Lack of carbon air filtration impacts early embryo development

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Abstract

Purpose To assess human fertilization and preimplantation embryo development in the presence and in the absence of carbon filtration

Methods This is a retrospective cohort analysis of fresh, controlled ovarian hyperstimulation cycles as well as previously cryopreserved pronuclear stage embryo transfer cycles in a single IVF center. Embryo development and cycle-based outcomes were compared among three groups: 1) when carbon filtration was present, 2) when carbon filtration was absent, and 3) when carbon filtration had been restored.

Results A total of 524 fresh cycles and 156 cryopreserved embryo cycles were analyzed. Fertilization, cleavage, and blastocyst conversion rates for fresh cycles all declined during the period of absent carbon filtration and recovered after the restoration of carbon filtration. Cryopreserved embryos that were thawed and cultured during the period of absent filtration did not have changes in cleavage or blastocyst conversion rates compared to periods where carbon filtration was present. Clinical pregnancy and live birth rates were unchanged among the three time periods.

Capsule The absence of carbon filtration in an IVF laboratory air handler is associated with poor fertilization and early embryo development for fresh cycles.

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Conclusions The absence of carbon filtration in an IVF laboratory air handler is associated with poor fertilization and early embryo development for fresh cycles. Because development of previously frozen pronuclear stage embryos was unaffected, the lack of carbon filtration may preferentially affect embryos in the peri-fertilization period. Carbon filtration is an integral part to a successful human in-vitro fertilization laboratory.

Keywords Embryo development · Air filtration · Assisted reproductive technology · Laboratory standards

Introduction

For decades, scientists have been studying in vitro growth of embryos, optimizing the laboratory environment to facilitate normal growth and development [1, 2]. In assisted reproductive technologies today, we attempt to replicate the conditions of the female reproductive tract in the IVF laboratory, with the hope that mimicking the natural environment will lead to the best outcomes.

Although many studies have examined the components of culture media and resultant gamete/embryo viability, substantially fewer studies have investigated the way in which ambient qualities such as temperature, collection systems equipment, and laboratory cleanliness/sterility affect embryo development. In particular, peer-reviewed data specifically about air quality in the IVF laboratory and the effect of it on gamete viability or embryo development are lacking. In one review of IVF laboratory air quality, Cohen et al. noted that during a temporary relocation of their research facilities, nearby construction events lethally affected their mouse embryo development, despite the presence of free-standing ionization and HEPA filtration units [3]. They also reported an anecdote

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about an IVF laboratory whose pregnancy rates dramatically decreased after the facility moved from a suburban environment to an urban one; the pregnancy rates returned to pre-move levels after the installation of a rudimentary air filter.

Most data describing air quality in the IVF suite are presented at national meetings only and rarely become peer-reviewed publications. The few published data indicate that new or upgraded facilities are often associated with improved air quality and improved IVF outcomes [4–6]. One program reported that the creation of a clean room and development of a new air-filtering system improved its IVF pregnancy outcomes after pregnancy rates decreased coincidentally with building construction adjacent to the IVF suite [4]. However, not all programs note an improvement in clinical outcomes when air filtration systems are employed [7]. In addition, studies may fail to assess the relationship, or demonstrate correlation, between improvements in laboratory parameters and clinical outcome. One bovine breeding program reported a 5 % increase in pregnancy rate with use of an intra-incubator air filter system [8] but no changes in blastocyst formation rate or embryo quality.

Other studies reported poor obstetric performance in women who are exposed to environmental pollutants around the time of their conception, with or without assisted reproduction. Perin et al. reported that pre-conception exposure to high levels of ambient fine particulate matter ($\leq 10 \mu\text{M}$) was associated with a 2.6-fold increase in early pregnancy loss, both in women with natural pregnancy and after IVF [9]. Legro et al. studied the air pollution levels based on US Environmental Protection Agency air quality monitors located around the homes and IVF clinics of 7403 women undergoing their first IVF cycles. The authors concluded that the effects of declining air quality on reproductive outcomes after IVF were variable, cycle dependent and complex, but that increased NO_2 concentration was consistently associated with lower birth rates [10].

Changes in clinical outcomes can be confounded by laboratory success that evolves with time as well as changes in stimulation and/or transfer protocols. In our program, we had the unique opportunity to observe embryo development in our IVF laboratory during a time in which the carbon filtration component of our laboratory's air-handling unit was missing. This filter was unknowingly removed by building maintenance in response to reports of low air pressure in our positive-pressure ventilation system; however, the maintenance staff that removed the filters did not report this action to supervisors or laboratory personnel. We anecdotally noted that early embryo performance seemed poor and were concerned that clinical outcomes were compromised. After eventually discovering the absence of the carbon filtration, we hypothesized that this factor may have contributed to the changes in early embryo development we had observed. To test our hypothesis, we designed a retrospective review

to assess embryo development and cycle outcomes before, during, and after this period of no carbon filtration.

Materials and methods

Population and participants

We retrospectively reviewed the embryo development and pregnancy outcomes of both fresh and cryopreserved embryo cycles occurring before, during, and after the unintentional removal of carbon filtration from the IVF laboratory. Included cycles were based on oocyte retrieval date (for fresh cycles) or cryopreserved pronuclear stage embryo thaw date (for frozen cycles) and were grouped into one of three categories: 1) January 27, 2010–July 31, 2010, when the IVF laboratory air handler contained carbon filtration (“Carbon 1”); 2) January 27, 2011–July 31, 2011, when carbon filtration was absent (“Absent”); and 3) January 27, 2012–July 31, 2012, after carbon filtration had been restored (“Carbon 2”). We deliberately chose the same time of year for all three groups to avoid potential differences in gamete and embryo performance owing to seasonal variations. Other air-filtration variables were consistent over the three periods. No additional or secondary laboratory space was used. HEPA filtration was continuously in place. No auxiliary (in-line or free-standing) air purification systems were employed. All embryo cultures were performed in sequential culture media (IVC-One and IVC-Three; InVivoCare, Frederick, MD) supplemented with 10 % Quinns Advantage Serum Protein Substitute (Cooper Surgical, Inc., Trumbull, CT) in 5.5–6.0 % CO_2 in air at 37 °C. Volatile organic compounds (VOC) in the laboratory air were not measured during any of these periods, as this was not a routine measurement in our laboratory.

Follicular stimulation was based on GnRH agonist or antagonist protocols and involved the use of recombinant follicle-stimulating hormone and human menopausal gonadotropins. Ovulation was triggered by human chorionic gonadotropin injection after 2 or more follicles reached a diameter of 18 mm. Transvaginal oocyte retrieval was performed 36 h after the administration of human chorionic gonadotropin, and oocytes were inseminated using the conventional method or intracytoplasmic sperm injection (ICSI) based on previous seminal fluid analyses.

Definitions

In fresh cycles, fertilization checks were performed 18 h after insemination; the fertilization rate was defined as the number of diploid zygotes (two pronuclei) divided by the number of inseminated oocytes. For frozen cycles, embryos had been previously cryopreserved after normal fertilization (i.e., a diploid zygote) was assured. These pronuclear stage embryos had

been cryopreserved by controlled-rate freezing 18–22 h after insemination in 1.5 mol/L 1,2-propanediol (Sigma) as previously described [11]. Embryos were thawed by warming the straw for 40 s in air followed by 10-s exposure to 30 °C sterile water. Cryoprotectants were removed in a step-wise dilutional fashion.

In both fresh and frozen cycles, the cleavage rate was defined as the number of embryos with at least two cells 42 h after insemination divided by the number of diploid zygotes in culture on day 1. The blastocyst conversion rate was determined by assessing the proportion of blastocyst-stage embryos, of all degrees of expansion, originating from diploid zygotes in culture. Blastocysts were graded using the classification system outlined by Gardner and Schoolcraft [12]. We calculated the percentage of good- or excellent-quality blastocysts (those having no worse than a grade B for either the trophectoderm or the inner cell mass) among all diploid zygotes cultured in each of the three periods.

Clinical outcomes assessed included rates of clinical intrauterine pregnancy (the presence of an intrauterine gestational sac on early obstetric ultrasonography per embryo transfer), implantation (number of gestational sacs per embryo transferred), and live birth (live births per embryo transfer). We also calculated the percentage of male infants born that resulted from cycles in each period as well as the proportion of live births that were twins. Our program has a mandatory day 5 single-embryo transfer policy, practiced across all providers, for patients with good prognosis defined by all the following criteria: female age less than 38 years, no history of failed fresh IVF cycles at our center, at least seven diploid zygotes to culture (six for women <35 years), and at least one good-/excellent-quality blastocyst on day 5 of culture. The criteria for this single-embryo transfer policy did not change over the 3-year period studied, and for those patients not fulfilling these criteria, double blastocyst transfer was performed.

Statistical analyses

For patient- and cycle-based comparisons, statistical analyses for rates and means ($\pm$ standard deviation) were performed using one-way analysis of variance with posthoc Bonferroni correction or Games-Howell test for equal or unequal variances, respectively. Proportions were compared using chi-square tests and the Fisher exact test where appropriate. *P* values <0.05 were considered statistically significant. For embryo-outcomes, we used a logistic regression model that was fitted using the generalized estimating equations method. This was performed to adjust for the correlation of embryos originating from the same parentage.

Ethical approval

Information about IVF cycles and outcomes were obtained from our clinical database and patients' medical records, as was approved by our Institutional Review Board.

Results

We analyzed the embryo development and clinical outcomes of 524 fresh cycles during the three periods defined above. In addition, to examine individual embryo development, we analyzed the outcomes of 3350 cultured diploid zygotes obtained from those 524 fresh cycles as well as 728 previously cryopreserved pronuclear stage embryos that were thawed and cultured for 156 cryopreserved embryo IVF cycles.

Fresh cycle characteristics

Fresh cycle characteristics for the Carbon 1, Absent, and Carbon 2 periods are given in Table 1. Mean female age, mean body mass index, race, and rates of male- or female-factor infertility did not differ significantly among the three periods. The mean duration of infertility prior to treatment for Absent cycles (40.8 months) was significantly longer than that for cycles in Carbon 1 (30.6 months, $p=0.016$), but not for cycles in Carbon 2 (36.3 months, $p=0.586$ Absent to Carbon 2, $p=0.167$ Carbon 1 to Carbon 2). Cycles in Carbon 2 were more likely to be repeat cycles than were cycles in Carbon 1 ($p=0.030$). Although the male-factor infertility rate did not significantly differ among the three groups, ICSI was used more often in cycles during Absent and Carbon 2 than in Carbon 1 ($p=0.004$). Total failed fertilizations, with no normal diploid zygotes, were also higher in Absent than in Carbon 1 or Carbon 2 ($p=0.017$); all failed fertilizations occurred after conventional insemination except in the one case in the Carbon 2 period. The percentage of cycles that resulted in a day 5 embryo transfer did not differ among the groups; however, the mean number of embryos transferred on day 5 during Absent (1.6) was significantly higher than that during Carbon 1 (1.3, $p=0.010$).

Fresh cycle embryo development

Fertilization, cleavage, and blastocyst conversion rates for fresh cycles all dropped during the Absent period and recovered during the Carbon 2 period (Fig. 1). For all cycles (Fig. 1a), the fertilization rate dropped from Carbon 1 to Absent ($p=0.053$) and recovered in the Carbon 2 period ($p=0.006$). Cleavage and blastocyst conversion rates also significantly declined from Carbon 1 to Absent ($p<0.001$) and recovered in Carbon 2 ($p=0.007$).

Table 1 Fresh cycles characteristics

Patient characteristic	Carbon 1 (<i>n</i> =152)	Absent (<i>n</i> =162)	Carbon 2 (<i>n</i> =174)	<i>p</i> value
Female age, y	33.6 (4.7)	33.3 (4.5)	33.7 (4.9)	0.742
BMI, kg/m ²	27.4 (6.8)	27.0 (6.7)	28.4 (7.6)	0.190
White, non-Hispanic, %	79.6	85.8	88.5	0.075
Duration of infertility, mo.	30.6 (22.5) ^a	40.8 (29.5) ^b	36.3 (28.3) ^{ab}	0.016
No. of IVF attempts	1.6 (1.1) ^a	1.7 (1.2) ^{ab}	2.0 (1.6) ^b	0.030
Couples with male-factor infertility, %	42.8	31.5	36.8	0.117
Female infertility factors, %				0.607
Age >38 years	18.0	14.6	16.9	
Uterine/Cervical	12.2	7.3	6.6	
Diminished ovarian reserve, ovarian insufficiency	7.9	9.9	7.8	
Endometriosis	12.9	16.6	10.8	
Polycystic ovaries/ovulatory factor	15.1	11.9	16.9	
Recurrent pregnancy loss	1.4	0.7	3.0	
Tubal factor	19.4	19.9	20.5	
Unexplained	12.9	19.2	17.5	
Cycle characteristic	Carbon 1 (<i>n</i> =160)	Absent (<i>n</i> =175)	Carbon 2 (<i>n</i> =189)	<i>p</i> value
No. of oocytes retrieved	15.1 (8.2) ^a	17.5 (9.1) ^b	18.1 (9.8) ^b	0.006
Cycles utilizing ICSI, %	52.5 ^a	69.7 ^b	64.0 ^b	0.004
Cycles with no normal (2PN) fertilization, %	0.2 ^a	1.3 ^b	0.2 ^a	0.017
D5 embryo transfers, %	60.0	59.0	57.7	0.907
No. of embryos transferred, D5	1.3 (0.5) ^a	1.6 (0.5) ^b	1.5 (0.5) ^{ab}	0.010
No. of embryos transferred, D3	2.3 (0.8)	2.2 (0.8)	2.4 (0.8)	0.257

All values are means (standard deviations) unless otherwise specified. Differences between letter labels (a vs. b) indicate significant difference within each category

Abbreviations: BMI Body Mass Index, ICSI intracytoplasmic sperm injection, D5 Day 5, D3 Day 3

Subgroup analyses of cycles based on insemination method (conventional (Fig. 1b) or ICSI (Fig. 1c)) revealed that the cleavage rate for embryos from conventional insemination during Absent (86.0±17.8 %) was significantly lower than that during Carbon 2 (94.9±11.8 %, *p*=0.007). There was a trend in the decline in cleavage rate from Carbon 1 to Absent. The fertilization, cleavage, and blastocyst conversion rates for ICSI cycles in the Absent period were almost all significantly lower than those in either Carbon 1 or Carbon 2, the only exception was fertilization rate showing a non-significant decline from Carbon 1 to Absent.

A subgroup analysis of first cycles (for patients who had not attempted IVF previously) (Fig. 1d) revealed that fertilization and cleavage rates in the Absent period were significantly lower than those in either Carbon 1 or Carbon 2 (*p*=0.010 and 0.032, respectively). The total number of blastocysts in this subgroup was too small to support a statistically significant difference, but the pattern of blastocyst conversion rates followed the trend noted above.

Embryo-specific outcomes

Embryo-based outcomes are illustrated in Fig. 2. The fresh embryo cleavage rate (Fig. 2a) for all fresh cycles in Absent (91.5 %) was significantly lower than that in the Carbon 1 (96.3 %) and Carbon 2 (96.3 %) (*p*<0.0001); this was also true when subanalyses of conventional insemination or ICSI cases were performed. Conversely, the cleavage rates for frozen embryos during the Carbon 1, Absent, and Carbon 2 periods did not differ significantly (*p*=0.523).

Like cleavage rates, blastocyst conversion rates also displayed different patterns among fresh and frozen cycles (Fig. 2b). Among all fresh cycles, the embryo blastocyst conversion rate during Absent (23.2 %) was significantly lower than those during Carbon 1 (36.4 %) and Carbon 2 (36.2 %) (*p*<0.0001). Subanalyses of conventional and ICSI cycles also revealed this difference. In contrast, blastocyst conversion rates in thawed pronuclear stage embryos during Carbon 1, Absent, and Carbon 2 did not differ significantly (*p*=0.167).

Good-/excellent-quality blastocyst conversion rate was significantly lower during the Absent period in fresh embryos

Fig. 1 Early embryo development in fresh cycles. Average fertilization, cleavage, and blastocyst conversion rates for each of the three defined periods. **a** All fresh cycles, **b** cycles using conventional insemination, **c** cycles using ICSI, and **d** only first-treatment cycles. Different letter labels

(a vs. b for fertilization rate, c vs. d for cleavage rate, and e vs. f for blastocyst conversion rate) indicate significant differences within each group ($p < 0.05$). Error bars indicate standard error of the mean

(Fig. 2c). The proportion of good-/excellent-quality blastocysts from all fresh cultured zygotes decreased from 21.8 % during Carbon 1 to 12.3 % during Absent and recovered to 21.1 % during Carbon 2 ($p < 0.0001$). Embryos resulting from both conventional insemination as well as ICSI had a similar and significant drop and recovery. The proportion of good-/excellent-quality blastocysts from frozen pronuclear stage embryos increased over the three periods (16.8 % in Carbon 1, 23.0 % in Absent, and 27.6 % in Carbon 2, $p = 0.033$ between Carbon 1 and Carbon 2).

Clinical outcomes

As fresh embryo development was substantially poorer in the absence of carbon filtration, we performed additional analyses to assess clinical outcomes associated with these embryos.

Interestingly, clinical outcomes, including pregnancy rates, implantation rates, and live birth rates, were statistically unchanged over the three periods (Table 2). Although we noted a trend of increased percentage of males born from successful cycles in the Absent period, the number of live births was too small to show statistical significance. The percentage of live births with twins increased during the Absent period but was not statistically significant. An analysis of only first-cycle outcomes showed no discrepancies in the above findings (data not shown).

Discussion

Presently, only a handful of case reports, review papers, and national meeting abstracts have discussed the relative

Fig. 2 Rates of cleavage and blastocyst conversion among all cultured embryos. Cleavage and blastocyst conversion rates, as well as the proportion of good- or excellent-quality blastocysts, for all fresh and frozen embryos in culture. **a** cleavage rate, per diploid zygote in culture; **b** day 5 blastocyst conversion rate, per diploid zygote in culture; and **c** proportion of good- or excellent-quality blastocysts among all diploid zygotes in culture. Notably, declines in cleavage and blastocyst conversion rates were noted during the Absent period only among fresh, not previously frozen, embryos. Differences between letter labels indicate significant differences ($p < 0.05$) within each group (a vs. b for all fresh embryos, c vs. d for conventional insemination, e vs. f for ICSI, and g vs. h for frozen embryos). Error bars indicate 95 % confidence intervals

importance of avoiding air contaminants in the IVF suite. Our study is the first to show a reversible decline in early human embryo development when carbon filtration is absent in the IVF suite air circulation and suggests that human embryos are preferentially affected in the peri-fertilization period.

We found that without solid media carbon filtration, fertilization, cleavage, and blastocyst conversion rates declined in fresh IVF cycles; these negative effects essentially disappeared when carbon filtration was restored. In most of our comparisons, the negative effects of this lack of filtration were more pronounced in embryos that had resulted from ICSI (Figs. 1c and 2b, c), which may be due to the fact that injected oocytes have already undergone a mechanical cellular stressor not experienced by conventionally inseminated oocytes and are thus more vulnerable. Additionally, cumulus cells present during conventional insemination may have provided a protective barrier to the effects present from the lack of carbon filtration.

Cleavage rates among IVF programs are routinely above 90 %, and rates of >95 % are indicative of good quality control in IVF laboratories.[13, 14] Thus, the finding of a significant drop in cleavage rates for conventional insemination embryos down to 86 %, associated with the lack of carbon filtration, was striking and has never been previously reported. Additionally, we reviewed cleavage rates in our program for the 4 years prior to, and 2 years after, the period of absent carbon filtration, and found this period of absent carbon filtration to be anomalous compared to the minor variation seen in all programs over time (Supplementary Fig. 1).

Our results showed that embryos that had been fertilized and cryopreserved prior to the Absent carbon filtration period,

but were thawed and cultured during the Absent period, showed no decrease in cleavage rate or blastocyst conversion rate. The absence of significant change in the cleavage rate, blastocyst conversion rate, or proportion of good-quality blastocysts developed from cryopreserved embryos while carbon filtration was absent suggests that this lack of carbon filtration affects embryos preferentially in the peri-fertilization period.

Maintaining optimal laboratory air quality through the use of positive air pressure, HEPA filtration, and VOC filtration has been addressed in limited scope in some best-practice guidelines [15] and identified as a common practice among high-performing IVF programs [16]. HEPA filtration removes almost 99.9 % of particles larger than approximately 0.3 μm [17] but cannot remove volatile compounds. An activated charcoal filter, such as that used in our laboratory's air handler, not only adsorbs VOCs, but it also can be impregnated with potassium permanganate to optimize VOC breakdown [17]. Given that the charcoal filter was missing, we suspect that the VOC concentration in the laboratory increased during that time. More than 20 years ago, Johnson et al. reported at a national meeting that volatile carbons, such as perfumes and colognes, were toxic to two-celled murine embryos in culture. VOCs may be directly embryotoxic, or they may react with other atmospheric molecules to form toxic compounds that cultured embryos, lacking epithelial protection, cannot defend against. The VOC-induced depletion of intracellular antioxidants, such as the decrease in glutathione noted in hepatocytes exposed to aldehydes, suggests that VOC exposure may decrease an embryo's ability to defend against oxidative damage [18].

Implantation rates for fresh cycles were unaffected, consistent with a study that reported that exposure of mouse embryos to acrolein (an unsaturated aldehyde) in the early cleavage period decreased numbers of blastocyst formed but that resulting blastocysts showed no difference in implantation rate [18]. We were able to maintain clinical pregnancy and live birth rates during the Absent period, likely owing to the increased number of embryos transferred. In a sense, our strict adherence to our embryo transfer guidelines, which called for more embryos to be transferred if the embryo quality was poor, protected our clinical pregnancy outcomes from the negative effects of poor air quality. Concordantly, along with a higher number of transferred embryos, we noted that the proportion of live births with dizygotic twins in the Absent period

Table 2 Clinical pregnancy outcomes of fresh embryo cycles

Outcome	Carbon 1	Absent	Carbon 2	<i>p</i> value
Clinical pregnancy rate	56.3	50.3	56.1	0.445
Implantation rate	36.6	34.7	34.6	0.848
Live birth rate	50.6	43.4	43.9	0.338
Proportion of males in all live births	44.6	53.3	46.7	0.471
Proportion of dizygotic twins in all live births	13.6	21.1	10.8	0.179

All values are percentages of cases

(21.1 %) was nearly twice that in the Carbon 1 (13.6 %) and Carbon 2 (10.8 %) periods. We suspect that this change would be statistically significant with more cases.

Certain differences in cycle characteristics can be explained by the changes in practice tied to the evolving trends noted in embryo development. The percent of cycles using ICSI was significantly increased during the Absent period, even though the percentage of couples with male-factor infertility was unchanged. This was due to the increased recommendation for ICSI owing to increased frequency of observing unanticipated failed or poor fertilizations during this period. And, as previously noted, the average number of embryos transferred was increased for day 5 transfers in the Absent period due to the fact that fewer patients qualified for single embryo transfer per the policy described in Methods.

The costs associated with this period of poor air quality were significant. Once the laboratory air issue had been identified, we knew that we could not in good conscience withhold this information from our patients, nor ignore the possibility that suboptimal air quality contributed to a cycle's failure. We were able to offer each patient, who received a fresh embryo transfer during the period of absent carbon filtration but did not conceive, one complimentary IVF cycle. Of the 81 complimentary cycles offered, 61 were performed; of the 20 patients who declined this offer, 9 had achieved a pregnancy prior to the offer. These 61 complimentary cycles resulted in 31 live births.

Our study had several potential limitations, most notably its retrospective nature. Evaluating the impact of air quality on early embryo development would ideally be studied in a randomized controlled fashion; however, this is very difficult to do due to its obvious ethical implications. Donated embryos may be used for this purpose, but the subsequent results could not be matched with clinical outcomes. While we suspect that altered VOC concentration was the cause of poor embryo development, this was not measured during our studied periods since it wasn't part of our quality assurance protocol, and the filter was replaced immediately when the absence was detected, without waiting to test an air sample from this period. Measurement of VOC concentration after the fact would not be meaningful, due to the lack of reference. Since the main purpose of activated carbon filter is VOC filtration, we believe it would be reasonable to speculate that the changes observed in the lack of this filter may be attributable to altered VOC concentrations in the IVF lab. Additionally, we caution that the lower numbers in the subanalyses of fresh-cycles, particularly conventional insemination and first cycles, might lead to a type II error (assuming no difference when, in fact, one exists).

We believe that the ability to control for seasonal variation as well as the large number of cultured embryos studied are the strengths of our study. Furthermore, the lack of knowledge about the removed carbon filter created a unique experimental design that provided coincidental randomization without any

change in the clinical and laboratory protocols, teams, and patient makeup. While recognizing that every laboratory experiences periods of suboptimal performance that wax and wane with time, this dramatic effect on embryo development, in only fresh and not cryopreserved embryos, has not been seen in our program before, or since.

Taken together, our results suggest that lack of carbon air filtration negatively affects early embryo development and that embryos are particularly sensitive to air quality changes in the peri-fertilization period, which leads to poorer in vitro development in the successive days. Our strict adherence to our transfer guidelines protected us from possibly worsened clinical outcome due to poor embryo quality. Given the dramatic reduction in embryo development, and the resolution of this phenomenon with the replacement of our air handler's carbon filtration, the importance of carbon filtration as an integral part to an efficient, successful embryology laboratory is clearly evident. The cost of such filtration seems insignificant compared to the potential costs that can result when it is not in place.

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Conflict of interest The authors declare that they have no conflict of interest.

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