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Follow up of children born after preimplantation genetic testing

Suivi des enfants nés après dépistage préimplantatoire

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Résumé. L'application du dépistage préimplantatoire pourrait-elle, en elle-même (*i.e.* indépendamment du résultat du test), exercer une influence sur la santé des enfants qui en naîtront ? Afin de répondre à cette interrogation, nous avons synthétisé quatorze études, publiées entre 2008 et 2018, dans lesquelles 1 794 enfant ont été suivis, jusqu'à l'âge de 9 ans, en comparés à deux groupes témoins : 39 709 enfants issus de l'assistance médicale à la reproduction, et 910 626 conçus naturellement. Nous n'identifions aucun effet défavorable du dépistage préimplantatoire sur la santé ou le développement des enfants. Néanmoins, certains auteurs soulignent la possibilité que le protocole exerce un effet subtile sur le développement de certains groupes d'enfants. Un suivi au long cours est donc nécessaire.

Mots clés: dépistage préimplantatoire, suivi, enfant, assistance médicale à la reproduction

Abstract. Could PGT itself affect the health of the children born after the application of this technique? This is a revision of 14 studies published between 2008 and 2018 in which a total of 1794 PGT children between new-borns to 9 years old were studied and compared to control groups (39,709 TRA and 910.626 naturally conceived children). To our knowledge, PGT itself does not seem to affect negatively the health and development of children. Nevertheless, some of the authors pointed out possible subtle effects on the development of some groups of PGT children that makes a long-term follow up of this population mandatory.

Key words : preimplantation genetic testing (PGT), follow up, children, TRA

Embryologists are passionate about embryos and, most of us are also fond of babies; thus babies are the result of our daily work. The objective of assisted reproduction techniques (ART) is to give a healthy baby at home to people that are not able to achieve it naturally.

In order to be able to reach our objective, gynaecologists and embryologists follow strict protocols and analyse in detail laboratory results and reproductive outcomes and scientists perform basic research to try to improve, day by day, our work.

But, do all the manipulations that embryos are suffering *in vitro* have any effect on the babies that are born lately? To answer this question, an accurate follow up of ART children is mandatory and, in fact, some studies have been published but a lot of future work is still necessary.

Although culture conditions and media are trying to simulate what happens *in vivo*, obviously, it is not exactly the same (*figure 1*). *In vitro* culture of embryos in one type of medium have been found to result in singletons with a lower weight during the first 2 years of age compared to the singletons born after the embryos being cultured using another medium [1]. On the other hand, another study concluded that the culture media did not affect the birth weight and length [2]. Thus, it is important to keep in mind that every single step of our work could have a consequence on the final result.

Assisted reproduction techniques vs. naturally conceived children

At present, 1.5 million ART cycles are performed and 350.000 babies born worldwide annually [3] and there are several studies comparing children born after natural conception (NC) with children born after the use of ART.

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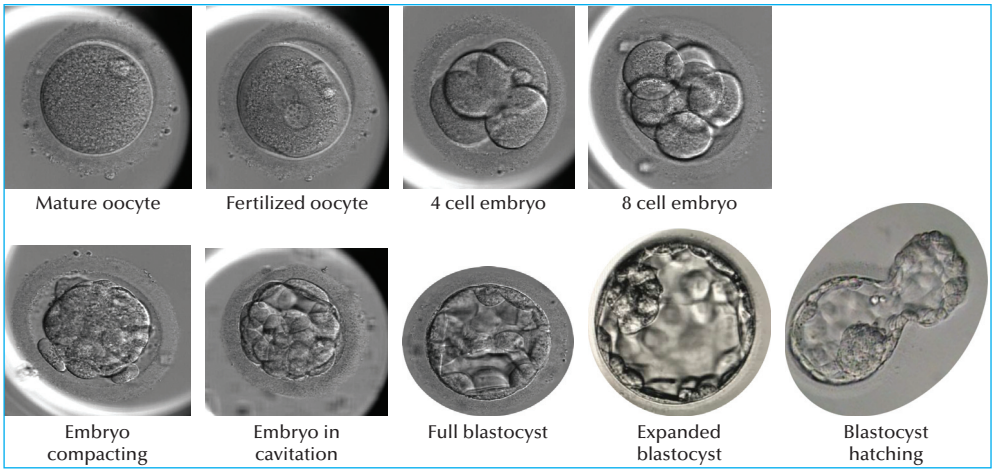

Figure 1. From oocyte to blastocyst.

Pandey and collegues [4] in a review article found that singleton pregnancies after *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) (*figure 2*) were associated with higher risks of obstetric and perinatal complications when compared with spontaneous conception. Concretely they reported more congenital anomalies, low birth weight, perinatal mortality, small for gestational age and preterm delivery. A similar conclusion was stated by another group when comparing 181.741 singletons born after IVF/ICSI with 4.636.508 singletons NC [5]; they found an increase in preterm birth, low birth weight, small for gestational age, perinatal mortality and congenital malformations.

Regarding children's development; a recent review of 24 studies has been published by Catfort [6] comparing ICSI and NC children. Cognitive and motor performance were analysed and also, in some cases, behaviour and family relations concluding that neurodevelopment in both groups was comparable. On the other hand, in the same study, pubertal development was comparable in both sexes but semen parameters were more likely to be altered in ICSI children. Also differences in general physical and metabolic health were described, so, an urgent need for longer follow-up was suggested.

The same research group also published a review article comparing children conceived by conventional IVF and by ICSI [7]. Neurodevelopment and growth and aspects of physical health were similar in childhood however, evaluation of health in adolescence and adulthood seemed still needed as well as studies about metabolic and reproductive points. This is interesting because potential epigenetic modifications could be induced by ICSI procedure, the consequences of which could not be visible until later in life.

Comparing ART to NC children, another study [8] found no detrimental effects on children's early cognitive outcomes up to the age of 11 years and highlighted the importance of parental characteristics in the ART children's development.

The health of children born following analysis of polar bodies (preimplantation genetic diagnosis using two polar bodies and preconception diagnosis using the first polar body)

Preconception genetic diagnosis (PGD) involving a biopsy of the first polar body has been practiced in our centre since 2001 and preimplantation genetic diagnosis (PIGD) involving a biopsy of the two polar bodies was developed in the 1990s. Literature is very sparse on studies monitoring children born following the use of these techniques, but the data that have been published are very reassuring. These practices involve making a hole in the zona pellucida before taking a biopsy of the polar bodies. The zona pellucida is opened mechanically, enzymatically, or with a laser. Regardless of the technique used, no particularly risks for descendants have been revealed based on more than 30 years of practice. In terms of our experience of PGD, 36 children have been born in the maternity ward of our hospital following this technique, and the clinical data are reassuring. Obstetric and neonatal monitoring indicate no particular complications.

In the United States, a team which carries out biopsies and analysis of the polar bodies (PBs), as part of preimplantation genetic diagnosis (PIGD) for monogenetic diseases and aneuploidy, has carried out obstetric and neonatal follow-up for around 100 children [9]. In 2000, the authors reported follow-up data on 109 children born following

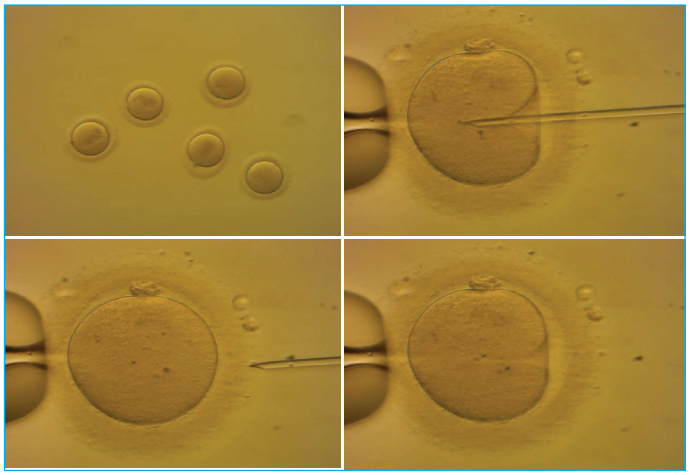

Figure 2. ICSI procedure.

PIGD. Discussions with paediatricians and parents, who all completed a birth questionnaire following childbirth, were supplemented by consultations when necessary. For children over the age of six months, a follow-up telephone interview was conducted to assess the child's development. For these 109 children, the authors assessed gestational age, birth method, perinatal mortality, weight and size at birth, the presence of congenital abnormalities, and stages of development. A total of 91 children were born following polar body analysis for chromosome anomalies, and 18 following analysis for monogenetic diseases. The results of PIGD for Mendelian traits were confirmed postnatally for all pregnancies and in all cases, the maternal allele present in the new-born had been correctly predicted. The same held for PIGD for aneuploidy; no cases of trisomy 21, 18 or 13 were detected at birth.

Of the pregnancies, 75% were carried to term. One neonatal death occurred due to placental abruption and the new-born died at six days due to multivisceral failure syndrome. The Caesarean rate for all births was 40%, which is comparable to that determined by other teams [10]. The percentage of low birth weights (lower than the 10th percentile) for 80 single pregnancies was 9%, indicating that the biopsy of polar bodies appears to have no impact on this parameter.

Regarding congenital abnormalities, no particular malformation profile was found in this cohort. Two cases of congenital haemangiomas were reported, *i.e.* an incidence of less than 2%, comparable to that found in the literature, between 1.1% and 2.6% [11]. Of the 109 children, two (2%) presented with congenital malformations with an impact on development, and four others (4%) presented with minor abnormalities. The same team, a few months earlier [12], had reported a rate of placenta praevia that was higher than that found in the general population (4%), but this remains to be confirmed as the authors had not taken into account any confounding risk factors in their population. For the 109 children followed, there was no increase in incidence of premature or difficult births, nor any significant difference in terms of weight or size at birth. No particular congenital abnormality profile was observed. Overall, polar body biopsy does not appear to have an impact on obstetric or neonatal outcomes. Another observational study of 413 births showed that there was no increase in the rate of anomalies at birth for children born following a biopsy of the blastomere or the polar body [13]. Finally, an important series was published in 2011 [14] consisting of 938 cycles of preimplantation genetic testing for monogenetic diseases with analysis of the two polar bodies. In total, 345 healthy children were born, once again demonstrating that this technique carries no particular risks for descendants. In conclusion, based on the literature, the biopsy of one or two polar bodies does not appear to have an impact on either pregnancy itself nor the state of health of the children subsequently born.

Preimplantation genetic testing

Preimplantation genetic testing (PGT) was first performed in 1990 [15] basically to avoid the transmission of some genetic diseases to the offspring. Nowadays, its use is continuously increasing [16] and the indications are wider.

PGT-A is a form of aneuploidy screening used to optimize pregnancy rates after IVF treatment, whereas PGT-SR is a diagnostic test for structural chromosome alterations and PGT-M is a diagnostic test for couples with an increased risk of offspring with a genetic disorder.

For many years PGT consisted of a blastomere biopsy of embryos at Day 3 and genetic analysis using

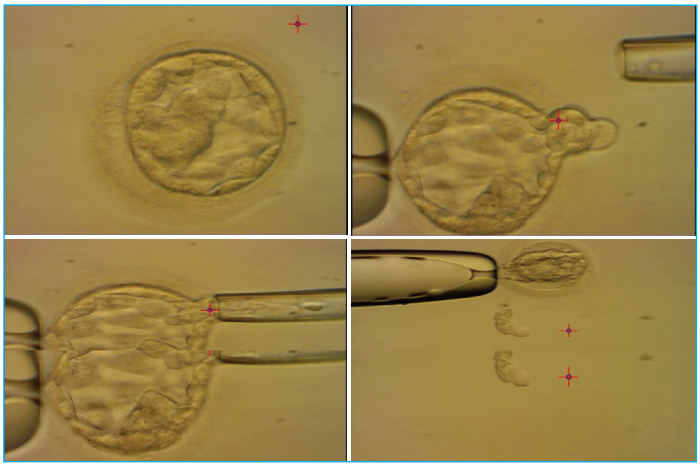

Figure 3. Blastocyst biopsy.

fluorescence in situ hybridization (FISH) or PCR. The utility of this technique for the analysis of aneuploidies (PGT-A) was discussed because while some authors found it useful to improve reproductive outcomes [17] others did not recommend its use [18].

Times have changed and nowadays PGT mostly consists of a trophoectoderm biopsy of blastocysts at Day 5/6 (*figure 3*), which seems to be less harmful for the embryos [19] and a genetic analysis using comprehensive chromosome screening allowing for the analysis of all the chromosomes. This new approach seems to be beneficial and cost effective for most of the patients [20].

PGT includes more extensive embryo manipulation than IVF and this has induced questions on its safety concerning child development. In 10 years of data collection, ESHRE reported 5.135 new-borns after the application of PGT [21].

Assisted reproduction techniques vs. preimplantation genetic testing children

Many studies have been published analysing the possible effect of PGT itself to the children born after the application of the technique.

A total of 14 studies performed between 2008 and 2018 are included in the present review (*table 1*). The number of children studied in all these works are: 1.794 from PGT; 39.709 from ART (FIV / ICSI without PGT) and 910.626 naturally conceived. They were from new-borns to 9-year-old children. There are 4 randomised control trials and 7 prospective, 1 pilot and 2 retrospective studies.

Many different characteristics of children have been analysed either regarding neonatal and physical health or regarding behaviour and other aspects of children necessities. Concretely, about physical aspects, the studies analysed adverse obstetric and neonatal outcomes; congenital malformations; neurodevelopment; blood pressure; anthropometrics; developmental neuropsychological profiles; minor and major morphologic abnormalities; physical development and also neurological characteristics and development. In a more psychological aspect, the studies reported results about cognitive and socio-emotional development; behaviour; psychosocial functioning; language development and also the received medical care.

Most of the authors did not find significant differences between the study groups although five of them could find increased risks in children born after PGT. Altered neurodevelopmental outcomes in PGT-A twins was reported by Schendelaar et al. [22]; the study compared 9 set of 4-year-old twins born after PGT-A with 11 born after TRA and a difference in brain function was found in twins but not in singletons; the neuromotor condition was affected negatively but the sequential processing was affected positively, pointing out to a different brain development in PGT-A twins. In another study [23], more perinatal death in multiple pregnancies of PGT was found; although the authors stated that their finding could be due to the low number of cases included in the analysis.

On the other hand, a trend to low cognitive skills [24] and an increased frequency of received paramedical care [25] were reported in children born after PGT. In the first study one fifth of the 31 studied children had moderate or low cognitive skills, thus the authors suggested an early screening performed by paediatricians in this group of children could be useful to allow an early detection of the problem. In the second study, children born after PGT-A were found to need more paramedical care although any other increased risk of this group of children could be

Table I. Summary of the studies included in the PGT review

Authors	Study design	N° children	Studied diseases	Conclusion
Heijligers M, *et al. Hum Reprod* 2018 [30]	Prospective PGT vs. ART vs. NC 5-year-old children	51 PGT; 52 ART; 35 NC	Cognitive and socio-emotional development	No differences between groups
Kuiper D, *et al. Hum Reprod* 2018 [32]	RCT PGT vs. ART 9-year-old children	43 PGT; 56 ART	Neurodevelopment. Cognitive development. Behaviour. Blood pressure. Anthropometrics	No differences between groups
Heijligers M, *et al. J Assist Reprod Genet* 2018 [31]	Retrospective PGT vs. NC Live born children	364 PGT	Congenital malformation. Adverse perinatal outcome	No differences between groups
Bay B, *et al. Fertil Steril* 2016 [26]	Retrospective PGT vs. ART vs. NC	149 PGT; 36.115 ART; 909.624 NC	Adverse obstetric and neonatal outcomes	Risk mainly related to parental condition rather than the PGT procedure
Sacks GC, *et al. Child Neuropsychol* 2016 [34]	Pilot PGT 4- to 5-year-old children	27 PGT	Developmental neuropsychological profiles	Normal neuropsychological development in study group
Winter C, *et al. Hum Reprod* 2015 [35]	Prospective PGT vs. ICSI vs. NC 5- to 6-year-old children	47 PGT; 50 ICSI; 55 NC	Psychosocial functioning	No differences between groups
Eldar-Geva T, *et al. Fertil Steril* 2014 [29]	Prospective PGT vs. ICSI vs. NC	242 PGT; 242 ICSI; 733 NC	Neonatal outcomes	PGT don't have an impact in pregnancy outcomes
Beukers F, *et al. Fertil Steril* 2013 [27]	RCT PGT vs. ART 2-year-old children	50 PGT; 72 ART; 66 NC	Minor and major morphologic abnormalities	No differences between groups
Seggers J, *et al. Pediatr Res* 2013 [25]	RCT PGT vs. ART 4-year-old children	49 PGT; 64 ART	Blood pressure. Anthropometrics. Received medical care	Higher frequency of received paramedical care in PGT group
Schendelaar P, *et al. Hum Reprod* 2013 [22]	RCT PGT vs. 4-year-old children	49 PGT; 64 ART	Neurological, cognitive and behavioural development	Altered neurodevelopmental outcomes in twins
Thomaidis L, *et al. World J Pediatr* 2012 [24]	Prospective PGT vs. ART	31 PGT	Physical development, neurological characteristics	No deterred growth and psychomotor development. Low cognitive skills in 1/5
Liebaers I, *et al. Hum Reprod* 2010 [23]	Prospective PGT vs. ICSI 2 month babies	581 PGT; 2889 ICSI	Term, birthweight, major malformations; perinatal death	More perinatal death in multiple pregnancies of PGT; but not in singletons
Desmyttere S, *et al. Hum Reprod* 2009 [28]	Prospective PGT vs. ICSI vs. NC Live born and 2-uear-old children	70 PGT; 70 ICSI; 70 NC	Weight, height, head circumference, congenital malformations, hospital interventions	No observable detrimental effects of the PGT procedure to children
Nekkebroeck J, *et al. Hum Reprod* 2008 [33]	Prospective PGT vs. ICSI vs. NC 2-year-old children	41 PGT; 35 ICSI; 43 NC	Socio-emotional and language development	No differences between groups

ART: assisted reproduction techniques; NC: natural conception; RCT: randomised control trial

found neither in their physical characteristics nor in needs of hospitalization. The reasons for their findings were not clear since the authors stated that they could be due to a selection bias or also an effect of PGT itself on subtle parameters of children's development.

Conclusions

With regard to the literature available nowadays, PGT itself does not seem to affect negatively the health of the children born after its application.

Nevertheless, the recent changes in the biopsy technique and the findings of some authors that point out to possible subtle effects in children's development make us conclude that long-term follow up of children born after TRA and, concretely, after PGT is mandatory to really elucidate if all the techniques we are applying in the laboratory and also the hormonal treatments that patients are carrying out when using ART, are affecting the health and development of the offspring. As far as we are concerned, it still remains to be seen.

Liens d'intérêt : Les auteurs déclarent n'avoir aucun lien d'intérêt en rapport avec cet article.

References

1. Kleijkers SH, van Montfoort AP, Smits LJ, *et al*. IVF culture medium affects post-natal weight in humans during the first 2 years of life. *Hum Reprod* 2014 ; 29(4) : 661-9.

2. Lin S, Li M, Lian Y, Chen L, Liu P. No effect of embryo culture media on birthweight and length of newborns. *Hum Reprod* 2013 ; 28(7) : 1762-7.

3. European IVF-Monitoring Consortium (EIM), European Society of Human Reproduction and Embryology (ESHRE), Kupka MS, *et al*. Assisted reproductive technology in Europe, 2011: results generated from European registers by ESHRE. *Hum Reprod* 2016 ; 31 : 233-48.

4. Pandey S, Shetty A, Hamilton M, Bhattacharya S, Maheshwari A. Obstetric and perinatal outcomes in singleton pregnancies resulting from IVF/ICSI: a systematic review and meta-analysis. *Hum Reprod Update* 2012 ; 18(5) : 485-503.

5. Qin JB, Sheng XQ, Wu D, *et al*. Worldwide prevalence of adverse pregnancy outcomes among singleton pregnancies after in vitro fertilization/intracytoplasmic sperm injection: a systematic review and meta-analysis. *Arch Gynecol Obstet* 2017 ; 295(2) : 285-301.

6. Catford SR, McLachlan RI, O'Bryan MK, Halliday JL. Long-term follow-up of ICSI-conceived offspring compared with spontaneously conceived offspring: a systematic review of health outcomes beyond the neonatal period. *Andrology* 2018 ; 6(5) : 635-53.

7. Catford SR, McLachlan RI, O'Bryan MK, Halliday JL. Long-term follow-up of intra-cytoplasmic sperm injection-conceived offspring compared with in vitro fertilization-conceived offspring: a systematic review of health outcomes beyond the neonatal period. *Andrology* 2017 ; 5(4) : 610-21.

8. Barbuscia A, Mills MC. Cognitive development in children up to age 11 years born after ART-a longitudinal cohort study. *Hum Reprod* 2017 ; 32(7) : 1482-8.

9. Strom CM, Levin R, Strom S, Masciangelo C, Kuliev A, Verlinsky Y. Neonatal outcome of preimplantation genetic diagnosis by polar body removal: the first 109 infants. *Pediatrics* 2000 ; 106 : 650-3.

10. Levy T, Goldman JA, Dicker D, Ashkenazi J, Feldberg D. Very early pregnancy wastage in in vitro fertilization and embryo transfer (IVF-ET). *J In Vitro Fert Embryo Transf* 1991 ; 8 : 250-3.

11. Silverman RA. Hemangiomas and vascular malformations. *Pediatr Clin North Am* 1991 ; 38 : 811-34.

12. Strom CM, Strom S, Levine E, Ginsberg N, Barton J, Verlinsky Y. Obstetric outcomes in 102 pregnancies after preimplantation genetic diagnosis. *Am J Obstet Gynecol* 2000 ; 182 : 1629-32.

13. Dawson A, Griesinger G, Diedrich K. Screening oocytes by polar body biopsy. *Reprod Biomed Online* 2006 ; 13 : 104-9.

14. Kuliev A, Rechitsky S. Polar body-based preimplantation genetic diagnosis for Mendelian disorders. *Mol Hum Reprod* 2011 ; 17 : 275-85.

15. Handyside AH, Kontogianni EH, Hardy K, Winston RML. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. *Nature* 1990 ; 344 : 768-70.

16. Sermon K, Capalbo A, Cohen J, *et al*. The why, the how and the when of PGS 2.0: current practices and expert opinions of fertility specialists, molecular biologists, and embryologists. *Mol Hum Reprod* 2016 ; 22 : 845-57.

17. Munné S, Chen S, Fischer J, *et al*. Preimplantation genetic diagnosis reduces pregnancy loss in women aged 35 years and older with a history of recurrent miscarriages. *Fertil Steril* 2005 ; 84(2) : 331-5.

18. Twisk M, Mastenbroek S, van Wely M, Heineman MJ, Van der Veen F, Repping S. Preimplantation genetic screening for abnormal number of chromosomes (aneuploidies) in in vitro fertilisation or intracytoplasmic sperm injection. *Cochrane Database Syst Rev* 2006 ; 1 : CD005291.

19. Scott RT, Upham KM, Forman EJ, Zhao T, Treff NR. Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. *Fertil Steril* 2013 ; 100 : 624-30.

20. Neal SA, Morin SJ, Franasiak JM, *et al*. Preimplantation genetic testing for aneuploidy is cost-effective, shortens treatment time, and reduces the risk of failed embryo transfer and clinical miscarriage. *Fertil Steril* 2018 ; 110(5) : 896-904.

21. Harper JC, Wilton L, Traeger-Synodinos J, *et al*. The ESHRE PGD Consortium: 10 years of data collection. *Hum Reprod Update* 2012 ; 18(3) : 234-47.

22. Schendelaar P, Middelburg KJ, Bos AF, *et al*. The effect of preimplantation genetic screening on neurological, cognitive and behavioural development in 4-year-old children: follow-up of a RCT. *Hum Reprod* 2013 ; 28(6) : 1508-18.

23. Liebaers I, Desmyttere S, Verpoest W, *et al*. Report on a consecutive series of 581 children born after blastomere biopsy for preimplantation genetic diagnosis. *Hum Reprod* 2010 ; 25(1) : 275-82.

24. Thomaidis L, Kitsiou-Tzeli S, Critselis E, *et al*. Psychomotor development of children born after preimplantation genetic diagnosis and parental stress evaluation. *World J Pediatr* 2012 ; 8(4) : 309-16.

25. Seggers J, Haadsma ML, Bastide-van Gemert Sl ML, *et al*. Blood pressure and anthropometrics of 4-y-old children born after preimplantation genetic screening: follow-up of a unique, moderately sized, randomized controlled trial. *Pediatr Res* 2013 ; 74(5) : 606-14.

26. Bay B, Ingerslev HJ, Lemmen JG, Degn B, Rasmussen IA, Kesmodel US. Preimplantation genetic diagnosis: a national multicenter obstetric and neonatal follow-up study. *Fertil Steril* 2016 ; 106(6) : 1363-1369.e1.

27. Beukers F, van der Heide M, Middelburg KJ, *et al*. Morphologic abnormalities in 2-year-old children born after in vitro fertilization/intracytoplasmic sperm injection with preimplantation genetic screening: follow-up of a randomized controlled trial. *Fertil Steril* 2013 ; 99(2) : 408-13.

28. Desmyttere S, De Schepper J, Nekkebroeck J, *et al*. Two-year auxological and medical outcome of singletons born after embryo biopsy applied in preimplantation genetic diagnosis or preimplantation genetic screening. *Hum Reprod* 2009 ; 24(2) : 470-6.

29. Eldar-Geva T, Srebnik N, Altarescu G, *et al*. Neonatal outcome after preimplantation genetic diagnosis. *Fertil Steril* 2014 ; 102(4) : 1016-21.

30. Heijligers M, Verheijden LMM, Jonkman LM, *et al*. The cognitive and socio-emotional development of 5-year-old children born after PGD. *Hum Reprod* 2018 ; 33(11) : 2150-7.

31. Heijligers M, van Montfoort A, Meijer-Hoogeveen M, *et al*. Perinatal follow-up of children born after preimplantation genetic diagnosis between 1995 and 2014. *J Assist Reprod Genet* 2018 ; 35(11) : 1995-2002.

32. Kuiper D, Bennema A, la Bastide-van Gemert S, *et al*. Developmental outcome of 9-year-old children born after PGS: follow-up of a randomized trial. *Hum Reprod* 2018 ; 33(1) : 147-55.

33. Nekkebroeck J, Bonduelle M, Desmyttere S, Van den Broeck W, Ponjaert-Kristoffersen I. Socio-emotional and language development of 2-year-old children born after PGD/PGS, and parental well-being. *Hum Reprod* 2008 ; 23(8) : 1849-57.

34. Sacks GC, Altarescu G, Guedalia J, *et al*. Developmental neuropsychological assessment of 4- to 5-year-old children born following Preimplantation Genetic Diagnosis (PGD): A pilot study. *Child Neuropsychol* 2016 ; 22(4) : 458-71.

35. Winter C, Van Acker F, Bonduelle M, Desmyttere S, Nekkebroeck J. Psychosocial development of full term singletons, born after preimplantation genetic diagnosis (PGD) at preschool age and family functioning: a prospective case-controlled study and multi-informant approach. *Hum Reprod* 2015 ; 30(5) : 1122-36.