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Birth of Thirty-One Healthy Babies following Transfer of Fresh and Frozen-Thawed Embryos Derived from Monopronuclear Zygotes: A Retrospective Study

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Abstract: Fertilized zygotes normally display 2 pronuclei (PN) but abnormal fertilization patterns (0, 1 or >2 PN) are daily observed in IVF labs. Multiple PN zygotes (>2) are generally discarded due to an increased risk of aneuploidy. However, the decision to transfer or not 1PN-derived embryos remains controversial. The aims of our study were to analyze the neonatal outcomes of fresh or frozen-thawed embryos derived from 1PN zygotes, and to evaluate the influence of the fertilization method. Data were retrospectively collected from cycles performed between January 2018 and December 2022. Fresh cycles were analyzed for the comparative fate of 1PN zygotes (n=1234) following conventional in vitro fertilization (cIVF; n=648) or intracytoplasmic sperm injection (ICSI; n=586), as well as the results of the 64 transfers of 1PN derived embryos (pregnancy rate (PR) and neonatal outcomes). This pregnancy follow-up was also applied to 167 transfers of frozen-thawed 1PN derived embryos. In fresh cycles, 46% of the 1PN zygotes in cIVF group gave rise to embryos of sufficient quality to be transferred or frozen (day 3 or 5/6). This rate decreased to 33% in the fresh ICSI cycles. Blastulation rate was also significantly higher in cIVF group (44%) in comparison to ICSI group (20%). The fresh embryo transfers (32 per group) allowed 7 pregnancies in the cIVF group (PR=21.9%) as compared to 4 pregnancies in the ICSI group (PR=12.5%). In the cIVF group, 4 deliveries of healthy newborns were achieved and only one in the ICSI group. In frozen/thawed cycles, 36 pregnancies were obtained out of the 167 transfers. A non-significative difference was observed between embryos derived from cIVF cycles (PR=26%) and ICSI cycles (PR=16%) with respectively 18 and 8 healthy babies born. In conclusion, we observed better outcomes for 1PN zygotes in cIVF cycles in comparison to ICSI cycles. Our center policy to transfer good quality 1PN-derived embryos allowed the birth of 31 healthy babies.

Keywords: monopronuclear; embryo transfer; live birth

1. Introduction

In assisted reproduction, fertilization success in *in vitro* fertilization (IVF) cycles for both conventional IVF (cIVF) or intracytoplasmic sperm injection (ICSI) is based on the assessment of pronuclei (PN) development and scoring.

Fertilization is the result of the fusion of a spermatozoon and an oocyte which is confirmed by the presence of two nuclei in the oocyte: a female nucleus (or maternal pronucleus: m-PN) from the oocyte, and a male nucleus (or paternal pronucleus: p-PN) from the spermatozoon. Therefore, a normal fertilized zygote displays 2 PN 16 to 18 hours post insemination while multiple PN zygotes (>2) are automatically discarded due to an increased risk of aneuploidy. The more controversy question is about 0 (no sign of fertilization) and 1PN (monopronuclear) zygotes. Indeed, embryos

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derived from such pronuclear stage are largely considered as abnormal and unsuitable for transfer. If we focus on 1PN zygotes, several hypotheses have been suggested to explain the origin of this "defective" fertilization: parthenogenesis leading to haploidy and abnormal embryos, asynchronization or fusion of m-PN and p-PN formation, or early pronuclear breakdown [1].

Although embryos derived from these types of zygotes have a normal morphology, the decision to use them or not varies considerably form one assisted reproductive technology (ART) center to another. Indeed, several studies showed that additional investigations could be required such as timelapse monitoring [2], preimplantation genetic testing (PGT) [1–3] or more morphometric correlation [4]. Nevertheless, other groups use to transfer 1PN embryos achieving good development without further analysis [5–8] .

In this retrospective study, we investigated embryos outcomes, live birth rate and neonatal outcomes of 1PN zygotes cohort in fresh and frozen-thawed cycles (cIVF and ICSI cycles) without comparison to 2PN derived embryos. The first aim of our study is to share our experience with 1PN-derived embryos and to help answer the question posed by several teams and recently reviewed by M Kemper « what happens to abnormally fertilized embryos ? » [9].

2. Materials and Methods

2.1. Study Design and patients

Our retrospective study was conducted at the Center for Reproductive Medicine of University of Liège, located at the Citadelle Hospital in Belgium, between January 2018 and December 2022. A total of 2762 fresh cycles (n= 2022 patients) and 167 frozen-thawed cycles (n= 150 patients) were included. The embryos transferred in the frozen-thawed cycles of this study were not systematically derived from the 2762 fresh cycles. All patients underwent cIVF or ICSI cycles and selection was performed according to the presence of at least one 1PN zygote as described in Figure 1 (a and b). Embryo and neonatal outcomes were studied for 648 and 586 1PN zygotes in cIVF and ICSI fresh cycles, respectively (Figure 1a). The study was approved by the Ethics Committee (number 2048) of the Citadelle Hospital of Liège, Belgium.

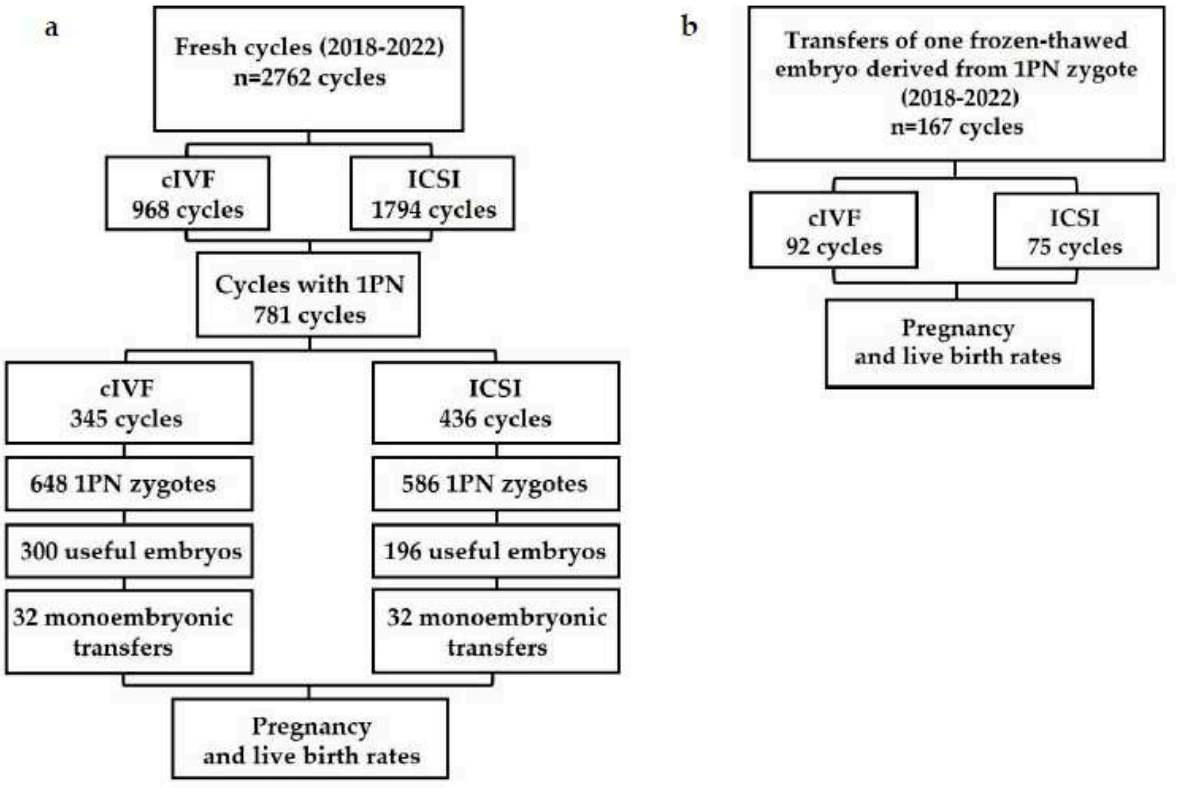

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Figure 1. (a) Analyzed cycles,1PN zygotes evolution and embryo transfers in fresh and (b) frozen-thawed cycles.

2.2. Ovarian stimulation and insemination

A total of 334 cycles in cIVF group and 401 cycles in ICSI group were carried out mainly using antagonist-controlled ovarian hyperstimulation protocols (in respectively in 96.8% and 92% of the cycles). Some other patients received a GnRH agonist in short or long protocols. Ovarian response to stimulation was monitored by hormonal blood tests and ultrasound assessment of follicular growth as previously described [10]. Oocyte pick-ups were performed by transvaginal aspiration under ultrasound guidance around 36 hours after human chorionic gonadotrophin (Ovitrelle®, 250 micrograms/0.5ml, Merck) injection, and retrieved oocytes were then fertilized using cIVF or ICSI. After embryo transfer, luteal phase was sustained by progesterone administration.

The present study included treatments with partner or donor sperm. For patients in the cIVF group, donor sperm was used in 244 cycles (70.7%) and partner ejaculated sperm in 101 cycles (29.3%). In the ICSI group, donor sperm was used in 45 cycles (10.3%) and partner sperm in 391 cycles (89.7 %) from either fresh or frozen samples.

2.3. Embryo culture, evaluation, and cryopreservation

After fertilization, oocytes were allocated for culture in 20 µl drops of Global® medium in dishes overlayed by Ovoil™ during 3 or 5/6 days [11]. For extended culture, medium was changed on day 3. Assessment of fertilization by evaluation of pronuclei (0, 1, 2 or >2 PN) was performed 18 hours post insemination (hpi) and followed by an early cleavage stage evaluation at 25 hpi. Oocytes showing more than 2PN were automatically discarded and embryos derived from 0, 1 or 2 PN zygotes were evaluated until day 3 or 5/6 of culture.

Day 3 and 5/6 embryos were assessed using our routine examination protocol based on Istanbul consensus criteria [12]. Day 3 embryos were classified considering blastomere number and symmetry, percentage of fragmentation and cytoplasmic appearance, as previously described [13]. Blastocyst quality was defined according to the degree of expansion, as well as the morphology of the inner mass and trophectoderm. Usable embryos were cryopreserved by vitrification.

2.4. Embryo thawing

After endometrium preparation by artificial stimulation or in spontaneous cycles, frozen-thawed embryos were transferred. Day 3 embryos were thawed one day before transfer, and day 5/6 embryos on the morning of transfer day.

2.5. Statistics

Data analysis was performed by using the Chi-square test (χ^2) to evaluate differences between proportions and group characteristics were compared using Student's t tests. Value of $p < 0.05$ was considered as significant.

3. Results

The present study included two groups of patients selected between 2018 and 2022 (Figure 1). In a first group (Figure 1a) of 2762 fresh cycles (968 cIVF and 1794 ICSI cycles), 781 cycles with at least one 1PN zygote were identified (345 cIVF and 436 ICSI cycles). We focused our investigation on this cohort of 1234 1PN zygotes of which 64 fresh monoembryonic transfers were performed. The second group (Figure 1b) comprised 167 transfers of frozen-thawed embryos derived from 1PN zygotes. These 1PN embryos originated either from cIVF (n=92) or ICSI cycles (n=75).

3.1. Study population

For fresh cycles, mean age of patients was 36.1 in cIVF group (345 cycles performed in to 321 patients) and 34.3 years in ICSI group (436 cycles performed in 410 patients), with significant

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difference between the two groups (p<0.001). Body Mass Index (BMI) as well as the mean cycle rank showed a significant difference between patient groups p<0.01 and p<0.001 respectively. Nevertheless, no differences were reported between the two groups regarding the number of retrieved oocytes or the hormonal status of E2 and P4 (Table 1). However, LH concentration on the day the decision to induce ovulation was taken was significantly higher in ICSI group in comparison to cIVF group (p<0.001) but without a clinical relevance.

Table 1. Study population of patients with at least 1PN zygote in fresh cycles. Hormonal dosages were performed on the day of the decision to induce ovulation, i.e., on the day of induction or 1-2 days before.

	cIVF n= 321 patients	ICSI n= 410 patients	P Value
Age	36.15±4.6	34.36±5.3	p<0.001
BMI	24.3±4.1	25.3±5	p<0.01
Cycle Rank	1.2±0.5	1.6±1.1	p<0.001
No of retrieved oocytes	12±7.6	11.4±7.8	NS
Serum [E2] pg/ml	2104±1431	2255±1875	NS
Serum [P4] ng/ml	0.75±0.51	0.75±0.5	NS
Serum [LH] mUI/ml	1.9±1.9	2.6±3.4	p<0.001

For frozen/thawed cycles, mean age of patients was 36.1 in cIVF group (92 cycles performed in to 79 patients) and 34.7 years in ICSI group (75 cycles performed in to 71 patients). Except differences for fresh cycle ranks (p<0.01) and numbers of retrieved oocytes (p<0.05), all patient characteristics were comparable between the two groups (Table 2). Frozen-thawed embryo transfers (FET) were performed using 2 different cycle regimens, either a spontaneous ovulatory (natural) cycle, or a protocol involving the use of estrogen and progesterone to artificially prepare the endometrium, commonly known as hormone therapy (HT) cycle. In cIVF FET group, natural cycle was applied in 32.6 % of the cycles, and HT cycle in 67.4 %. In comparison, figures in the ICSI FET group were respectively 40 % and 60 %.

Table 2. *Study population of patients with at least 1PN zygote in frozen-thawed cycles*. *Correspond to the fresh cycle rank of frozen embryos. ** Correspond to the rank of frozen/thawed embryo transfer.

	cIVF n= 79 patients	ICSI n= 71 patients	P Value
Age	36.1±5	34.7±5,8	NS
BMI	24.6±11.8	25.5±11.9	NS
Fresh cycle Rank*	1.2±0.6	2.2±1.5	p<0.01
No of retrieved oocytes	15.3±9	12.3±8.6	p<0.05
Embryo transfer rank**	2.2±1.5	2.2±1.4	NS

3.2. Embryo outcomes

The fate of a total of 1234 1PN zygotes (648 cIVF and 586 ICSI) was analyzed. Forty-six percent of them gave rise to embryos of sufficient quality to be transferred or frozen (day 3 or 5/6) in the cIVF group. However, this rate decreased significantly to 33% in the ICSI group (p<0.001). In terms of embryo quality, no difference was observed between the two groups for either the A, B or C score. Nevertheless, blastulation rate was significantly more important in cIVF group (44%) in comparison to ICSI group (20%) (p<0.001) (Table 3).

Table 3. Embryo outcomes per group in fresh cycles. Usable embryos correspond to transferred or frozen embryos.

	cFIV n=648 zygotes	ICSI n=586 zygotes	P value
Usable embryos (D3 and D5-6)	300/648 (46.3%)	196/586 (33.4%)	p<0.001

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Score A	103/300 (34.3%)	67/196 (34.2%)	NS
Score B	90/300 (30%)	45/196 (22.9%)	NS
Score C	107/300 (35.7%)	84/196 (42.9%)	NS
Transferred embryos	52/648 (8%)	55/586 (9.4%)	NS
Discarded embryos	348/648 (53.7%)	390/586 (66.6%)	p<0.001
Blastulation rate	195/ 439 (44.4%)	66/329 (20.1%)	p<0.001

3.3. Pregnancy and clinical outcomes of cleaved 1PN embryos in fresh and frozen-thawed cycles

The fresh embryo transfers (32 in the cIVF group and 32 in the ICSI group) allowed 7 pregnancies in the cIVF group (PR=21.9%) as compared to 4 pregnancies in the ICSI group (PR=12.5%). The 7 cIVF pregnancies ended in 4 deliveries (LBR= 12.5%) of healthy newborns, 2 miscarriages (28.6%) and one pregnancy still ongoing. In the ICSI group, 1 birth of a healthy newborn (LBR=3.1%) and 3 miscarriages (75%) were observed. In frozen-thawed cycles 36 pregnancies were achieved out of the 167 transfers. A non-significative difference was observed in pregnancy and live birth rates between embryos derived from cIVF cycles (PR=26.1%, LBR=18.5%) and ICSI cycles (PR=16%, LBR=10.7%). One pregnancy in the cIVF group delivered twins for a total of 18 babies in this group (Table 4).

Table 4. Pregnancy and clinical outcomes of cleaved 1PN embryo in fresh and frozen-thawed cycles.

	Fresh cleaved 1PN embryo		**Frozen-thawed cleaved 1PN embryo**	
	cIVF n=32	ICSI n=32	cIVF n=92	ICSI n=75
Pregnancy rate	7/32 (21.9%)	4/32 (12.5%)	24/92 (26.1%)	12/75 (16%)
Miscarriage rate	2/7 (28.6%)	3/4 (75%)	7/24 (29.2%)	4/12 (33.3%)
Live birth rate	4/32 (12.5%)	1/32 (3.1%)	17/92 (18.5%)	8/75 (10.7%)

Neonatal data showed that 8 births have been achieved before 37 weeks of gesta-tion and only 1 before 32 weeks of gestation of all births of our study. The gender balance was the same in all groups, with an overall sex ratio of 1.06 for the 31 births. However, birth weight was higher in the frozen-thawed cycles than in the fresh cycles without signification (Table 5).

Table 5. Neonatal outcomes of cleaved 1PN embryo in fresh and frozen-thawed cycles.

	Fresh cleaved 1PN embryo		**Frozen-thawed cleaved 1PN embryo**	
	cIVF n=4	ICSI n=1	cIVF n=18	ICSI n=8
Weeks of gestation	38.3±1,4	39.5	37.8±2.7	38.3±1.5
Gender	2♀/2♂	1♂	10♀/8♂	3♀/5♂
Birth weight (g)	2718±548	2950	3336±564	3581±341
Average lenght (cm)	46±4	49	50±3	48±6

The Non-Invasive Prenatal Test (NIPT) performed by 18 patients at the end of the first trimester of gestation showed no abnormalities for chromosomes 13, 18 or 21. In addition, no congenital anomalies were reported in any of the babies born.

4. Discussion

The transfer of embryos developed from monopronuclear zygotes has resulted in the birth of 31 healthy babies without malformation in our center between 2018 and 2022. The decision to transfer such embryos has been widely debated for several years in assisted reproduction groups, but no consensus has yet been reached.

Regarding embryo outcomes in fresh cycles, our analysis showed that percentage of 1PN embryos reaching the blastocyst stages is almost two-fold in cIVF group (44%) in comparison to ICSI group (20%). The same observation was reported by other studies : 21.4% versus 10.7% in the publication of Itoi [5], 26% versus 13.8 % in the data reported by Bradley et al. [1] and 41.6 versus 23.25% in the study published by Li et al [7]. As for the Araki study, the blastulation rate was 32.2%

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in 1PN-derived embryos but without distinction for the used insemination technique [4]. However, in a study analyzing the genome-wide haplotype of embryos derived from 0PN, 1PN and 2PN, blastulation rate in 1PN ICSI embryos was better compared to our results, approximately 45.5% [14].

This low potential for blastulation and production of good-quality embryos of 1PN zygotes is generally linked to a chromosomal defect, particularly after ICSI rather than cIVF [15]. Indeed, Mateo *et al* had shown in 2013 by studying a small cohort of 54 embryos derived from 1PN ICSI zygotes that most of them were chromosomally abnormal [16]. Observation of 1PN zygotes after cIVF is more associated to an inappropriate timepoint on fertilization control. Indeed, it is documented that embryos development kinetics and pronuclei formation are different in zygotes arising from IVF in comparison to those from ICSI [17]. It is also important to highlight in our results that the better blastulation rate in the cIVF group compared to the ICSI group was observed in a population of significantly older patients ($p<0.001$), confirming the efficiency of cIVF method.

In this study, we investigate the fate of 1PN embryos without comparison to 2PN embryos. Nevertheless, when such comparison is performed, it is confirmed that 2PN derived embryos blastulate better than 1PN embryos [1,4,5,6]. Nevertheless, 1PN zygotes that developed into blastocysts had reasonable implantation and live birth rates. In the present study, live birth rates (LBR) were better in frozen cycles (cIVF 18.5 % and ICSI 10.7 %) in comparison to fresh cycles (cIVF 12.5% and ICSI 3.1 %). The same trend of LBR, but at lower rates, has already been reported by Li et al with 32.1% for cIVF and 15.25% for ICSI in frozen cycles in comparison to 8% for cIVF and 0% for ICSI in fresh cycles [7]. To our knowledge, few studies have compared live birth rates after embryo transfer developed from 1PN in fresh and frozen-thawed cycles. Indeed, a recent review authored by Kemper et al [9], confirmed that most publications concern fresh cycles.

There were, in total, 31 live babies born after 1PN-derived embryo transfer without difference in gender balance between the different analyzed groups of our study with a sex ratio male/female of 1.06. Few publications investigating neonatal outcomes after 1PN embryo transfer with sex and gender data are available. Nevertheless, in the study of Li et al, they showed that the sex ratio was also not significantly different between the 1PN and 2PN groups in cIVF cycles[7]. Neonatal outcomes investigation showed a slightly elevated birth weight in the frozen-thawed cleaved 1PN group in comparison the fresh cycles group. Such difference between fresh and frozen cycles was documented in several study as reviewed by Zaat et al in 2021 [18].

Decision to transfer or not 1PN embryos has evolved over the last few decades. Indeed, less recent studies analyzing the chromosomal composition of embryos derived from 1PN zygotes suggest that these embryos should not be used for transfer or cryopreservation after IVF or ICSI treatment [16,19,20,21]. Nevertheless, more recently, the clinical use of these embryos has been reconsidered thanks to the development of extended culture at the blastocyst stage, time-lapse technique and PGT [1,2,3,4,14,22,23].

In the present study, decision to transfer each 1PN embryo was based on its capacity to develop up to the blastocyst stage with sufficient morphological quality. This kind of selection was previously chosen by Itoi and Li teams [5,7,24]. Transfer of days 3 embryos derived from 1PN zygotes was realized only in the absence of normally fertilized embryos and when no other ones were available.

5. Conclusions

In conclusion, we observed better outcomes for 1PN zygotes in cIVF cycles in comparison to ICSI cycles. Our center policy to transfer good quality 1PN-derived embryos allowed the birth of 31 healthy babies which may increase chance of pregnancy in some patients. Considering these results, the discard of 1 PN zygotes should perhaps not be automatic but based on blastocyst development and quality, and ideally on their ploidy analysis.

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Institutional Review Board Statement: The study was approved by the Ethics Committee (number 2048) of the Citadelle Hospital of Liège, Belgium.

Informed Consent Statement: As our study is retrospective, patient consents are "Not applicable".

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Bradley CK, Traversa M V., 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. https://doi.org/10.1016/j.rbmo.2017.03.013.
2. Mateo S, Vidal F, Parriego M, Rodríguez I, Montalvo V, Veiga A, et al. Could monopronucleated ICSI zygotes be considered for transfer? Analysis through time-lapse monitoring and PGS. *J Assist Reprod Genet*, **2017**,34:905–11. https://doi.org/10.1007/s10815-017-0937-z.
3. Capalbo A, Treff N, Cimadomo D, Tao X, Ferrero S, Vaiarelli A, et al. Abnormally fertilized oocytes can result in healthy live births: improved genetic technologies for preimplantation genetic testing can be used to rescue viable embryos in in vitro fertilization cycles. *Fertil Steril* **2017,** 108, 1007-1015.e3. https://doi.org/10.1016/j.fertnstert.2017.08.004.
4. Araki E, Itoi F, Honnma H, Asano Y, Oguri H, Nishikawa K. Correlation between the pronucleus size and the potential for human single pronucleus zygotes to develop into blastocysts: 1pn zygotes with large pronuclei can expect an embryo development to the blastocyst stage that is similar to the development of 2pn . *J Assist Reprod Genet* **2018**, 35, 817–23. https://doi.org/10.1007/s10815-018-1137-1.
5. 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. https://doi.org/10.1007/s10815-015-0518-y.
6. Chen X, Shi S, Mao J, Zou L, Yu K. Developmental Potential of Abnormally Fertilized Oocytes and the Associated Clinical Outcomes. *Front Physiol* **2020**, 11, 1–7. https://doi.org/10.3389/fphys.2020.528424.
7. 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**, 1–6. https://doi.org/10.1017/S096719942000009X.
8. Li M, Huang J, Zhuang X, Lin S, Dang Y, Wang Y, et al. Obstetric and neonatal outcomes after the transfer of vitrified-warmed blastocysts developing from nonpronuclear and monopronuclear zygotes: a retrospective cohort study. *Fertil Steril* **2021**, 115, 110–7. https://doi.org/10.1016/j.fertnstert.2020.07.019.
9. M Kemper J, Liu Y, Afnan M, Mol BWJ, Morbeck DE. What happens to abnormally fertilised embryos? A scoping review. *Reprod Biomed Online* **2023**, 46, 802–7. https://doi.org/10.1016/j.rbmo.2023.02.005.
10. Lédée N, Gridelet V, Ravet S, Jouan C, Gaspard O, Wenders F, et al. Impact of follicular G-CSF quantification on subsequent embryo transfer decisions: A proof of concept study. *Hum Reprod* **2013**, 28, 406–13. https://doi.org/10.1093/humrep/des354.
11. Labied S, Jouan C, Wenders F, Ravet S, Gaspard O, Thonon F, et al. Comparison between paraffin and mineral oil covering on early human embryo culture: a prospective randomized study. *Syst Biol Reprod Med* **2019**, 65. https://doi.org/10.1080/19396368.2018.1492645.
12. Balaban B, Brison D, Calderón G, Catt J, Conaghan J, Cowan L, et al. Istanbul consensus workshop on embryo assessment: Proceedings of an expert meeting. *Reprod Biomed Online* **2011**, 22, 632–46. https://doi.org/10.1016/j.rbmo.2011.02.001.
13. Labied S, Jouan C, Wenders F, Ravet S, Gaspard O, Thonon F, et al. Comparison between paraffin and mineral oil covering on early human embryo culture: a prospective randomized study. *Syst Biol Reprod Med* **2019**, 65, 81–6. https://doi.org/10.1080/19396368.2018.1492645.
14. 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. https://doi.org/10.1093/humrep/dey325.
15. Rosenbusch B. The Chromosomal Constitution of Embryos Arising from Monopronuclear Oocytes in Programmes of Assisted Reproduction. *Int J Reprod Med* **2014**, 2014, 1–8. https://doi.org/10.1155/2014/418198.
16. Mateo S, Parriego M, Boada M, Vidal F, Coroleu B, Veiga A. In vitro development and chromosome constitution of embryos derived from monopronucleated zygotes after intracytoplasmic sperm injection. *Fertil Steril* **2013**, 99. https://doi.org/10.1016/j.fertnstert.2012.11.014.
17. Bodri D, Sugimoto T, Serna JY, Kondo M, Kato R, Kawachiya S, et al. Influence of different oocyte insemination techniques on early and late morphokinetic parameters: Retrospective analysis of 500 time-lapse monitored blastocysts. *Fertil Steril* **2015**, 104, 1175-1181.e2. https://doi.org/10.1016/j.fertnstert.2015.07.1164.
18. Zaat T, Zagers M, Mol F, Goddijn M, van Wely M, Mastenbroek S. Fresh versus frozen embryo transfers in assisted reproduction. *Cochrane Database Syst Rev* **2021**, 2021. https://doi.org/10.1002/14651858.CD011184.pub3.

19. Staessen C, Janssenswillen C, Devroey P, Steirteghem ACV. Cytogenetic and morphological observations of single pronucleated human oocytes after in-vitro fertilization. *Hum Reprod* **1993**, 8, 221–3. https://doi.org/10.1093/oxfordjournals.humrep.a138026.
20. 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. https://doi.org/10.1093/humrep/12.2.321.
21. Azevedo AR, Pinho MJ, Silva J, Sá R, Thorsteinsdóttir S, Barros A, et al. Molecular cytogenetics of human single pronucleated zygotes. *Reprod Sci* **2014**, 21, 1472–82. https://doi.org/10.1177/1933719114530185.
22. Kroener L, Ambartsumyan G, Briton-Jones C, Dumesic D, Surrey M, Munné S, et al. The effect of timing of embryonic progression on chromosomal abnormality. *Fertil Steril* **2012**, 98, 876–80. https://doi.org/10.1016/j.fertnstert.2012.06.014.
23. 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. https://doi.org/10.1016/j.fertnstert.2014.04.026.
24. 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. https://doi.org/10.1016/j.fertnstert.2019.04.045.