 (second wave) and 2022 (milder third wave) will give us more information on the IVF outcomes. Such data will also help to study the effects of the early phase of the pandemic with the later waves on pregnancy outcomes. Furthermore, future studies on the pregnancy outcomes in COVID-recovered women undergoing ART will help determine long-term effect of the virus on fertility.

COVID-19 will likely be a part of our lives for the near future and accepting to live with it and performing ART with necessary modifications and measures for patient safety will be future norms. Fertility treatment is an urgent, essential service that can be performed safely and responsibly during this pandemic without untoward effect on the pregnancy outcome with the emerging safety evidence if all necessary precautions, screening measures and safety protocols are maintained.

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Data availability

Data are available on request (drparul20arora@gmail.com).

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Conflicts of interest

Dr. Manish Banker is currently an associate editor of the Journal of Human Reproductive Sciences. He has not participated in the blinded external review or the editorial review process.

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