Original Investigations

Comparison of the rates for reaching the blastocyst stage between normal and abnormal pronucleus embryos monitorized by time-lapse system in IVF patients
Uzun et al. Blastocyst development and pronucleus

Kübra Nur Uzun[1], Mehmet Cıncık[2], Belgin Selam[3], Özgüç Takmaz[3], Esra Uyar[4]

[1]Medipol University, Institute of Health Sciences, İstanbul, Turkey
[2]Maltepe University School of Medicine, Department of Histology and Embryology, İstanbul, Turkey
[3]Acibadem Mehmet Ali Aydinlar University, School of Medicine, Department of Obstetrics and Gynecology, İstanbul, Turkey
[4]Acibadem Altunizade Hospital, Unit of ART, İstanbul, Turkey

Adress for Correspondence: Belgin Selam
bselam@hotmail.com

DOI: 10.4274/jtgga.galenos.2020.2020.0033

Received: 2 Mar, 2020 **Accepted:** 20 Jul, 2020

Abstract
Objective: To compare the rates of blastocyst stage development between the embryos fertilizing after one (MPN) or more than two pronucleus (3PN, 4PN - MultiPN) with those after two pronucleus (2PN) in the same patients. The embryos were observed by the time-lapse system.
Material and Methods: As a total of 140 patients's embryos who had both abnormal PN (MPN, 3PN or 4PN) and normal fertilized (2PN) embryos after fertilization were followed with time-lapse system following ICSI procedure. The ratios for reaching the blastocyst stage were compared between normal and abnormally fertilized embryos.
Results: 1820 oocytes were collected from 140 patients and 1280 (70.3%) of them were fertilized. MPN, 2 PN and 3PN, 4PN (MultiPN) ratios of the embryos in the pronuclear stage were 11.4%, 83.13% and 5.47%, respectively. The rates of reaching the blastocyst stage among these embryos were 17.1%, 60.8% and 42.8% for MPN, 2 PN and MultiPN, respectively. Rate of blastocyst development was significantly higher following 2PN compared to those after MPN and MultiPN ($p<0,05$). Embryos developing after MultiPN had significantly higher rates of reaching the blastocyst stage compared to those after the MPN ($p<0.01$).
Conclusion: The majority of abnormally pronucleated embryos arrest without reaching the blastocyst stage. MultiPN embryos have higher ratios of blastocyst development than MPN embryos.
Keywords: Blastocyst, ICSI, MPN, MultiPN, time-lapse

Introduction

Successful fertilization is the first parameter that is checked following the combination of oocyte and sperm in assisted reproduction treatments. Two pronucleus (PN) and two polar bodies (PB) are observed 16-18 hours after fertilization under normal conditions. However, an abnormal number of pronucleus (1, 3 or 4) can be seen if abnormal fertilization has occurred.

The reasons for the presence of only one pronucleus (MPN) in the embryo after fertilization have been examined and elucidated by many researchers. MPN and one or two PB's are observed in 2.7-17% of the embryos after IVF and ICSI procedures (1). Embryos with MPN have usually two PB's (1). Plachot reported that the incidence of MPN as around 1%, in IVF or ICSI cycles (2). The zygotes with MPN were thought to be due to parthenogenetic activation rather than spermatozoon fertilization (3). However, sperm penetration findings were observed in approximately 45% of MPN zygotes, providing evidence for their development after fertilization (4). The presence of Y chromosome in the genetic structure of MPN zygotes with preimplantation genetic diagnosis (PGD) was recorded as a proof of fertilization (5). Most embryos that develop from MPN have been detected to be aneuploid even though they contain Y chromosomes (6).

The presence of MPN may be due to errors in the formation or fusion of pronucleus during fertilization (7). Failure of male or female chromatids to form pronucleus causes MPN. Flaherty et al observed, swollen sperm head as (52%), intact, undecondensed sperm head (28%), ejection of the spermatozoon (20%) in one-pronuclear oocytes (8). The formation of MPN embryos was also based on the asynchronous occurrence of pronucleus, the union of male and female pronucleus, and male or female parthenogenesis (9). The asynchronous view of pronucleus has been assessed as the first possibility to explain the presence of biparental diplodia in embryos developing from MPN and some of these embryos were reported to be transferable (10).

The presence of more than two pronucleus in the fertilization control stage rather than the expected 2PN is called MultiPN. Rate of three pronucleus (3PN) formation was 1% and 5% after ICSI and IVF procedures, respectively. (11). "Feenan and Herbert" considered the presence of more than two pronucleus to be associated with genetic disorders (12). They concluded that out of 3PN embryos, 61.8% had a triploid chromosome component, 25.2% had mosaic sequencing, and only 12.6% carried a diploid chromosome set (12). More than two pronucleus formation may be triggered by inability to extrude the second polar body, incomplete chromatid separation to the extruded polar body, and dispersion of oocyte chromatids in the second polar body formation. (13).

Early cleavage may appear normal in 3PN zygotes, but progression may cease or aneuploidy may occur later (14). 3PN zygotes after IVF may develop into different embryo stages with variable chromosomal component (triploidy; XXY, XXX, XYY, diploidy, mosaic) (15).

Time-lapse system observes embryonic development in detail and provides algorithms to select high quality embryos according to the kinetic and morphologic changes. It is important to select embryos with high implantation potential for transfer in assisted reproduction. Abnormally fertilized oocytes, depending on how they are formed, either develop to different stages or arrest usually from the first day. Those who develop until the fifth day are not preferred for transfer because they have low scores. We aimed to compare the rates of reaching the blastocyst stage among abnormally fertilized embryos developing after MPN and MultiPN with those of the normally fertilized embryos after 2PN by time-lapse system, in the current study.

Material and Methods

Study design

This was a retrospective study conducted on IVF patients treated at IVF Center, between January 2013 and December 2017. The study protocol was approved by the institutional review board of University. The study included 140 patients. Consent forms were obtained from the patients. Inclusion criteria were patient age 25-40 years, patients who had both abnormal PN (MPN, 3PN or 4PN) and normal fertilized (2PN) embryos after fertilization, with all embryos followed with time-lapse system following ICSI procedure. The development of fertilized oocytes from the first day to the fifth day were examined. We compared the ratios for reaching the blastocyst stage between normal and abnormally fertilized embryos.

Ovarian Stimulation

The standard ovarian stimulation protocol of our clinic has been reported previously (16). Pituitary downregulation was performed either by GnRHa leuprolide acetate (Lucrin 0,5mg/ml, Abbott, Spain) or GnRH antagonist cetrorelix acetate (Cetrotide, Baxter Oncology GmbH). GnRHa leuprolide acetate was used daily in late luteal phase before the treatment cycle and GnRH antagonist cetrorelix acetate was started daily on the 5th day of the treatment and were continued until the ovulation was triggered. Gonadotropin injections were started on the cycle days 2 or 3, if >2 cm cysts were not observed by baseline ultrasound in patients. The daily dosage for gonadotropin stimulation were individualized between 150 and 300 IU. Patiens were monitorized by ultrasound until the trigger criteria including three follicles with maximum diameter >17 mm. HCG 10000 U (Choriomon, IBSA, Italy) and 5000 U hCG (Choriomon, IBSA, Italy) plus 0.2 mg triptorelin acetate (Gonapeptyl, Ferring GmbH Liel, Germany) were used for oocyte maturation in the agonist cycles and the antagonist cycles, respectively. Patients underwent transvaginal ultrasound-guided oocyte retrieval with a 17-gauge needle, under general anesthesia, 35–36 h after the hCG administration. The oocyte-corona complexes were denuded, incubated for 2 hours and ICSI was performed. We perform ICSI routinely in all patients as our clinical policy.

Preparing EmbryoScope Dish

Micro wells of EmbryoScope dish was filled with pre-heated culture solution. Wells were filled with ~25µl solution without any bubble and were covered with 1.4 ml Ovoil. The dishes were incubated at 37ºC. Each injected oocyte was placed in a well without any bubble and slides were placed into EmbryoScope.

Embryo Selection

Patient information, the qualities of embryos observed were recorded every day by the embryoscope. Main issue for selection of an embryo for transfer was to be in appropriate stage according to post-insemination time. Size and number of PNs were considered. Size and evenness of blastomere, fragmentation (> 10%), multinucleation and vacuole (> 14 µm), cleavage times were evaluated at the early cleavage stages. Cell numbers that join the cell compaction were considered for the next stage, and then, inner cell mass (ICM) and trophectoderm (TE) were notated for the blastocyst stage embryo.

Statistical Analysis

All analyses were carried out by NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA). Univariable and mulivariable Generalized Linear Mixed Models were used to examine the effect of PN number on the blastocyst development and the effect of age and etiologies of infertility on PN number. P value less than 0.05 was considered as statistically significant.

Results

Characteristic data of the patients are summarized in Table 1.

The number of oocytes collected from 140 (N) patients in the study was 1820. Fertilization was observed in 70.3% of the oocytes (n = 1280) (Table 2). Among the fertilized oocytes, 146 were MPN (11,4%), 1064 were 2PN (%83,1) and 70 were MultiPN (%5,5). (Table 2). Abnormal fertilization (MPN,MultiPN) was observed in 216 embryos with abnormal fertilization rate as 16.9%. Out of 1280 fertilized embryos, 702 (54.8%) reached the blastocyst stage.

Three groups as MPN, 2PN and MultiPN were formed according to the number of pronucleus observed on the first day and the ratios of blastocyst development were calculated for each group. Blastocyst development was detected in 17.1%, 60.8% and 42.9% of the embryos developing after MPN, 2PN and MultiPN, respectively (Table 3).

Generalized Linear Mixed Models was used to examine the effect of PN number on blastocyst development. The model obtained was statistically significant, thus the number of PN had a statistically significant effect on the success of blastocyst development ($F=43,731$; $p<0,001$) (Table 4). When binary evaluations were examined, blastocyst development percentage was higher in 2PN group oocytes than MPN and multiPN groups ($p<0,001$; $p=0,015$, respectively). When abnormally fertilized groups were analyzed, blastocyst development rate in MultiPN group was significantly higher than MPN group ($p<0.001$).

We also analyzed the relevance of abnormal PN incidence and patients characteristics. We did not observe any significant differences in the MPN/multiPN incidence between age groups as <35 years and ≥35 years of age ($p>0.05$) (Table 5).

We analyzed the the ratio of abnormal PN between different infertility etiology groups. We did not observe any significant difference for the ratio of multiPN among the infertility etiology groups by Post-hoc analysis ($p>0.05$). The ratio of MPN in POR group was significantly higher than those of the other etiology groups ($p<0,05$).

DISCUSSION

The current study demonstrated that most of the embryos developing after MPN and MultiPN could not reach the blastocyst stage. The proportion of those which reached the blastocyst stage was higher in MultiPN embryos than in MPN embryos. The development of blastocyst from embryos with two pronucleus was significantly higher when compared to the other two groups with abnormal fertilization. According to this data, selection of the embryos with an abnormal number of pronucleus for transfer is not preferred in routine practice. However, a small number of embryos that fertilized with an abnormal number of pronucleus oocyte, can reach to blastocyst stage in some patients. Transfer of these embryos should be considered carefully. This was a retrospective study and we evaluated patients having both normal and abnormally fertilized embryos. As patients already had 2 PN embryos, further tests including trophoectoderm biopsy and PGS were not performed for abnormal PN embryos which reached the blastocyst stage to check the possibility of self-cerrection.

We evaluated patients having both normal and abnormally fertilized embryos in the current study. As the patients already had blastocysts developing from 2 PN embryos, blastoscysts developing from abnormal PN embryos were not transferred. Blastocysts developing from 2PN embryos were preferred for transfer. Therefore, final IVF outcome between abnormal and 2PN transferred blastocysts were not evaluated.

Chromosomal abnormalities are observed in 31.4% of the 3PN zygotes (17). Most of the embryos with MultiPN are usually triploid and usually result with abortion (17). In our study, 42.8% of

MultiPN embryos reached the blastocyst stage. Despite this high rate, the risk of abnormality may not be estimated without genetic screening before embryo transfer.

Development potentials of MPN zygotes with wide pronuclear area or diameter are similar to those for 2PN zygotes (18). This may be observed due to early fusion of the male and female pronucleus. Embryos with MPN and two polar bodies can be transferred only if a PGS diagnosis of euploidy is obtained and there is no other normal fertilized embryo (19). The risk of chromosomal abnormalities in cells with MPN after ICSI is clearly higher than those after IVF and there are arguments that these embryos should not be transferred (20, 21).

A study on abnormal pronucleus, compared embryos with 0 PN and MPN to embryos with 2PN on the fifth and sixth day of development and found no significant difference (22). In another similar study, implantation was not observed following transfer of the embryos developing from 0 PN and MPN (6). The embryos developing after MPN had the lowest rates of reaching blastocyst stage in our study. There is no consensus on the transfer of embryos with MPN in the literature, due to the limited number of studies.

Abnormal pronucleus may be iatrogenic by the methods used in assisted reproductive techniques. The incidence of MultiPN is increased in cases of short incubation of gametes and early clearance of cumulus cells in oocytes (23). Embryos from MPN after IVF have higher rate of development on day 3, 5 and 6 compared to embryos from MPN after ICSI (24). Aneuploidy increases with maternal age, and abnormalities that occur after meiosis, such as mosaicism, polyploidy may occur at a similar rate in all age groups (25). Kang et al. reported that MPN and 3PN incidence increases in hyperstimulated cycles (26). It is controversial whether the factors other than oocyte and sperm may also have effect on abnormal pronucleus.

Different techniques and methods have been searched for utilization of abnormal pronucleated embryos. One of these procedures is removing the excess pronucleus by enucleation from the zygotes with 3PN. A diploid embryo with two pronucleus is obtained after the procedure (27). The main limitation of this practice is the determination of which pronucleus to remove. Removal of the wrong pronucleus may cause the embryo to carry two sets of maternal or paternal chromosomes. In a related study, one pronucleus of 3PN zygotes were removed in the first group, and they were compared with a second group with 3PN zygotes followed without performing any procedure (28). The enucleation group had an increased potential for embryo formation, although the ratio was still low when compared with 2PN zygotes (28). When karyotype analysis was performed on part of the enucleated group, 44.4% were identified as diploid and 55.5% as aneuploid.

Abnormal embryos can also be used to obtain human embryonic stem cells (hESC). hESC are cells with potential for self-renewal and differentiation into three germ layers (29). They can be used as a renewable source in cell transplantation for serious degenerative diseases. Huan et al., obtained hESC from embryos with abnormal pronucleus (0 PN, MPN, 3PN) and normal pronucleus (2PN) (30). All hESCs had normal chromosome content according to karyotype analysis. There was no structural difference among the obtained hESC cells, demonstrating distinct identity and karyotypic stability (30). Therefore, abnormal fertilized embryos can be used as a source for healthy hESC production.

However, genetic disorders can be observed in embryos developing after abnormal pronucleus. An embryo with mosaicism can correct itself by moving the mosaic cells towards the trophoblast during the development process, through the self-repairing mechanism of the cells (31). Tetraploid cells may be excluded from the primitive ectoderm lineage at an early stage. Another self-correcting mechanism is apoptosis and chromosomally abnormal embryos may be suffering from apoptosis (32).

Time-lapse system provides valuable morphokinetic data for embryo in assisted reproduction techniques and each IVF laboratory should determine its own embryo selection criteria based on its own data (33). There is no consensus in the literature, whether abnormally fertilized embryos detected by time-lapse system are suitable for transfer.

Staessen et al., reported the birth of two healthy children and one biochemical pregnancy after transfer of abnormally fertilized embryos (5). Grass and Trounson, similarly recorded a healthy newborn in their study (34). One of the major successes was the birth of nine healthy infants from the transfer of embryos from MPN after IVF and implantation was observed in four of the embryos developing from MPN after ICSI (24). It is not right to declare that abnormally fertilized embryos should not be transferred. However, all abnormally fertilized embryos who have reached the blastocyst stage may not be eligible for transfer. In cases, there is no other embryo reaching the blastocyst stage, they can be transferred if no abnormality is detected after PGT-A.

Mutia et al analyzed 30 embryos developing from 3PN using Next Generation Sequencing (NGS). They detected normal chromosomal array in one third of them while the rest had abnormal chromosome and the highest percentage of abnormality was triploidy (35). In vitro culture and chromosomal analysis of clinically discarded human embryos were investigated by Yao et al (36). Blastocyst formation rates of 2PN embryos were higher than those for the abnormal 1PN, 3PN, and ≥4PN embryos (36). Following PGT-A, 3 healthy live births were obtained by transfer of 4 euploid 0PN-derived blastocysts to 4 patients by Lim and Lee (37). One on-going pregnancy was achieved by transfer of four euploid 1PN-derived blastocysts to 4 patients (37). Similar study by Capalbo et al reported 3 live births by transfer of abnormally fertilized oocyte-derived blastocysts after performing PGT-A (38). Hondo et al evaluated the rates of clinical pregnancy and live birth following transfer of frozen-thawed 1PN- and 0PN-derived blastocysts (39). The pregnancy and live birth rates for 0PN-derived embryos obtained by ICSI were similar to 2PN-derived blastocysts. However, 1PN-derived blastocysts had significantly lowest rates (39).

We evaluated the status of the oocytes only on the first and fifth days. All data were analyzed from one center in which the protocol was applied till day 5. Therefore the study does not involve the data for day 6. If more extensive information is obtained, all data from the first day to the fifth day can be listed, considering the stage of the oocytes on each day. Thus, development of embryos after abnormal fertilization; according to pronucleus numbers, stages, abnormalites in cell numbers until they reach the blastocyst stage can be further evaluated. Scoring the embryos of MPN and MultiPN origin reaching the blastocyst stage and comparing them with embryos of 2PN origin may further illuminate data on the quality of abnormally fertilized embryos.

In conclusion, rate of blastocyst development after 2PN is significantly higher compared to those of the abnormal pronucleated embryos. MultiPN embryos have higher chance to develop blastocytes than MPN embryos. Large-scale research is difficult on this subject, because abnormal fertilization is not very common. Development of abnormally fertilized embryos are usually stalled without reaching the fifth day. Owing to low prevalence of abnormal fertilization, multicenter studies would delineate the effect of abnormal fertilization on embryonic development.

Ethics Committee Approval: Approved by Univesity Ethics Commitee on 22/09/2017-78

References

1. Azevedo AR, Pinho MJ, Silva J, Sa R, Thorsteinsdottir S, et al. Molecular cytogenetics of human single pronucleated zygotes. Ann NY Acad Sci 2014; 21(12).
2. Plachot M. Fertilization. Hum Reprod 2000; 15(4): 19-30.
3. Kaufman MH. Early Mammalian Development: Parthenogenetic Studies. UK: Cambridge University Press; 1983
4. Balakier H, Squire J, Casper RF. Characterization of abnormal one pronuclear human oocytes by morphology, cytogenetics and in-situ hybridization. Hum Reprod 1993; 8(3): 402-408.
5. Staessen C, Janssenwillen C, Devroey P, Steirteghem AC. Cytogenetic and morphological observations of single pronucleated human oocytes after in-vitro fertilization. Hum Reprod 1993; 8(2): 221-22.
6. Noyes N. Fino ME, Krey L, McCaffrey C, Adler A, Grifo J. Embryo biopsy: the fate of abnormal pronuclear embryos. Reprod BioMed Online 2008; 17(6): 782-788.
7. Prados FJ, Debrock S, Lemmen JG, Angerholm I. The cleavage stage embryo. In: ESHRE: Atlas of Human Embryology.
8. Flaherty SP, Dianna P, Swann NJ, Matthews CD. Aetiology of failed and abnormal fertilization after intracytoplasmic sperm injection. Hum Reprod 1995; 10(10): 2623-262.
9. Flaherty SP, Payne D, Matthews CD. Fertilization failures and abnormal fertilization after intracytoplasmic sperm injection. Hum Reprod 1998; 13: 155-164.
10. Rosenbusch B. The chromosomal constitution of embryos arising from monopronuclear oocytes in programmes of assisted reproduction. Reprod Med 2014; 8.
11. Papale L, Fiorentino A, Montag M, Tomasi G. The zygote. Hum Reprod 2012; 27: 22-49.
12. Feenan K, Herbert M. Can abnormally fertilized zygotes give rise to viable embryos?. Hum Fertil 2006; 9(3): 157-169.
13. Rosenbusch BE. A preliminary concept, deduced from cytogenetic analyses, for explaining different types of multipronuclear oocytes obtained after intracytoplasmic sperm injection. Fertil Steril 2010; 94(6): 2479-2481.
14. Moore KL, Persaud TVN, Torchia MG. The Developing Human: Clinically Oriented Embryology. 9th. Philadelphia: Elsevier Saunders; 2013
15. Chen Z, Yan J, Feng HL. Aneuploid analysis of tripronuclear zygotes derived from in vitro fertilization and intracytoplasmic sperm injection in humans. Fertil Steril 2005; 83(6): 1845-1848.
16. Uyar E, Usal D, Selam B, Cincik M, Bagis T. IVF outcomes after hysteroscopic metroplasty in patients with T- shaped uterus. Fertil Res Pract. 2019; 5: 15.
17. Rosenbusch B, Schneider M, Sterzik K. The chromosomal constitution of multipronuclear zygotes resulting from in-vitro fertilization. Hum Reprod 1997; 12(10): 2257-2262.
18. 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. J Assist Reprod Genet 2018; 35(5): 817-823.
19. Mateo S, Vidal F, Parriego M, Rodriguez I, Montalvo V. et al. Could monopronucleated icsi zygotes be considered for transfer? Analysis through time-lapse monitoring and pgs. J Assist Reprod Genet 2017; 34(7): 905-911.
20. Greuner M, Montag M. Morphological Selection of Gametes and Embryos: 2PN/Zygote. In: Montag M, editor. A practical guide to selecting gametes and embryos. CRC press; 2014 p. 97-113.

21. 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: 897-902.
22. Yin BL, Hao HY, Zhang YN, Wei D, Zhang CL. Good quality blastocyst from non-/monopronuclear zygote may be used for transfer during IVF. Syst Biol Reproductive Medicine 2016; 62(2): 139-145.
23. Zhou L, Wang J, Xiao L, Sun H, Wang Y et al. Differential effects of short co-incubation of gametes and early removal of cumulus cells in patients with different fertilizing capabilities. Reprod BioMed Online 2016; 32: 591-596.
24. Itoi F, Asano Y, Shimizu M, Honnma H, Murata Y. Birth of nine normal healthy babies following transfer of blastocyst derived from human single-pronucleate zygotes. Assist Reprod Genet 2015; 32(9): 1401-1407.
25. Munne S. Chromosomal abnormalities and their relationship to morphology and development of human embryos. Reprod BioMed 2005; 12(2): 234-253.
26. Kang H, Neri QV, Wang A, Rosenwaks Z, Palermo GD. Withdrawal of fsh during controlled ovarian superovulation impairs fertilization with icsi. Fertil Steril 2006; 86(3): 320.
27. Suss-Toby E, Nir SG, Amit M, Manor D, Itskoviz-Eldor J. Derivation of a diploid human embryonic stem cell line from a mononuclear zygote. Hum Reprod 2004; 19(3): 670-675.
28. Jin H, Dai S, Song W, Yao G, Shi S, Sun Y. Embryo developmental potential of microsurgically corrected human three-pronuclear zygotes. Syst Biol Reprod Med 2015; 61(2): 96-102.
29. Reubinoff BE, Pera MF, Fong CY, Trounson A, Bongso A. Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro. Nat Biotechnol 2000; 18: 399-404.
30. Huan Q, Gao X, Wang Y, Shen Y, Ma W, Chen ZJ. Comparative evaluation of human emryonic stem cell lines derived from zygotes with normal and abnormal pronuclei. Dev Dyn 2010; 239: 425-438.
31. James RM, West JD. A chimaeric animal model for confined placental mosaicism. Hum Genet 1994; 93(5): 603-604.
32. Hardy K. Cell death in the mammalian blastocyst. Mol Hum Reprod 1997; 3(10): 919-925.
33. Yalçınkaya E, Ergin EG, Calışkan E, Oztel Z, Ozay A, Ozörnek H Reproducibility of a time-lapse embryo selection model based on morphokinetic data in a sequential culture media setting. J Turk Ger Gynecol Assoc 2014; 15(3):156-160.
34. Grass L, Trounson AO. Pregnancy and birth resulting from transfer of a blastocyst observed to have one pronucleus at the time of examination for fertilization. Hum Reprod 1999; 14(7): 1869-1871.
35. Mutia K, Wiweko B, Iffanolida PA, Febri RR, Muna N. et al. The frequency of chromosomal euploidy among 3PN embryos. J Reprod Infertil 2019; 20(3): 127–131.
36. Yao G, Xu J, Xin Z, Niu W, Shi S. et al. Developmental potential of clinically discarded human embryos and associated chromosomal analysis. Sci Rep 2016; 6: 23995.
37. Lim AYX, Lee CSS. Embryos Arising from Apronuclear (0PN) and Unipronuclear (1PN) Have Similar Euploidy Rates with Those from 2PN and Should be Considered for Transfer. Fertility & Reproduction 2019;1(2): 73-77.
38. Capalbo A, Treff N, Cimadomo D, Tao X, Ferrero S. 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(6): 1007-1015.e3.

39. Hondo S, Arichi A, Muramatsu H, Omura N, Ito K. et al. Clinical outcomes of transfer of frozen and thawed single blastocysts derived from nonpronuclear and monopronuclear zygotes. Reprod Med Biol 2019; 18(3): 278-283.

Table 1. Characteristic data of the patients		
N=146		
Age (year)	Range	25-40
	Mean and standard deviation	32,74±4,32
BMI	Range	16,2-41,5
	Mean and standard deviation	23,48±4,48
Indications		**n (%)**
	Unexplained	46 (31,5)
	Male	46 (31,5)
	Anovulation	14 (9,6)
	Tubal factor	23 (15,8)
	Endometriosis	15 (10,3)
	Poor ovarian reserve	16 (11,0)
Number of previous embryo transfer	**0**	37 (25,2)
	1	9 (6,2)
	2	96 (65,8)
	3	2 (1,4)
	4	2 (1,4)

Table 2. Fertilization and blastocyst development among the study group			
		n	**%**
Fertilization	No	540	29,7
	Yes	1280	70,3
PN (n=1280)	MPN	146	11,4
	2PN	1064	83,1
	MultiPN	70	5,5
Blastocyst development (n=1280)	No	578	45,2
	Yes	702	54,8

Table 3. Development of blastocyst according to pronucleus number (PN)			
		Total	**Development of blastocyte**
		n	**n (%)**
PN	MPN	146	25 (17,1)
	2PN	1064	647 (60,8)
	MultiPN	70	30 (42,9)

Table 4. Generalized Linear Mixed Models for the effect of PN number on blastocyst development			
	Model		
	Beta	**Exp(Beta) (95% CI)**	***p***
PN (MPN)	-	1	**-**
PN (2PN)	2,148	8,570 (5,405; 13,588)	***<0,001****
PN (MultiPN)	1,504	4,501 (2,294; 8,828)	***<0,001****
MPN group is used as reference for PN variable			

Table 5: Generalized Linear Mixed Models (GLMM) for the effect of age on PN number				
	MPN	**2PN**	**MultiPN**	**F, p**
	n (%)	**n (%)**	**n (%)**	
Age				F=2.266
<35	94 (10.5)	744 (83.3)	55 (6.2)	p=0.104
≥35	52 (13.4)	320 (82.7)	15 (3.9)	