BMJ Open

Effects of air pollution on clinical pregnancy rates after in vitro fertilisation (IVF): a retrospective cohort study

Marie Tartaglia ,[1,2] Lucie Chansel-Debordeaux,[3] Virginie Rondeau,[2] Agnès Hulin,[4] Alexandre Levy,[5] Clément Jimenez,[3] Patrick Bourquin,[4] Fleur Delva ,[1,2] Aline Papaxanthos-Roche[3]

To cite: Tartaglia M, Chansel-Debordeaux L, Rondeau V, *et al*. Effects of air pollution on clinical pregnancy rates after in vitro fertilisation (IVF): a retrospective cohort study. *BMJ Open* 2022;**12**:e062280. doi:10.1136/bmjopen-2022-062280

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2022-062280).

Received 23 February 2022
Accepted 04 November 2022

[1]Environmental Health Platform Dedicated to Reproduction, ARTEMIS Center, CHU Bordeaux GH Pellegrin, Bordeaux, France
[2]Bordeaux Population Health Research Center, Inserm UMR1219-EPICENE, University of Bordeaux, Bordeaux, France
[3]Department of Reproductive Medicine, CHU Bordeaux GH Pellegrin, Bordeaux, France
[4]Partnerships and Innovation Department, ATMO Nouvelle Aquitaine, Bordeaux, France
[5]Eurofins, Jean Villar Polyclinic, Bruges, France

Correspondence to
Marie Tartaglia;
marie.tartaglia@u-bordeaux.fr

ABSTRACT

Objective To evaluate the effect of air pollution, from oocyte retrieval to embryo transfer, on the results of in vitro fertilisation (IVF) in terms of clinical pregnancy rates, at two fertility centres, from 2013 to 2019.

Design Exploratory retrospective cohort study.

Setting This retrospective cohort study was performed in the Reproductive Biology Department of Bordeaux University Hospital localised in Bordeaux, France and the Jean Villar Fertility Center localised in Bruges, France.

Participants This study included 10 763 IVF attempts occurring between January 2013 and December 2019, 2194 of which resulted in a clinical pregnancy.

Primary and secondary outcome measures The outcome of the IVF attempt was recorded as the presence or absence of a clinical pregnancy; exposure to air pollution was assessed by calculating the cumulative exposure of suspended particulate matter, fine particulate matter, black carbon, nitrogen dioxide and ozone (O_3), over the period from oocyte retrieval to embryo transfer, together with secondary exposure due to the presence of the biomass boiler room, which was installed in 2016, close to the Bordeaux University Hospital laboratory. The association between air pollution and IVF outcome was evaluated by a random-effects logistic regression analysis.

Results We found negative associations between cumulative O_3 exposure and clinical pregnancy rate (OR=0.92, 95% CI = (0.86 to 0.98)), and between biomass boiler room exposure and clinical pregnancy rate (OR=0.75, 95% CI = (0.61 to 0.91)), after adjustment for potential confounders.

Conclusion Air pollution could have a negative effect on assisted reproductive technology results and therefore precautions should be taken to minimise the impact of outdoor air on embryo culture.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This cohort study contains a vast amount of data with few missing observations about all in vitro fertilisation (IVF) or intracytoplasmic sperm injection attempts performed between 1 January 2013 and 31 December 2019, at two fertility centres, in Bordeaux, France.
⇒ The exposure of suspended particulate matter, fine particulate matter, nitrogen dioxide, ozone and black carbon was measured by air quality monitoring stations.
⇒ The effects on IVF outcomes of a biomass boiler room installation, emitting pollutants, located 100 m from the IVF laboratory of Bordeaux University Hospital (center 1), were also studied.
⇒ Random-effects logistic regression was used to analyse the longitudinal aspects of the data, as each couple could have several IVF attempts.

CONTEXT

Air pollution is defined by the Environmental Protection Agency as a mixture of solid particles and gases in the air.[1] Car emissions, chemicals from factories, dust, pollen and mould spores may be suspended as particles. These pollutants, therefore, affect air quality. Several classes of air pollutants have been defined, including gaseous pollutants (sulphur dioxide SO_2, nitrogen dioxide NO_2, carbon monoxide CO), organic compounds (solvents, dioxins), heavy metals (lead, copper), fine particulate matter ($PM_{2.5}$) and suspended particulate matter (PM_{10}). Indoor air quality is influenced by outdoor air pollution. The factors promoting this transfer to the interior may be specific to the outdoors or specific to the building concerned.[1]

Epidemiological studies investigating the effects of air pollution on reproduction have revealed an impact on various female and male reproductive parameters.[2] Toxicological studies have revealed negative effects of diesel exhaust particles and exposure to $PM_{2.5}$ on the development of mouse embryos obtained by in vitro fertilisation (IVF).[2]

For a number of years, it was suspected that environmental factors had an influence on

the outcome of IVF, but few studies on the specific effects of air pollution on IVF were published.

Epidemiological studies on exposure to air pollution throughout the IVF cycle and over various independent periods (online supplemental figure S1), performed in the USA and China, have shown that: (A) exposure to $PM_{2.5}$, NO_2 and CO over the period from oocyte retrieval to embryo transfer (period 3 in online supplemental figure S1) decreases the rate of biochemical and clinical pregnancies in women under the age of 35 years[3]; (B) exposure to NO_2 and SO_2 over the same period is associated with lower clinical pregnancy rates[4 5] and (C) exposure to black carbon (BC) decreases the rate of IVF success.[6] Exposure to SO_2 during this period has been shown to be associated with a significant increase in the male/female sex ratio,[7] and exposure to SO_2 and ozone (O_3) significantly decreases live birth rates in frozen/thawed embryo transfer cycles.[8] Contradictory results were obtained in two studies performed in the USA and in China, in which exposure to O_3 slightly increased the rates of implantation, live birth[9] and clinical pregnancy.[10] Other studies performed in China and Korea found no such associations over the period of interest.[11 12] Since the air quality was described to be better in France than in the countries mentioned above,[13] it would therefore appear pertinent to investigate the effects of exposure of gamete and embryos cultured in the IVF laboratory to air pollution on the results of IVF in terms of clinical pregnancy.

The objective of this exploratory study was to evaluate the effect of air pollution, from oocyte retrieval to embryo transfer, on the results of IVF in terms of clinical pregnancy rates, at two fertility centres, from 2013 to 2019. Moreover, in the local context of Bordeaux University Hospital, a biomass boiler room was installed in 2016, to supply heat and hot water. Like any other combustion reaction, biomass combustion emits various atmospheric pollutants, principally fine particles (PM_{10}, $PM_{2.5}$) and organic compounds.[14] The biomass boiler room is located 100 m from the Reproductive Biology Department of Bordeaux University Hospital. The secondary objective was to evaluate the effect of the presence of the biomass boiler room on the results of IVF, in terms of clinical pregnancy rates, at the Reproductive Biology Department of Bordeaux University Hospital, from 2013 to 2019.

MATERIALS AND METHODS

Population and study design

This exploratory retrospective cohort study included IVF or intracytoplasmic sperm injection (ICSI) attempts performed between 1 January 2013 and 31 December 2019, at the Reproductive Biology Department of Bordeaux University Hospital (center 1) or the Jean Villar Fertility Centre in Bruges, France (center 2). The exclusion criteria were: couples with a first IVF attempt before 1 January 2013, serodiscordant couples, IVF in a context of fertility preservation, IVF with oocyte donation or sperm donation.

Outcome

The early pregnancy consists of βHCG>100 UI/L (human chorionic gonadotropin) 2 weeks after embryo transfer. The outcome studied was clinical pregnancy following embryo transfer. We choose clinical pregnancy because we consider that this first clinical IVF outcome is the one which is the most influenced by air pollution during embryo culture. Clinical pregnancy was defined as the presence of an intrauterine gestational sac with cardiac activity between four and 6 weeks after embryo transfer. These data were collected during patient management and were provided by the two centers.

Estimation of exposure

Air pollution was considered to be the principal exposure and was assessed by determining the concentrations of PM_{10}, $PM_{2.5}$, NO_2, O_3 and BC. The concentrations of these pollutants are measured by air quality monitoring stations and were provided by ATMO Nouvelle Aquitaine; the data for the Talence station were used here (online supplemental figure S2). This station monitors background air quality. Background pollution corresponds to pollution levels representative of the air quality of a large geographical area. Thus, it characterises the minimum pollution to which the population of this sector is exposed. This is the closest pollution monitoring station to our centers and is a good representation of the ambient pollution to which fertility centers are exposed. It is located well away from traffic and provides an indication of the background pollution in the geographic area. Cumulative exposure to these pollutants was studied over the period from oocyte retrieval to embryo transfer, corresponding to a period of 2–6 days.

The biomass boiler room provides a source of secondary exposure. We assessed the effects of such exposure by comparing data before and after 2016 (exactly before and after 15 December 2015). This analysis was restricted to data from Bordeaux University Hospital, the IVF laboratory of which is located very close (100 m) to the biomass boiler room.

Covariate data

Confounding factors were identified on the basis of published studies and our own knowledge, with a directed acyclic graph (online supplemental figure S3). The season (spring, summer, fall, winter) of IVF procedure affects the pregnancy rate.[15] The ovarian reserve (continuous),[16] woman's age (<30 years, 30–35 years, 35–40 years, >40 years),[17] body mass index (BMI) (underweight, normal, overweight, obese),[18] smoking status (smokers, ex-smokers, non-smokers),[19] the oocyte fertilisation method (IVF, ICSI)[20] and site of residence (urban, non-urban) are also known to affect IVF success. The number of transferred embryos is also a confounding factor for the relationship between biomass boiler room-related pollution and clinical pregnancy. The data were provided by the Reproductive Biology Department of Bordeaux University Hospital and by the Jean Villar Fertility Center.

IVF procedures

Ovarian stimulation was achieved by daily subcutaneous gonadotropin injections for approximately 10 days. Depending on the ovarian reserve and the clinical context of the patient, the gynaecologist decided to perform a GnRH (gonadotropin-releasing hormone) agonist or antagonist protocol. Stimulation was monitored regularly by ovarian ultrasound, to observe the growth of the follicles, and by biological monitoring of plasma 17 β-estradiol and luteinising hormone concentrations. Ovulation was triggered by an injection of recombinant hCG (Ovitrelle, Merck, Lyon, France) or GnRH agonist (Decapeptyl, Ipsen pharma, Boulogne Billancourt, France) when the ultrasound and biological criteria were met. The follicles were punctured 36 hours after ovulation triggering, in the operating room, under local anaesthesia, with the patient conscious or unconscious under sedation. Progesterone was administered by the vaginal route to support the luteal phase, initiated in the evening of follicle puncture. Depending on sperm quality, the spermatozoa were selected by density gradient (>1 million fresh mobile spermatozoa/mL) or wash-centrifugation techniques (frozen sperm or <1 million fresh mobile spermatozoa/mL). For selection by density gradient centrifugation, semen was placed on top a gradient of colloid solution (PureSperm, NidaCon Laboratories AB, Gothenburg, Sweden) prepared in a centrifuge tube and then subject to centrifugation. Subsequently, motile spermatozoa were recovered from the tube bottom containing the fraction with higher density of colloid. The wash-centrifugation technique consisted in two successive centrifugations of semen, washed with a clean medium free of cells. Finally, the supernatant was discarded and the pellet of spermatozoa was resuspended in 0.1 mL clean medium. Oocyte fertilisation was performed by conventional IVF or ICSI with fresh ejaculated sperm depending on the number of spermatozoa selected after semen preparation techniques or ICSI with frozen sperm. All oocytes and embryos were cultured and incubated in trigas incubators at 37°C. Fertilisation was observed a mean of 16–18 hours after insemination by conventional IVF or ICSI, as the presence of two pronuclei. Embryonic development was evaluated on day 2 (44–48 hours post-insemination or post-injection) or day 3 (68–72 hours post-insemination or post-injection) and for some embryos at blastocyste stage (114–118 hours postinsemination or postinjection). The decision to transfer fresh embryos, to vitrify embryos or to not preserve embryos was taken on day 2 or 3, or at the blastocyst stage, depending on embryo quality, assessed with the following morphological criteria for cleavage stage embryos: number and regularity of blastomeres and percent fragmentation evaluated according to standard procedure[21] (<20%, 20%–50%, >50%). For blastocysts, the Gardner classification was used.[22]

Laboratory air quality control

The IVF laboratories are equipped with an air treatment unit. External air is filtered by a HEPA (high-efficiency particulate air) system before its release into the laboratories. The incubation atmosphere composition is characterised by a concentration of 6% CO_2 and 5% O_2, confirmed by independent measurements of the air in the incubators.

The laboratories conform to EN/ISO 14644 standards ISO (International Organization for Standardization) 8 class D.

Statistical analysis

For descriptive analysis, results are expressed as means and SD for quantitative variables and as numbers and percentages for qualitative variables.

For the principal analysis, we used random-effects logistic regression to analyse the longitudinal aspects of the data, as each couple could have several IVF attempts. We used the *glmer* function of the *lme4* package of R: two random intercepts were integrated into the model, one to take the correlation of events in the same woman into account, and the other taking fertility center into account.[23] The secondary analysis focused exclusively on IVF attempts at center 1, because of its proximity to the biomass boiler room. We used the same methods as for the principal analysis, but we integrated only one random intercept into the model, to take the correlation of events in the same woman into account.[23]

We took missing data for IVF attempts (BMI, tobacco use, AMH (anti-müllerian hormone) rate: a reflection of the ovarian reserve, site of residence) into account by multiple imputation. Indeed, as these data are clinical, we hypothesised that they would conform to a missing at random distribution.[24] We used the multiple imputation method based on chain equations with the *mice* package in R software to generate five tables, by five iterations. Outliers (BMI, AMH rate, number of the days of embryo culture) were considered as missing data and imputed as described above. A linear interpolation was performed with the *zoo* package of R software for missing data on air pollutants.[25]

The statistical power of the study was calculated for the different cumulative exposures to PM_{10}, $PM_{2.5}$, BC, NO_2, O_3, based on a first-species risk α of 5% (online supplemental table S1).

Analyses were performed with R Core Team (2020) software. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.Rproject.org/.

Patient and public involvement

This study is a retrospective non-interventional research. In this context, it does not concern individuals but health data.

RESULTS

Population selection

Between 1 January 2013 and 31 December 2019, 12 992 IVF attempts were performed at the two centers. We

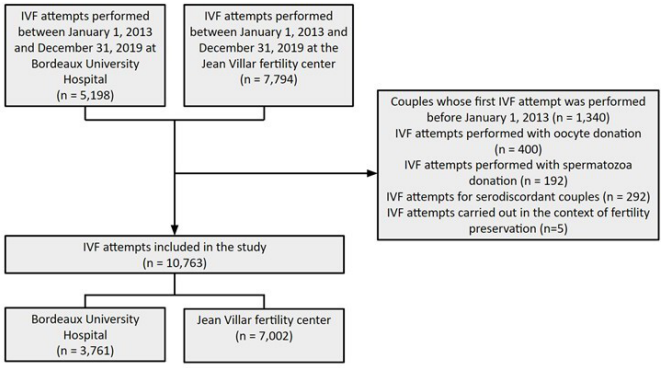

Figure 1 Flow chart of the selection procedure. France, 2013–2019. IVF, in vitro fertilisation.

excluded 2229 of these IVF attempts. The study population therefore consisted of 10 763 IVF attempts (figure 1).

Descriptive analysis

Of the 10 763 IVF attempts included, 24.9% resulted in an early pregnancy, 20.4% resulted in a clinical pregnancy, 18.9% resulted in a delivery and 2.7% ended in early miscarriage (online supplemental figure S4).

More than half the couples resided in the urban residence. For the men, clinical pregnancy rates differed significantly with age, with a lower likelihood of clinical pregnancy for older men (mean age 37.5 years vs 36 years, SD=6.2 years, p<0.001). The men were predominantly overweight (39.3% for the group achieving clinical pregnancy and 41% for those without clinical pregnancy, p=0.16) and non-smokers (41.3% for those achieving clinical pregnancy and 42.4% for those without clinical pregnancy, p=0.55). For the women, age also had a significant effect on the likelihood of achieving a clinical pregnancy, with a favourable outcome of IVF less likely among older women (mean age 35.1 years vs 33.5 years, SD=4.8 years, p<0.001). More than half the women were of normal weight (58.5% for those achieving clinical pregnancy and 58.8% for those without clinical pregnancy, p=0.01) and non-smokers (55.5% for those achieving clinical pregnancy and 56.4% for those without clinical pregnancy, p=0.15). For IVF attempts, there was no statistically significant difference between attempts resulting and not resulting in clinical pregnancy in terms of sperm origin (mostly fresh sperm), and the season in which IVF was performed (mostly in the fall). By contrast, statistically significant differences between attempts resulting and not resulting in clinical pregnancy were observed for other characteristics, with a higher AMH level (3.1 ng/mL vs 2.6 ng/mL, SD=2.4 ng/mL, p<0.001), number of oocytes obtained by puncture (9.8 vs 8.7, SD=4.8, p<0.001), number of oocytes inseminated in conventional IVF (9.5 vs 8.6, SD=4.8, p<0.001), number of oocytes injected in ICSI (7.2 vs 6.4, SD=2.4, p<0.001), number of embryos obtained (6.2 vs 5.0, SD=3.4, p<0.001), number of transferred embryos (1.6 vs 1.0, SD=0.5, p<0.001) and number of frozen embryos (1.8 vs 1.3, SD=2.1, p<0.001) for the IVF attempts resulting in clinical pregnancy. The rank of the IVF attempt (1.8 vs 1.6, SD=1.1, p<0.001) and the number of the days of embryo culture (3.0 days vs 2.9 days, SD=1.1 days, p<0.001) were greater for attempts that did not result in clinical pregnancy (table 1). The characteristics of the IVF attempts performed at center 1 are provided in online supplemental table S2.

An analysis of the cumulative exposure of pollutants over the period from oocyte retrieval to embryo transfer showed that PM_{10}, $PM_{2.5}$, BC, NO_2 and O_3 concentrations were higher for attempts that did not result in a clinical pregnancy than for those that did (table 2), but this difference was not statistically significant in univariate analysis (table 3).

Regarding exposure to pollutants from the biomass boiler room, 47.3% of clinical pregnancies were obtained while the biomass boiler room was in operation (table 1).

Principal analysis

Univariate analyses showed no significant association between cumulative exposure to pollutants during the period from oocyte retrieval to embryo transfer and clinical pregnancy (table 3). Multivariate analyses showed a significant association between O_3 exposure from oocyte retrieval to embryo transfer and clinical pregnancy. Indeed, the odds of a clinical pregnancy were decreased by 8% for an increase of 83 $\mu g/m^3$ in O_3 concentration, after adjustment for the season in which IVF was performed, age, BMI, smoking status, AMH level, oocyte fertilisation method and place of residence (95% CI = (0.86 to 0.98)) (table 3).

Secondary analysis

The univariate analysis showed a significant association (p<0 .001) between the presence of the biomass boiler room and clinical pregnancy rates. Indeed, the odds of clinical pregnancy were significantly lower (42% lower) when the biomass boiler room was present than before its construction (95% CI = (0.49 to 0.69)) (table 4). Similarly, the odds of a clinical pregnancy were significantly decreased, by 25%, by the presence of the biomass boiler room after adjustment for the season in which IVF took place, age, BMI, smoking status, oocyte fertilisation method, AMH level, place of residence and number of embryos transferred (95% CI = (0.61 to 0.91)) (table 4).

DISCUSSION

The objective of this study was to evaluate the effect on IVF outcomes of air pollution over the period from oocyte retrieval to embryo transfer. We found a significant association between O_3 exposure during the period of interest and clinical pregnancy rates, after adjustment for age, BMI, smoking status, AMH level, oocyte fertilisation method, season in which IVF took place and place of residence. Also, a significant association was found between exposure to biomass boiler room and clinical pregnancy.

We found a significant inverse association between exposure to O_3 between oocyte retrieval and embryo

Table 1 Characteristics of the IVF attempts included in the study, according to outcome (presence or absence of clinical pregnancy), in the two IVF laboratories (centre 1 and centre 2), France, 2013–2019, (n=10 763)

	Clinical pregnancy (n=2194)		No clinical pregnancy (n=8569)		P value*
	% (N)	Mean±SD	% (N)	Mean±SD	
Couple characteristics					
Site of residence					0.048
Rural	40.0 (878)		37.3 (3198)		
Urban	58.2 (1276)		60.5 (5187)		
Missing data	1.8 (40)		2.1 (184)		
Characteristics of the men					
Age (years)		36.0±5.7		37.5±6.2	<0.001
BMI (kg/m^2)		25.6±3.9		25.7±4.1	0.16
Underweight	0.7 (15)		0.7 (60)		
Normal	38.9 (853)		39.2 (3355)		
Overweight	39.3 (863)		41 (3512)		
Obese	11.9 (261)		13.0 (1117)		
Missing data	9.2 (202)		6.1 (525)		
Smoking status					0.55
Smokers	32.3 (708)		31.7 (2716)		
Ex-smokers	21.0 (460)		22.3 (1909)		
Non-smokers	41.3 (906)		42.4 (3634)		
Missing data	5.5 (120)		3.6 (310)		
Characteristics of the women					
Age (years)		33.5±4.4		35.1±4.8	<0.001
BMI (kg/m^2)		23.4±4.4		23.6±4.6	0.01
Underweight	7.1 (156)		0.7 (597)		
Normal	58.5 (1283)		58.8 (5036)		
Overweight	19.3 (423)		19.7 (1685)		
Obese	10.5 (231)		12.0 (1027)		
Missing data	4.6 (101)		2,6 (224)		
Smoking status					0.15
Smokers	21.9 (480)		20.5 (1758)		
Ex-smokers	18.7 (410)		20.8 (1785)		
Non-smokers	55.5 (1217)		56.4 (4834)		
Missing data	4.0 (87)		2.2 (192)		
Cycle characteristics					
No of IVF cycles		1.6±1.0		1.8±1.1	<0.001
Stimulation protocol					0.45
Agonist	892 (40.7)		3635 (42.4)		
Antagonist	1296 (59.1)		4885 (57.0)		
Missing data	6 (0.2)		49 (0.6)		
AMH rate (ng/mL)		3.1±2.4		2.6±2.2	<0.001
Missing data	17.0 (374)		13.4 (1152)		
No of oocytes retrieved		9.8±4.8		8.7±5.6	<0.001
Frozen sperm	6.5 (143)		6.1 (526)		0.92
Fertilisation method					0.11
IVF	35.7 (784)		37.3 (3195)		

Continued

Table 1 Continued

	Clinical pregnancy (n=2194)		No clinical pregnancy (n=8569)		P value*
	% (N)	Mean±SD	% (N)	Mean±SD	
ICSI	64.3 (1410)		62.7 (5374)		
No of oocytes inseminated in conventional IVF		9.5±4.8		8.6±5.4	<0.001
No of oocytes injected in ICSI		7.2±2.4		6.4±2.0	<0.001
Embryos cultured in the embryoscope time lapse†	31.1 (683)		27.0 (2,311)		0.002
No of days of embryo culture		2.9±0.9		3.0±1.1	<0.001
Missing data	0.1 (2)		15.6 (1335)		
No of embryos obtained		6.2±3.4		5.0±4.0	<0.001
No of embryos transferred		1.6±0.5		1.0±0.8	<0.001
No of embryos frozen		1.8±2.1		1.3±2.0	<0.001
Season in which IVF was performed					0.24
Spring	27.8 (611)		25.4 (2175)		
Summer	19.5 (427)		19.9 (1702)		
Fall	34.6 (759)		36.9 (3159)		
Winter	18.1 (397)		17.9 (1533)		
Presence of the biomass boiler room‡					
Yes	47.3 (433)		35.1 (998)		
No	52.7 (482)		64.9 (1848)		

*P value for the Wald test.
†Time lapse embryoscope: a specific type of incubator.
‡Only at center 1 (n=3761) where n=915 IVF attempts resulted in a clinical pregnancy.
AMH, Anti-müllerian hormone; BMI, body mass index; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilisation.

transfer and clinical pregnancy rate. This association was also reported in three previous studies. Wang *et al*, in a study performed in China in 2019, showed that O_3 exposure resulted in poorer IVF outcomes in terms of the live birth rate (OR=0.66, 95% CI = (0.56 to 0.78)).[8] By contrast, Boulet *et al* obtained conflicting results in a study performed in the USA in 2019: exposure to O_3 resulted in higher implantation and live birth rates (OR=1.01, 95% CI = (1.001 to 1.02) and OR=1.01, 95% CI = (1.004 to 1.02)); but no significant results on clinical pregnancy (OR=1.01, 95% CI = (1.00 to 1.02).[9] Jin *et al* obtained same results in a study conducted in China in 2022: exposure to O_3 resulted in higher clinical pregnancy (OR=1.081, 95% CI = (1.03 to 1.133)).[10] Other studies found no association between exposure to O_3 and clinical pregnancy rates,[4 11 12] biochemical pregnancy,[11] live birth,[6] sex ratio.[7]

Table 2 Characteristics of pollutants according to cumulative exposure over the period from oocyte retrieval to embryo transfer, by IVF outcome, France, 2013–2019, (n=10 763)

Pollutants (µg/m³)	Cumulative exposure, Mean±SD		
	All (N=10 763)	Clinical pregnancy (N=2194)	No clinical pregnancy (N=8569)
PM_{10}	56.5±32.2	55.9±30.5	56.7±32.6
$PM_{2.5}$	36.4±27.4	35.7±26.1	36.6±27.7
BC	4.3±3.6	4.2±3.3	4.4±3.6
NO_2	56.2±34.1	55.7±33.0	56.3±34.4
O_3	148±83.1	144±73.0	149±85.5

BC, black carbon; IVF, in vitro fertilisation; NO_2, nitrogen dioxide; O_3, ozone; PM_{10}, suspended particulate matter; PM2.5, fine particulate matter.

O_3 is a secondary pollutant that forms in the atmosphere when primary pollutants react. It is a highly reactive molecule with a low solubility in water, so it cannot easily pass through the oil layer of the embryo culture into the medium. However, O_3 can interact with the surfaces of material and pollutants in the interior of building, thereby generating secondary pollutants, such as aldehydes, which are more likely to penetrate through the oil layer of the embryo culture.[26] Indoor formaldehyde concentrations have been show to increase with outdoor O_3 concentration. Formaldehyde is classified as a carcinogen by the International Agency for Research on Cancer, and it is also embryotoxic.

By contrast, we found no association between exposure to other pollutants and clinical pregnancy rates. Previous

Table 3 Relationship between clinical pregnancy and exposure to PM_{10}, $PM_{2.5}$, BC, NO_2 and O_3 during gametes and embryos culture in the two IVF laboratories (center 1 and centrer2)

	Univariate model		Adjusted model*	
	OR (95% CIs)	P value†	OR (95% CIs)	P value†
PM_{10} (for an increase of 32 µg/m^3)	1.02 (0.97 to 1.07)	0.43	0.99 (0.95 to 1.05)	0.98
$PM_{2.5}$ (for an increase of 27 µg/m^3)	0.99 (0.95 to 1.05)	0.89	0.99 (0.94 to 1.04)	0.60
BC (for an increase of 4 µg/m^3)	0.98 (0,93 to 1.04)	0.50	0.99 (0.93 to 1.05)	0.74
NO_2 (for an increase of 34 µg/m^3)	1.02 (0.98 to 1.08)	0.31	1.02 (0.96 to 1.08)	0.60
O_3 (for an increase of 83 µg/m^3)	0.99 (0.94 to 1.04)	0.62	0.92 (0.86 to 0.98)	0.008

Random-effects logistic regression. France, 2013–2019, (n=10 763).
*Adjusted for season, age, BMI, smoking status, AMH rate, oocyte fertilisation method, place of residence.
†P value for the Wald test.
AMH, Anti-müllerian hormone; BC, black carbon; BMI, body mass index; IVF, in vitro fertilisation; NO2, nitrogen dioxide; O3, ozone; PM10, suspended particulate matter; PM2.5, fine particulate matter.

studies have reported associations between BC exposure from oocyte retrieval to embryo transfer and IVF failure rate (OR=1.41 and 95% CI = (1.1 to 1.8))[6]; between NO_2 exposure from oocyte retrieval to embryo transfer and clinical pregnancy rates (OR=0.95 and 95% CI = (0.90 to 0.99), p=0.033)[4 5] ; and between PM_{10} from oocyte retrieval to embryo transfer and the number of available embryos (beta=0.06, 95% CI = (0.02 to 0.07), p<0.001).[27] There are several possible explanations for these discrepancies between studies. First, the studies reporting links with exposure to these chemicals were performed in China[4] and the USA,[9] where the pollution levels are higher than in France. Indeed, the air quality index is 203 in China and 93 in the USA, whereas it is 70 in France and only 21 in Bordeaux.[13] Moreover, in our study, pollutant concentrations were measured at the Talence air quality monitoring station, which measures background levels: it is located far from traffic and its measurements therefore correspond to representative background pollution levels for the geographic sector in which it is located. The values recorded by this station correspond to the minimum level of pollution to which the population of the sector is subjected, regardless of their activities, over long periods. Finally, the filtration systems used in IVF laboratories may differ between laboratories. They are designed to remove particles from the air by passage through several filters of increasing efficiency (ie, through a succession of filters of decreasing pore size). Simple air filtration removes the largest particles, such as dust particles, and HEPA filtration then removes the smallest particles, such as PM.[28 29] Filters containing activated carbon are more effective for removing volatile organic compounds (VOCs), as is the CODA (carbon activated air filtration) system in the incubators, which limits gas influx.[29] Finally, the implementation of a CODA system and HEPA filtration with activated carbon and potassium permanganate was found to decrease particulate matter, and aldehyde levels in an IVF laboratory.[30]

In term of IVF results, the installation of a HEPA filtration system and VOC filtration with potassium permanganate has been reported to improve pregnancy rates.[31] The installation of a CODA system was shown to improve live birth rate,[32] and pregnancy rate, but no improvement in embryo quality was observed.[33] Several studies have reported an improvement of IVF outcomes following a change in the filtration system. Indeed, the installation of a 'clean' room (ie, an ISO class 5 laboratory, with a maximum of 3530 particles larger than 0.5 µm/m^3) has been shown to improve both fertilisation and pregnancy rates.[34] In 2018, the Cairo Consensus on IVF laboratory environment and air quality issued recommendations on air quality, concerning, in particular, filtration systems

Table 4 Relationship between clinical pregnancy and exposure to biomass boiler room pollutants during gametes and embryos culture in the center 1 IVF laboratory

	Univariate model		Adjusted model*	
	OR (95% CIs)	P value†	OR (95% CIs)	P value†
Presence of the biomass boiler room				
No	ref		ref	
Yes	0.58 (0.49 to 0.69)	<0.001	0.75 (0.61 to 0.91)	0.004

Results of random-effects logistic regression. France, 2013–2019, (n=3761).
*Adjusted for season, age, BMI, smoking status, AMH rate, oocyte fertilisation method, place of residence and number of embryos transferred.
†P value for the Wald test.
AMH, Anti-müllerian hormone; BMI, body mass index; IVF, in vitro fertilisation.

(an ISO class 7 laboratory with an activated carbon filter preceding a HEPA filter of at least operating room quality), the renewal of laboratory air (15 renewals per hour, including 3 renewals with new air per hour), temperature (20°C–24°C) and humidity (40%–45%).[35]

However, a study in 2018 showed that even the most advanced air treatment systems (high-efficiency HEPA particle filters and activated carbon filters) were not sufficient to limit the entry into IVF laboratories of certain air pollutants known to have an impact on in vitro culture.[36] Indeed, the efficiency of HEPA filters decreases by 25% over 6 months in a lightly polluted environment. The decrease in efficiency is likely to be even greater in a heavily polluted environment, during pollution peaks, for example. A Canadian study showed that HEPA filters remove only 68% of ultrafine particles and 63% of fine particles. Pollution peaks would be expected to decrease this efficiency further, particularly during O_3 peaks in our case, with filter systems reaching saturation and no longer performing their air filtration function correctly. As a result, air pollutants not removed from the laboratory air would have the potential to act as endocrine disruptors, inducing oxidative stress, changes to DNA or epigenetic modifications.[37] For this reason, it is recommended to clean the filters every 3–4 weeks, to replace them at least once per year, and to change them once or twice per month during pollution peaks.[38]

Regarding our secondary analysis, this study is, to our knowledge, the first to investigate the effects on IVF outcomes of a biomass boiler room installation, located 100 m from the IVF laboratory in which the embryos are cultured.

The biomass boiler room operates throughout the year to provide hot water and heating. The combustion of biomass leads to the emission of pollutants, principally fine particles and organic compounds. The presence of the biomass boiler room would, therefore, indirectly lead to high levels of exposure to PM_{10}, $PM_{2.5}$, BC and VOCs, because these pollutants are emitted by the biomass combustion process. In other words, this structure contributes to pollution specific to the vicinity of the fertility department of center 1 rather than background pollution measured by the Talence station.

The fine particles emitted by the biomass burning process may have adverse effects on gametes and embryos. Indeed, mechanistic studies have shown that cytochrome CYP450 transforms PM through the quinone reductase activity, catalysing electron transfer reactions and stimulating the production of reactive oxygen species (ROS).[39] ROS production plays an important role in the physiological processes of spermatozoa, contributing, in particular, to sperm mobility, oocyte fertilisation and sperm/oocyte fusion. This ROS production is regulated by the antioxidant capacity of the ROS generated, which interrupts the chain reactions leading to ROS production. However, an imbalance, with excessive ROS production, leads to oxidative stress, a decrease in sperm motility, fertilisation capacity and impaired embryonic development.[11 12 39] Excessive ROS production during folliculogenesis leads to oxidative stress that can impair ovarian functions. One of the consequences of excessive ROS production is an induction of cellular inflammation, leading to DNA alterations, with the hypomethylation and hypermethylation of certain genes. Concerning the VOCs emitted by the biomass combustion process, in 1998, Hall *et al* measured VOCs in seven IVF laboratories equipped with HEPA filters and incubators with 5% CO_2-containing atmospheres. They showed that VOC levels increased from the exterior (533 µg/m^3) to the interior (2862 µg/m^3) of the building, including the incubators (2769 µg/m^3).[40] These compounds can be deposited in fatty media (such as oil used for embryo culture), and VOCs can transform components of the medium into compounds toxic to embryos.[36] VOCs are directly or indirectly embryotoxic following their reaction with other molecules in the atmosphere, leading to a decrease in intracellular levels of antioxidant agents, such as glutathione, exposing the embryo to the effects of ROS. VOCs have antiestrogenic effects at the cellular level. They can also bind to the aryl hydrocarbon receptor, activating xenobiotic response elements located in the promoter region of several important genes and leading to the expression of these genes.

We studied a retrospective cohort of more than 10 000 IVF attempts at two fertility centres, for which detailed and confirmed clinical information was available. However, the retrospective nature of the study precluded adjustment for some potential confounding factors, such as alcohol consumption, for which no data were available in the medical records. Indeed, the consumption of alcohol in women leads to a development of embryos of inferior quality[18]; and in the long term a decrease in the ovarian reserve (AMH rate).[41 42] In the principal analysis, pollutant levels were measured at the Talence air quality monitoring station, to provide a proxy for background pollution at centers 1 and 2, despite the large distance between these two centres (online supplemental figure S2). Thus, during pollution peaks, the pollutant concentrations at center 2 will have been underestimated, due to the location of this site close to the ring road where pollutant concentrations are more higher, potentially leading to an underestimation of the ORs. In terms of health impacts, for the same duration of exposure, peak pollution levels have greater impacts than background levels. Another point is that we characterised the exposure to pollutants from the biomass boiler room through a before/after study relative to the date of installation of the biomass boiler room. It is therefore possible that other changes, including changes not specific to the laboratory, occurred during the 2013–2015 and 2016–2019 periods, and that these changes had an effect on outcomes. Finally, a large number of statistical tests were performed because we generated a model for each pollutant. This multiplicity of testing may have increased the risk of incorrectly identifying a statistically significant difference. However, one of the strengths of this study is the inclusion of all IVF attempts by a given couple. Indeed, couples

may have gone through several IVF attempts during the study period, and the correlation between the data for these attempts was taken into account with appropriate statistical models. Also, the data on IVF attempts were obtained from two centres, an element that we took into account in the statistical model since we put a random intercept.

CONCLUSIONS

In conclusion, this exploratory study of 10 763 IVF attempts revealed a negative association between exposure to air pollution, particularly O_3 and clinical pregnancy rates, and a negative association between exposure to biomass boiler room pollution and clinical pregnancy rates specifically for centre 1.

However, as this study was exploratory, it would now be of interest to continue the research by measuring the concentration of pollutants, particularly for O_3, VOC and PM, in the IVF laboratory and analyse data from newborns, and study the development over a 5 years or more time period to evaluate the impact on their development. Such studies could lead to recommendations for improvements in the air filtration systems of IVF laboratories, leading to an improvement in IVF results.

Acknowledgements We thank Julie Sappa from Alex Edelman & Associates for proofreading the English version of this article.

Contributors MT, LC-D, VR, AH, PB, CJ, FD and AP-R contributed to the conception and design of the study. LC-D, AP-R, AL, AH and PB contributed to data acquisition. MT, VR and FD contributed to data analysis and interpretation. All authors contributed to the drafting of the article or to its critical revision for important intellectual content. All the authors have read and approved the final submitted manuscript. MT act as guarantor.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval This study is a retrospective non-interventional research. In this context, it does not concern individuals but health data. According to deliberation no 2018-154 of 3 May 2018 approving the reference methodology for the processing of personal data implemented in the context of research in the health field not requiring the collection of the consent of the person concerned (MR-003) and repealing deliberation no 2016-263 of 21 July 2016, the reference methodology MR-003 has been respected. The individual information to the patient is done via the admission file. Patients attending the hospital were informed of the possibility of the reuse of their data for research and given an opportunity to object.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

ORCID iDs
Marie Tartaglia http://orcid.org/0000-0003-0396-2259
Fleur Delva http://orcid.org/0000-0001-5594-5405

REFERENCES

1 Environmental Protection Agency. Criteria Air Pollutants, [online]. Available: https://www.epa.gov/criteria-air-pollutants
2 Conforti A, Mascia M, Cioffi G, *et al*. Air pollution and female fertility: a systematic review of literature. *Reprod Biol Endocrinol* 2018;16:117.
3 Zeng X, Jin S, Chen X, *et al*. Association between ambient air pollution and pregnancy outcomes in patients undergoing in vitro fertilization in Chengdu, China: a retrospective study. *Environ Res* 2020;184:109304.
4 Li L, Zhou L, Feng T, *et al*. Ambient air pollution exposed during preantral-antral follicle transition stage was sensitive to associate with clinical pregnancy for women receiving IVF. *Environ Pollut* 2020;265:114973.
5 Wu S, Zhang Y, Wu X, *et al*. Association between exposure to ambient air pollutants and the outcomes of in vitro fertilization treatment: a multicenter retrospective study. *Environ Int* 2021;153:106544.
6 Gaskins AJ, Fong KC, Abu Awad Y, *et al*. Time-Varying Exposure to Air Pollution and Outcomes of *in Vitro* Fertilization among Couples from a Fertility Clinic. *Environ Health Perspect* 2019;127:77002.
7 Wang X, Cai J, Liu L, *et al*. Ambient outdoor air pollutants and sex ratio of singletons born after in vitro fertilization: the effect of single blastocyst transfer. *Fertil Steril* 2020;113:140–8.
8 Wang X, Cai J, Liu L, *et al*. Association between outdoor air pollution during *in vitro* culture and the outcomes of frozen–thawed embryo transfer. *Human Reproduction* 2019;34:441–51.
9 Boulet SL, Zhou Y, Shriber J, *et al*. Ambient air pollution and in vitro fertilization treatment outcomes. *Human Reproduction* 2019;34:2036–43.
10 Jin H-X, Guo Y-H, Song W-Y, *et al*. Effect of ambient air pollutants on in vitro fertilization-embryo transfer pregnancy outcome in Zhengzhou, China. *Environ Toxicol Pharmacol* 2022;90:103807.
11 Choe SA, Jun YB, Lee WS, *et al*. Association between ambient air pollution and pregnancy rate in women who underwent IVF. *Hum Reprod* 2018;33:1071–8.
12 Qiu J, Dong M, Zhou F, *et al*. Associations between ambient air pollution and pregnancy rate in women who underwent in vitro fertilization in Shenyang, China. *Reprod Toxicol* 2019;89:130–5.
13 World Air Quality Index. Air quality in the world: ranking based on air quality, [online]. Available: https://aqicn.org/rankings/fr/
14 Dalkia. Inauguration of the biomass heating plant of the CH. Ch Perrens and CHU of Bordeaux, [online]. Available: https://www.chu-bordeaux.fr/Espace-m%C3%A9dia/Communiqu%C3%A9s-de-presse/DP-Chaufferie-biomasse.pdf/
15 Zhao M, Zhang H, Waters THB, *et al*. The effects of daily meteorological perturbation on pregnancy outcome: follow-up of a cohort of young women undergoing IVF treatment. *Environ Health* 2019;18:103.
16 Sun XY, Lan YZ, Liu S, *et al*. Relationship between anti-Müllerian hormone and in vitro Fertilization-Embryo transfer in clinical pregnancy. *Front Endocrinol* 2020;11:595448.
17 Cimadomo D, Fabozzi G, Vaiarelli A, *et al*. Impact of maternal age on oocyte and embryo competence. *Front Endocrinol* 2018;9:327.
18 Hornstein MD. Lifestyle and IVF outcomes. *Reprod Sci* 2016;23:1626–9.
19 Feichtinger W, Papalambrou K, Poehl M, *et al*. Smoking and in vitro fertilization: a meta-analysis. *J Assist Reprod Genet* 1997;14:596–9.
20 Peultier A-S, Fréour T, Cazenave N, *et al*. [Fertilization failure in IVF and ICSI]. *J Gynecol Obstet Biol Reprod* 2015;44:380–6.
21 Balaban B, Brison D, Calderon G, *et al*. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. *Human Reproduction* 2011;26:1270–83.
22 Gardner DK, Schoolcraft WB. Culture and transfer of human blastocysts. *Current Opinion in Obstetrics and Gynaecology* 1999;11:307–11.

23 Commenges-Ducos M, Tricaud S, Papaxanthos-Roche A, *et al*. Modelling of the probability of success of the stages of in-vitro fertilization and embryo transfer: stimulation, fertilization and implantation. *Hum Reprod* 1998;13:78–83.
24 Pedersen AB, Mikkelsen EM, Cronin-Fenton D, *et al*. Missing data and multiple imputation in clinical epidemiological research. *Clin Epidemiol* 2017;9:157–66.
25 Ottosen T-B, Kumar P. Outlier detection and gap filling methodologies for low-cost air quality measurements. *Environ Sci Process Impacts* 2019;21:701–13.
26 Nicolas M. Ozone et qualité de l'air intérieur: interactions avec les produits de construction et de décoration.
27 Liu J, Zhao M, Zhang H, *et al*. Associations between ambient air pollution and IVF outcomes in a heavily polluted City in China. *Reprod Biomed Online* 2022;44:49–62.
28 Khoudja RY, Xu Y, Li T, *et al*. Better IVF outcomes following improvements in laboratory air quality. *J Assist Reprod Genet* 2013;30:69–76.
29 Esteves SC, Bento FC. Implementation of air quality control in reproductive laboratories in full compliance with the Brazilian cells and germinative tissue Directive. *Reprod Biomed Online* 2013;26:9–21.
30 Forman M, Sparks A, Degelos S. Statistically significant improvements in clinical outcomes using engineered molecular media and genomically modeled ultraviolet light for comprehensive control of ambient air (AA) quality. *Fertil Steril. sept* 2014;1:91.
31 Jindal SK, Polotsky AJ, Buyuk ER, *et al*. Improved pregnancy rates following introduction of engineering controls of lab air quality. *Fertil Steril* 2008;90:S403.
32 Heitmann RJ, Hill MJ, James AN, *et al*. Live births achieved via IVF are increased by improvements in air quality and laboratory environment. *Reprod Biomed Online* 2015;31:364–71.
33 Merton JS, Vermeulen ZL, Otter T, *et al*. Carbon-activated gas filtration during in vitro culture increased pregnancy rate following transfer of in vitro-produced bovine embryos. *Theriogenology* 2007;67:1233–8.
34 Boone WR, Johnson JE, Locke AJ, *et al*. Control of air quality in an assisted reproductive technology laboratory. *Fertil Steril* 1999;71:150–4.
35 Mortimer D, Cohen J, Mortimer ST, *et al*. Cairo consensus on the IVF laboratory environment and air quality: report of an expert meeting. *Reprod Biomed Online* 2018;36:658–74.
36 Gauthier G, Sunol J, Novo S. *Air pollution decreases the rate of blastocysts in in vitro fertilization cycles*. Marquès Inst, 2018.
37 Carré J, Gatimel N, Moreau J, *et al*. Does air pollution play a role in infertility?: a systematic review. *Environ Health* 2017;16:82.
38 Pollet-Villard X, Levy R. [Impact of air quality on practices and results in the IVF laboratory]. *Gynecol Obstet Fertil Senol* 2018;46:713–28.
39 Jurewicz J, Radwan M, Sobala W, *et al*. Effects of occupational exposure - is there a link between exposure based on an occupational questionnaire and semen quality? *Syst Biol Reprod Med* 2014;60:227–33.
40 Hall J, Gilligan A, Schimmel T, *et al*. The origin, effects and control of air pollution in laboratories used for human embryo culture. *Hum Reprod* 1998;13 Suppl 4:146–55.
41 Firns S, Cruzat VF, Keane KN, *et al*. The effect of cigarette smoking, alcohol consumption and fruit and vegetable consumption on IVF outcomes: a review and presentation of original data. *Reprod Biol Endocrinol* 2015;13:134.
42 Li N, Fu S, Zhu F, *et al*. Alcohol intake induces diminished ovarian reserve in childbearing age women. *J Obstet Gynaecol Res* 2013;39:516–21.