Perinatal outcomes in singleton live births after blastocyst transfer: an analysis of 60,926 in vitro fertilization cycles from the United Kingdom

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Objective: To compare perinatal outcomes between singleton live births after blastocyst-stage and cleavage-stage fresh embryo transfer using data from all United Kingdom licensed fertility clinics.
Design: A cohort study.
Setting: Not applicable.
Patient(s): A total of 60,926 in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycles resulting in a singleton live birth after blastocyst-stage and cleavage-stage fresh embryo transfer between 2012 and 2018.
Intervention(s): Baseline characteristics between IVF/ICSI blastocyst and cleavage-stage transfer groups were compared using the χ^2 test for categorical/dichotomized variables and the Mann-Whitney test for continuous variables. Statistical significance was set at $<.05$. Association between perinatal outcomes and blastocyst transfer compared with cleavage-stage transfer was assessed using multinomial logistic regression, adjusting for confounders selected using directed acyclic graphs (95% confidence interval [CI], adjusted relative risk ratio [aRRR]). A subgroup analysis included cycles in women undergoing their first IVF/ICSI cycle.
Main Outcomes Measure(s): Gestational age at birth and birth weight.
Result(s): The blastocyst group comprised 42,677 IVF/ICSI cycles and cleavage-stage group 18,249 cycles. There was likely little to no difference in the risk of preterm (aRRR, 1.07; 95% CI, 1.00–1.15) and very preterm birth (aRRR, 1.05; 95% CI, 0.91–1.21) in singleton live births after fresh blastocyst and cleavage-stage transfer. Risks of low birth weight (aRRR, 1.02; 95% CI, 0.95–1.09), very low birth weight (aRRR 0.96; 95% CI, 0.83–1.11), high birth weight (aRRR, 0.97; 95% CI, 0.90–1.04), and very high birth weight (aRRR, 0.91; 95% CI, 0.77–1.08) were likely similar between the groups. The findings were consistent in the subgroup analysis.
Conclusion(s): Fresh blastocyst transfer does not appear to have a negative impact on gestational age at birth and birth weight in singleton live births compared with fresh cleavage-stage transfer. (Fertil Steril® 2023;120:312–20. ©2023 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Blastocyst, cleavage, perinatal outcome, singleton, and in vitro fertilization

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Use of extended embryo culture (transfer of embryos at the stage of blastocyst 5 or 6 days after oocyte fertilization) in in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycles has increased steadily over the last decade and has been shown to provide higher pregnancy and live birth rates per embryo transfer than cleavage-stage embryos (2 or 3 days after oocyte fertilization) (1). Extended culture is

currently acknowledged as the best method to select most viable embryos for elective single embryo transfer to reduce the risk of multiple pregnancies while maintaining high live birth rates (1, 2). Nevertheless, some would argue that prolonged exposure of embryos to different laboratory chemicals adversely impacts perinatal safety in resulting singleton pregnancies (3, 4). A recent and up-to-date systematic review and cumulative meta-analysis conducted by our group (5) has shown that extended culture is associated with increased risks of large for gestational age singletons and preterm delivery in comparison with cleavage-stage embryos regardless the use of fresh or frozen-thawed embryos (5).

A previous study comparing perinatal outcomes between singleton live births after fresh blastocyst and cleavage-stage embryo transfer using Human Fertilisation and Embryology Authority (HFEA) national data from the United Kingdom failed to show consistent increased risks with extended culture compared with cleavage-stage transfer (6). This study was limited to data up to 2011 and the meta-analysis has shown that effect sizes are still evolving for most outcomes in recent years (7), possibly as a result of advancement in embryo culture techniques and clinical protocols (8, 9).

As new United Kingdom data have become available, we decided to analyze new anonymized data (HFEA) to compare perinatal outcomes between singleton live births after fresh blastocyst transfer and those after fresh cleavage-stage transfer. The analysis of more recent data (up to 2018) from a large national dataset, controlling for a number of different confounders, would provide a better understanding of the possible impact of extended embryo culture on perinatal outcomes of singleton pregnancies given the sustained advances in laboratory and clinical protocols.

MATERIALS AND METHODS

Study Design and Data Collection

We conducted a retrospective cohort study using anonymized HFEA data recorded from 2012 to 2018 (available at https://www.hfea.gov.uk/about-us/our-data/). Anonymized HFEA data are cycle-based and freely available on the HFEA website.

Ethics

As this is a retrospective analysis of publicly available anonymized data, no institutional review board approval was required. There was no requirement to seek the approval of the Caldicott Guardian as patients' identifiable information was not being accessed.

Inclusion and Exclusion Criteria

Inclusion criteria were all fresh IVF/ICSI cycles resulting in a singleton live birth after blastocyst or cleavage-stage embryo transfer. Exclusion criteria were cycles with frozen embryo transfer (day of embryo transfer is not reported for frozen cycles in the HFEA anonymized dataset); cycles resulting in a multiple pregnancy; donor/surrogate, unstimulated, and preimplantation genetic testing cycles; cycles with contradictory or implausible data for the purpose of cycle selection and missing information in outcome measures. Exposure groups included cycles transferring blastocyst-stage embryos (exposed) and cycles transferring cleavage-stage embryos (non exposed).

Baseline Characteristics and Outcomes of Interests

Baseline characteristics recorded in the HFEA dataset (maternal age at treatment, causes of infertility, previous pregnancies from IVF/ICSI cycles, IVF/ICSI, number of oocytes collected, number of embryos transferred, elective single embryo transfer, number of gestational sacs, gender of the baby) were compared between the groups. The investigators selected all relevant baseline characteristics among those recorded in the HFEA dataset. The following perinatal outcomes were compared between the groups: gestational age at delivery (full term birth $\geq$37 weeks, preterm birth [PTB] 32-36 weeks + 6 days and very preterm birth [VPTB] <32 weeks), birth weight at delivery (very low birth weight [VLBW] <1,500 g, low birth weight [LBW] 1,500 g–2,499 g, normal birth weight 2,500 g–3,999 g, high birth weight [HBW] 4,000 g–4,499 g, and very high birth weight [VHBW] $\geq$4,500 g). The categories of LBW and VLBW also were combined into the category of total low birth weight (TLBW), and HBW and VHBW were combined into total high birth weight (THBW).

Statistical Analyses

Comparison of baseline characteristics between the blastocyst and cleavage-stage groups was performed using the χ^2 test (categorical/dichotomized covariates) and the Mann-Whitney test (continuous covariates; level of significance set at <.05). Multinomial logistic regression was used to establish relationships between embryo transfer stage and categorical outcomes (PTB, VPTB, LBW, VLBW, HBW, VHBW, TLBW, and THBW) to estimate relative risk ratios (RRRs), adjusted RRRs (aRRRs), and their 95% confidence interval (CI).

Relevant confounders were identified using directed acyclic graphs (DAGs; reported in Supplemental Figures 1, 2, available online) (10, 11). The creation of DAGs and selection of confounders was done using the DAGitty browser-based software (available at: http://www.dagitty.net/dags.html). A DAG is a graphical representation of causal relationships between exposures, outcomes, and covariates that are related to or modify the association with the outcome (11). Clinical expertise and evidence from literature informed discussions among investigators to agree on causal relationships between variables in DAGs. Confounders selected using DAGs were included in adjustment models even if their distribution was not statistically significantly different between the groups (P>.05, Table 1) but not if they had missing data. Covariates identified as mediators between the exposure and the outcome in DAGs were not included for adjustment to avoid the risk of "overadjustment" (11–13). The methods used to create DAGs, including strengths and limitations, are described in Supplemental Table 1 (available online). The assumption of linearity between continuous covariates and

TABLE 1

Background characteristics of included IVF/ICSI cycles.

	All cycles analysis			Subgroup analysis of first cycles		
Characteristics	**Blastocyst-stage (n = 42,677)**	**Cleavage-stage (n = 18,249)**	**χ^2 Test* *P* Value**	**Blastocyst-stage (n = 18,640)**	**Cleavage-stage (n = 8,321)**	**χ^2 Test* *P* Value**
Maternal age at treatment (y)	N (%)	N (%)	<.001	N (%)	N (%)	<.001
18–34	25,492 (59.7)	8,778 (48.1)		12,284 (65.9)	4,600 (55.3)	
35–37	10,223 (24)	4,598 (25.2)		3,994 (21.4)	1,963 (23.6)	
38–39	4,545 (10.7)	2,777 (15.2)		1,630 (8.7)	1,099 (13.2)	
40–42	2,238 (5.2)	1,860 (10.2)		685 (3.7)	589 (7.1)	
>42	179 (0.4)	236 (1.3)		47 (0.3)	70 (0.8)	
Cause of infertility						
Tubal disease	5,575 (13.1)	2,308 (12.6)	.16	2,526 (13.6)	1,066 (12.8)	.10
Ovulatory disorder	6,404 (15)	2,083 (11.4)	<.001	2,896 (15.5)	1,018 (12.2)	<.001
Male factor	16,718 (39.2)	7,706 (42.2)	<.001	7,116 (38.2)	3,435 (41.3)	<.001
Endometriosis	2,932 (6.9)	1,298 (7.1)	0.28	1,309 (7)	592 (7.1)	.80
Unexplained	13,274 (31.1)	5,322 (29.2)	<.001	5,943 (31.9)	2,469 (29.7)	<.001
Previous pregnancy			<.001	N/A	N/A	N/A
Yes	3,162 (11)[a]	1,858 (12.2)[a]				
No	25,561 (89)[a]	13,341 (87.8)[a]				
Missing	13,954 (32.7)[b]	3,050 (16.7)[b]				
Type of fertilization			<.001			.01
IVF	18,751 (43.9)	7,576 (41.5)		9,086 (48.7)	3,916 (47.1)	
ICSI	23,926 (56.1)	10,673 (58.5)		9,554 (51.3)	4,405 (52.9)	
No. of oocytes collected			.001			.001
Median	12	7		12	7	
Interquartile range	9–16	5–11		8–16	5–11	
Missing	13,954 (32.7)[b]	3,050 (16.7)[b]		0 (0)	0 (0)	
No. of embryos transferred			.001			.001
1	31,407 (73.6)	5,915 (32.4)		15,123 (81.1)	3,322 (39.9)	
2	10,963 (25.7)	11,612 (63.6)		3,432 (18.4)	4,805 (57.8)	
≥3	307 (0.7)	722 (4)		85 (0.5)	194 (2.3)	
Elective single embryo transfer			.001			.001
Yes	26,698 (62.6)	3,174 (17.4)		12,843 (68.9)	2,024 (24.3)	
No	15,979 (37.4)	15,075 (82.6)		5,797 (31.1)	6,297 (75.7)	
No. of gestational sacs			<.001			<.001
1	41,585 (97.4)	17,611 (96.5)		18,312 (98.2)	8,077 (97.1)	
>1	1,092 (2.6)	638 (3.5)		328 (1.8)	244 (2.9)	
Gender of the baby			<.001			.002
Female	20,639 (48.4)	9,222 (50.5)		9,054 (48.6)	4,216 (50.7)	
Male	22,038 (51.6)	9,027 (49.5)		9,586 (51.4)	4,105 (49.3)	

IVF = in vitro fertilization; ICSI = intracytoplasmic sperm injection.
* Test of difference between Blastocyst-stage and Cleavage-stage groups using Pearson χ^2 test.
[a] Percentage calculated on the number of cycles with complete data.
[b] Percentage calculated on all cycles.

Marconi. Perinatal outcomes of blastocyst. Fertil Steril 2023.

outcomes was evaluated using the Box-Tidwell procedure (14). Where the assumption was violated, we planned to include appropriate higher terms to model the function of the continuous variable.

As the inability to identify clustering of cycles within women (cycle-based anonymized dataset) could lead to spurious associations between exposures and outcomes (15, 16), we conducted a subgroup analysis in women undergoing their first IVF/ICSI cycle. This subgroup analysis was limited to data up to 2016 as information on previous IVF cycles was not available in the period 2017–2018.

We performed a sensitivity analysis in which all confounders, including those with missing data, with a different distribution between the groups were included in adjustments in 'all cycles' and subgroup analyses. Similarly, to address differences in baseline characteristics between the groups, we conducted additional propensity score matching analyses. The confounder "duration of infertility" was not included in any adjustments as almost 100% of data were missing. We used sensitivity and propensity score matching analyses to further explore the issue of confounding arising from potential errors in the assumptions used to create DAGs and from the fact that DAGs do not account for sampling variation of the included IVF/ICSI cycles (10, 17, 18).

Statistical analyses were performed using IBM SPSS Statistics 24.0 (IBM Corp., Armonk, NY). STATA 17 Software (StataCorp 2021, Stata Statistical Software: Release 17; StataCorp Co., College Station, TX) was used for propensity score matching analyses.

RESULTS

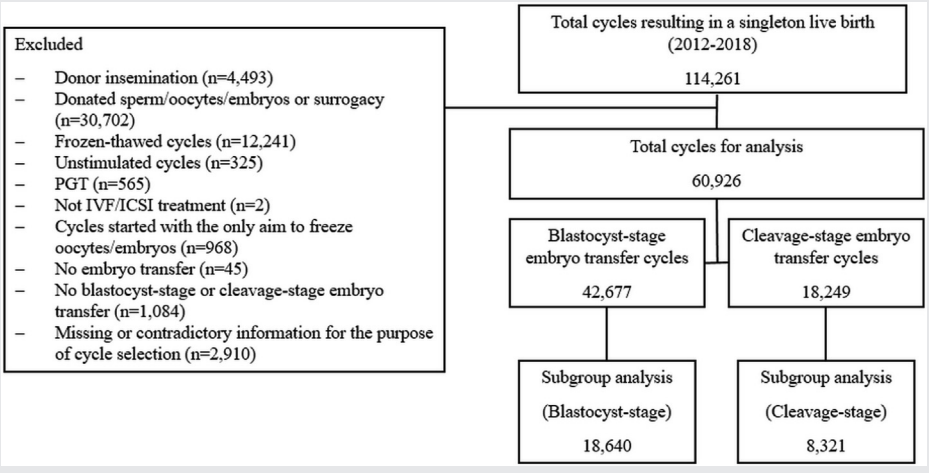
The total number of assisted reproduction technology cycles resulting in a singleton live birth and recorded in the HFEA dataset between 2012 and 2018 was 114,261 (Fig. 1). The number of IVF/ICSI cycles resulting in a singleton live birth that were included in the analysis was 60,926 (Fig. 1). Blastocysts were transferred in 42,677 cycles and cleavage-stage embryos in 18,249 (Fig. 1). The number of cycles transferring blastocyst-stage embryos was always higher than the number of those transferring cleavage-stage embryos, with the difference steadily increasing from 2012 to 2018 (Supplemental Fig. 3, available online).

Analysis of All Eligible IVF/ICSI Cycles

Comparison of baseline characteristics between blastocyst-stage and cleavage-stage groups is reported in Table 1.

Data on the duration of infertility were not used, and therefore not reported, because of the high proportion of missing data (99.6%). There was a significant association between the groups and maternal age at treatment ($P<.001$) with younger and older women having blastocyst and cleavage-stage embryo transfer more frequently, respectively (Table 1). Causes of infertility were significantly different between the groups ($P<.001$) with the exception of tubal factor infertility ($P=.16$) and endometriosis ($P=.28$; Table 1). The number of IVF/ICSI cycles in people who had already achieved at least one pregnancy before the current cycle was higher in the cleavage-stage group ($P<.001$), but there was a high proportion of missing data in both groups (blastocyst, 32.7%; cleavage-stage, 16.7%; Table 1). The use of IVF as method of fertilization was more common in blastocyst transfer cycles, whereas ICSI in cleavage-stage embryo transfer cycles ($P<.001$; Table 1). The median number of oocytes collected was higher in the blastocyst group ($P=.001$), which was also characterized by a higher number of cycles transferring a single embryo ($P=.001$; Table 1). The number of pregnancies resulting in visualization of >1 gestational sac at early pregnancy ultrasound assessment was significantly higher in the cleavage-stage group ($P<.001$; Table 1). The proportion of male singleton live births was higher in the

FIGURE 1



Cycle selection diagram.

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blastocyst group, while that of female singleton births was higher in the cleavage-stage group ($P<.001$; Table 1).

Gestational Age at Birth

There was a higher proportion of PTB (7.8% vs. 7.2%) in singleton live births after fresh blastocyst-stage embryo transfer than after fresh cleavage-stage embryo transfer (Table 2). The proportion of VPTB singleton live births was similar between the blastocyst group and the cleavage-stage group (1.6% vs. 1.6%). After adjusting for potential confounders, there was probably little to no difference in the risk of PTB (aRRR, 1.07; 95% CI, 1.00–1.15) and VPTB (aRRR, 1.05; 95% CI, 0.91–1.21) between singleton live births after fresh blastocyst-stage embryo transfer and those after fresh cleavage-stage embryo transfer (Table 2).

Birth Weight

There was a marginally higher proportion of LBW (7.2% vs. 7.1%) singleton live births after fresh blastocyst-stage embryo transfer than after fresh cleavage-stage embryo transfer (Table 2). The proportions of HBW (6.2% vs. 6.6%) and VHBW (1.1% vs. 1.2%) singleton live births were slightly lower in the blastocyst-stage group than in the cleavage-stage group, while the proportion of VLBW singletons was similar between the groups (1.6% vs. 1.6%; Table 2). After adjusting for potential confounders, there was probably little to no difference in the risk of LBW (aRRR, 1.02; 95% CI, 0.95–1.09), VLBW (aRRR, 0.96; 95% CI, 0.83–1.11), HBW (aRRR, 0.97; 95% CI, 0.90–1.04), VHBW (aRRR, 0.91; 95% CI, 0.77–1.08), TLBW (aRRR, 1.00; 95% CI, 0.94–1.07), and THBW (aRRR, 0.96; 95% CI, 0.90–1.03) in singleton live births after fresh blastocyst-stage embryo transfer compared with those after fresh cleavage-stage embryo transfer (Table 2).

Subgroup Analysis of Women Undergoing Their First IVF/ICSI Cycle

The number of cycles in women undergoing their first IVF/ICSI cycle was 26,961 (Fig. 1). Comparison of baseline characteristics between blastocyst-stage and cleavage-stage groups is reported in Table 1. There was a significant association between the groups and maternal age at treatment ($P<.001$) with younger and older women having blastocyst and cleavage-stage embryo transfer more frequently, respectively (Table 1). Causes of infertility were significantly different between the groups ($P<.001$) with the exception of tubal factor infertility ($P=.10$) and endometriosis ($P=.80$). The use of IVF as method of fertilization was more common in blastocyst transfer cycles, whereas ICSI in cleavage-stage embryo transfer cycles ($P=.01$; Table 1). The median number of oocytes collected was higher in the blastocyst group ($P=.001$), which was also characterized by a higher number of cycles transferring a single embryo ($P=.001$; Table 1). The proportion of pregnancies that started with >1 gestational sac was significantly higher in the cleavage-stage group ($P<.001$; Table 1). The proportion of male singleton live births was higher in the blastocyst group, while that of females was higher in the cleavage-stage group ($P=.002$; Table 1).

Gestational Age at Birth

The proportion of PTB singleton live births was similar (7.5% vs. 7.5%) between the blastocyst and cleavage-stage groups in the subgroup analysis (Table 3). The proportion of VPTB singleton live births was marginally lower in the blastocyst group than in the cleavage-stage group (1.6% vs. 1.7%; Table 3). We found little to no difference in the risk of PTB (aRRR, 0.99; 95% CI, 0.89–1.11) and VPTB (aRRR, 0.83; 95% CI, 0.67–1.03) in singleton live births after blastocyst

TABLE 2

Perinatal outcomes – all cycles analysis: 60,926 cycles.

Outcomes	Blastocyst-stage (n = 42,677)	Cleavage-stage (n = 18,249; %)	Blastocyst-stage versus Cleavage-stage Unadjusted RRR (95% CI)	Blastocyst-stage versus Cleavage-stage Adjusted RRR[c] (95% CI)[e]
Gestational age at birth[a]	**N (%)**	**N (%)**		
Full term	38,640 (90.6)	16,646 (91.2)	1[d]	1[d]
PTB	3,337 (7.8)	1,322 (7.2)	1.09 (1.02–1.16)	1.07 (1.00–1.15)
VPTB	700 (1.6)	281 (1.6)	1.07 (0.93–1.23)	1.05 (0.91–1.21)
Birth weight[b]				
NW	35,803 (83.9)	15,239 (83.5)	1[d]	1[d]
LBW	3,088 (7.2)	1,285 (7.1)	1.02 (0.96–1.10)	1.02 (0.95–1.09)
VLBW	676 (1.6)	289 (1.6)	1.00 (0.87–1.14)	0.96 (0.83–1.11)
HBW	2,645 (6.2)	1,210 (6.6)	0.93 (0.87–1.00)	0.97 (0.90–1.04)
VHBW	465 (1.1)	226 (1.2)	0.88 (0.75–1.03)	0.91 (0.77–1.08)
Summarized Birth weight[b]				
NW	35,803 (83.9)	15,239 (83.5)	1[d]	1[d]
TLBW	3,764 (8.8)	1,574 (8.6)	1.02 (0.96–1.08)	1.00 (0.94–1.07)
THBW	3,110 (7.3)	1,436 (7.9)	0.92 (0.86–0.98)	0.96 (0.90–1.03)

RRR = relative risk ratio; PTB = preterm birth; VPTB = very preterm birth; NW = normal weight; LBW = low birth weight; VLBW = very low birth weight; HBW = high birth weight; VHBW = very high birth weight; TLBW = total low birth weight; THBW = total high birth weight.
[a] Adjusted for the cause of infertility (endometriosis, male factor, ovulatory disorder, unexplained), IVF/ICSI, maternal age at treatment, year of treatment.
[b] Adjusted for the cause of infertility (endometriosis, tubal disease, male factor, ovulatory disorder, unexplained), IVF/ICSI, maternal age at treatment, year of treatment.
[c] Multinomial logistic regression.
[d] Reference category.
[e] No continuous covariates were included in adjustment models so the assessment of the linearity of the logit assumption was not performed.

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TABL.E 3

Perinatal outcomes–subgroup analysis of first cycles: 26,961 cycles.

Outcomes	Blastocyst-stage (n = 18,640)	Cleavage-stage (n = 8,321)	Blastocyst-stage versus Cleavage-stage Unadjusted RRR (95% CI)	Blastocyst-stage versus Cleavage-stage Adjusted RRR[c] (95% CI)[e]
Gestational age at birth[a]	**N (%)**	**N (%)**		
Full term	16,944 (90.9)	7,552 (90.8)	1[d]	1[d]
PTB	1,391 (7.5)	623 (7.5)	1.00 (0.90–1.10)	0.99 (0.89–1.11)
VPTB	305 (1.6)	146 (1.7)	0.93 (0.76–1.14)	0.83 (0.67–1.03)
Birth weight[b]				
NW	15,623 (83.8)	6,950 (83.5)	1[d]	1[d]
LBW	1,357 (7.3)	623 (7.5)	0.97 (0.88–1.07)	0.98 (0.88–1.09)
VLBW	286 (1.6)	140 (1.7)	0.91 (0.74–1.12)	0.85 (0.68–1.07)
HBW	1,163 (6.2)	509 (6.1)	1.02 (0.91–1.13)	1.06 (0.94–1.19)
VHBW	211 (1.1)	99 (1.2)	0.95 (0.75–1.21)	0.87 (0.67–1.13)
Summarized Birth weight[b]				
NW	15,623 (83.8)	6,950 (83.5)	1[d]	1[d]
TLBW	1,643 (8.8)	763 (9.2)	0.96 (0.88–1.05)	0.95 (0.86–1.05)
THBW	1,374 (7.4)	608 (7.3)	1.01 (0.91–1.11)	1.03 (0.92–1.15)

RRR = relative risk ratio; PTB = preterm birth; VPTB = very preterm birth; NW = normal weight; LBW = low birth weight; VLBW = very low birth weight; HBW = high birth weight; VHBW = very high birth weight; TLBW = total low birth weight; THBW = total high birth weight.
[a] Adjusted for the cause of infertility (endometriosis, male factor, ovulatory disorder, unexplained), IVF/ICSI, maternal age at treatment, number of oocytes collected, and year of treatment.
[b] Adjusted for the cause of infertility (endometriosis, tubal disease, male factor, ovulatory disorder, unexplained), IVF/ICSI, maternal age at treatment, year of treatment.
[c] Multinomial logistic regression.
[d] Reference category.
[e] Assumption of linearity of the logit met and no square or polynomial components were included in the adjustement models.

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transfer compared with those after cleavage-stage transfer (Table 3).

Birth Weight

There was a marginally lower proportion of LBW (7.3% vs. 7.5%), VLBW (1.6% vs. 1.7%), and VHBW (1.1% vs. 1.2%) singleton live births after fresh blastocyst-stage embryo transfer than after fresh cleavage-stage embryo transfer (Table 3). The proportion of HBW (6.2% vs. 6.1%) singleton live births was marginally higher in the blastocyst-stage group than in the cleavage-stage group (Table 3). After adjusting for potential confounders, we found little to no difference in the risk of LBW (aRRR, 0.98; 95% CI, 0.88–1.09), VLBW (aRRR, 0.85; 95% CI, 0.68–1.07), HBW (aRRR, 1.06; 95% CI, 0.94–1.19), VHBW (aRRR, 0.87; 95% CI, 0.67–1.13), TLBW (aRRR, 0.95; 95% CI, 0.86–1.05), and THBW (aRRR, 1.03; 95% CI, 0.92–1.15) between the groups (Table 3).

Supplemental Analyses

Our results did not change in the sensitivity analysis where we adjusted for all baseline covariates, which were statistically significantly different between the groups (Supplemental Tables 2, 3, available online). In the sensitivity analysis including all cycles, there was little to no difference in the risk of PTB (aRRR, 1.04; 95% CI, 0.95–1.13), VPTB (aRRR, 0.95; 95% CI, 0.79–1.14), LBW (aRRR, 1.02; 95% CI, 0.93–1.11), VLBW, (aRRR, 0.97; 95% CI, 0.80–1.17), HBW (aRRR, 0.99; 95% CI, 0.90–1.09), VHBW (aRRR, 0.94; 95% CI, 0.76–1.15), TLBW (aRRR, 1.01; 95% CI, 0.93–1.09), and THBW (aRRR, 0.98; 95% CI, 0.90–1.07; Supplemental Table 2). Results were similar in the sensitivity subgroup analysis of first IVF/ICSI cycles (Supplemental Table 3).

The propensity score matching analysis including all cycles showed little to no difference in the risk of PTB (aRRR, 1.05; 95% CI, 0.95–1.15), VPTB (aRRR, 0.98; 95% CI, 0.81–1.20), LBW (aRRR, 0.99; 95% CI, 0.90–1.09), VLBW (aRRR, 0.95; 95% CI, 0.78–1.16), HBW (aRRR, 1.07; 95% CI, 0.97–1.19), VHBW (aRRR, 0.96; 95% CI, 0.77–1.21; Supplemental Table 4 and Supplemental Fig. 4, 5, available online). Results were similar in the propensity score matching subgroup analysis (Supplemental Table 5 and Supplemental Figs. 6, 7, available online).

DISCUSSION

Principal Findings

This cohort study analyzing United Kingdom national data does not demonstrate increased risks of PTB/VPTB or LBW/HBW in singleton live births after fresh blastocyst transfer compared with fresh cleavage-stage transfer.

Strengths

The main strength of this study was the use of robust data from a national register collating information from all the licensed United Kingdom fertility centers. A subgroup analysis of cycles in women undergoing their first IVF/ICSI cycle was conducted to address the inability to adjust for clusters of cycles in the same women. Sensitivity and propensity score matching analyses were performed to assess the validity of the results.

Limitations

A major limitation was the use of cycle-based anonymized data. As it was not possible to identify clusters of cycles performed in the same women, spuriously narrow standard errors

might have been computed in the analysis including all eligible IVF/ICSI cycles.

No data were collected on several confounders in the anonymized HFEA dataset, such as previous medical/obstetrical history, smoking status, and body mass index. Due to the extremely high proportion of missing data, covariates, such as duration of infertility, could not be included in the analysis. Comparisons on other relevant outcomes, such as small for gestational age babies and congenital anomaly, could not be performed due to the lack/imprecision of information in the anonymized HFEA dataset.

We decided to use DAGs as they offer the opportunity to present causal associations between variables as seen by investigators, providing a rationale for the identification of confounders (19). However, DAGs have limitations as well (17) and there is no accepted guidance on their creation and use (19). As causal assumptions to build DAGs can be inaccurate and vary according to researchers' perceptions, there can be different versions of a DAG to address the same research question (17, 20). Statistical approaches to mitigate against confounding, such as the change-in-estimate method or stepwise selection, involve the use of significance criteria ($P<.05$ or $<.20$) to select confounders. They are useful when relationships among exposure, outcome, and covariates are unknown, but they cannot clarify the nature of a covariate as a confounder, mediator, or collider (17, 18, 21–23).

Clinical expertise and evidence from literature were used in discussions to select covariates and relationships among variables in DAGs. Some covariates, such as causes of infertility, are proxies for other unmeasured covariates (such as smoking status or medical background of women undergoing IVF). Therefore, we decided to exclude unmeasured covariates from our DAGs with the aim to keep their structures as much simple as possible (22).

We tested the robustness of our findings by conducting a sensitivity analysis adjusting results for all covariates (including those with missing data) that were distributed differently between the groups ($P<.05$; Table 1) and the results did not change (Supplemental Tables 2, 3). Propensity score matching analyses also showed similar results to those of primary analyses (Supplemental Tables 4, 5). However, these approaches have limitations, such as the possible inclusion of mediators/colliders in adjustment models and residual confounding (21, 24, 25).

As it is recommended to integrate DAGs with statistical methods to select confounders (17), we decided to conduct a further complete case analysis in which results were adjusted for all covariates identified as confounders in DAGs and that were also unevenly distributed ($P<.20$) between the blastocyst and cleavage-stage groups (Supplemental Tables 6–8, available online). Improved DAGs with main unmeasured covariates were reported for this analysis (Supplemental Figs. 8, 9 and Supplemental Table 9, available online). It was reassuring that, again, results did not change (Supplemental Tables 7, 8). However, the integration of statistical methods in DAGs has been criticized as at risk of introducing bias by mistakenly including mediators or colliders in adjustments as well (17, 18, 21, 22).

Comparison with Other Studies

Our results are reassuring with regard to perinatal safety in singleton live births after extended embryo culture and are similar to the findings of our previous systematic review (5). Interestingly, our findings are consistent with those of a similar study from our group using anonymized HFEA data and limited to the period 1999–2011 (6). The present and the older retrospective cohort study included a similar number of cycles. However, the subgroup analysis of first IVF/ICSI cycles in the 2019 study suggested the probability of a lower risk of LBW in singleton pregnancies after blastocyst transfer than in those after cleavage-stage embryo transfer (aRR, 0.86; 95% CI, 0.76–0.98) (6). This was not confirmed in the subgroup analysis of the present study (LBW: aRRR, 1.01; 95% CI, 0.89–1.13) and this discrepancy may reflect technological advances in the laboratory. Our findings are consistent with those of another single center study (26) comparing perinatal outcomes between blastocyst and cleavage-stage fresh embryo transfer and using more recent data up to 2015. Only a large register-based Nordic study (27), including data up to 2015, showed an increased risk of PTB in singletons after fresh blastocyst transfer compared with fresh cleavage-stage transfer (adjusted odds ratio, 1.14; 95% CI, 1.01–1.29), whereas no increased risk was found in our study. We did not find any difference in all the remaining outcomes in the main and subgroup analyses, as in previous studies using data from different periods of time (2006–2015, 1997–2015, and 2001–2004) and from different countries (26–28). In contrast, much older observational studies have reported higher risks of PTB and VPTB in singleton pregnancies resulting from fresh blastocyst transfer (29, 30).

Implications of Clinical Practice

Our findings provide reassurance for the current default position of embryo transfer at the blastocyst stage. It is worth noting that, unlike cleavage-stage transfer, blastocyst transfer is generally undertaken in good prognosis patients. Despite this, perinatal outcomes limited to IVF/ICSI cycles resulting in a singleton live birth seem to be similar in both groups. It could be argued that single cleavage-stage transfer in good prognosis patients might result in better perinatal outcomes, but this is speculation and must be proven.

Implications for Research

We highlight the value of continual evaluation of maternal and perinatal safety in addition to accurate and validated reporting of outcomes, including congenital anomalies.

Assessment of perinatal outcomes after blastocyst transfer is based mostly on observational data, which, as in the present study, have several limitations undermining the validity of results (31). Analysis of follow-up data from randomized controlled trials, focused on reproductive outcomes of extended culture, is an option but it would be limited as the advantages of randomization are lost when selecting subgroups of IVF/ICSI cycles resulting in a live birth and, in most cases, participants did not consent to collection of follow-up data. Another option might be to perform an

individual patient data meta-analysis (IPD-MA) of large register-based studies from different countries. In the meta-analytic approach, raw data on each patient included in the original studies are obtained from the investigators and combined in the meta-analysis instead of aggregated data from the published studies (32). This method provides several advantages that might improve the quality of observational data analyses: deeper knowledge of patients' pre-existing characteristics (potential confounders); analysis of different subgroups of patients; analysis of follow-up data, and lower risk of publication bias, bearing in mind that original investigators might be aware of unpublished or ongoing studies (32). However, conclusions from IPD-MAs remain subject to a degree of uncertainty (still some residual confounding). Furthermore, efforts in the context of time, costs, authorization and coordination among different investigators/institutions are required to perform an IPD-MA (32).

In conclusion, fresh blastocyst transfer does not appear to have a negative impact on gestational age at birth and birth weight compared with fresh cleavage-stage embryo transfer. However, it is important to keep monitoring perinatal outcomes in pregnancies after blastocyst-stage embryo transfer in view of advancement in laboratory and clinical procedures.

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Resultados perinatales en nacimientos únicos después de transferencia de blastocisto: análisis de 60,926 ciclos de fecundación in vitro en Reino Unido.

Objetivo: Comparar los resultados perinatales entre los nacidos de embarazo único tras transferencia en estadio de blastocisto y tras transferencia de embriones en células utilizando datos de todas las clínicas de fertilidad autorizadas en Reino Unido.

Diseño: Estudio de cohortes.

Entorno: No aplica.

Paciente(s): Un total de 60.926 ciclos de fecundación in vitro (FIV)/inyección intracitoplasmática de espermatozoides (ICSI) que resultaron en nacimientos únicos después de la transferencia de embriones frescos en estadio de blastocisto y en estadio de células entre 2012 y 2018.

Intervención(es): Se compararon las características basales entre los grupos de transferencia en blastocisto y en estadio de células en ciclos de FIV/ICSI utilizando el test χ^2 para variables categóricas/dicotomizadas y el test de Mann-Whitney para variables continuas. La significación estadística se estableció en $<0,05$. Se evaluó la asociación entre resultados perinatales de embriones transferidos en estadio de blastocisto comparados con resultados perinatales de embriones transferidos en células aplicando regresión logística multinomial.

Medida(s) del resultado principal: edad gestacional en el parto y peso al nacer.

Resultado(s): El grupo de blastocistos estaba compuesto por 42.677 ciclos de FIV/ICSI y el grupo de transferencia en células por 18.249 ciclos. Se observó muy poca o no diferencia en el riesgo de parto pretérmino (aRRR, 1,07; 95% CI, 1,00–1,15) y prematuridad extrema (aRRR, 1,05; 95% CI, 0,91–1,21) en nacidos vivos de embarazos únicos después de la transferencia en fresco de blastocistos y embriones en estadio de células. El riesgo de bajo peso al nacer (aRRR, 1,02; 95% CI, 0,95–1,09), muy bajo peso al nacer (aRRR 0,96; 95% CI, 0,83–1,11), alto peso al nacer (aRRR, 0,97; 95% CI, 0,90–1,04) y peso muy alto al nacer (macrosomía) (aRRR, 0,91; 95% CI, 0,77–1,08) fue similar entre los grupos. Los hallazgos presentaron consistencia en el análisis de subgrupos.

Conclusión(es): La transferencia embrionaria en fresco de blastocistos no parece tener un impacto negativo sobre la edad gestacional en el momento del parto ni en el peso de nacidos vivos de embarazo único cuando se comparan con embriones transferidos en fresco en estadio de células.