ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Perinatal outcomes in singleton pregnancies after in vitro fertilization cycles over 24 years

Jaimin S. Shah, M.D.,[a,b,c] Denis A. Vaughan, M.D.,[a,b,c] Angela Leung, M.D.,[a,b,c] Ann Korkidakis, M.D., M.P.H.,[a,b,c] Francesc Figueras, M.D., Ph.D.,[d,e,f] Desiree Garcia, Ph.D.,[g] Alan S. Penzias, M.D.,[a,b,c] and Denny Sakkas, Ph.D.[c]

[a] Department of Obstetrics and Gynecology, Beth Israel Deaconess Medical Center, Boston, Massachusetts; [b] Department of Obstetrics, Gynecology and Reproductive Biology, Harvard Medical School, Boston, Massachussetts; [c] Boston IVF, Waltham, Massachussetts; [d] Department of Maternal-Fetal Medicine, BCNatal, Barcelona Center for Maternal-Fetal and Neonatal Medicine, Hospital Sant Joan de Déu and Hospital Clínic, Universitat de BarcelonaBarcelona, Spain; [e] Institut de Recerca August Pi Sunyer, Barcelona, Spain; [f] Center for Biomedical Network Research on Rare Diseases, Instituto de Salud Carlos III, Barcelona, Spain; and [g] EUGIN, Barcelona, Spain.

Objective: To determine how a shift in clinical practice along with laboratory changes has impacted singleton perinatal outcomes after autologous in vitro fertilization (IVF) cycles.
Design: Retrospective cohort.
Setting: Single academic fertility clinic.
Patient(s): Singleton live births resulting from all IVF cycles (n = 14,424) from August 1, 1995 to October 31, 2019.
Intervention(s): None.
Main Outcome Measure(s): Live birth weight, large for gestational age (GA), small for GA, and preterm birth.
Result(s): The entire cohort consisted of 9,280 fresh and 5,144 frozen IVF cycles. Maternal age, parity, body mass index, neonatal sex, and GA at delivery were similar in both groups. There was a decrease in adjusted birth weight per year over the study period for the entire cohort of IVF cycles (−4.42g, 95% confidence interval [CI]: −6.63g to −2.22g). Rates of large for GA newborns decreased by 1.7% (95% CI: 2.9% to 0.6%) annually across the entire cohort of IVF cycles. Furthermore, there was a decrease in annual rates of preterm birth before 32 weeks by 3.2% (95% CI: 5.9% to 0.5%) across the entire cohort of IVF cycles. Trends were also seen in annual reduction of rates of preterm birth before 37 and 28 weeks.
Conclusion(s): With the gradual evolution of clinical and IVF laboratory practices, there has been a decrease in birth weight over 24 years for the entire cohort of IVF cycles. Concurrently, noteworthy practice changes have resulted in an improvement in IVF outcomes with decreased rates of large for GA newborns and preterm birth before 32 weeks for the entire cohort of IVF cycles. (Fertil Steril® 2021;■:■–■. ©2021 by American Society for Reproductive Medicine.)
Key Words: Birthweight, in vitro fertilization, large for gestational age, preterm birth, small for gestational age

Since the first successful in vitro fertilization (IVF) live birth in 1978 (1), IVF has become a dominant mode of treatment for infertility globally. Numerous clinical and laboratory innovations have led to significant improvements in infertility treatment efficacy and efficiency.

In recent years, markers of infertility treatment success have shifted from clinical pregnancy rates to focus on live birth rates that include perinatal outcomes. The Barker hypothesis (2) emphasizes the importance of the intrauterine milieu over the external environment on the long-term health of the offspring. Therefore, attention has been focused on birth weight (BW) as an important neonatal outcome parameter. Understanding how IVF affects perinatal outcomes, specifically live BW, has been of interest, but it is complex owing to the many variables in fertility treatment and pregnancy that can affect BW (3). Irrespective of these limitations, BW continues to be widely used as a metric for fetal health and a marker for obstetrical and neonatal outcomes. In addition, BW has been used as a validated marker for assessing IVF optimization (4–8).

Significant changes have occurred within the IVF laboratory over the last

Received December 15, 2020; revised January 12, 2021; accepted January 21, 2021.
J.S.S. has nothing to disclose. D.A.V. has nothing to disclose. A.L. has nothing to disclose. A.K. has nothing to disclose. F.F. has nothing to disclose. D.G. has nothing to disclose. A.S.P. has nothing to disclose. D.S. received personal fees from EMD Serono and is on the scientific advisory board for Igenomix.
This study was internally funded.
Reprint requests: Denny Sakkas, Ph.D., 130 Second Avenue, Waltham, Massachussetts 02451 (E-mail: dsakkas@bostonivf.com).

Fertility and Sterility® Vol. ■, No. ■, ■ 2021 0015-0282/$36.00

https://doi.org/10.1016/j.fertnstert.2021.01.043

24 years, including improvements in culture media, cryopreservation methods, incubators, and other laboratory equipment (9–11). Clinically, changes in stimulation protocols (12), medications, cycle monitoring, day of embryo transfer (ET), and most importantly, the number of embryos being transferred (13) have played an important role in the improvement of IVF outcomes.

The utilization of frozen ETs has drastically increased in the past decade because of improvements in cryopreservation methods with the adoption of vitrification (14). In fact, the number of frozen ETs performed has now surpassed that of fresh ETs in a number of countries, including the United States (15, 16). Frozen ETs have been shown to have some improved perinatal outcomes compared with those of fresh ETs (17) but have possibly increased rates of large for gestational age (LGA) neonates (18, 19). However, the live BWs resulting from frozen ETs were comparable to those resulting from spontaneously conceived pregnancies (7). When comparing singleton live births from IVF to those from natural conception, studies have shown an increased risk of low BW (LBW), very LBW, prematurity, and extreme prematurity (20, 21). It is thought that these poor perinatal outcomes may be related to the patients' underlying infertility rather than the IVF treatment itself (20, 21).

Our institution, a single large academic fertility clinic in the United States, has previously demonstrated stable BWs in newborns after IVF treatment from 1996 to 2013 when comparing the mean BW for each 6-month period between fresh and frozen cycles (22). In contrast, Castillo et al. (23) reported that live BW associated with fresh and frozen IVF cycles had gradually increased during the period from 1991 to 2015 in the United Kingdom. Differences in laboratory and clinical practices as well as the demographics of the patient population may have influenced these trends in BW. The assessment of this data over a longer term also provides clinics with some key quality assurance parameters.

In light of continued advancements in IVF, in particular the recent increase in frozen ET use, understanding the continued impact on perinatal outcomes provides critical and relevant information. The purpose of this study was to characterize the singleton perinatal outcomes of autologous fresh and frozen IVF cycles over an extended period of time during which a significant increase in frozen ET use occurred.

MATERIALS AND METHODS

This retrospective cohort study consisted of singleton live births from autologous fresh and frozen IVF cycles at a single large academic fertility clinic from August 1, 1995 to October 31, 2019. Institutional Review Board approval (2019D000895) was obtained from the Committee on Clinical Investigations at Beth Israel Deaconess Medical Center. Exclusion criteria consisted of gestational carriers (n = 234), reciprocal IVF cycles (n = 52), donated embryos (n = 11), or gamete intrafallopian transfers (n = 155). Patients were included if they had ≥ 1 fetal sac and a singleton delivery.

Baseline maternal and neonatal characteristics including age, body mass index (BMI), gravidity, parity, neonatal sex, gestational age (GA) at delivery, and live BW were collected for both groups. Clinical characteristics, such as number of oocytes retrieved, mode of fertilization, number of embryos transferred, and day of ET, were included. Perinatal outcomes of small for gestational age (SGA), LGA, and preterm birth (PTB) rates were calculated for all patients (24). At our institution, outcome data of any IVF cycle and live birth data are documented and reported to the Society for Assisted Reproductive Technology and Center for Disease Control and Prevention (CDC).

Several protocol changes for the laboratory and clinical aspects of IVF have occurred over the past 24 years and have been closely documented (Supplemental Table 1, available online). During this time, our institution has had 3 laboratory location changes. There has been a steady increase in patient volume, with currently >6,000 fresh and frozen autologous IVF cycles per year. Clinical changes evolved from the initial use of urinary gonadotropins to recombinant forms, increased use of leuprolide acetate triggers, change in luteal support protocols, and more blastocyst transfers. In the laboratory, the cryopreservation technique has changed from slow cooling at both the cleavage and blastocyst stages to vitrification of blastocysts alone. Furthermore, embryo culture media has progressed from the use of human tubal fluid (25) to complex sequential media (26, 27) to a single-step embryo culture medium that is currently in use (28). Protein sources in embryo culture media changed from patient serum to various forms of purified albumin. Other laboratory changes included different incubators and the gas phase used for embryo culture. Our institution has been certified by the International Organization for Standardization since 2008.

Statistical analysis was performed with descriptive statistics for the study population. A one-way analysis of variance was performed to compare the differences in live BW for cycle type, method of fertilization, day of transfer, and neonatal sex. A P value $<.05$ was considered statistically significant. The primary outcome was adjusted BW (maternal parity, GA at delivery, and neonatal sex) (29) with secondary outcomes of PTB before 37, 32, and 28 weeks; SGA (BW <10th %) and LGA (BW >90th %). The effects of the year of delivery on several perinatal outcomes were tested by linear or logistic regression. In the multivariate analysis, adjustment was performed for the following covariates: maternal age at cycle start; BMI at cycle start (imputation performed in 6,911 patients by linear regression with age as a predictor and using residuals for estimation adjustment with randomness of the missing values checked by Little's test); maternal parity; day of ET; fresh versus frozen IVF; and number of embryos transferred.

Additionally, a time series analysis was performed by aggregating data by the quarter of the year from 1995 Q4 to 2019 Q3. Mean and median values for quantitative outcomes and proportions for categorical outcomes were calculated within each quarter. The time series data were assumed to have no seasonality. To investigate for possible key changes across the timeline, exogenous break points were also tested by the Chow test when new technologies replaced older ones and included: vitrification at the blastocyst stage (in August 2011), benchtop incubators (June 2012), and single-step embryo culture medium (October 2013). The Chow test

evaluates if a single regression line or 2 separate regression lines fit the data best (30).

RESULTS

A total of 14,424 singleton live births resulted from fresh (n = 9,280) and frozen (n = 5,144) IVF cycles. Patient demographics, treatment factors, and clinical outcomes are shown in Table 1. Maternal age, BMI, GA at delivery, and neonatal sex were similar between the fresh and frozen IVF cycles groups. Preimplantation genetic testing was performed in 31.5% of frozen ET cycles with normal results. Figure 1 shows the number of deliveries and mean live BWs resulting from fresh and frozen IVF cycles over 24 years. Figure 1B demonstrates the increase in frozen ET use since 2015 at our institution. The mean (± SD) live BWs for fresh and frozen ETs were 3,275 g (± 605) and 3,430g (± 579), respectively; the BWs of singletons from fresh ETs were 155 g less than those of frozen ETs ($P<.001$). When comparing neonatal sex by cycle type (female fresh vs. female frozen ET and male fresh vs. male frozen ET), there was a statistically significant difference of 151 g for females and 161 g for males ($P<.001$). Eighty-five percent of fresh and 89% of frozen IVF cycles had term deliveries with 91% of fresh and 95% of frozen IVF cycles having a live BW >2,500 g. No significant differences were seen in mean BW when comparing day of ET (cleavage stage versus

TABLE 1

Patient and treatment factors in outcomes of fresh and frozen in vitro fertilization cycles.

Patient and treatment factors	Fresh ET	Frozen ET
Singletons	9,280	5,144
Patient factors		
Patient age (y)	35 (4.1) [20.7–45.7]	35.2 (3.8) [22.2–46.8]
Body mass index (kg/m^2)[a]	26.1 (5.5) [16.3–48.3]	25.8 (5.5) [15.8–50.3]
Maternal parity		
0	6,471 (69.7)	3,219 (62.6)
1	2,364 (25.5)	1,644 (32)
2	366 (3.9)	233 (4.5)
3+	79 (0.9)	48 (0.9)
Primary infertility diagnosis		
Endometriosis/uterine	551 (6.0)	212 (4.1)
Male factor	2,009 (21.6)	1,107 (21.5)
Ovarian/ovulatory	1,266 (13.6)	835 (16.2)
Recurrent pregnancy loss	19 (0.2)	102 (2)
Tubal	881 (9.5)	290 (5.6)
Unexplained	2,737 (29.5)	1,026 (19.9)
More than 1 reason	892 (9.6)	541 (10.5)
Other/unknown	925 (10.0)	1,031 (20.1)
Total oocytes collected	11.9 (6.7) [1–49]	—
Total number of 2 pronuclei	7.4 (4.7) [1–35]	—
Treatment factors		
Any preimplantation genetic testing	8 (0.1)	1,622 (31.5)
Intracytoplasmic sperm injection	3,320 (35.8)	—
Type of embryo transfer		
Cleavage (day 1–3)	6,582 (70.9)	879 (17.1)
Blastocyst (day 5–7)	2,698 (29.1)	4,265 (82.9)
Total embryos transferred		
1	2,601 (28)	3,834 (74.5)
2	3,681 (39.7)	937 (18.2)
3	1,818 (19.6)	278 (5.4)
4+	1,180 (12.7)	95 (1.8)
Outcomes		
Unadjusted BW (g)	3,275 (605) [380–5,812]	3,430 (579) [468–5,670]
Adjusted BW[b] (g)	3,363 (587) [640–6,703]	3,514 (568) [738–5,859]
Gestational age (wk)	38.4 (2.1) [22.1–42.9]	38.8 (1.9) [23.4–43.1]
Preterm birth		
<37 wk	1,450 (15.6)	617 (12)
<32 wk	173 (1.9)	60 (1.2)
<28 wk	51 (0.5)	23 (0.4)
Number of male neonates[c]	4,725 (51.3)	2,612 (51)
Number of female neonates[c]	4,493 (48.7)	2,511 (49)
Small for gestational age <10%	678 (7.3)	259 (5)
Large for gestational age >90%	976 (10.5)	836 (16.3)
Spontaneous fetal reduction	671 (7.2)	132 (2.6)

Note: Continuous variables are presented as mean (SD) [range] and categorical variables as row total (%). BW = birth weight; ET = embryo transfer.

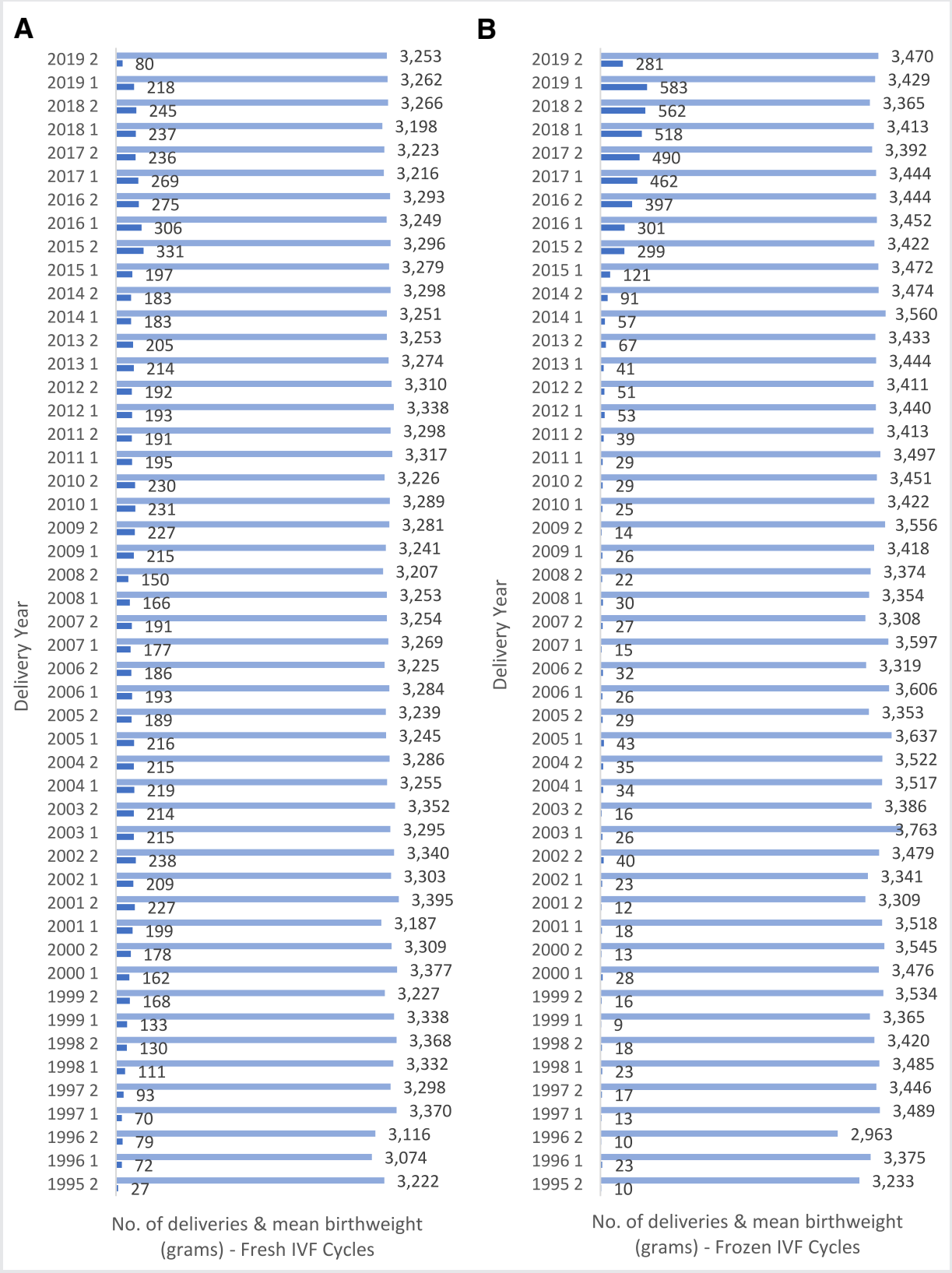
[a] Body mass index was not listed for 6,911 patients.

[b] Adjusted for parity, gestational age at delivery, and neonatal sex.

[c] Neonatal sex was not listed for 62 fresh and 21 frozen births.

Shah. Perinatal outcomes after IVF cycles. Fertil Steril 2021.

FIGURE 1



Mean birth weights for autologous fresh (A) and frozen (B) in vitro fertilization IVF cycles. Mean singleton birth weights are provided in the *light blue bar* whereas the number of singleton deliveries is specified in the *dark blue bar* in 6-month intervals.

Shah. Perinatal outcomes after IVF cycles. Fertil Steril 2021.

TABLE 2

Linear regression estimated annual trends in outcome measures for the entire cohort of in vitro fertilization cycles (n = 14,424).

	Univariate	Multivariate[a]
Patient and treatment factors	**Coefficient/Odds ratio (95% confidence interval)**	
Gestational age (wk)	**0.01 (0.005, 0.015)**[b]	0.001 (−0.007, 0.008)
Unadjusted birth weight (g)	**3.38 (1.94, 4.82)**[b]	−1.7 (−3.99, 0.52)
Adjusted birth weight (g)	**2.46 (1.05, 3.88)**[b]	**−4.42 (−6.63, -2.22)**[b,c]
Preterm birth <37 wk	**0.988 (0.981, 0.995)**[b]	0.992 (0.981, 1.002)
Preterm birth <32 wk	**0.968 (0.941, 0.975)**[b]	**0.968 (0.941, 0.995)**[d]
Preterm birth <28 wk	**0.959 (0.929, 0.99)**[d]	0.971 (0.925, 1.02)
Small for gestational age <10%	0.996 (0.987, 1.006)	1.011 (0.996, 1.026)
Large for gestational age >90%	1.03 (0.996, 1.011)	**0.983 (0.971, 0.994)**[e]
Spontaneous fetal reduction	**0.931 (0.921, 0.94)**[b]	1.013 (0.998, 1.028)

[a] Adjusted for age at cycle start, body mass index (imputed in 6,911 patients by linear regression), parity (0 vs. 1, 2, 3+), day of embryo transfer (cleavage vs. blastocyst), fresh vs. frozen embryo transfer, number of transferred embryos (1 vs. 2, 3, 4+).
[b] Statistically significant values are bolded with $P \leq .001$.
[c] Adjusted for the same covariates except for parity (as the dependent variable is already adjusted for it).
[d] Statistically significant values are bolded with $P \leq .02$.
[e] Statistically significant values are bolded with $P < .005$.

Shah. Perinatal outcomes after IVF cycles. Fertil Steril 2021.

blastocyst) in both fresh ($P=.31$) and frozen ($P=.20$) groups and when comparing IVF versus intracytoplasmic sperm injection in fresh IVF cycles ($P=.23$).

Table 2 shows a multivariate analysis estimating annual trends of different perinatal outcomes by linear regression. The adjusted BW decreased over the entire study period for the entire cohort of IVF cycles (−4.42g, 95% confidence interval [CI]: −6.63g to −2.22g; $P<.001$). Similarly, when analyzing by cycle type separately, there was a decrease in adjusted BW per year over the study period for fresh (−3.51g, 95% CI: −5.98g to −1.05g; $P=.005$) and frozen (−8.64g, 95% CI: −14.05g to −3.24g; $P=.002$) IVF cycles. There was a 1.7% decrease annually in rates of LGA newborns ($P=.004$) for the entire cohort of IVF cycles. Furthermore, the rate of PTBs before 32 weeks decreased 3.2% annually ($P=.02$) for the entire cohort of IVF cycles.

The effects of delivery year and reproductive parameters on BW, LGA, and PTB before 32 weeks were tested by linear or logistic regression for the entire cohort of IVF cycles (Supplemental Table 2, available online). A parity of 1 or 2 was significantly correlated with a larger BW ($P<.001$) and fresh versus frozen ET was significantly correlated with a smaller BW ($P<.001$). Increasing parity was significantly associated with an increasing rate of LGA. Significant lower rates of LGA were noted in fresh compared with frozen IVF cycles. None of the parameters significantly affected the rate of PTB before 32 weeks.

Exogenous break points for the 3 major changes in our laboratory were also tested for temporal associations in our entire cohort of IVF cycles (Table 3). The 3 changes in the laboratory were significant break points for LGA and GA at delivery. Furthermore, vitrification at the blastocyst stage and

TABLE 3

Testing of exogenous break points over the timeline of 1995 to 2019 for the entire cohort of in vitro fertilization cycles (n = 14,424)

	August 2011	June 2012	October 2013
Date and change	**Vitrification at blastocyst stage**	**Benchtop incubators**	**Single-step embryo culture media**
Perinatal outcomes	F-test values[a]		
Gestational age at delivery	**12.3**[b]	**7.87**[b]	**4.28**[c]
Birth weight	**3.16**[c]	2.94	2.81
PTB <37 wk	2.71	1.39	0.54
PTB <32 wk	0.08	0.23	0.05
PTB <28 wk	1.73	0.5	0.07
Small for gestational age <10%	**6.09**[d]	**4.13**[c]	1.43
Large for gestational age >90%	**5.38**[d]	**5.61**[d]	**4.8**[c]

Note: PTB = preterm birth.
[a] Chow test evaluates if a single regression line or 2 separate regression lines fit the data best.
[b] Statistically significant values are bolded with $P \leq .001$.
[c] Statistically significant values are bolded with $P < .05$.
[d] Statistically significant values are bolded with P value $< .007$.

Shah. Perinatal outcomes after IVF cycles. Fertil Steril 2021.

introduction of benchtop incubators were significant break points for SGA, with just vitrification at the blastocyst stage a significant break point for BW. PTB was not significantly affected by the aforementioned laboratory changes.

DISCUSSION

In this large retrospective cohort study, we noted a significant annual decrease in rates of LGA newborns and PTB before 32 weeks for the entire cohort of IVF cycles over the course of 24 years. This is clinically relevant because both LGA (31) and PTB (32) have direct perinatal consequences and delayed effects into infancy through adulthood. The improved outcomes were observed over the study period in which numerous clinical and laboratory changes were made, but in which the adjusted BW decreased by a few grams every year for the entire cohort of IVF cycles.

Consistent with previous research (7, 17, 33–37), our study, over 24 years, showed singletons born from fresh ETs were on average 155 g lighter than those from frozen ETs, a difference of just under 5% of mean total BW. Our previous study showed a difference of 173 g (22). The precise cause of the fetal weight difference is unknown but speculation includes the impact of supraphysiologic hormone levels, caused by ovarian stimulation in fresh IVF cycles, on the uterine environment (38).

Evaluating changes in the laboratory and how these affect perinatal outcomes can be difficult given the many factors that are involved. We identified key time points in our study period when new technologies replaced older ones. The change from slow cooling of cleavage stage embryos to vitrification of blastocysts and the introduction of solid state, triple gas-controlled benchtop incubators and single-step embryo culture media are examples.

The method of embryo cryopreservation has been scrutinized because of the increasing role that cryopreserved embryos play in fertility treatment worldwide. Ernstad et al. (39) compared perinatal outcomes of vitrified blastocysts with those of slow-cooled cleavage stage embryos and reported a higher risk of PTB for vitrified blastocysts but no differences in LBW newborns, SGA newborns, fetal macrosomia, or LGA newborns. In contrast, Gu et al. (40) reported no difference in PTB when comparing vitrification with slow cooling of cleavage stage embryos while affirming no difference in LBW, SGA, LGA, or macrosomia. In our study, replacing slow cooling with vitrification appears to have contributed to the reduction in LGA while also reducing SGA births and had a positive impact on BW and GA at delivery for the entire cohort of IVF cycles.

A number of papers have investigated the impact of the embryo culture media on BW with no conclusive results (10, 41–47). However, changing to a single-step embryo culture media and introducing high-performance benchtop incubators may have also played a role in the decrease in LGA.

Although adoption of vitrification in lieu of slow cooling, introduction of benchtop incubators, and single-step embryo culture media impacted certain perinatal outcomes, they did not seem to impact PTB. The etiology of the decrease in PTB before 32 weeks in our study is unclear.

Our results are concordant with 2 meta-analyses (48, 49) comparing fresh and frozen IVF cycles in demonstrating that frozen ETs result in a decreased risk of SGA, LBW, and PTB but our findings differ with respect to the risk of LGA. In contrast, our study showed a rate of 1.7% annual reduction in LGA for both fresh and frozen IVF cycles. Our study has the advantage of a longer study period than previously published work and a robust sample size of frozen ETs because of a large increase in the use of frozen embryos over the past 5 years at our center. Whereas other studies have shown a decrease in PTB only for frozen ET cycles, we showed a significant annual decrease in PTB before 32 weeks with decreasing trends for PTB before 37 and 28 weeks for the entire cohort of IVF cycles.

Maternal parity is an independent determinant of BW (50). Our study was consistent with previous reports (29, 51) showing heavier newborns at birth with increasing parity. This also supported the postulate that LGA is correlated with parity. In our study, 8.7% of newborns from fresh and 14% from frozen ETs were macrosomic (>4,000 g). In contrast, the CDC (52) reported in 2018 a 7.9% incidence of macrosomia in infants born nationally to women aged 20–44 years. The significant decline in our LGA incidence over the study period suggests that the different incidence of macrosomia in our study population compared with national data may reflect delivery at later GAs among our patients.

We reported fewer cases of LBW newborns after frozen ET (5.3%) than after fresh ET (9.1%), which is comparable to CDC national data (8.7%) (52). Finally, the percentage of term deliveries for fresh (85%) and frozen (89%) IVF cycles mirrored the CDC national average (89%) for women 20–44 years old (52).

We reported a mean decrease of 4.4 g in BW per year over the 24-year study period using a multivariate analysis for the entire cohort of IVF cycles. In contrast, a cross sectional study from 1991 to 2015 in the United Kingdom by Castillo et al. (23) included 2,780 singleton live births after autologous IVF cycles and reported a 7.4 g increase in BW per year. The different findings may stem from varied patient populations and practice patterns. The decrease in adjusted BW correlates with the decreased rate of LGA in our entire cohort. Future analysis may allow us to discern if recent laboratory, clinical, or demographic changes can explain these trends.

Our study has several notable strengths. Our large cohort represents a reliable dataset from a single institution with a well-documented timetable of changes in the clinic and laboratory over the 24-year study period. To our knowledge, this is the first and largest study to show a decrease in rates of LGA newborns and PTB before 32 weeks for all autologous IVF cycles, fresh and frozen. Having carefully documented key changes in our laboratory including adoption of vitrification of blastocysts in lieu of slow cooling of cleavage stage embryos; extended culture of embryos in a single-step culture medium; and use of high-performance benchtop incubators, we were able to perform a detailed analysis to test for temporal associations that may hint at causality for some of our significant findings. Given certain patients may have had more than one newborn in our cohort, linear regression of BW was performed with maternal parity to avoid confounding

error. Furthermore, we were able to validate previous documented findings of BW differences in neonatal sex, cycle type, and as a function of maternal parity.

Several limitations, however, need to be acknowledged. As a retrospective study, there remains the possibility of unknown confounders limiting conclusions about cause and effect. Changes in obstetrical management could not be accounted for; however, the incidence in PTB before 37 and 34 weeks stayed consistent per the national CDC data for all deliveries from 2010 to 2017 (52). Imputation of BMI was performed for the patients with missing data by linear regression with randomness checked by Little's test. This is relevant as maternal BMI is a known risk factor for LGA newborns. When examining for significant break points in the time series analysis, a limitation is that the exact reason, clinical or laboratory related, may not be truly apparent despite differences in time points being indicated. The data were from a single institution in the northeast of the United States and may not be fully generalizable to all geographic locations and patient populations.

CONCLUSION

In conclusion, our study showed annual reductions in the rates of LGA newborns and PTB before 32 weeks for the entire cohort of IVF cycles over a 24-year period. Both findings were important positive surrogate markers of neonatal health. We also demonstrated a small decrease in live BW per year despite the many clinical and laboratory changes. These data provided reassurance that changes in IVF over many years have positively impacted perinatal outcomes. Further research is needed to study the effects of IVF on perinatal outcomes in other patient populations and investigate how specific changes in clinical or laboratory management may individually impact perinatal outcomes with the goal of continued improvement.

REFERENCES

1. Steptoe PC, Edwards RG. Birth after the reimplantation of a human embryo. Lancet 1978;2:366.
2. Barker DJ. The fetal and infant origins of adult disease. Br Med J 1990;301:1111.
3. Kondapalli LA, Perales-Puchalt A. Low birth weight: is it related to assisted reproductive technology or underlying infertility? Fertil Steril 2013;99:303–10.
4. Dumoulin JC, Land JA, Van Montfoort AP, Nelissen EC, Coonen E, Derhaag JG, et al. Effect of in vitro culture of human embryos on birthweight of newborns. Hum Reprod 2010;25:605–12.
5. Baker VL, Brown MB, Luke B, Conrad KP. Association of number of retrieved oocytes with live birth rate and birth weight: an analysis of 231,815 cycles of in vitro fertilization. Fertil Steril 2015;103:931–8.e2.
6. Griesinger G, Kolibianakis EM, Diedrich K, Ludwig M. Ovarian stimulation for IVF has no quantitative association with birthweight: a registry study. Hum Reprod 2008;23:2549–54.
7. Shih W, Rushford DD, Bourne H, Garrett C, McBain JC, Healy DL, et al. Factors affecting low birthweight after assisted reproduction technology: difference between transfer of fresh and cryopreserved embryos suggests an adverse effect of oocyte collection. Hum Reprod 2008;23:1644–53.
8. Nelissen EC, Van Montfoort AP, Coonen E, Derhaag JG, Geraedts JP, Smits LJ, et al. Further evidence that culture media affect perinatal outcome: findings after transfer of fresh and cryopreserved embryos. Hum Reprod 2012;27:1966–76.
9. Zandstra H, Van Montfoort APA, Dumoulin JCM. Does the type of culture medium used influence birthweight of children born after IVF? Hum Reprod 2015;30:530–42.
10. Kleijkers SHM, Mantikou E, Slappendel E, Consten D, Van Echten-Arends J, Wetzels AM, et al. Influence of embryo culture medium (G5 and HTF) on pregnancy and perinatal outcome after IVF: a multicenter RCT. Hum Reprod 2016;31:2219–30.
11. Chronopoulou E, Harper JC. IVF culture media: past, present and future. Hum Reprod Update 2015;21:39–55.
12. Horsthemke B, Ludwig M. Assisted reproduction: the epigenetic perspective. Hum Reprod Update 2005;11:473–82.
13. De Sutter P, Gerris J, Dhont M. A health-economic decision-analytic model comparing double with single embryo transfer in IVF/ICSI: a sensitivity analysis. Hum Reprod 2003;18:1361.
14. Rienzi L, Gracia C, Maggiulli R, LaBarbera AR, Kaser DJ, Ubaldi FM, et al. Oocyte, embryo and blastocyst cryopreservation in ART: systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance. Hum Reprod Update 2017;23:139–55.
15. Centers for Disease Control and Prevention. 2016 Assisted reproductive technology fertility clinic success rates report. Atlanta (GA): US Department of Health and Human Services. Available at: ftp://ftp.cdc.gov/pub/Publications/art/ART-2016-Clinic-Report-Full.pdf. Accessed March 22, 2021.
16. Centers for Disease Control and Prevention, American Society for Reproductive Medicine, Society for Assisted Reproductive Technology. 2016 Assisted reproductive technology national summary report. Atlanta (GA): US Department of Health and Human Services; 2018. Available at: https://www.cdc.gov/art/pdf/2018-report/ART-2018-Clinic-Report-Full.pdf. Accessed March 22, 2021.
17. Pelkonen S, Koivunen R, Gissler M, Nuojua-Huttunen S, Suikkari AM, Hydén-Granskog C, et al. Perinatal outcome of children born after frozen and fresh embryo transfer: the Finnish cohort study 1995–2006. Hum Reprod 2010;25:914–23.
18. Berntsen S, Pinborg A. Large for gestational age and macrosomia in singletons born after frozen/thawed embryo transfer (FET) in assisted reproductive technology (ART). Birth Defects Res 2018;110:630–43.
19. Korosec S, Frangez HB, Steblovnik L, Verdenik I, Bokal EV. Independent factors influencing large-for-gestation birth weight in singletons born after in vitro fertilization. J Assist Reprod Genet 2016;33:9–17.
20. Luke B, Gopal D, Cabral H, Stern JE, Diop H. Pregnancy, birth, and infant outcomes by maternal fertility status: the Massachusetts Outcomes Study of Assisted Reproductive Technology. Am J Obstet Gynecol 2017;217:327.e1–14.
21. Ombelet W, Martens G, Bruckers L. Pregnant after assisted reproduction: a risk pregnancy is born! 18-years perinatal outcome results from a population-based registry in Flanders, Belgium. Facts Views Vis Obgyn 2016;8:193–204.
22. Maas K, Galkina E, Thornton K, Penzias AS, Sakkas D. No change in live birthweight of IVF singleton deliveries over an 18-year period despite significant clinical and laboratory changes. Hum Reprod 2016;31:1987–96.
23. Castillo CM, Horne G, Fitzgerald CT, Johnstone ED, Brison DR, Roberts SA. The impact of IVF on birthweight from 1991 to 2015: a cross-sectional study. Hum Reprod 2019;34:920–31.
24. Alexander GR, Himes JH, Kaufman RB, Mor J, Kogan M. A United States national reference for fetal growth. Obstet Gynecol 1996;87:163–8.
25. Quinn P. Enhanced results in mouse and human embryo culture using a modified human tubal fluid medium lacking glucose and phosphate. J Assist Reprod Genet 1995;12:97–105.
26. Gardner DK, Lane M. Culture of viable human blastocysts in defined sequential serum-free media. Hum Reprod 1998;13(Suppl 3):148–59.
27. Quinn P. Culture systems: sequential. Methods Mol Biol 2012;912:211–30.

28. Machtinger R, Racowsky C. Culture systems: single step. Methods Mol Biol 2012;912:199–209.
29. Gardosi J, Francis A. A customized standard to assess fetal growth in a US population. Am J Obstet Gynecol 2009;201:25.e1–7.
30. Nielsen B, Whitby A. A joint Chow test for structural instability. Econometrics 2015;3:156–86.
31. Boulet SL, Alexander GR, Salihu HM, Pass MA. Macrosomic births in the United States: determinants, outcomes, and proposed grades of risk. Am J Obstet Gynecol 2003;188:1372–8.
32. Saigal S, Doyle LW. An overview of mortality and sequelae of preterm birth from infancy to adulthood. Lancet 2008;371:261–9.
33. Li Z, Wang YA, Ledger W, Sullivan EA. Birthweight percentiles by gestational age for births following assisted reproductive technology in Australia and New Zealand, 2002–2010. Hum Reprod 2014;29:1787–800.
34. Hwang SS, Dukhovny D, Gopal D, Cabral H, Diop H, Coddington CC, et al. Health outcomes for Massachusetts infants after fresh versus frozen embryo transfer. Fertil Steril 2019;112:900–7.
35. Ozgur K, Berkkanoglu M, Bulut H, Humaidan P, Coetzee K. Perinatal outcomes after fresh versus vitrified-warmed blastocyst transfer: retrospective analysis. Fertil Steril 2015;104:899–907.e3.
36. Roy TK, Bradley CK, Bowman MC, McArthur SJ. Single-embryo transfer of vitrified-warmed blastocysts yields equivalent live-birth rates and improved neonatal outcomes compared with fresh transfers. Fertil Steril 2014;101:1294–301.
37. Litzky JF, Boulet SL, Esfandiari N, Zhang Y, Kissin DM, Theiler RN, et al. Effect of frozen/thawed embryo transfer on birthweight, macrosomia, and low birthweight rates in US singleton infants. Am J Obstet Gynecol 2018;218:433.e1–10.
38. Weinerman R, Mainigi M. Why we should transfer frozen instead of fresh embryos: the translational rationale. Fertil Steril 2014;102:10–8.
39. Ginström Ernstad E, Spangmose AL, Opdahl S, Henningsen AA, Romundstad LB, Tiitinen A, et al. Perinatal and maternal outcome after vitrification of blastocysts: a Nordic study in singletons from the CoNARTaS group. Hum Reprod 2019;34:2282–9.
40. Gu F, Li S, Zheng L, Gu J, Li T, Du H, et al. Perinatal outcomes of singletons following vitrification versus slow-freezing of embryos: a multicenter cohort study using propensity score analysis. Hum Reprod 2019;34:1788–98.
41. Thompson J, Davies M, Pool T, Rienzi L, Nagy PZ, Hardarson T, et al. Birthweight and the effects of culture media. Hum Reprod 2017;32:717–8.
42. Kleijkers SHM, Van Montfoort APA, Smits LJM, Coonen E, Derhaag JG, Evers JLH, et al. Age of G-1 PLUS v5 embryo culture medium is inversely associated with birthweight of the newborn. Hum Reprod 2015;30:1352–7.
43. Lemmen JG, Pinborg A, Rasmussen S, Ziebe S. Birthweight distribution in ART singletons resulting from embryo culture in two different culture media compared with the national population. Hum Reprod 2014;29:2326–32.
44. Vergouw CG, Kostelijk EH, Doejaaren E, Hompes PGA, Lambalk CB, Schats R. The influence of the type of embryo culture medium on neonatal birthweight after single embryo transfer in IVF. Hum Reprod 2012;27:2619–26.
45. Lin S, Li M, Lian Y, Chen L, Liu P. No effect of embryo culture media on birthweight and length of newborns. Hum Reprod 2013;28:1762–7.
46. Eskild A, Monkerud L, Tanbo T. Birthweight and placental weight; do changes in culture media used for IVF matter? Comparisons with spontaneous pregnancies in the corresponding time periods. Hum Reprod 2013;28:3207–14.
47. De Vos A, Janssens R, VandeVelde H, Haentjens P, Bonduelle M, Tournaye H, et al. The type of culture medium and the duration of in vitro culture do not influence birthweight of ART singletons. Hum Reprod 2015;30:20–7.
48. Maheshwari A, Pandey S, Shetty A, Hamilton M, Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in vitro fertilization treatment: a systematic review and meta-analysis. Fertil Steril 2012;98:368–77.e1–e9.
49. Maheshwari A, Pandey S, Amalraj Raja E, Shetty A, Hamilton M, Bhattacharya S. Is frozen embryo transfer better for mothers and babies? Can cumulative meta-analysis provide a definitive answer? Hum Reprod Update 2018;24:35–58.
50. Halileh S, Abu-Rmeileh N, Watt G, Spencer N, Gordon N. Determinants of birthweight: gender based analysis. Matern Child Health J 2008;12:606–12.
51. Nelson SM, Lawlor DA. Predicting live birth, preterm delivery, and low birth weight in infants born from in vitro fertilisation: a prospective study of 144,018 treatment cycles. PLoS Med 2011;8:e1000386.
52. Martin JA, Hamilton BE, Osterman MJK, Driscoll AK. Births: final data for 2018. Natl Vital Stat Rep 2019;68:1–47.