Reproductive BioMedicine Online (2015) ■■, ■■–■■

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ARTICLE

Live births achieved via IVF are increased by improvements in air quality and laboratory environment

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Abstract Infertility is a common disease, which causes many couples to seek treatment with assisted reproduction techniques. Many factors contribute to successful assisted reproduction technique outcomes. One important factor is laboratory environment and air quality. Our facility had the unique opportunity to compare consecutively used, but separate assisted reproduction technique laboratories, as a result of a required move. Environmental conditions were improved by strategic engineering designs. All other aspects of the IVF laboratory, including equipment, physicians, embryologists, nursing staff and protocols, were kept constant between facilities. Air quality testing showed improved air quality at the new IVF site. Embryo implantation (32.4% versus 24.3%; $P < 0.01$) and live birth (39.3% versus 31.8%, $P < 0.05$) were significantly increased in the new facility compared with the old facility. More patients met clinical criteria and underwent mandatory single embryo transfer on day 5 leading to both a reduction in multiple gestation pregnancies and increased numbers of vitrified embryos per patient with supernumerary embryos available. Improvements in IVF laboratory conditions and air quality had profound positive effects on laboratory measures and patient outcomes. This study further strengthens the importance of the laboratory environment and air quality in the success of an IVF programme.

Published by Elsevier Ltd on behalf of Reproductive Healthcare Ltd.

http://dx.doi.org/10.1016/j.rbmo.2015.04.011
1472-6483/Published by Elsevier Ltd on behalf of Reproductive Healthcare Ltd.

Please cite this article in press as: Ryan J. Heitmann, Micah J. Hill, Aidita N. James, Tim Schimmel, James H. Segars, John M. Csokmay, Jacques Cohen, Mark D. Payson, Live births achieved via IVF are increased by improvements in air quality and laboratory environment, Reproductive BioMedicine Online (2015), doi: 10.1016/j.rbmo.2015.04.011

KEYWORDS: air quality, implantation, IVF, laboratory environment, live birth

Introduction

Infertility is a common disease, affecting about 10–15% of all couples (Healy et al., 1994), causing a majority of them to seek treatment with assisted reproduction techniques. In 2010, over 146,000 assisted reproduction technique cycles were carried out in the USA (Society for Assisted Reproductive Technologies, 2010). Many factors contribute to the success of assisted reproduction techniques, which include, but are not limited to, age, cause and duration of infertility, number of embryos transferred, day of embryo transfer and embryo quality. Laboratory environment and air quality are two other important metrics that have been shown to influence the success of assisted reproduction techniques. Previous studies have shown the negative effect of poor air quality on embryo development and ultimately on pregnancy outcomes (Boone et al., 1999; Cohen et al., 1997, 1998; Hall et al., 1998; Khoudja et al., 2013; Legro et al., 2010). The laboratory environment, especially air quality improvements, was also identified as a critical factor in top performing IVF laboratories in the USA (Van Voorhis et al., 2010).

In 2005, the Base Realignment and Closing Act identified military installations, which would either be closed or merged, with other installations to streamline services. As a result, all tertiary medical care services at Walter Reed Army Medical Center (WRAMC) were combined with Naval Medical Center Bethesda to form Walter Reed National Military Medical Center (WRNMMC) in August 2011. The Division of Reproductive Endocrinology and Infertility along with the ART Institute of Washington, Inc. was therefore transferred to a new state-of-the-art IVF facility constructed to accommodate this move.

The objective of the present study was to compare patient cycle outcomes along with laboratory measures for the last year of IVF cycles at the old facility and the first year at the new facility. No changes occurred in physician personnel, laboratory equipment or protocols during this time period. This offered a unique opportunity to compare outcomes between two different laboratory environmental conditions. Positive IVF outcomes, defined as live birth, was hypothesized to increase as a result of the new IVF facility and improved environment.

Material and methods

Study design

This retrospective cohort analysis of all fresh, autologous, IVF and intracytoplasmic sperm injection (ICSI) cycles was carried out at WRAMC, Washington DC, USA and WRNMMC, Bethesda, MD, USA, between January 2011 and October 2012. The last IVF cycle was carried out in August 2011 and the first cycle in the new facility was carried out in January 2012. This retrospective review and analysis of data without patient identifiers was collected as part of routine clinical care and approved International Review Board (IRB) exempt by the IRB at WRNMMC.

Laboratory conditions

The assisted reproduction technique clinic and laboratory at WRAMC was retrofitted into an available operating room space in 1996 when the military assisted reproduction technique programme was opened. The IVF laboratory, oocyte retrieval and embryo transfer rooms were each an operating room connected by two internal hallways. The air environment for the laboratory was provided by the hospital operating room air handling system and the laboratory often had negative pressure relative to outside common areas. The heating, ventilation and air conditioning (HVAC) air intake was located on the roof of the hospital; 10 feet from the cafeteria exhaust port. The volatile organic compound (VOC) level at the air intake was 184.6 $\mu g/m^3$. The old unit had an extremely unreliable HVAC system that did not always maintain the proper air balance along with proper temperature, humidity, or both.

In attempts to improve the air environment four Coda® tower filter units (Life Global, Guelph, Canada) were installed in the laboratory and two Coda® Loboy filter units in the operating room space. Each Coda® filter unit was maintained according to manufacturer's protocol with main stage filter changes occurring before each IVF series and HEPA filter changes occurring once a year. No further Coda® filter systems were installed into the embryo transfer room. Further information regarding the Coda® filter system can be found in the **Supplementary materials and methods**. The entire laboratory and operating room unit lacked a humidity control system; therefore, relative humidity was consistent with outside conditions. The internal environment of the incubators and isolettes was maintained by individual gas cylinders that were housed in the hallway directly adjacent to the laboratory space or to the particular pieces of equipment using the gas. The gasses were fed to each piece of equipment using sterilized tygon tubing that was run from one room to the next through a hole in the wall, filtered by Coda® Inline filter cartridges (Life Global). Each filter unit was maintained according to manufacturer's protocol with changes occurring before each IVF series. Lastly, the procedural and laboratory areas were lit by surgical lamps and fluorescent lighting. The lighting level was kept low to minimize detrimental effects.

Environmental conditions were improved by strategic engineering decisions during the planning phase for the construction of the new IVF suite at WRNNMC. The new air filtration system included clean steam to control humidity and air that was passed through paper pre-filters, an ultraviolet light section, two chemical beds of activated carbon mixed with potassium permanganate (Purafil, Doraville, GA) and a bank of high efficiency filters; air ultimately entered the suite through the final HEPA filters. Air temperature and humidity were controlled before reaching the laboratory and procedure room environments by a programmed control system. Further information detailing the HVAC unit and IVF laboratory set up can be found in the **Supplementary materials and methods.**

The new assisted reproduction technique suite consisted of four rooms separated by magnetic lock controlled doors with sealing designed to help maintain a positive pressure

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environment. The IVF laboratory had the highest level of positive air pressure with a decreasing pressure gradient as the air flows to each subsequent space until the external portions of the IVF suite are reached. The entire suite was built floor to ceiling to create a sealed box environment to prevent contamination from inter-floor or wall sources. All materials used were approved by an environmental specialist (Alpha Environmental, Jersey City, NJ, USA) to be low or no VOC, and the unit was off gassed by using a burn-off period of 6 weeks after construction was complete. Details about specific materials used are provided in **Supplementary materials and methods**. Access to the suite is limited to assisted reproduction technique personnel. During IVF cycles, only essential personnel in appropriate clean attire may enter the laboratory. All entrances are equipped with an adhesive mat to remove any dust or dirt from the soles of shoes. All personnel entering the laboratory spaces during an IVF cycle are required to wear clean scrubs, dedicated shoes or booties and a scrub cap. The same average number of personnel (four to five) worked in the laboratory area at both locations.

The entire suite undergoes 15 air changes per hour; temperature is maintained at 22.0–25.0°C, and relative humidity at 30–35%. The air supply for the laboratory is maintained at 50% outside air and 50% recirculated air. The air supplied to the IVF suite HVAC unit from the outside is provided from a region of the building that was previously determined to have the cleanest air (15.1 $\mu g/m^3$ total VOC content) around the hospital building. Recirculated air has been filtered by the hospital's HVAC unit. Both the outside and recirculated air is then pushed through our HVAC system starting after the prefilters. In the event of poor outside environmental conditions (i.e. construction, high pollution day), the air-handling unit can be 'submarined' and adjusted to run on 100% recirculated air.

The gas cylinders that supply the incubators and isolettes used for embryology procedures were confined to a dedicated tank room outside the IVF suite. This allowed the potentially unclean exteriors of the gas cylinders to be maintained separately from the laboratory environment. Gas was transported from the cylinder banks to various pieces of equipment through fluorinated ethylene propylene tubing that was installed as continuous lines during construction. This prevented potential contamination of gassed environments because there are no entry points created by connections.

All other aspects of the IVF laboratory, including equipment, physicians, nursing staff and protocols, were kept constant from the old facility (WRAMC) to the new facility (WRNMMC). The same culture media, fishes and needles were used at both the old and new facility. A new embryologist was added after the move to the new laboratory. A sub-analysis evaluating the effect of this addition was carried out. No new laboratory equipment was purchased as part of the move.

Air quality testing

Air quality testing was carried out at WRAMC and before construction at WRNMMC by Alpha Environmental Specialists. Assessed parameters included, but were not limited to, total VOC and total aldehyde counts.

Patients

All women, who were military healthcare beneficiaries, aged 18–42 years undergoing fresh, autologous, IVF cycles during the study time period were included in the analysis. Those who underwent embryo vitrification transfer cycles were excluded from the study. No donor oocyte or donor embryo cycles were performed at our facility.

Stimulation protocol

Ovarian stimulation protocols primarily used a mixed FSH and LH protocol under pituitary suppression with either gonadotrophin releasing hormone (GnRH) agonist long luteal suppression (Porter et al., 1984) or GnRH agonist microdose flare (Leondires et al., 1999; Scott and Navot, 1994). A mixed gonadotrophin protocol using recombinant FSH (follitropin-alfa) (gonal-F, EMD Serono, Rockland, MA, USA) and human menopausal gonadotrophin (Menopur, Ferring Pharmaceuticals, Parsippany, NJ, USA) was used for ovarian stimulation. Starting gonadotropin dose was based on patient age, day 3 FSH, previous cycle history and antral follicle count. Dosage adjustments during stimulation were made after assessment of transvaginal ultrasound and serum oestradiol assays by the IVF team. All changes were given final approval by the IVF director. Final oocyte maturation was triggered with 10,000 units of human chorionic gonadotrophin (HCG) when the two lead follicles were greater than or equal to 18 mm (mean) diameter. Those patients with oestradiol levels above 5000 pg/ml either had their cycle cancelled or underwent 'antagonist rescue' as previously described (Hill et al., 2012) and triggered with 5000 units of HCG. Ultrasound-guided transvaginal oocyte retrieval was carried out 36 h later. Insemination was by conventional IVF or ICSI, as clinically indicated. Between 80 and 85% of cycles had ICSI performed.

Ultrasound-guided embryo transfer using the afterload technique (Neithardt et al., 2005) was carried out either 3 or 5 days after oocyte retrieval. Luteal phase progesterone supplementation was started on the night of oocyte retrieval with either 50 mg daily progesterone-in-oil intramuscular injection (Freedom Pharmacy, Bayfield, MA, USA) or 100 mg three-times daily vaginal progesterone (endometrin, Ferring Pharmaceuticals, Parsippany, NJ, USA) and continued until positive serum HCG. After two positive and appropriately rising serum HCG measurements luteal support was switched to 100 mg twice-daily vaginal progesterone until 8 weeks estimated gestational age.

The number of embryos transferred was determined on the basis of clinic policy, age, cycle history and embryo morphology in accordance with the guidelines set by the American Society for Reproductive Medicine (ASRM) and the Society for Assisted Reproductive Technology (SART) (The Practice Committee of the American Society for Reproductive Medicine and the Practice Committee of the Society for Assisted Reproductive Technology, 2013). Starting in 2010, all patients undergoing blastocyst transfer were evaluated for a 'mandatory' single blastocyst transfer (Csokmay et al., 2011) and, if criteria were met, underwent single blastocyst transfer. Serum HCG levels were drawn 2 weeks after oocyte retrieval and ultrasound confirmation of all positive pregnancies was carried out at about 7 weeks gestation.

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Embryo culture and grading

In both locations, embryo culture was carried out in one of a number of Sanyo multi-gas incubators (Model MCO-5M) with internal HEPA filters as well as a gas supply that is filtered through Coda® Inline filter cartridges (LifeGlobal). All embryos were evaluated by an experienced embryologist using the grading systems described previously by Veeck (1986) for day 3 embryos and by Gardner and Schoolcraft (Gardner DK, 1999) for day 5 embryos. All embryos were graded on day 3 and again on day 5 if criteria for extended culture were met and permission was given by the couple to undergo extended embryo culture. The laboratory criteria for extended culture include at least three embryos of high quality (grade 1 or 2) on day 3. No cycle cancellations were attributed to extended embryo culture, and all patients meeting these criteria had an embryo available to transfer on day 5.

Comparison of day 3 and day 5 post-oocyte retrieval embryology was made to analyse any differences in the laboratory as a result of the new facility. Embryos on day 3 were evaluated for the number of cells and percentage of fragmentation. Embryos were grouped in the following categories: two to three cells; four to five cells; six to seven cells; and eight cells or more. The total number of embryos containing six or more cells with less than 20% fragmentation was also analysed. On day 5, the embryos were analysed for their degree of blastulation. The inner cell mass (ICM) and trophectoderm grades were compared for embryos graded as blastocyst, expanding blastocyst, and hatching or hatched blastocyst.

Embryo cryopreservation

Patients with supernumerary embryos remaining after ultrasound-guided transfer and meeting cryopreservation criteria were offered embryo cryopreservation. Criteria for cryopreservation include at least one day 5 or day 6 blastocyst of grade B/B morphology or better. Cryopreservation was carried out using the Fast Freeze® vitrification techniques described elsewhere (Stachecki et al., 2008). Embryos were stored at the IVF facility until a future vitrified and warmed embryo transfer cycle, a patient request for disposition or indefinitely.

Outcomes

The primary outcome evaluated was live birth. Secondary outcome measures included implantation, clinical pregnancy, multiple gestations, biochemical pregnancy, spontaneous abortion, number of embryos transferred, number of day 5 transfers and number of embryos cryopreserved. All calculations were made on a per-embryo transfer basis. Implantation was calculated by dividing the number of gestational sacs seen by the number of embryos transferred. Each individual percentage was then averaged to determine the composite implantation percentage for the cycle. Biochemical pregnancy was defined as any positive pregnancy followed by a spontaneous decrease to zero before ultrasound confirmation of pregnancy. Clinical pregnancy was defined as a rising serum HCG value and a gestational sac seen on transvaginal ultrasound. Spontaneous abortion was defined as pregnancy loss (up to 24 weeks) after a clinical pregnancy was diagnosed. Live birth was defined as any live infant born after 24 weeks estimated gestational age.

In August 2012, the long-standing IVF director left the uniformed service. This aspect is of potential importance given the extensive involvement of the IVF director in this IVF programme. All cycles are reviewed daily by the IVF team in conjunction with the IVF director and assistant IVF director. All medication changes and cycle decisions are made with the approval of the IVF director or assistant director. The assistant IVF director, of 3 years, was promoted to the IVF director. The new IVF assistant director was a recent Reproductive Endocrinology and Infertility (REI) fellow graduate. Both the new IVF director and assistant director were graduates of the National Institutes of Health/Walter Reed fellowship programme, and therefore had vast experience and knowledge of the IVF programme before taking their new respective roles. Given the transient nature of our population, patients are 'batched' into large IVF cycles once a quarter (four cycles per year). Only the last IVF cycle (October) of 2012 was carried out with a different IVF director. To evaluate any differences the IVF director may have had on the cycle outcomes, analysis of just the first three IVF cycles (January, April and July) in 2012 was compared with the 2011 cycles, during the time period the IVF director was constant.

Statistical analysis

Statistical analysis was conducted using Microsoft Excel, Vassar Stats (http://vassarstats.net) and Statistical Package for Social Sciences (SPSS) version 17 (IBM Inc., USA). Chi-squared analysis was used to compare implantation, clinical pregnancy and live birth between the two groups. Fisher's exact test was used to compare categorical day 3 and day 5 embryology data. Continuous parameters were compared using either a student's *t*-test or Mann-Whitney U test. Univariate regression analysis was used to correlate cycle year and other variables with assisted reproduction technique outcomes. Multivariate regression analysis was carried out to control for other variables that correlated with live birth such as age, cycle year, number of embryos transferred, day of embryo transfer and embryology grading metrics. $P < 0.05$ was considered statistically significant.

Results

Air quality testing was carried out at the old facility and again in the new IVF space at WRNMMC. Results showed improved air quality metrics and decreased environmental contaminants at the new IVF facility site. Total VOC (819.4 $\mu g/m^3$ versus 32 ug/m^3) and aldehyde (13.69 ug/m^3 versus 5.2 ug/m^3) concentrations in the IVF laboratory space were reduced in 2012 compared with 2011. The average temperature and humidity in the IVF suite at WRAMC was 20°C and 51.8%, whereas the new air handling system at WRNMMC now conditions the air to 25°C and 30%.

A total of 820 fresh cycles, 388 in 2011 at the old facility and 432 in 2012 at the new facility, were identified for the

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study time period. No differences were found in baseline demographics between the cohorts (Table 1). The average age for patients undergoing IVF in 2011 and 2012 was 33.9 ± 4.6 and 34.1 ± 4.7 years, respectively. No difference was found in the number of patients who underwent pituitary down regulation with either a luteal lupron or microdose flare protocol, the average days of stimulation, the average daily ampoules of gonadotrophins, average peak oestradiol level or use of antagonist rescue between the two study years (Table 2).

Comparison of the average number of oocytes retrieved (2011: 13.6 versus 2012: 13.0), mature oocytes (2011: 10.1 versus 2012: 9.93), and fertilized oocytes (2011: 6.7 versus 2012: 6.9) revealed no difference between the two study years. When the mature and fertilized oocyte percentage per patient was analysed, a higher percentage of fertilized oocytes per patient was observed in 2012 (2011: 65.7% versus 2012: 69.0%; $P = 0.04$). The use of ICSI was no different between the two study years (2011: 81.3% versus 2012: 85.6%) (Table 3).

A total of 377 transfers were carried out in 2011 and 406 transfers carried out in 2012. No differences were observed in the percentage of day 3 (2011: 50.9% versus 2012: 44.8%) and day 5 (2011: 49.1% versus 2012: 55.2%) transfers between the two cohorts. More single blastocyst transfers were carried out in 2012 compared with 2011 (2011: 36.8% versus 2012: 49.6%, $P = 0.01$). No differences were observed in the total number of patients who had at least one embryo available for cryopreservation (2011: 21.0% versus 2012: 25.6%) but, in patients with embryos available, there were more embryos vitrified per patient in 2012 compared with 2011 (2011: 1.8 versus 2012: 2.2, $P = 0.04$) (Table 4).

Implantation (2011: 24.3% versus 2012: 32.4%, $P < 0.01$), clinical pregnancy (2011: 40.8% versus 2012: 50.2%, $P = 0.01$), and live birth (2011: 31.8% versus 2012: 39.3%, $P = 0.03$) were significantly increased in 2012 compared with 2011. The percentage of both biochemical pregnancies (2011: 22.1% versus 2012: 19.6%, $P = 0.14$) and spontaneous abortions (2011: 12.3% versus 2012: 10.3%) were similar in 2011 and 2012. Multiple gestations were decreased in 2012 also. In 2011, 29.2% of the live births were twins compared with only 17.5% in 2012 ($P = 0.03$). No triplet or higher order multiple births took place in either cohort (Figure 1).

Table 1 Baseline demographics of the two study populations.

Category	*Old facility*	*New facility*
Age years	33.9 ± 4.6	34.1 ± 4.7
Cycle number	1.53	1.53
Day 3 FSH (U/L)	6.6 ± 2.2	7.23 ± 2.2
Primary infertility diagnosis		
Male factor	131 (35.2)	126 (29.4)
Tubal factor	102 (27.4)	103 (24.0)
Unexplained	59 (15.9)	111 (25.9)
Anovulation	32 (8.6)	45 (10.5)
Endometriosis	19 (5.1)	21 (4.9)
Diminished ovarian reserve	13 (3.5)	17 (4.0)
None listed	14 (3.8)	6 (1.4)
Uterine	2 (0.5)	0

No statistically significant differences were found between the two groups. Values presented as mean ± SD or number (%).

Table 2 Cycle characteristics.

Category	*Old facility*	*New facility*
Stimulation protocol		
Long luteal	24 (6.2)	25 (5.8)
Microdose flare	364 (93.8)	407 (94.2)
Mean days of stimulation	11.3 ± 2.6	11.3 ± 1.6
Mean total amps of gonadotrophins		
FSH (gonal-F)	26.6 ± 13.2	26.2 ± 13.1
Menotropins (Menopur)	18.9 ± 10.1	19.3 ± 11.4
Mean ampules/day of gonadotrophins	4.6 ± 2.9	4.5 ± 3.5
Mean peak oestradiol (pg/ml)	4052 ± 2097	4172.8 ± 2123
Antagonist rescue	52 (13.4)	65 (15)

No statistically significant differences were found between the two groups. Values presented as absolute value ± SD. Percentages listed in parenthesis.

Table 3 Laboratory outcomes.

Category	*Old facility*	*New facility*
Number of oocytes	5287	5607
Average per patient	13.6 ± 7.7	13.0 ± 7.3
Mature oocytes	3924	4271
Mean per patient	10.1 ± 6.0	9.93 ± 5.9
Fertilized oocytes	2576	2985
Mean per patient	6.7 ± 4.6	6.9 ± 4.8
Mature oocytes per patient (%)	74.5	76.9
Fertilization per patient (%0	65.7	69.0[a]
Fertilization via IVF	72 (18.7)	61 (14.4)
Fertilization via ICSI	314 (81.3)	363 (85.6)

[a] $P = 0.04$. Values presented as absolute value ± S.D. Percentages listed in parenthesis.
ICSI = intracytoplasmic sperm injection.

Table 4 Embryo transfer characteristics.

Category	*Old facility*	*New facility*	*P-value*
Number of transfers	377	406	
Day 3 transfer	192 (50.9)	182 (44.8)	NS
Mean number per patient	2.05	2.08	
Day 5 transfer	185 (49.1)	224 (55.2)	NS
Mean number per patient	1.69	1.61	
Day 5 single embryo transfer	68 (36.8)	111 (49.6)	0.01
Patients with ≥1 vitirfied embryos	79	104	NS
Mean number per patient	1.8	2.2	0.04

Listed as absolute value. Percentages listed in parenthesis.
NS = non-significant.

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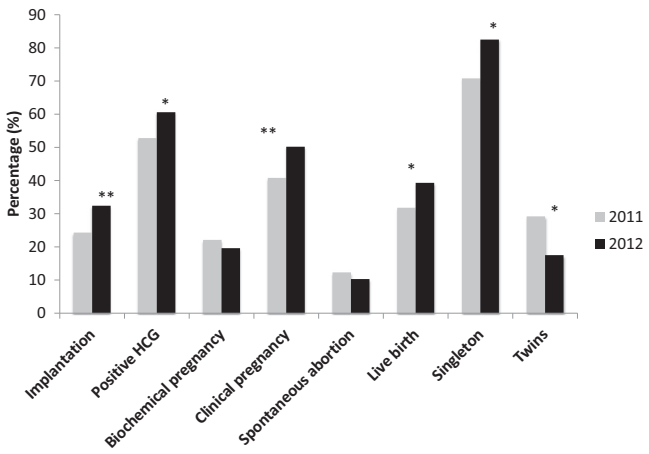

Figure 1 Comparison of IVF cycle outcomes per embryo transfer by cycle year (2011 versus 2012). Implantation reported as average implantation per patient. Chi-squared test was used for analysis. *$P < 0.05$; **$P < 0.01$.

Table 5 Comparison of embryology on day 3 according to the number of cells present.

Category	*2011*	*2012*
Total day 3 embryos	2548	2955
Total day 3 two-to-three cell	88 (3%)	120 (4%)
Mean	0.23	0.28
Total day 3 four-to-five cell	328 (13%)	341 (12%)
Mean	0.85	0.79
Total day 3 six-to-seven cell	517 (20%)	594 (20%)
Mean	1.34	1.38
Total day 3 eight + cell	1615 (63%)	1900 (64%)
Mean	4.17	4.4
Day 3 six-cell or greater 20% or less fragmentation	2044 (80%)	2408 (81%)
Mean	5.28	5.57

Expressed as absolute value (%). Mean value is mean number of embryos per patient. No statistically significant differences were found using Fisher's exact or chi-squared.

Comparison of day 3 embryology was conducted to assess any difference in laboratory embryology metrics between the two years. A total of 2548 embryo in 2011 and 2955 embryos in 2012 were analysed. No differences were observed in cellularity in 2011 compared with 2012. Similarly, no difference was observed in the number of embryos with six or more cells with 20% or more fragmentation on day 3 (**Table 5**). Blastulation improved in 2012 compared with 2011. This difference was seen in the number of expanding blastocysts (2011: 9% versus 2012: 20%, $P = 0.01$). No difference was found between the two cohorts when comparing the number of transferred embryo graded blastocyst or better (blastocyst, expanded blastocyst, hatching blastocyst) (2011: 64% versus 2012: 65%). No difference was observed when comparing the ICM grade (≥B) between transferred embryos (2011: 73% versus 79%). Improved trophectoderm cells grading (≥B) was seen in 2012 compared with 2011 (2011: 45% versus 2012: 57%, $P = 0.02$) (**Table 6**). No difference was observed in other

Table 6 Comparison of embryology of transferred embryos on day 5.

Category	*2011 n (%)*	*2012 n (%)*	*P-value n (%)*
Total number embryos transferred	300	333	
HB	0	1 (<1)	0.01[a]
XB	28 (9)	66 (20)	0.01
Blast	163 (54)	149 (45)	NS[b]
EB	95 (32)	95 (29)	
M	14 (5)	22 (7)	
ICM Grade			NS[a]
A	20 (10)	21 (10)	NS[c]
B	121 (63)	151 (69)	
C	51 (27)	47 (21)	
TE Grade			
A	5 (3)	1 (<1)	0.02[d]
B	81 (43)	123 (56)	
C	104 (55)	94 (43)	

Expressed as absolute value (%). Fisher's exct or chi-squared test used for analysis.
[a]Fisher's Exact Test comparing all groups.
[b]comparing transfer ≥blastocyst with EB and lower.
[c]comparing ICM ≥B to ICM C.
[d]comparing TE ≥B to TE C.
EB = early blastocyst; HB = hatching/hatched blastocyst; ICM = inner cell mass; M = morula; NS = non-significant; TE = trophectoderm; XB = expanding blastocyst.

laboratory outcomes (e.g. fertilization rate and ICSI rate) between embryologists (data not shown).

Univariate regression analyses demonstrated age (OR 0.93, 95% CI 0.89 to 0.95) and cycle year (OR 1.34, 95% CI 1.01 to 1.80) to be significantly correlated with live birth. Additional univariate analysis also demonstrated embryo stage, number of embryos transferred and supernumerary embryos to also be positively correlated with live birth. In multivariate regression controlling for the variables of patient age, cycle year, number of embryos transferred, and day of embryo transfer, IVF cycle year (OR 1.38, 95% CI 1.02 to 1.88) remained significant.

When accounting for embryo quality, univariate analysis demonstrated age (OR 0.93, 95% CI 0.89 to 0.95), cycle year (OR 1.34, 95% CI 1.01 to 1.80), embryo stage (OR 2.6, 95% CI 1.95 to 3.90), number of eight-cell embryos (1.11, 95% CI 1.07 to 1.12), number of high-quality day 3 embryos (1.09, 95% CI 1.05 to 1.12) and embryo expansion (OR 1.43, 95% CI 1.03 to 1.33) to be significantly correlated with live birth. In multivariate regression analysis accounting for embryo quality, age (OR 0.93, 95% 0.89 to 0.97), embryo stage (OR 1.62, 95% CI1.03 to 2.42), number of embryos transferred (OR 1.57, 95% CI 1.12 to 2.22) and embryo expansion (OR 1.38, 95% CI 1.02 to 1.87) were all significantly correlated with live birth.

Again, given the change in IVF directors in August 2012, a secondary analysis was carried out excluding the last IVF cycle in 2012 to analyse any potential influence the IVF director may have had in the results. No difference was found in the analysis when comparing the 2011 cycles with either all 2012 cycles or just the first three cycles of 2012 (data not shown). Given the addition of a new embryologist, each outcome measure was evaluated in the new facility with each embryologist in

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respect to ICSI, oocyte retrieval and embryo transfer. No differences were found in pregnancy outcomes with the analysis (data not shown).

Discussion

In this study, we have observed that, as a result of a new IVF laboratory facility built with consideration of environmental factors and a state-of-the-art air handling system, IVF outcomes were improved. Potentially confounding variables (patient variables, staff, protocols, medications, media, and laboratory equipment) remained unchanged. We have shown an increase in implantation and live birth without an increase in biochemical pregnancies or spontaneous abortion. The improvements in pregnancy were seen in conjunction with an increased use of single blastocyst transfer. More patients had improved blastulation with significantly more patients having a single blastocyst transfer in 2012. It is well established that multiple gestation pregnancies place both the mother and fetus at higher risk for adverse pregnancy and birth events compared with their singleton counterparts (Pinborg, 2005). Increasing single blastocyst transfer is an important aspect for clinics, and the field of IVF as a whole, to maximize pregnancy while minimizing twin and higher order multiple pregnancies.

Although, statistically, the total number of patients with cryopreserved embryos did not increase, those patients with supernumerary embryos had more embryos vitrified in 2012 compared with 2011. This may be partially explained by the increase in single blastocyst transfers during 2012 compared with 2011 but may also be a reflection of the improved laboratory settings for embryo growth and development. An increase in the percentage of fertilized mature oocytes was also observed. This makes plausible sense, as fertilization of oocytes through IVF or ICSI is the first time in the process of assisted reproduction techniques in which the effects of the laboratory setting and air quality can be observed and attributed to the laboratory setting.

Not too surprisingly, assessment of embryo quality showed increased embryo quality with the new facility. No difference was observed in the cellularity or number of high-quality embryo on day 3 after retrieval. The improvements, however, were seen in the increased blastulation and high-quality day 5 embryos in 2012. Interestingly, when comparing the ICM and trophectoderm cells grading, only trophectoderm cells grading was significantly improved. It has been recently reported that trophectoderm cells grade is a better predictor of live birth than ICM (Ahlstrom et al., 2011; Hill et al., 2013; Honnma et al., 2012). Trophectoderm cells grade in our multivariate regression analysis was not significantly correlated with live birth, perhaps owing to a smaller number of patients compared with these recent studies.

Air quality was shown to be improved with the construction and focus on a custom-built facility and air handling system. It is rather common for IVF laboratories to have higher VOC concentrations in indoor air. The source in the laboratory can vary by facility, but is related to gasses, sterile plasticware, people, equipment and off-gassing from construction. These results further point to the dramatic effect that the environment carries in the success of an IVF programme. The importance of properly designing and constructing an IVF laboratory are emphasized by these results.

The IVF laboratory environment and air quality are two important metrics commonly overlooked when assessing IVF performance settings. There can be profound positive or negative consequences on IVF success based on laboratory and air quality settings. Both the American Society for Reproductive Medicine and the European Society of Human Reproduction and Embryology recognize air quality and the laboratory environment as key factors in the guidelines for IVF laboratories (The Practice Committee of the American Society for Reproductive Medicine and the Practice Committee of the Society for Assisted Reproductive Technology, 2008; Magli et al., 2008). No recommendations, standards or requirements, however, are suggested in either document.

Cohen et al. (1997) examined the effect of ambient air in the IVF laboratory. They showed that outside air may actually be a better source than laboratory air because of the accumulation of VOC in outside air. Off gassing from sterile petri dishes, incubators, cleaning supplies, monitors, microscopes and even furniture all contribute compounds, which may negatively affect air quality. This study showed the importance of good air handling and filtration to minimize these effects. In 2012, Esteves and Bento (2013) compared IVF outcomes after ICSI between 1999 and 2010 in an IVF laboratory after implementation of Brazilian national clean room standards. The investigators showed an improvement in live birth with a decrease in spontaneous abortion between the two study periods. The large time frame of the study periods, however, allowed for other advancements in laboratory and assisted reproduction techniques to be potential confounders. In early 2013, a study from China also reported on improved IVF outcomes with air quality improvements (Khoudja et al., 2013). This study compared two filtration systems to one another. The study compared three different time periods with the two different systems: no carbon change filter change in the old system; the time period after carbon filter change in the old system; and the time period after instillation of the new system. The differences were seen between the time period of no carbon filter change and the new filtration system. When comparing the time period after carbon filter change to the new system, no differences were found. Despite their limitations, both studies are important in demonstrating proper air filtration and quality is critical in IVF outcomes.

The strengths of this study include its large patient volume, single-centre design and programme consistency. Laboratory environment along with personnel and staff consistency was cited as a common attribute of high performing IVF programmes in a survey by Van Voorhis et al. (2010). The stimulation protocols, staff, patient population, embryologists and embryology laboratory techniques and equipment remained constant throughout both time periods minimizing potential confounders. In our secondary analysis of the data looking at just cycles with a constant IVF director, we failed to show changes in any of the previously observed outcomes, proving that any changes were not solely due to personnel and can be eliminated as a potential confounder.

Weaknesses of this study include its retrospective design and the inherent biases associated with these studies. We also recognize the potential drawback of comparing these cohorts during two distinct time periods; however, the main difference in these two time periods was the move to the

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new laboratory with an updated air handling system. We recognize that measurements of air quality (VOCs) would ideally be made multiple times; unfortunately, the move between the two IVF sites was not carried out with a research study in mind. In this retrospective design, however, we cannot exclude the effect of undetected biases on the results. Given the difficulty in conducting randomized controlled trials to compare two different laboratory environments, analysing retrospective data is the next best alternative. As previously stated, most of the other aspects of the programme, to include the physicians, nurses, stimulation protocols, transfer protocols and laboratory equipment, remained constant. We recognize that, although other smaller changes (gas line tubing) took place, most of the changes between the two IVF laboratories involved the air handling system and other environmental factors. We feel this strengthens the argument, which is limited in the literature, that environmental quality greatly influences IVF laboratory and outcome results. We further controlled for potential confounding variables correlating with live birth to minimize baseline potential bias between the two cohorts.

In conclusion, our data suggest positive effects on IVF outcomes for patients. This study strengthens the available evidence on improvements in IVF outcomes after improvements in the IVF laboratory.

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.rbmo.2015.04.011.

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Declaration: The authors report no financial or commercial conflicts of interest.

Received 29 October 2014; refereed 24 April 2015; accepted 28 April 2015.