Obstetric and neonatal outcomes after the transfer of vitrified-warmed blastocysts developing from nonpronuclear and monopronuclear zygotes: a retrospective cohort study

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Objective: To evaluate the obstetric and neonatal outcomes after the transfer of vitrified-warmed single blastocysts developing from nonpronuclear (0PN) and monopronuclear (1PN) zygotes.
Design: Cohort study.
Setting: Affiliated hospital.
Patient(s): This study was a retrospective analysis of 435 0PN and 281 1PN vitrified-warmed single blastocyst transfers, and 151 0PN and 75 1PN singletons, compared with 13,167 two-pronuclear (2PN) vitrified-warmed single blastocyst transfers and 4,559 2PN singletons, respectively.
Intervention(s): None.
Main Outcome Measure(s): Pregnancy rate (PR), abortion rate (AR), live birth rate (LBR), and singleton birthweight were the primary outcome measures.
Result(s): PR, AR, and LBR were similar when compared between the 0PN and 2PN groups after vitrified-warmed blastocyst transfer. However, the 0PN group had a higher birthweights, higher *z* scores, and a greater proportion of very large for gestational age newborns. When comparing the 1PN and 2PN groups, we found that the PR was similar whereas the AR was higher and the LBR was lower. No differences were detected in the other neonatal outcomes.
Conclusion(s): The results of the present study show that the transfer of 2PN blastocysts should be prioritized because of a higher AR and a lower LBR after 1PN blastocyst transfers and a higher birthweight after 0PN blastocyst transfers when compared with 2PN blastocyst transfers. Our data indicate the need for concern about the safety of 1PN and 0PN embryo transfers. (Fertil Steril® 2021;115: 110-17. ©2020 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Live birth rate, monopronuclear zygotes, nonpronuclear zygotes, very large for gestational age, *z* score

In the field of human assisted reproductive technology, normal fertilization is confirmed by the presence of a two-pronuclear (2PN) zygote showing two polar bodies (PBs) or the fragmentation of PBs 16 to 18 hours after insemination. Nonpronuclear (0PN) and monopronuclear (1PN) zygotes are considered to arise from abnormal or failed fertilization (1). Some 0PN and 1PN zygotes can develop into embryos and exhibit

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morphology that is similar to that of high-quality 2PN embryos, even to the blastocyst stage. Numerous studies have shown that the transfer of 0PN or 1PN embryos can result in healthy babies (2–5), although the efficiency of transfer is low (5, 6). Therefore, in current clinical practice, it is not recommended to transfer embryos derived from 0PN or 1PN zygotes (7).

With the development of vitrification technology for blastocysts, some studies have reported that the culture of both 0PN and 1PN embryos to the blastocyst stage, after transfer in vitrified-warmed cycles, can result in similar outcomes as transfers involving 2PN blastocysts (8–10). These outcomes include clinical pregnancy rate (PR), abortion rate (AR), live birth rate (LBR), congenital malformations, and defects in psychomotor development. If these findings can be confirmed, then it does not seem necessary to prioritize the transfer of 2PN blastocysts. However, some limitations are associated with these previous studies. First, the number of blastocyst transfers is insufficient (82 cycles for 0PN and 134 cycles for 1PN); this creates serious limitations with regard to obstetric conclusions relating to PR, AR, and LBR (8, 10). Second, research methods involving "cycle matches" may also have limitations that could influence the conclusions (9, 10). Third, previous studies lack information relating to birthweight; this parameter is a major standard used to assess neonatal outcome because birthweight is associated with both short-term and long-term health (11).

Consequently, there are many unanswered questions. For example, is there any real difference in the outcomes of transfers involving 0PN, 1PN, and 2PN transfers? Must we prioritize the transfer of 2PN blastocysts? In the absence of 2PN blastocysts, do we prioritize 0PN or 1PN blastocysts? To address these issues, we designed a retrospective analysis in a single infertility center of the obstetric and neonatal outcomes of 0PN and 1PN embryos after vitrified-warmed single blastocyst transfers and compared these 2PN embryos. PR, AR, LBR, and the birthweights were the main focus in the current study.

MATERIALS AND METHODS

Patients and Cycles

This was a retrospective analysis of the clinical outcomes of transfer of vitrified-warmed single blastocysts showing zygotic stages with 1PN, 0PN, and 2PN embryos. Cases of single blastocyst transfer and singletons born from vitrified-warmed cycles were included in the study. We excluded cases involving 1PN zygotes arising from intracytoplasmic sperm injection (ICSI), sperm or oocyte donation cycles, in vitro maturation cycles, rescue ICSI, artificial oocyte activation cycles, and any case involving preimplantation genetic testing. Our final dataset included 13,167 2PN, 435 0PN, and 281 1PN single blastocyst transfers, along with 151 0PN, 75 1PN, and 4,555 2PN live singleton newborns. The study was carried out between March 2012 and March 2019 in the Reproductive Medical Center of Peking University Third Hospital. The Ethics Committee of Beijing University Third Hospital approved this study (Reference no. 2019SZ-071).

Fresh Cycles

All women underwent controlled ovarian hyperstimulation by the use of GnRH antagonist or GnRH agonist protocols, as described previously (12). Between 36 and 38 hours after human chorionic gonadotropin administration, oocytes were retrieved and fertilized by conventional in vitro fertilization (IVF) or ICSI. In conventional IVF cycles, oocytes were inseminated 3 to 4 hours after oocyte retrieval. Spermatozoa were collected by the swim-up technique with 50,000 motile sperm per milliliter in the insemination dish. For ICSI, the removal of cumulus cells from oocytes was performed 2 hours after retrieval, and ICSI was performed as previously described (13). Normal fertilization was assessed by the presence of two pronuclei 16 to 18 hours after insemination. Embryos were classified as either 2PN, 1PN, >2PN, or nonpronuclear (0PN). Available embryos were transferred 72 hours after oocyte retrieval. Supernumerary cleavage-stage embryos (including 0PN and 1PN embryos) that remained after transfer or vitrification of cleavage-stage embryos 72 hours after oocyte retrieval were further cultured to the blastocyst stage, and blastocyst vitrification was performed after a blastocyst was formed. The vitrification protocols were performed according to standard procedures, as previously described (14). To assess the ability of cleavage-stage 1PN and 0PN embryos to develop to a blastocyst, the study compared transferable blastocyst rates between cleavage-stage 0PN, 1PN, and 2PN embryos in fresh cycles.

Vitrified-Warmed Cycles

Embryo transfers for vitrified-warmed cycles were performed in either artificial hormone replacement or natural monitored cycles. Natural cycles were used for women with regular ovulatory cycles. Thawed embryo transfer was scheduled 5 days after ovulation. Luteal support was provided by intramuscular injections of progesterone (Shanghai General Pharmaceutical Company, Shanghai, PR China) in oil (20–40 mg) from the night of transfer to day 12, at which time serum hCG levels were assessed. In artificial hormone replacement cycles, we used oral estradiol to prepare the endometrium. Progesterone was provided when the endometrial thickness and estradiol concentrations were suitable. Embryo transfer was performed on day 7 after the administration of progesterone. Hormone replacement therapy was continued until pregnancy test results were available. The serum hCG concentration was measured 12 days after embryo replacement. One week later, transvaginal ultrasound was performed to confirm an intrauterine pregnancy. In cases of pregnancy, steroid supplementation was maintained until 12 weeks of gestation.

Outcome Parameters

The number of transfer times was calculated by taking this time into account (for example, this is the first transfer and the number of transfer times is 1). Pregnancies ending in miscarriages were classified as early abortions ($\leq$12 weeks) and late abortions (>12 weeks). Gestational age was calculated by adding 19 days from the date of blastocyst transfer. Premature delivery was defined as delivery at <37 gestational

weeks. Postmature delivery was defined as delivery at ≥42 gestational weeks. Very low birthweight (LBW), LBW, and high birthweight were defined as birthweights <1,500 g, < 2,500 g, and > 4,500 g, respectively. Very small for gestational age (SGA) and SGA newborns were defined as birthweights <3rd and <10th percentiles, respectively. Large for gestational age (LGA) and very LGA were defined as birthweights >90th and >97th percentiles, respectively. Birthweight was strongly affected by gestational age; thus, we used birthweight z scores to eliminate this effect. We calculated the birthweight z scores adjusted for neonatal gender and gestational age according to the INTERGROWTH-21st reference (15). The diagnoses of congenital malformations at birth were performed on the basis of the Chinese Birth Defects Monitoring Program. All data relating to patient characteristics and transfer outcomes were obtained from electronic medical records.

Statistical Analysis

Patient characteristics, along with treatments and obstetric/neonatal outcomes (continuous variables) were compared by one-way analysis of variance, and comparisons of rates were performed by a χ^2 test, with a continuity correction test or Fisher's exact test as appropriate. Multiple linear regression analysis was performed to compare 0PN, 1PN, or 2PN zygotes with regard to gestational age, birthweight, and *z* score while accounting for the following potential confounders: maternal age, maternal body mass index, type of infertility, duration of infertility, paternal age, cause of infertility (Supplementary Table 1), fertilization method (including IVF and ICSI), vitrified-warmed transfer endometrial preparation, gestational age (used just for birthweight), and newborn sex (used just for gestation age and birthweight). Logistic regression analysis was used to analyze 0PN, 1PN, or 2PN zygotes with regard to obstetric outcomes (including PR, AR, early AR, late AR, BR, and LBR) and neonatal outcomes (including premature delivery, postmature delivery, LBW, high birthweight SGA, LGA, and very LGA) while accounting for the following potential confounders: maternal age, maternal body mass index, type of infertility, duration of infertility, number of transfer times, paternal age, cause of infertility (Supplemental Table 1), fertilization method (including IVF and ICSI), and vitrified-warmed transfer endometrial preparation. Statistical significance was defined as a two-sided *P* value <.05 or corrected when multiple comparisons occurred. All statistical analyses were performed with Statistical Package for Social Sciences software (SPSS, Inc.).

RESULTS

In this study, a total of 1,143,116 oocytes were retrieved in 102,064 cycles and 2,174 0PN, 1,495 1PN, and 44,161 2PN blastocysts were vitrified (Supplemental Fig. 1). Finally, 435 0PN, 281 1PN, and 13,167 2PN vitrified-warmed single blastocyst transfers were performed. These cycles resulted in 151, 75, and 4,555 live singleton newborns from 0PN, 1PN, and 2PN, respectively, vitrified-warmed single blastocyst transfers. In fresh cycles, supernumerary cleavage-stage 1PN and 0PN embryos that were further cultured to blastocyst stage had a lower rate of transferable blastocysts than 2PN embryos (Supplementary Table1).

The characteristics of patients and treatments in vitrified-warmed blastocysts transfer (VBT) are presented in Supplementary Table 2. PR, AR, BR, and LBR, were similar when compared between the 0PN and 2PN groups after VBT, based on logistic regression analysis (Table 1). When the 1PN and 2PN groups were compared, there were no differences in PR, but the AR (odds ratio [OR] 1.764, 95% confidence interval [CI] 1.065–2.974), especially in late AR (OR 3.231, 95% CI 1.413–6.639), was higher, and BR (OR 0.697, 95% CI 0.493–0.987) and LBR (OR 0.701, 95% CI 0.496–0.992) were lower in the 1PN group than in the 2PN group after logistic regression analysis (Table 1).

The characteristics of patients and treatments in live singletons are presented in Supplementary Table 3. The proportion of very LGA singletons was greater in the 0PN group than n the 2PN group, and all other parameters were similar when determined by crosstabs analysis (Table 2). No differences were found in live singletons between the 1PN and 2PN groups when analyzed by crosstabs analysis (Table 2). Furthermore, after regression analysis and adjustment for other potential confounding factors, we found that the mean birthweight was significantly greater (β 106.400, 95% CI 23.101–189.699), the *z* score was higher (β 0.202, 95% CI 0.015–0.389) (Table3), and the proportion of very LGA singletons was greater (OR 1.976, 95% CI 1.191–3.279) (Table 4) in the 0PN group than in the 2PN group; there were no significant differences for any of the other neonatal outcomes. There were no differences between the 1PN and 2PN groups with respect to any of the neonatal outcomes, as determined by regression analysis (Table 3 and Table 4).

DISCUSSION

During assisted reproductive technology cycles, 0PN and 1PN zygotes are commonly observed at the time of fertilization assessment. However, there has been significant debate over the past 20 years as to whether or how we can transfer 0PN and 1PN embryos. In this study, we found that the PR, AR, and LBR of 0PN zygotes were similar to those of 2PN zygotes after VBT, whereas singletons had a higher birthweight, higher *z* score, and a greater proportion of very LGA newborns in the 0PN group. Compared with 2PN zygotes, 1PN zygotes had a similar PR but a higher AR and a lower LBR. These data suggest that 0PN and 1PN zygotes can be transferred, although we should prioritize 2PN zygotes when they are available.

Early studies showed that the outcomes of 0PN or 1PN embryo transfer were very poor (5, 6). However, more recent studies have reported similar outcomes to those of 2PN zygotes after VBT. These results may be due to the fact that blastocyst culture can select more "normal" embryos for transfer (16, 17). However, two chromosomal risks need to be considered when embryos are selected from blastocyst culture only (18). First, chromosomal abnormalities (haploid, mosaic, aneuploid, or chaotic karyotypes) still exist, and they may occur in either 0PN, 1PN, or 2PN zygotes. Second, embryos

TABLE 1

Results of logistic regression analysis of obstetric outcomes in nonpronuclear zygotes, monopronuclear zygotes, and two-pronuclear zygotes.

				0PN vs. 2PN		1PN vs. 2PN	
Result	0PN (n = 435)	1PN (n = 281)	2PN (n = 13,167)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
PR	45.52 (198/435)	41.28 (116 /281)	46.23 (6,087/13,167)	0.973 (0.802–1.177)	1.012 (0.806–1.270)	0.818 (0.643–1.039)	0.792 (0.580–1.082)
AR	21.72 (43/198)	33.62 (39/116)	23.84 (1,451/6,087)	0.914 (0.64–01.305)	1.003 (0.616–1.546)	1.668 (1.111–2.505)	1.764 (1.065–2.974)
Early AR	17.17 (34/198)	22.41 (26/116)	19.98 (1,216/6,087)	0.952 (0.665–1.361)	0.945 (0.615–1.481)	1.370 (0.876–2.142)	1.415 (0.767–2.497)
Late AR	4.55 (9/198)	11.21 (13/116)	3.86 (235/6,087)	1.295 (0.697–2.407)	0.557 (0.163–1.759)	3.266 (1.807–5.903)	3.231 (1.413–6.639)
BR	35.63 (155/435)	27.40 (77/281)	35.20 (4,636/13,167)	1.019 (0.834–1.244)	1.052 (0.830–1.333)	0.695 (0.533–0.906)	0.697 (0.493–0.987)
LBR	35.63 (155/435)	27.40 (77/281)	35.13 (4,626/13,167)	1.022 (0.837–1.248)	1.056 (0.833–1.338)	0.697 (0.535–0.908)	0.701 (0.496–0.992)

Note: Data presented as % (n/N) or odds ratio (95% confidence interval), unless stated otherwise. 0PN = nonpronuclear zygotes; 1PN = monopronuclear zygotes; 2PN = two-pronuclear zygotes; AR = abortion rate; BR = birth rate; CI = confidence interval; LBR = live birth rate; OR = odds ratio; PR = clinical pregnancy rate.

Li. Outcomes of 0PN and 1PN transfer. Fertil Steril 2020.

that are uniparental diploid cannot be excluded, thus creating the need to consider the presence of 0PN and 1PN zygotes. We found that PR, AR, and LBR in the 0PN group were similar to those in the 2PN group; this is consistent with a previous report by Li et al. (10). These results may be related to the genetic status of the blastocyst transferred. Yin et al. (19) found that 64.71% of 0PN zygotes had a normal chromosomal status compared with 69.39% in the 2PN group. Further studies showed that the proportions of uniparental diploid zygotes were similar when compared between 0PN (75.51%) and 2PN (80.13%) groups (20). These results may indicate that 0PN blastocysts can be used in clinical practice because they exhibit a chromosomal composition that is similar to that of 2PN zygotes.

When comparing 1PN and 2PN zygotes, we found that PR was similar, but AR was higher in the 1PN group. The higher AR in the 1PN group may be related to the genetic status of the blastocysts transferred. Notably, just 1PN-IVF blastocysts were included in the current study, and the research on chromosomal composition of 1PN-IVF blastocysts is very limited. In a previous study, Yin et al. (19) reported that the proportion of 1PN blastocysts with a normal chromosomal status was lower than that of 2PN blastocysts (50.00% vs. 69.39%, respectively). However, another study showed that there was no difference in the proportion of zygotes with a normal chromosomal status when compared between 1PN and 2PN blastocysts (39.3 vs, 36.5%, respectively); furthermore, analysis of 15 embryos failed to detect any uniparental diploids (21). Many studies have reported that 1PN zygotes or embryos have a uniparental composition (22–25). Lin et al. (26) further confirmed that 1PN blastocysts may be derived from a parthenogenetic origin because haploid oocytes might undergo spontaneous diploidization during blastocyst development. Given the data arising from these studies, it is possible that 1PN-IVF blastocysts may be derived from uniparental diploid embryos. Therefore, further studies are now needed to investigate the chromosomal status of 1PN-IVF blastocysts, particularly with regard to uniparental diploid status. When the high AR associated with 1PN zygotes is considered, it does appear feasible to transfer 1PN embryos only when 2PN embryos are not available. Furthermore, it is mandatory to provide patients with genetic counseling and ensure that they are fully informed of the potential risks before to 1PN embryo transfer. Preimplantation genetic testing is also recommended.

Another concern is the birthweight of 0PN and 1PN newborns; only one case report has been published thus far (27). In this study, we found no differences in birthweight between 1PN and 2PN zygotes; 0PN singletons had a higher birthweight and *z* score compared with 2PN singletons, and the proportion of very LGA newborns was significantly greater in the 0PN group than in the 2PN group. In other words, 0PN was associated with increased birthweight and a higher risk for very LGA newborns. Several studies have reported that epigenetic changes can affect neonatal outcomes (28, 29). Other studies have shown that neonatal methylation patterns are related to birthweight (30, 31), particularly in the high birthweight centile (32). Previous research has confirmed that DNA demethylation occurs in the male and female

TABLE 2

Neonatal outcomes of singleton live births in nonpronuclear zygotes, monopronuclear zygotes, and two-pronuclear zygotes.

Outcome	0PN (n = 151)	1PN (n = 75)	2PN (n = 4,555)	*P* value (0PN vs. 2PN)	*P* value (1PN vs. 2PN)
Mean gestational age (wk)	38.30 ± 1.52	38.25 ± 1.42	38.34 ± 1.77	0.792[a]	0.685[a]
<36	14 (9.27)	9 (12.00)	432 (9.48)	0.930[b]	0.462[b]
37-41	136 (93.59)	65 (89.69)	4101 (90.55)	0.989[b]	0.336[b]
≥42	1 (0.66)	1 (1.33)	22 (0.48)	1.000[c]	0.833[c]
Birthweight (g)	3,438.31 ± 548.66	3,362.27 ± 520.51	3,379.58 ± 521.22	0.169[a]	0.775[a]
z score	0.83 ± 1.00	0.67 ± 1.10	0.69 ± 0.96	0.176[a]	0.608[a]
Very LBW (<1,500 g)	0 (0.00)	0 (0.00)	25 (0.55)	1.000[d]	1.000[d]
LBW (<2,500 g)	5 (3.31)	3 (4.00)	182 (4.00)	0.832[b]	1.000[c]
HBW (>4,500 g)	4 (2.64)	2 (2.67)	51 (1.12)	0.182[c]	0.483[c]
Very SGA (<3rd percentile)	0 (0.00)	1 (1.33)	27 (0.59)	1.000[d]	0.944[c]
SGA (<10th percentile)	2 (1.32)	2 (2.67)	80 (1.77)	0.934[c]	0.880[c]
LGA (>90th percentile)	46 (30.46)	17 (22.67)	1,221 (26.80)	0.319[b]	0.422[b]
Very large for gestational age (>97th percentile)	26 (17.21)	8 (10.66)	486(10.66)	0.011[b]	0.999[b]
Newborn sex				0.591[b]	0.496[b]
Male	88	45	2,554		
Female	63	30	2,001		
Sex ratio, male/female	1.39	1.50	1.28	NA	NA
Malformations	2 (1.32)	0 (0.00)	57 (1.25/4555)	1.000[c]	1.000[d]

Note: Data are presented as the number (%) or mean ± standard deviation, unless stated otherwise. Statistical significance was defined as $P < .025$. 0PN = nonpronuclear zygotes; 1PN = monopronuclear zygotes; 2PN = two-pronuclear zygotes; HBW = high birthweight; LBW = low birthweight; LGA = large for gestational age; NA = not applicable; SGA = small for gestational age.
[a] One-way analysis of variance.
[b] Pearson χ^2 test.
[c] Continuity correction test.
[d] Fisher's exact test.

Li. Outcomes of 0PN and 1PN transfer. Fertil Steril 2020.

pronuclei of the zygote after fertilization (33). On the basis of the above findings, we may make an assumption: if the origin of a 0PN zygote is related to some "abnormal" zygotic demethylation processes, and that these "abnormalities" can influence the birthweight of newborns. Further research is required to address this possibility. The increased birthweight and a higher risk for very LGA associated with 0PN zygotes raise significant concern about the long-term safety of newborns derived from 0PN, including cardiovascular disease and diabetes mellitus (11). Therefore, the transfer of 2PN embryos still remains the first choice.

Our analysis clearly indicates that the priority method for transfer is to use 2PN embryos. However, if 2PN blastocysts are not available, would we transfer a 0PN or 1PN blastocysts? According to current research, it is very difficult to give a definite answer to these questions. The risk of 0PN lies in the health of the offspring, whereas the risk of 1PN lies in the high AB, particularly late abortion, which is very harmful to the physical and mental health of the gravida. In view of the insufficient number of 1PN singleton cases, our findings related to the birthweights of 1PN embryos need further confirmation. We therefore suggest preferring to transfer 0PN blastocysts between 0PN and 1PN when 2PN blastocysts are unavailable.

To our knowledge, this is the first study to investigate a sufficient number of 0PN newborns and includes the largest dataset relating to 0PN and 1PN blastocyst transfer. Our findings are reliable and have significant value for clinical practice. Another advantage of our study is that we included only cases involving single embryo transfer; this practice excludes the potential effects arising from multiple embryo transfer. For example, twin pregnancies arising from multiple embryo transfer are associated with a higher AR and a higher incidence of singletons of vanishing twins with a lower birthweight (34); these factors could affect AR and birthweight, respectively. Therefore, single embryo transfer data are likely

TABLE 3

Results of multiple regression analysis of gestation age, birthweight, and *z* score in singleton live births.

	0PN vs. 2PN				1PN vs. 2PN			
Variable	β	95% CI	Std. error	*P* value	β	95% CI	Std. error	*P* value
Gestation age (wk)	0.081	−0.258 to 0.421	0.173	0.639	0.249	−0.229 to 0.727	0.244	0.308
Birthweight (g)	106.400	23.101 to 189.699	42.489	0.012	−48.462	−165.824 to 68.901	73.103	0.994
z score	0.202	0.015 to 0.389	0.095	0.034	−0.138	−0.401 to 0.125	0.134	0.304

Note: Statistical significance was defined as a P value $< .05$. 0PN = nonpronuclear zygotes; 1PN = monopronuclear zygotes; 2PN = two-pronuclear zygotes; CI = confidence interval; Std. = standard.

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TABLE 4

Results of logistic regression analysis of abnormal delivery and abnormal birthweight in singleton live births.

	0PN vs. 2PN		1PN vs. 2PN	
Variable	**Unadjusted OR (95% CI)**	**Adjusted OR (95% CI)**	**Unadjusted OR (95% CI)**	**Adjusted OR (95% CI)**
Premature delivery	0.975 (0.558–1.705)	0.681 (0.313–1.483)	1.301 (0.644–2.630)	0.763 (0.272–2.142)
Postmature delivery	1.374 (0.184–10.258)	1.898 (0.243–14.817)	2.784 (0.370–20.930)	5.757 (0.697–47.550)
LBW (<2,500 g)	0.823 (0.333–2.031)	0.379 (0.047–3.086)	1.001 (0.312–3.208)	2.269 (0.475–10.838)
HBW (>4,500 g)	2.403 (0.857–6.737)	2.180 (0.652–7.280)	2.420 (0.578–10.127)	1.438 (0.188–11.005)
SGA (<10th percentile)	0.751 (0.183–3.083)	0.549 (0.075–4.003)	1.533 (0.370–6.353)	1.097 (0.148–8.147)
LGA (>90th percentile)	1.196 (0.841–1.702)	1.301 (0.853–1.982)	0.800 (1.464–1.380)	0.586 (0.283–1.215)
Very large for gestational age (>97th percentile)	1.741 (1.130–2.685)	1.976 (1.191–3.279)	1.000 (0.477–2.094)	0.700 (0.252–1.994)

Note: 0PN = nonpronuclear zygotes, 1PN = monopronuclear zygotes, 2PN = two-pronuclear zygotes, CI = confidence interval; HBW = high birthweight; LBW = low birthweight; LGA = large for gestational age; OR = odds ratio; SGA = small for gestational age.

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to yield more accurate results than multiple embryo transfer data, particularly with regard to abortion and birthweight. However, some limitations to our study need to be considered. First, the number of infants born after 1PN was relatively small; our results relating to 1PN birthweight need to be further confirmed with a larger number of cases. The lack of time-lapse technology to assess zygote status may also represent another limitation. However, at present, the majority of IVF cycles are performed without the use of time-lapse systems (35), and the majority of studies relating to 0PN or 1PN zygotes involved confirmation at the time of fertilization assessment (8–10, 20, 21, 27). Therefore, the results of this study may provide a more valuable suggestion for clinical practice.

In conclusion, our findings indicate that the transfer of 2PN blastocysts should be prioritized because 0PN and 1PN zygotes are associated with additional risk. 0PN and 1PN blastocysts can be transferred if 2PN blastocysts are not available, but in such cases, genetic evaluation and provision of information to patients with regard to the associated risks are mandatory.

REFERENCES

1. Feenan K, Herbert M. Can 'abnormally' fertilized zygotes give rise to viable embryos? Hum Fertil (Camb) 2006;9:157–69.
2. Burney RO, Gebhardt J, Shu Y, Behr B, Westphal LM. Normal pregnancy resulting from a non-pronuclear oocyte at the time of examination for fertilization. Clin Exp Obstet Gynecol 2008;35:170–1.
3. Dasig D, Lyon J, Behr B, Milki AA. Monozygotic twin birth after the transfer of a cleavage stage embryo resulting from a single pronucleated oocyte. J Assist Reprod Genet 2004;21:427–9.
4. Reichman DE, Jackson KV, Racowsky C. Incidence and development of zygotes exhibiting abnormal pronuclear disposition after identification of two pronuclei at the fertilization check. Fertil Steril 2010;94:965–70.
5. Staessen C, Janssenswillen C, Devroey P, Van Steirteghem AC. Cytogenetic and morphological observations of single pronucleated human oocytes after in-vitro fertilization. Hum Reprod 1993;8:221–3.
6. Manor D, Kol S, Lewit N, Lightman A, Stein D, Pillar M, et al. Undocumented embryos: do not trash them, FISH them. Hum Reprod 1996;11:2502–6.
7. ESHRE Guideline Group on Good Practice in IVF Labs, De los Santos MJ, Apter S, Coticchio G, Debrock S, Lundin K, et al. Revised guidelines for good practice in IVF laboratories (2015). Hum Reprod 2016;31:685–6.
8. Si J, Zhu X, Lyu Q, Kuang Y. Obstetrical and neonatal outcomes after transfer of cleavage-stage and blastocyst-stage embryos derived from monopronuclear zygotes: a retrospective cohort study. Fertil Steril 2019;112:527–33.
9. Li M, Dang Y, Wang Y, Li J, Liu P. Value of transferring embryos derived from monopronucleated (1PN) zygotes at the time of fertilization assessment. Zygote 2020;28:241–6.
10. Li M, Lin S, Chen Y, Zhu J, Liu P, Qiao J. Value of transferring embryos that show no evidence of fertilization at the time of fertilization assessment. Fertil Steril 2015;104:607–11.e2.
11. Belbasis L, Savvidou MD, Kanu C, Evangelou E, Tzoulaki I. Birth weight in relation to health and disease in later life: an umbrella review of systematic reviews and meta-analyses. BMC Med 2016;14:147–61.
12. Liu N, Ma Y, Li R, Jin H, Li M, Huang X, et al. Comparison of follicular fluid amphiregulin and EGF concentrations in patients undergoing IVF with different stimulation protocols. Endocrine 2012;42:708–16.
13. Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 1992;340:17–8.
14. Zheng X, Chen Y, Yan J, Wu Y, Zhuang X, Lin S, et al. Effect of repeated cryopreservation on human embryo developmental potential. Reprod Biomed Online 2017;35:627–32.
15. Villar J, Cheikh Ismail L, Victora CG, Ohuma EO, Bertino E, Altman DG, et al. International standards for newborn weight, length, and head circumference by gestational age and sex: the Newborn Cross-Sectional Study of the INTERGROWTH-21st Project. Lancet 2014;384:857–68.
16. Kroener L, Ambartsumyan G, Briton-Jones C, Dumesic D, Surrey M, Munne S, et al. The effect of timing of embryonic progression on chromosomal abnormality. Fertil Steril 2012;98:876–80.
17. Vega M, Breborowicz A, Moshier EL, McGovern PG, Keltz MD. Blastulation rates decline in a linear fashion from euploid to aneuploid embryos with single versus multiple chromosomal errors. Fertil Steril 2014;102:394–8.
18. Xie PY, Tang Y, Hu L, Ouyang Q, Gu YF, Gong F, et al. Identification of biparental and diploid blastocysts from monopronuclear zygotes with the use of a single-nucleotide polymorphism array. Fertil Steril 2018;110:545–54.e5.
19. Yin BL, Hao HY, Zhang YN, Wei D, Zhang CL. Good quality blastocyst from non-/mono-pronuclear zygote may be used for transfer during IVF. Syst Biol Reprod Med 2016;62:139–45.
20. Destouni A, Dimitriadou E, Masset H, Debrock S, Melotte C, Van Den Bogaert K, et al. Genome-wide haplotyping embryos developing from 0PN and 1PN zygotes increases transferrable embryos in PGT-M. Hum Reprod 2018;33:2302–11.
21. Bradley CK, Traversa MV, Hobson N, Gee AJ, McArthur SJ. Clinical use of monopronucleated zygotes following blastocyst culture and preimplantation genetic screening, including verification of biparental chromosome inheritance. Reprod Biomed Online 2017;34:567–74.
22. Staessen C, Van Steirteghem AC. The chromosomal constitution of embryos developing from abnormally fertilized oocytes after intracytoplasmic sperm

injection and conventional in-vitro fertilization. Hum Reprod 1997;12:321–7.
23. Yan J, Li Y, Shi Y, Feng HL, Gao S, Chen ZJ. Assessment of sex chromosomes of human embryos arising from monopronucleus zygotes in in vitro fertilization and intracytoplasmic sperm injection cycles of Chinese women. Gynecol Obstet Invest 2010;69:20–3.
24. Jamieson ME, Coutts JR, Connor JM. The chromosome constitution of human preimplantation embryos fertilized in vitro. Hum Reprod 1994;9:709–15.
25. Sultan KM, Munne S, Palermo GD, Alikani M, Cohen J. Chromosomal status of uni-pronuclear human zygotes following in-vitro fertilization and intracytoplasmic sperm injection. Hum Reprod 1995;10:132–6.
26. Lin G, OuYang Q, Zhou X, Gu Y, Yuan D, Li W, et al. A highly homozygous and parthenogenetic human embryonic stem cell line derived from a one-pronuclear oocyte following in vitro fertilization procedure. Cell Res 2007;17:999–1007.
27. Itoi F, Asano Y, Shimizu M, Honnma H, Murata Y. Birth of nine normal healthy babies following transfer of blastocysts derived from human single-pronucleate zygotes. J Assist Reprod Genet 2015;32:1401–7.
28. Banik A, Kandilya D, Ramya S, Stunkel W, Chong YS, Dheen ST. Maternal factors that induce epigenetic changes contribute to neurological disorders in offspring. Genes (Basel) 2017;8:150–74.
29. Whitelaw N, Bhattacharya S, Hoad G, Horgan GW, Hamilton M, Haggarty P. Epigenetic status in the offspring of spontaneous and assisted conception. Hum Reprod 2014;29:1452–8.
30. Agha G, Hajj H, Rifas-Shiman SL, Just AC, Hivert MF, Burris HH, et al. Birth weight-for-gestational age is associated with DNA methylation at birth and in childhood. Clin Epigenetics 2016;8:118–29.
31. Engel SM, Joubert BR, Wu MC, Olshan AF, Haberg SE, Ueland PM, et al. Neonatal genome-wide methylation patterns in relation to birth weight in the Norwegian Mother and Child Cohort. Am J Epidemiol 2014;179:834–42.
32. Haworth KE, Farrell WE, Emes RD, Ismail KM, Carroll WD, Hubball E, et al. Methylation of the FGFR2 gene is associated with high birth weight centile in humans. Epigenomics 2014;6:477–91.
33. van Montfoort AP, Hanssen LL, de Sutter P, Viville S, Geraedts JP, de Boer P. Assisted reproduction treatment and epigenetic inheritance. Hum Reprod Update 2012;18:171–97.
34. Magnus MC, Ghaderi S, Morken NH, Magnus P, Bente Romundstad L, Skjaerven R, et al. Vanishing twin syndrome among ART singletons and pregnancy outcomes. Hum Reprod 2017;32:2298–304.
35. Dolinko AV, Farland LV, Kaser DJ, Missmer SA, Racowsky C. National survey on use of time-lapse imaging systems in IVF laboratories. J Assist Reprod Genet 2017;34:1167–72.

Resultados obstétricos y neonatales tras las transferencias de un único blastocisto vitrificado-desvitrificado desarrollados a partir de cigotos sin pronúcleos y monopronucleares: un estudio de cohorte retrospectivo.

Objetivo: Evaluar los resultados obstétricos y neonatales después de las transferencias de un único blastocisto vitrificado-desvitrificado desarrollados a partir de cigotos sin pronúcleos (0PN) y monopronucleares (1PN).

Diseño: Estudio de cohorte.

Entorno: Hospital afiliado.

Paciente(s): Este estudio fue un análisis retrospectivo de las transferencias de un único blastocisto vitrificado-desvitrificado procedentes de 435 0PN y 281 1PN y de los nacidos únicos de 151 0PN y 75 1PN, comparados con las 13167 transferencias de un único blastocisto vitrificado-desvitrificado con dos pronúcleos (2PN) y 4559 2PN nacidos únicos, respectivamente.

Intervención(es): Ninguna.

Principales medidas de resultado: La tasa de embarazo (PR), la tasa de aborto (AR), la tasa de nacido vivo (LBR) y el peso del recién nacido único al nacer fueron las principales medidas de resultado.

Resultado(s): Las PR, AR y LBR fueron similares cuando se compararon entre los grupos 0PN y 2PN después de la transferencia de blastocisto vitrificado- descongelados. Sin embargo, el grupo 0PN tuvo un mayor peso al nacer, puntuaciones z más altas y una mayor proporción de recién nacidos muy grandes para la edad gestacional. Al comparar los grupos 1PN y 2PN, encontramos que la PR fue similar mientras que la AR fue mayor y la LBR fue menor. No se detectaron diferencias en los otros resultados neonatales.

Conclusión(es): Los resultados del presente estudio muestran que la transferencia de blastocistos 2PN debe priorizarse debido a una AR más alta y una LBR más baja tras la transferencia de un blastocisto 1PN y un mayor peso al nacer después de la transferencia de un blastocisto 0PN en comparación con la transferencia de un blastocisto 2PN. Nuestros datos indican la necesidad de preocuparse por la seguridad de la transferencia de embriones 1PN y 0PN.