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Abstract

Before 1990, options were limited for couples who were at risk for transmitting a genetic disease or a structural chromosomal abnormality to their children. Couples traditionally underwent invasive procedures such as amniocentesis and chorionic villus sampling, after which termination was offered if the fetus was found to be affected. Many couples chose not to have children at all. Since then, technological advances have allowed preimplantation genetic testing (PGT) to be offered to these couples. Couples who choose PGT undergo in-vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI), where the oocyte is injected by a single sperm and is then implanted into the mother a few days later. However, in PGT, a few cells are removed and genetically analyzed before implantation to determine whether the embryo has a specific genetic defect or aneuploidy. The purpose of this paper is to determine whether PGT causes adverse clinical outcomes by critically analyzing PGT research studies. Current research does not seem to show any major adverse clinical outcomes after PGT especially in cases of singleton pregnancies. It is important to continue to examine the effects of an embryo biopsy in terms of neonatal and obstetric outcomes, as well as future development.

Introduction

Preimplantation genetic testing (PGT) has become an integral part of assisted reproductive technology (ART) and over a third of ART Centers in the United States are utilizing PGT technology (Kuliev, Rechitsky, 2017). There are three kinds of PGT. The first, PGT-M, analyzes the embryo for monogenic diseases. This is generally used when one or both parents carry a mutation, such as those linked to Huntington's disease or cystic fibrosis. Testing is performed to ensure the single-gene trait has not been passed to the embryo. It is often used after a previous child has been diagnosed with a specific genetic condition. PGT-M may also be used for sex selection, such as when a parent is a carrier of an x-linked disorder (Pastore, et. al. 2019). The second, PGT-A, and third, PGT-SR, are not standard procedures and were developed to improve the success rate of IVF. PGT-A is used to look for embryonic aneuploidy and PGT-SR is used to look for chromosomal structural rearrangements such as inversions or translocations. PGT-A and PGT-SR are usually only recommended in cases of previous failed rounds of IVF, severe male infertility, recurrent pregnancy loss, in cases where one or both parents have a balanced chromosome structural rearrangement, or for patients at high risk for embryo aneuploidy, such as those of advanced maternal age. While all forms of PGT come with many ethical questions, in general PGT-M is considered more acceptable, especially when it is used to prevent severe genetic diseases with few treatment options. Genetic counseling is recommended before any form of PGT to ensure that the couple understands the risks and limitations of the procedure (Eskew, Jungheim, 2017).

There are multiple methods of performing PGT. Polar body biopsy (PBB) is a common method for genetically analyzing an embryo. Polar bodies are formed during meiosis of an oocyte and are not required for fertilization or embryo development. Therefore, they can be removed safely and screened without harming the embryo. Additionally, PBB avoids errors due to the presence of mosaicism that other methods of PGT incur. Mosaicism is when different cells have different genotypes within one organism and is not present at the zygote stage. PBB is considered a less invasive procedure and is a good option for patients who view more invasive procedures as unethical. However, PBB can only provide maternal genetic information. Because PBB does not include paternal genetic information and cannot be used to determine gender, this method can only work in certain cases (Schenk, et. al. 2018).

PGT can also be done through a blastomere biopsy during the cleavage stage. This is done three days after fertilization, when the embryo is between six to eight cells. A blastomere biopsy is an invasive procedure where cell-to-cell adhesions are loosened and one or two blastomeres are aspirated. The blastomeres are then genetically analyzed for either aneuploidy or specific genetic mutations (Kalma, et. al. 2018). A blastomere biopsy allows both maternal and paternal genetic information to be analyzed, which makes determining the gender of the embryo possible. However, given that this is an invasive procedure, this method may affect the growth and development of the embryo. While there is evidence that a day three embryo can tolerate and overcome the possible resultant damage, it is likely that embryos that would otherwise progress to implantation will be lost at this stage of embryo development. Additionally, a blastomere biopsy may not always be reliable since it is affected by both the technical and biological problems associated with single cell analysis. Specifically, mosaicism, which is at the highest level at this stage of development, can lead to false positive and false negative errors. In order to compensate, two blastomeres can be removed. While this may increase the accuracy of the genetic testing, around 25% of the embryonic mass is removed, which may impact clinical outcomes (Cimadomo, et. al. 2016).

A third method of PGT is a blastocyst biopsy. It is

usually done five to six days after fertilization, when the embryo is about one hundred cells. During a blastocyst biopsy, five to six cells of the trophectoderm are removed and analyzed. This method allows more cells to be tested, compared to the only one or two cells that can be removed during a blastomere biopsy, and allows for improved accuracy of the genetic testing. Additionally, this procedure removes a smaller proportion of embryo cell mass when compared to the day three biopsy and only removes cells from the trophectoderm, not the inner cell mass. However, blastocyst biopsies have limitations as well. Only 50% of IVF embryos develop to the blastocyst stage and waiting for a day five biopsy may result in no transfer at all. Additionally, following a day five biopsy, embryos typically need to be cryopreserved and then thawed which precludes the transfer of a fresh embryo. While there are many methods of performing PGT, each method has its own benefits and limitations (Wang, et. al. 2018). Once the cells are removed, they are genetically analyzed by either polymerase chain reaction (PCR), fluorescence in situ hybridization (FISH), array-comparative genomic hybridization, and more recently, next-generation sequencing, in order to determine if there are any genetic defects (Heijligers, et. al. 2018).

Because PGT is an invasive procedure, researchers have wondered if it increases the risk of adverse clinical outcomes. It is especially important to monitor the safety of PGT since the majority of the PGT couples, specifically couples undergoing PGT-M, have no fertility issues and have the alternative of a natural conception with or without invasive prenatal testing. This review is aimed at determining whether PGT increases the risk of adverse obstetric and neonatal clinical outcomes as well as future development.

Methods

The research discussed in this paper was collected using EBSCO, ProQuest, PubMed and Google Scholar with access provided by the Touro College Library. All articles included are original, peer reviewed research papers that were analyzed to ensure accurate data.

Discussion

Neonatal Outcomes

Malformation/ Perinatal Death

A study was aimed at evaluating the safety of PGT and focused on the rate of congenital malformations as well as other adverse perinatal outcomes. In this study, embryos for PGT analysis were produced by intracytoplasmic sperm injection (ICSI) and subjected to blastomere biopsy. Parents filled out a questionnaire regarding their pregnancy and the health of their child. Medical information, such as age of both parents at embryo transfer, gravidity, parity, number of previous in vitro fertilization (IVF)/ PGT cycles, whether the embryo(s) was fresh or frozen/ thawed, how many blastomeres had been removed, and how many embryos were transferred, was also obtained from their doctor. The largest proportion of couples in this study opted for PGT-M, in order to avoid passing an autosomal dominant disease to the child. In this study, more girls than boys were born after PGT with a ratio of 1.2. This may be due to sex-selection, where a female embryo is transferred to reduce the risk of inheriting an X-linked condition. Major congenital malformations were found in nine of the 364 live births (2.5%). Four of these children had multiple congenital anomalies and five children (1.4%) had minor malformations. Three pregnancies were terminated because of diagnoses of exencephaly, trisomy 18, and trisomy 21. The major malformation rate when including pregnancy terminations due to congenital malformations was 3.3%. A report by the European Surveillance of Congenital Anomalies stated a prevalence of 261.45 major and minor birth anomalies per 10,000 births (2.6%) between 2008 and 2012, which is similar to the rate in this study. According to these results, the risk of major malformations in children born after PGT does not seem to be increased when compared to the general population. The study also found that perinatal deaths were reported in 3 out of 364 PGT pregnancies studied. Two of the pregnancies were of a twin and a triplet. Additionally, at 37 weeks gestational age, a singleton was stillborn, after an uncomplicated pregnancy. With a perinatal mortality rate of 0.8%, no evidence for a potential increased risk in fetal or neonatal death was found after PGT (Heijligers, et. al. 2018).

Similar results were found in a study that looked at the health of 49 children conceived after PGT compared to 66 naturally conceived (NC) controls. Control children were matched for age, sex, ethnicity, maternal educational level and socioeconomic status. A majority of PGT subjects had undergone PGT-A, however the study did not distinguish between subjects who had undergone PGT-M, PGT-A, or PGT-SR. However, all PGT subjects were born after an embryo biopsy at the eight to ten cell stage. Pediatricians that assessed the children were blinded to the conception status of the children, strengthening the results of this study. The study found that two children born after PGT had congenital anomalies, one with a minor ear deformity and the other with mild hypospadias (Banerjee, et. al. 2008).

Another study examined whether PGT blastomere biopsies impacted the health of infants up to two months of age by comparing the data of 995 children born after PGT

and 1507 children born after IVF and ICSI. Twenty-three PGT children (2.3%) and 40 ICSI children (2.7%) presented major malformations. Major genital malformations were recorded in seven PGT children and 9 ICSI children. Four stillborns conceived after PGT and seven stillborns conceived after ICSI presented major malformations. The total major malformation rate, including stillborn and live born, was comparable in the PGT group (2.6%) and the ICSI group (3%). These results do not indicate that the added cleavage-stage biopsy procedure increases the risk of major birth defects compared to the ICSI procedure (Desmyttere, et. al. 2012).

In another study, data from the PGT pregnancies of 158 singletons, 42 pairs of twins, and 1 set of triplets was compared to data obtained from 242 children born after IVF/ ICSI and 733 randomly selected NC children born during the same time period. The mothers in all groups were matched for age, preconception body mass index, and parity. Data collected included parental demographic information, type of biopsy performed (polar body and/ or blastomere biopsy), number of embryos transferred, whether the embryo(s) were fresh or frozen/ thawed, gestational age and mode of delivery. At two to four months, parents also filled out a questionnaire regarding any malformations that had not been diagnosed at birth. In both single and multiple pregnancies, the type of embryo biopsy had no significant influence the neonatal outcome. Four of the PGT children (1.7%) presented congenital malformations. One intrauterine fetal death occurred at 33 weeks with a subsequent diagnosis of thrombophilia. The congenital malformation rate for PGT pregnancies is similar to the rates found in other studies (Eldar-Geva, et. al. 2014). Additionally, in another Israeli study completed around the same time of 213,288 NC births, the rate of congenital malformations was 1.9% which is similar to the rate of malformation after PGT in this study (Farhi, et. al. 2013).

Another study looked at the health of 581 children born after a blastomere biopsy. Questionnaires were sent to both physicians and parents at conception and delivery and children were examined at two months of age, usually by a clinical geneticist. The researchers followed 484 pregnancies, with three terminations for major malformations seen on prenatal ultrasounds. Of these, 385 were singleton pregnancies, 92 were twin pregnancies and four were triplet pregnancies leading to a total of 581 PGT children. There were no differences in any of the studied properties between biopsies done for PGT-M or PGT-A and the results were therefore combined. As seen in many other studies, sex distribution of live born children was uneven with 54% girls for 46% boys and is due to sex selection for X-linked diseases. Of the 581 children in the PGT cohort, eighteen were stillborn and nine died neonatally. Of these 27 perinatal deaths, four were in singleton pregnancies and 23 in multiple pregnancies. The rate of perinatal deaths in singletons is comparable to the ICSI cohort, however the ICSI multiple birth cohort had a higher perinatal death rate. Major malformations were seen in 17 PGT fetuses which led three to be terminated. This led to a malformation rate in born and unborn children of 2.9%. Of the fourteen children with malformations, two were stillborn and both were from a multiple pregnancy. This leaves one inherited and eleven sporadic mutations in the 563 PGT children born alive with a rate of 2.13%. The major malformation rate in the ICSI cohort was 3.13%. The main finding of this study is that a day three embryo biopsy does not seem to increase the risk of major malformations. When these results are compared with the data collected from IVF/ ICSI children born within the same timeframe, the rate of malformations is comparable. (Liebaers, et. al. 2010)

Gestational Age/ Birth Weight

Studies also looked at the gestational age and birthweight of children conceived through PGT. In the study by Heijligers et al. (2018), eighty percent of the PGT children were born full term. Eight children, all from twin pregnancies (2.2%), were born very premature (<32 weeks). The study distinguished very premature children from premature children to show that the very premature children were all from twin pregnancies. Less than 15% of the PGT children had a low birth weight and were either twins or triplets. Only one singleton had a very low birth weight. The child was born at 35 weeks through caesarian section because of HELLP syndrome in the mother. A z-score of +0.17 was calculated for the singletons which indicates a comparable birth weight between this cohort and the rest of the Dutch population. In concordance with other studies on PGT there is an evident increase in prematurity and low and very low birth weight in multiples when compared to singletons. This strongly supports the current Dutch single embryo transfer policy. Overall, data from this study on pregnancy duration and birth weight in the Dutch PGT population, especially in the singletons, seems similar to the published data on naturally conceived children.

In the study performed by Eldar-Geva et. al. (2014), the difference in mean birth weight for singleton pregnancies between the three groups was statistically significant. Singleton NC children had a significantly higher birth weight than those born after ICSI (P=.006) but not compared to the PGT singletons. Low birth weight was also more frequent in the ICSI group than in the PGT and

NC singletons. Also, significantly more ICSI twins (58%) presented with low birth weight compared to 41.0% of PGT twins and 44.2% of NC twins. Very low birth weight (<1,500 g) was rare in all groups. There was also a statistically significant difference among the groups when examining intrauterine growth for singleton pregnancies (P=.001). Intrauterine growth restriction was more frequent in ICSI pregnancies (9.5%) than in NC (5.5%) or PGT pregnancies (5.1%). Children born large for their gestational age was more frequent in the PGT group (16.5%) than the NC group (8.8%). The mean gestational age, rates of preterm birth and intrauterine growth restriction for twin and triplet pregnancies were similar for the three groups.

These results show that there are no increased risks of intrauterine growth restriction or low birth weight in singleton or twin pregnancies after PGT compared to NC. However, ICSI pregnancies did show an increased risk for both of these complications. These results remained true even after controlling for factors such as maternal age, parity, BMI, number of embryos transferred and whether the embryo was cryopreserved, which can affect pregnancy outcomes. The increased likelihood of adverse outcomes in ICSI pregnancies may be due to the fertility status of the parents. Infertile women are more prone to adverse outcomes even when conceiving naturally, indicating that infertility itself is what increases the risk of adverse outcomes such as low birth weight and preterm delivery (Basso, Baird, 2003). This may explain the similarity of the results in birth weight and intrauterine growth from PGT and NC pregnancies, since the majority of PGT couples usually do not struggle with fertility.

The difference in pregnancy duration for singleton pregnancies between the three cohorts was also statistically significant. NC pregnancies were longer, than both the PGT and the ICSI pregnancies. However, for the PGT group, these findings had no clinical significance because the frequency of preterm deliveries, both <37 (7.4%) and <34 weeks (1.3%) was comparable with NC pregnancies (5.7% and 2.0%, respectively). However, 11.4% of the ICSI cohort were born prematurely. PGT and ICSI pregnancies may have been shorter for different reasons. Women who undergo PGT are at high risk for autosomal recessive, X-linked, or dominant genetic disease and therefore have a higher incidence of previous pregnancy terminations for affected fetuses. Complications associated with induced abortions include premature delivery of future children and cervical incompetence. Additionally, some of the PGT women in the study had autosomal dominant diseases such as myotonic dystrophy, achondroplasia, neurofibromatosis and tuberous sclerosis and because of this chose to have a cesarean delivery at 37 to 38 weeks. In fact, the PGT cesarean delivery rate was more than double in PGT pregnancies. Additionally, some of the families had critically ill children which may have placed an emotional and physical burden on the family and pregnant mother (Eldar-Geva, et. al. 2014). The preterm birth rate for the IVF/ ICSI group is unsurprising. As discussed above many studies have found that preterm birth is associated with children conceived through IVF/ ICSI because of the parents underlying fertility issues (Wisborg, et. al. 2010).

Another study looked at the health of 49 PGT children and 66 NC children. The PGT cohort had a significantly lower gestational age at birth (P = 0.0001) and more preterm births than the NC group. The PGT group was also more likely to have a lower birth weight and a higher number of births with a birth weight of less than 2500 grams. Interestingly, this finding is consistent with other studies of assisted reproduction outcomes such as IVF/ ICSI. In most cases PGT conception is closest to natural conception and not assisted reproduction conception, with regard to the reproductive health of parents. Parents who opt to undergo the most common form of PGT, PGT-M, usually do not have fertility issues but are concerned with passing a genetic disease to their children. However, in this study, the majority of PGT patients had undergone PGT-A in which parents bear closer risk and resemblance to couples undergoing other assisted reproductive conception. PGT-A is usually used after failed IVF cycles or because of other fertility issues, such as advanced maternal age. It is therefore unsurprising that the age of the PGT mothers was significantly higher than the NC mothers, (P = 0.0001) as was the rate of preterm birth and low birth weight in the PGT group, which is commonly seen in other assisted reproduction outcome studies (Banrjee, et. al. 2008).

The study performed by Desmyttere et. al. (2012), found that the average birthweight for PGT singletons and PGT multiples with a very low birth weight (<1500 g) was comparable with the ICSI children. However, significantly more ICSI multiples presented a low birthweight (<2500 g), more specifically 268 (17.8%) ICSI compared to 161 (16.2%) PGT babies. Again, this may be due to the fertility status of the parents, since the ratio of infertile couples was higher in the ICSI cohort than in the PGT cohort. Measurements of height and head circumference showed no significant differences between the two groups. Mean gestational age at birth for PGT singletons, twins and triplets showed no difference compared to the ICSI group. Additionally, the number of PGT singletons and multiples born prematurely (<37 weeks) showed no differences compared with their ICSI counterparts

(all P-values > 0.05). Twenty-one ICSI and four PGT singletons versus 67 ICSI and 31 PGT multiples were born very prematurely (<32 weeks) which is not significant (P = 0.056 and P = 0.43 for singletons and multiples). Admission after delivery to the neonatal intensive care unit was comparable for both the PGT and ICSI groups for singletons and multiples. These results show that singletons and multiples born after a PGT embryo biopsy had similar neonatal outcomes in terms of auxological data, gestational age and neonatal hospital admission, to the control group of singletons and multiples with no embryo biopsy. Additionally, in this study, PGT multiples appear to be at a lower risk for low birthweight when compared to IVF/ ICSI multiples.

The study by Liebaers et al. (2010) found that in the PGT cohort, 11.5% of singletons and 65.7% of multiples were born premature. In the ICSI cohort, 8.4% of singletons and 57.9% of multiples were born premature. Low birthweight was observed in 7.4% of PGT singletons and very low birth weight in 3 (0.8%) PGT children. Multiple PGT births of a low birth weight (62.5%) were significantly lower than ICSI multiple births (49.4%). Very low birth weight was observed in 19 of the PGT multiples. These results suggest that a three day PGT biopsy does not seem to add significant health risks for singleton PGT children since when these results were compared to the data collected from IVF/ ICSI children born within the same years, gestational ages and birthweights were similar. However, PGT multiples appeared to be at an increased risk of low birthweight, preterm birth, and perinatal death compared to ICSI multiples. Multiple pregnancies should be avoided when possible and may potentially solve problems especially regarding perinatal death.

A similar study looked at whether women who conceived after PGT and their children have greater risks of adverse pregnancy and birth outcomes compared with children conceived spontaneously or after IVF with or without intracytoplasmic sperm injection (ICSI). The study looked at factors such as pre-eclampsia, preterm primary rupture of membranes, placenta previa, abruption of placenta, preterm birth, low birth weight, major malformations, and neonatal admission. It was found that compared to women conceiving spontaneously, women who had undergone PGT or IVF/ ICSI were older, more often uniparous, had a higher BMI and smoked less often during pregnancy. The children conceived after IVF/ ICSI had a lower birth weight, shorter gestational age, longer neonatal hospital admission and an increased risk of preterm birth and malformations. Children born after PGT had a comparable risk of the same complications, however, the results were just short of statistical significance for many of the outcomes. Nonetheless, PGT children were found to have a significant increased risk of preterm birth, shorter gestation, and longer neonatal hospital admission. The study also looked at the difference in outcomes between PGT-M, PGT-A and PGT-SR. When compared to NC children, PGT-SR and PGT-A children did not have an increased risk of adverse neonatal outcomes. When compared to IVF/ ICSI children, PGT-SR and PGT-A children had comparable neonatal outcomes and were found to have a higher mean birth weight. However, compared to NC children, children born after PGT-M had a significantly lower birth weight, shorter gestation and increased risk for longer neonatal hospital admission. These results show an increased risk of neonatal complications in PGT pregnancies when compared to spontaneous pregnancies. However, the risk of adverse outcomes was generally comparable to IVF/ ICSI pregnancies, indicating that the actual embryo biopsy does not add additional risks. Additionally, when separating PGT-SR and PGT-A pregnancies from PGT-M pregnancies, adverse neonatal outcomes were only found in children conceived through PGT due to a parental monogenetic disorder (PGT-M) and not in children born after PGT-SR and PGT-A. These results make it likely that the risk of adverse outcomes is not related to PGT itself, but to the underlying condition of the parents. These factors can include the known genetic disorder, associated comorbidities or any medications taken during pregnancy. (Bay, et. al. 2016)

A study compared the growth data at birth and two years for 70 singletons born after PGT, ICSI or natural conception. Children were matched for gender, language, birth order and maternal education level. At birth, height and head circumference data were comparable for the PGT, IVF/ ICSI and NC cohorts. While the PGT singletons tended to have a lower birthweight and gestational age compared with the NC children, these differences did not reach statistical significance. When comparing children born after a biopsy of one or two blastomeres, weight, height and head circumference measurements were comparable for the two groups. Additionally, admission to a neonatal ward was comparable in the three conception groups and PGT children did not experience more hospital stays for medical reasons than the ICSI and NC groups. PGT children were also reported to have undergone more complementary examinations (with normal results) compared with NC and ICSI babies. However, this is probably due to precautionary measures for 'specially conceived' children (Desmyttere, et. al. 2009).

Obstetric Outcomes

In the study performed by Eldar-Geva et. al. (2014), the incidence of pregnancy complications such as hypertension

and diabetes were similar in the PGT, IVF/ ICSI and NC groups. Of the PGT mothers 1% had hypertension and 2% had gestational diabetes. Of the ICSI and NC mothers, 1% had hypertension and 6% had diabetes, and 3% had hypertension and 4% had diabetes, respectively. Additionally, the differences in mode of delivery for singleton pregnancies was statistically significant. The cesarean delivery rates were 28.5% in the PGT group, 31.6% in the ICSI group, and 11.0% in the NC group (P<.005). As discussed earlier, the cesarean rate for the PGT cohort was more than double the rate of the NC group in this study and was probably due to the fact that women with autosomal dominant diseases in the PGT cohort opted to have a cesarean delivery at 37 to 38 weeks.

In the study performed by Desmyttere et. al. (2009), increased rates of cesarean births were found when PGT mothers were compared to IVF/ ICSI mothers. Results also showed that when compared to NC mothers, PGT mothers experienced more pregnancy complications such as gestational diabetes, thyroid pathology, pregnancy-induced hypertension, placental pathology and premature contractions. However, there were no differences regarding pregnancy complications when comparing PGT and IVF/ ICSI mothers.

In the study performed by Bay et. al. (2016) the IVF/ ICSI cohort showed an increased risk of placental disorders, including placenta previa, pre-eclampsia, placental abruption, preterm primary rupture of membranes, and induction of labor or cesarean section. The women who gave birth after PGT had a comparable risk for most of the same complications when compared to the NC cohort, although for most of the outcomes the results were just short of statistical significance. However, the PGT cohort did show a significant increased risk of placenta previa and cesarean section. Because the risk of adverse outcomes was generally comparable to IVF/ ICSI pregnancies in many of these studies, it seems like the actual embryo biopsy does not add additional risks.

When the study separated PGT-M subjects from PGT-A and PGT-SR subjects interesting results emerged. PGT-SR and PGT-A children did not have an increased risk of any adverse obstetric outcomes, except for a higher risk of placenta previa when compared to NC controls. When compared to IVF/ ICSI children, PGT-SR and PGT-A children had comparable obstetric outcomes. However, compared to NC children, children born after PGT-M had a significantly increased risk for preterm primary rupture of membranes, cesarean section and placenta previa. Again, these results make it likely that the risk of adverse outcomes is not related to PGT itself, but to the underlying condition of the parents. However, there was a consistent increased risk of placenta previa after both PGT and IVF/ICSI, which suggests that parental factors do not explain all the adverse outcomes.

Follow up Study

Growth

A study assessed whether PGT causes adverse outcomes by comparing findings at birth and at 2 years of age for singletons born after PGT, IVF/ ICSI, and NC. The study also investigated whether the body size of children born after biopsy of one blastomere was different from that of children born after biopsy of two blastomeres. Subjects in all groups were matched for gender, maternal educational level, mother tongue and birth order. A strength to this study is that all children were examined by the same pediatrician in a standardized way. At a two year follow up, weight, height, head circumference, and waist and arm measurements were comparable for the three cohorts. These results show that PGT singletons do not appear to be at a higher risk of growth retardation compared with IVF/ ICSI and NC singletons. In PGT children, the mean BMI was statistically significantly lower compared with NC children. Growth parameters of the PGT children born after biopsy of one blastomere were comparable to children born after a biopsy of two blastomeres. (Desmyttere, et. al. 2009)

The study performed by Banerjee et. al. (2008) found similar results. When assessed at the mean age of 18 months, growth parameters for all PGT children were within the normal range including the children who had been born preterm and/ or with a low birthweight. Furthermore, Desmyttere et. al. (2009) found that in their follow up study that rates of chronic disease and chronic use of medication were similar between PGT and NC children.

Socio-emotional and Language Development

A study was performed to assess the socio-emotional and language development of children at age two born after PGT, IVF/ ICSI, and NC, as well as parental wellbeing. A small number of children (n= 10) that were born before between 33-36 weeks gestations were included in the study and were equally distributed among the cohorts. Most of these children had a normal birth weight (<2500 g), and none of them had a very low birth weight or obtained an Apgar score of less than nine after ten minutes. Twins were excluded from the study because developmental outcome is affected by prematurity and low birth weight, which are known to be more common in twins and triplets. NC and ICSI controls were matched for gender, maternal education level, native language, and birth order. All members of the PGT cohort

had a blastomere biopsy at the eight-cell stage. Parents were asked to complete the Short Temperament Scale for Toddlers (STST) and the Child Behavioral Checklist (CBCL) in order to assess the child's socio-emotional development. The STST, placed children into one of three temperament categories, easy, average or difficult. The CBCL answers questions about the child's emotional and behavioral problems. Parents answered if the problem presented is 'not true', 'somewhat or sometimes true' or 'very true or often true' of their child, with item scores of 0, 1 or 2. A total score of 60 is at the bottom of the clinical range, and a score of 64 or more represents larger issues. Language comprehension and production were rated according to the McArthur Communicative Developmental Inventories.

The CBCL scores showed no difference in the proportion of children above the clinical threshold points according to mothers and fathers. After controlling for socio-demographic variables, PGT and ICSI mothers reported significantly fewer problems than the NC cohort. According to the STST scores, a similar proportion of parents from all three conception groups reported their child's temperament as easy, average or difficult. This remained true even after controlling for socio-demographic variables. Additionally, the mean Language Comprehension score and Language Production score did not differ significantly among the cohorts.

This study had some weaknesses. Firstly, results were obtained exclusively from parental reports and more valid reports could have been obtained from a multiple informant approach. Additionally, the PGT cohort had members that had undergone PGT-M and PGT-A. Since these procedures are usually done for different reasons, PGT-M and PGT-A populations have different medical histories and family backgrounds, which may influence socio-emotional and language results (Nekkebroeck, et. al. 2008a).

Additionally, the study by Banerjee et al. (2008) found that the PGT cohort had significantly higher scores on the Hearing and Language subscale, than the NC group. These studies suggest that PGT does not cause adverse neurodevelopmental outcomes.

Mental and Psychomotor Development

In the study by Banjeree et al. (2008), children up to age four were evaluated with a focus on neurodevelopmental screening which was measured using the Griffiths Scales of Mental Development. The mean Griffiths quotient for both the PGT and NC groups were in the normal range and did not differ significantly. The only significant differences were for the Locomotor subscale, where the PGT group was significantly lower than the NC group.

A similar study aimed at assessing the mental and psychomotor developmental outcomes in two-year-old children conceived through PGT compared to children born after IVF/ ICSI and natural conception (NC). ICSI and NC controls were matched for gender, maternal education, birth order, and native language. All PGT subjects had a blastomere biopsy at the eight-cell stage of the embryo. At two years of age, the children were all tested by a psychologist using the Dutch version of the Bayley Scales of Infant Development (BSID). The psychologist was blinded to the status of the subject's conception while conducting the evaluation. Parents were questioned regarding socio-demographic characteristics. The BSID consists of two major scales. The mental scale measures visual and auditory information procession, imitation, memory, hand-eye coordination, and problem solving. The motor scale appraises control of gross and fine motor skills.

There were no significant group differences regarding mental and motor scale scores. Additionally, equal numbers of PGT, ICSI, and NC subjects were represented in each level (accelerated, normal, delayed) of psychomotor and mental development. Interestingly, when compared across all three cohorts, psychomotor and mental development scores were very similar for males and females. However, when compared within each cohort, ICSI boys obtained lower scores on both scales than the ICSI girls ($P = 0.061$). The mode of delivery had no impact on psychomotor or mental development even after controlling for sociodemographic factors. (Nekkebroeck, et. al. 2008b).

From the results of these two studies, it can be concluded that the embryo biopsy done in PGT has no impact on the mental and psychomotor development of two-year-old children, compared to ICSI and NC children

Parent-Child Relationship

The study by Banerjee et. al. (2008), used the Parental Stress Index and the Parental Acceptance-Rejection Questionnaire to assess differences in the parent-child relationship. The Parental Stress Index, which asked parents about parental distress, parent–child dysfunctional interaction, and the difficulty of the child, showed no significant difference between the PGT and NC groups. In the Parental Acceptance-Rejection Questionnaire, the PGT group had significantly higher scores on the warmth-affection subscale, and significantly lower scores on the aggression-hostility and rejection subscales than the NC group.

In another study parental stress and health status were measured with the Parent Stress Index and the General Health Questionnaire (GHQ). No differences in parental stress were found for mothers and fathers among the

three groups. However, after controlling for socio-demographic variables, the ICSI mothers and fathers reported less stress from parenting (P = 0.048). These results are similar to findings in other studies and may be because greater efforts are made by ICSI parents to have a child compared to parents who conceive naturally. Another theory is that ICSI parents may be inclined to underreport behavioral issues because of their need to demonstrate their abilities as parents and move on from the issue of infertility where they struggled. On the other hand, there was an equal proportion from all three cohorts that experienced low, moderate or high levels of parenting stress. Scores on the GHQ measuring parental health were not significantly different, even after controlling for socio-demographic factors. Parents from all three conception groups obtained similar scores on the subscales: somatic symptoms, anxiety, social dysfunction and severe depression. The results from these studies imply that parents seem to cope with the extra stress of PGT without it affecting the parent-child relationship (Nekkebroeck, et. al. 2008a).

Conclusion

Overall, it does not seem that preimplantation genetic testing causes adverse clinical outcomes. This is especially important since the majority of couples who undergo PGT usually do not have fertility issues and have the option of natural conception with invasive prenatal testing. The results of these research studies show no significant increased risk of perinatal death or malformations, especially when compared to IVF/ ICSI births, indicating that the embryo is able to recover from the cells removed during the biopsy and it therefore adds no additional risk. Furthermore, children conceived through PGT seem to be on the same developmental level as their peers and show no growth retardation in follow up studies. While some studies show an increased risk of preterm delivery, low birth weight and some obstetric outcomes, it is important to determine whether this is because of the embryo biopsy or because of the underlying health condition of the parents such as the fertility issues or the genetic disease for which they chose to undergo PGT in the first place. However, since this technology is fairly new, there are few follow up studies that investigate the long-term effects of PGT. Additional follow up studies are necessary to ensure the long-term safety of this technology. Future studies can also investigate the specific outcomes for each method of PGT, since most of the research is either regarding a blastomere biopsy or combines all methods of PGT in the PGT cohort. Furthermore, future studies should focus on determining the outcome differences between PGT-M, PGT-A and PGT-SR since parents who undergo the different forms of PGT have different medical backgrounds which can affect the results of these studies. Couples considering PGT should consult their physician or a genetic counselor to determine whether PGT is the correct option as well as which method of PGT should be used.

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