**human
reproduction
update**

Obstetric, neonatal, and child health outcomes following embryo biopsy for preimplantation genetic testing

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GRAPHICAL ABSTRACT
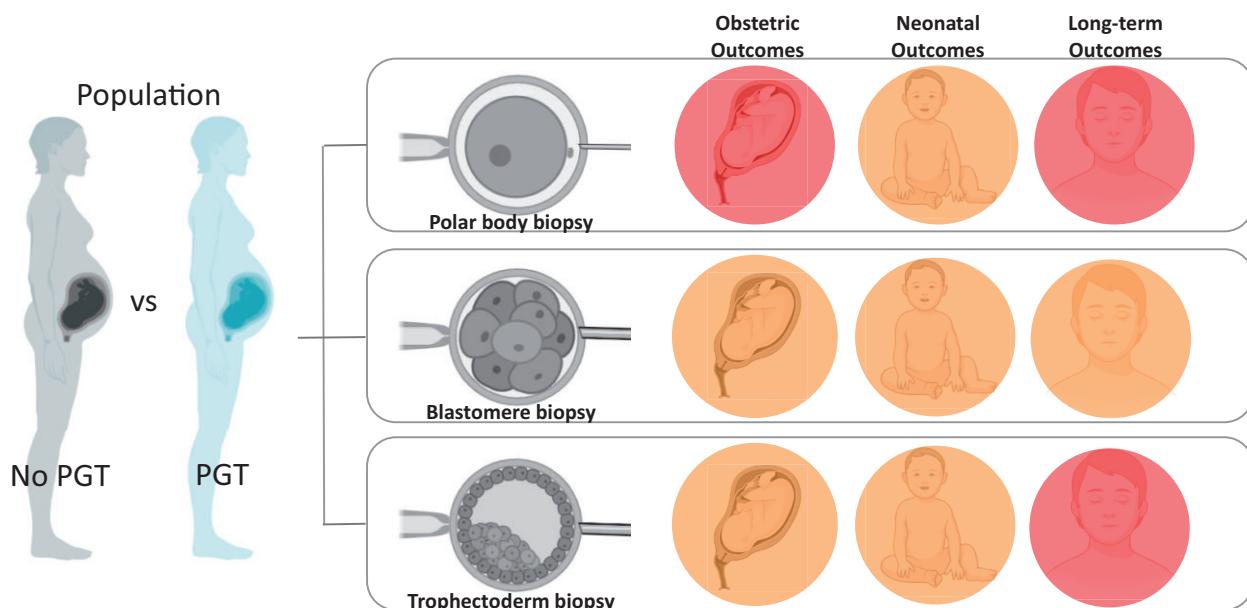
No conclusive evidence for an adverse effect of PGT on outcomes. The amber symbol represents weak and/or a controversial body of safety evidence for which further research is required. The red symbol warns that no evidence of safety exists. PGT, preimplantation genetic testing.

BACKGROUND: Preimplantation genetic testing (PGT) of embryos developed *in vitro* requires a biopsy for obtaining cellular samples for the analysis. Signs of cell injury have been described in association with this procedure. Thus, the consequences of the biopsy on obstetric and neonatal outcomes have been the subject of some quantitative analyses, although the reliability of data pooling may be limited by important issues in the various reports.

OBJECTIVE AND RATIONALE: The present review identifies evidence for whether pregnancies conceived after embryo biopsy are associated with a higher risk of adverse obstetric, neonatal, and long-term outcomes. Available evidence has been summarized considering manipulation at various stages of embryo development.

SEARCH METHODS: We used the scoping review methodology. Searches of article databases were performed with keywords pertaining to the embryo biopsy technique and obstetric, neonatal, and postnatal outcomes. Studies in which embryos were biopsied at different stages (i.e. both at the cleavage and blastocyst stages) were excluded. We included data on fresh and frozen embryo transfers. The final sample of 31 documents was subjected to qualitative thematic analysis.

OUTCOMES: Sound evidence is lacking to fully address the issues on the potential obstetric, neonatal or long-term consequences of embryo biopsy. For polar body biopsy, the literature is too scant to draw any conclusion. Some data, although limited and controversial, suggest a possible association of embryo biopsy at the cleavage stage with an increased risk of low birthweight and small for gestational age neonates compared to babies derived from non-biopsied embryos. An increase in preterm deliveries and birth defects in cases of trophectoderm biopsy was suggested. For both biopsy methods (at the cleavage and blastocyst stages), an increased risk for hypertensive disorders of pregnancy was found. However, these findings may be explained by confounders such as other embryo manipulation procedures or by intrinsic patient or population characteristics.

WIDER IMPLICATIONS: Since there is inadequate evidence to assess obstetric, neonatal, and long-term health outcomes following embryo biopsy, an invasive PGT strategy should be developed with a cautious approach. A non-invasive approach, based on the analysis of embryo cell-free DNA, needs to be pursued to overcome the potential limitations of embryo biopsy.

Introduction

The rapid diffusion of medically assisted reproduction (MAR) in the past three decades has been favoured by the development of technologically sophisticated procedures (Waynforth, 2018). There is, however, growing concern that some MAR-related technologies or processes may increase the risk of obstetric, perinatal, and congenital anomalies. In this context, concern over increased adverse outcomes has been raised for babies born after the collection of samples needed for preimplantation genetic testing (PGT). PGT aims to detect genetic disorders and aneuploidies in *in vitro*-conceived embryos to avoid the transfer of a chromosomally abnormal or affected conceptus. Three different invasive strategies have been proposed over the years to collect cells to be analysed: polar body (PB) biopsy; blastomere biopsy; and blastocyst stage biopsy (Cimadomo *et al.*, 2020). Recently, data from Europe collected by the ESHRE PGT Consortium showed that cleavage stage biopsy is the most widely used approach for monogenic/single-gene defects (PGT-M) and/or for chromosomal structural rearrangements (PGT-SR) (78% and 67%, respectively). Conversely, in the case of PGT for aneuploidies (PGT-A), the majority of biopsies are performed at the blastocyst stage (87%) (van Montfoort *et al.*, 2021).

Rationale

The aims here were to collect and analyse existing data to provide a review of the short- and long-term risks of embryo biopsy in prenatal or postnatal human development and health. To achieve this goal, we have summarized the available evidence on the obstetric, neonatal, and long-term outcomes of conceptions after MAR and a biopsy at different embryonic stages compared to pregnancies obtained from non-biopsied embryos.

To correctly assess these outcomes, it is vital to consider some critical aspects. This fits well within the framework of a scoping review that should identify gaps and biases in the literature.

Potential damaging effects of embryo biopsy

The mammalian embryo has been shown in a wide range of species to be able to regulate the loss of cells without compromising or affecting post-implantation development and the birth of normal-sized offspring. In 1968, Gardner and Edwards were able to sex rabbit embryos using a sex-specific chromatin pattern in blastocyst biopsies before their transfer to the uterus. Preimplantation testing of embryos is also used routinely in animal husbandry to produce animals of the preferred sex (Braude *et al.*, 2002). However, in humans, the safety of the procedure still presents a concern. PB biopsy on metaphase II oocytes and/or zygotes is potentially less invasive than in any other stage of preimplantation development, since it involves the removal of the 'waste products' of meiosis. However, it is a demanding and time-consuming procedure, and it only provides maternal genetic information, not considering parental or mitotic division abnormalities (Greco *et al.*, 2020).

Embryo biopsy at the cleavage stage requires the collection of blastomeres at an early stage through an invasive procedure (Mastenbroek *et al.*, 2007; Alteri *et al.*, 2019). Interestingly, blastomere loss at early stages of development as a consequence of cryopreservation has been reported to be associated with a lower number of cells in resultant blastocysts (Archer *et al.*, 2003). However, even if the removal of one or two cells at the eight-cell stage reduces the inner cell mass (ICM)

of the blastocyst (Hardy *et al.*, 1990), embryos with fragmented or damaged blastomeres are routinely transferred, resulting in live births.

The blastocyst biopsy, characterized by the removal of 5–10 trophectoderm (TE) cells without affecting the ICM lineage, has been shown to be safer than blastomere biopsy and, consequently, it is gradually replacing both PB and cleavage-stage biopsy approaches (van Montfoort *et al.*, 2021). However, the real impact of TE biopsy is still unclear. The only randomized controlled trial (RCT) designed to investigate the safety of the TE biopsy procedure, performed by removing a maximum of five cells, demonstrated the safety of this procedure in terms of implantation potential and live birth rate (Scott *et al.*, 2013).

Based on the results of the study by Munné *et al.* (2019), a mathematical model supported the idea that the indiscriminate application of PGT-A leads to a loss of potentially competent embryos, possibly a result of biopsy-related embryo damage (Pagliardini *et al.*, 2020). Notably, it is possible that the deleterious effects of blastocyst biopsy may not manifest until later in pregnancy. He *et al.* (2019) analysed the neonatal outcomes of babies born after TE biopsy, dividing them into two groups according to the number of biopsied cells (<10 and ≥10 biopsied cells); the frequency of neonatal macrosomia and the sex ratio were different between the two groups. The limitations of the study are the small sample size and the borderline statistical significance, but this finding might be worth further investigation to understand the effects of different numbers of biopsied cells.

Since during the biopsy a sample of 5–10 cells is removed from the TE, which ultimately gives rise to the placenta, it is plausible that its disruption might result in abnormalities in placentation. In line with this, some studies reported an association between TE biopsy and abnormal placentation conditions, such as pre-eclampsia and an increased risk of preterm birth (Zhang *et al.*, 2019; Li *et al.*, 2021). Furthermore, serum β-hCG levels on day 12 after the transfer of biopsied blastocysts were found to be statistically significantly lower than the levels of β-hCG after frozen embryo transfer (FET) without blastocyst biopsy (Lu *et al.*, 2020). Since maternal β-hCG is produced by villous syncytiotrophoblast cells as pregnancy progresses, TE biopsy might affect the secretion of β-hCG. Similarly, the β-hCG concentration secreted by a biopsied blastocyst in the culture medium seems to be inversely proportional to the number of TE cells removed (Dokras *et al.*, 1991). In disagreement with this observation, Wu *et al.* (2021) recently showed no impact of TE biopsy on serum β-hCG levels 14 days after embryo transfer.

The confounding effect of infertility status

Many studies have evaluated the effects of embryo biopsy without distinction among PGT-M, PGT-SR, and PGT-A. Although the technical procedures performed by embryologists are identical, the indications are different, leading to significant heterogeneity in the characteristics of the patients involved. PGT-M is generally applied for fertile individuals. Conversely, PGT-A is used for infertile individuals undergoing MAR to optimize the efficiency of MAR cycles. Similarly, PGT-SR is offered to individuals to avoid a pregnancy with a chromosomally unbalanced product of the translocation and, hence, to reduce the risk of miscarriage. Infertility *per se* (rather than MAR treatments) is related to adverse obstetric and perinatal outcomes (Thomson *et al.*, 2005; DoPierala *et al.*, 2016), implying a higher risk after biopsies for PGT-A compared to PGT-M. This represents an important source of bias affecting the studies. Two recent systematic reviews and meta-analyses

have evaluated obstetric and neonatal outcomes from PGT pregnancies considering pooled data derived from PGT-M, PGT-SR, and PGT-A cases and comparing them to pregnancies derived from natural conceptions or fresh and FET ART cycles without embryo biopsy (Hou et al., 2021; Zheng et al., 2021). Overall, PGT pregnancies were shown to be associated with lower rates of low birthweight (LBW), very low birthweight (VLBW), and very preterm delivery (VPTD) compared to those obtained without embryo biopsy and with increased risks of LBW, preterm delivery (PTD), and hypertensive disorders of pregnancy (HDP) compared to spontaneous conceptions (Hou et al., 2021; Zheng et al., 2021). It is likely that these findings can be simply attributed to the pooling of very different PGT populations in the analysis.

The confounding effect of embryo freezing
Analysis of biopsy samples using the most recent analytical technologies requires time. Therefore, manipulated embryos, particularly at the blastocyst stage, are often vitrified after biopsy and warmed before transfer, allowing the analysis to be completed. However, freezing has been reported to potentially influence obstetric and neonatal outcomes. Recent evidence supports a higher risk of pre-eclampsia in pregnancies after FET compared to fresh cycles (Roque et al., 2019; Wei et al., 2019). Atypical placentation with a reduced uterine artery pulsatility index from 6 to 37 weeks has been reported in singleton pregnancies conceived after the transfer of frozen blastocysts (Cavoretto et al., 2020). Moreover, FETs have been associated with greater foetal crown-rump length at 6–14 weeks and greater foetal growth (with a lower risk of small for gestational age (SGA) and greater estimated foetal weight or birthweight) (Cavoretto et al., 2021a,b).

An RCT demonstrated a higher risk of obstetric complications after frozen vs fresh embryo transfers in women with PCOS (Zhang et al., 2018). Specifically, women with PCOS showed an increased risk of large for gestational age (LGA) babies in singleton pregnancy and a higher risk of pre-eclampsia in twin pregnancies after FET (Zhang et al., 2018). Moreover, placentas from FET pregnancies showed a higher rate of anatomic and vascular pathology than those from fresh transfers, with a significantly higher risk of marginal cord insertion, accessory lobes, foetal vascular malperfusion characteristics with cord anomalies, and subchorionic thrombi after adjustment for potential confounding variables (Sacha et al., 2020). The extent to which TE biopsy might exacerbate the possible impact of embryo cryopreservation on obstetric and neonatal complications remains to be established.

Objectives

The research questions guiding this exploratory review were:

- What are the obstetric, neonatal, and long-term outcomes after transfers of biopsied embryos?
- What knowledge gaps should be bridged with future research to help ensure safe treatment for patients?

Methods

Protocol and registration

We followed the five stages of the updated scoping review protocol developed by Arksey and O'Malley (2005), Colquhoun et al. (2014),

Levac et al. (2010), Peters et al. (2015), and Tricco et al. (2016). This scoping review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analysis-scoping review extension (PRISMA-ScR) (Tricco et al., 2018). The rationale, eligibility criteria, and study selection were defined a priori. The scoping review protocol was recorded a priori and is available at OSF registries (Registration doi:10.17605/OSF. IO/26X49, https://osf.io/registries).

Eligibility criteria

The intervention group consisted of pregnancies following the transfer of biopsied embryos compared with pregnancies following the transfer of non-biopsied embryos. We included reported data about manipulated embryos with PB, blastomere, and TE, but not morula, biopsy. Studies in which embryos were biopsied at different stages (i.e. at the cleavage and blastocyst stages) were excluded. We included data about both fresh embryo transfers and FETs. All study designs were considered, with the exception of case reports and case series. We excluded studies focused on psychosocial outcomes. This search was limited to articles in English.

Information sources and search

The bibliographical PubMed, Embase, and Cochrane Library databases were searched for studies including combinations of the following terms, alone or combined with Boolean operators: 'embryo biops*', 'blastomere biops*', 'blastocyst biops*', 'polar body biops*', 'trophectoderm biops*', 'preimplantation genetic', 'longterm', 'natal', 'obstetric', and 'children'. The scoping review included all studies published until April 2021.

Selection of sources of evidence

The authors did not use strict inclusion and exclusion criteria (Arksey and O'Malley, 2005). Two authors (A.A. and G.C.C.) independently examined the potentially relevant studies by checking the titles, abstracts, and full texts of the chosen studies using the same inclusion criteria, as suggested by research methodology guidelines (Arksey and O'Malley, 2005). The authors resolved disagreements on study selection and data extraction by discussion with other reviewers (G.G. and M.P.) if needed. When authors identified reviews, the references that potentially met the inclusion criteria were selected and checked for missed studies. The PRISMA flow chart depicts the study selection process, and the entire screening process is shown in Fig. 1.

Data-charting process

A data-charting form was jointly developed by two reviewers (A.A. and M.P.) to determine which sensitive variables to extract. Three of the co-authors (A.A., G.C.C., and G.G.) independently extracted and charted data from each study and discussed differences until they reached consensus. The reviewers continuously updated the data-charting form and discussed the results with other reviewers when in doubt.

The selected clinical outcomes are defined as follows. PTD is defined as any delivery resulting in a vital neonate occurring before the completion of 37 weeks of pregnancy (Blencowe et al., 2012). Gestational hypertension is hypertension arising de novo after 20 weeks of gestation in the absence of proteinuria and without biochemical or
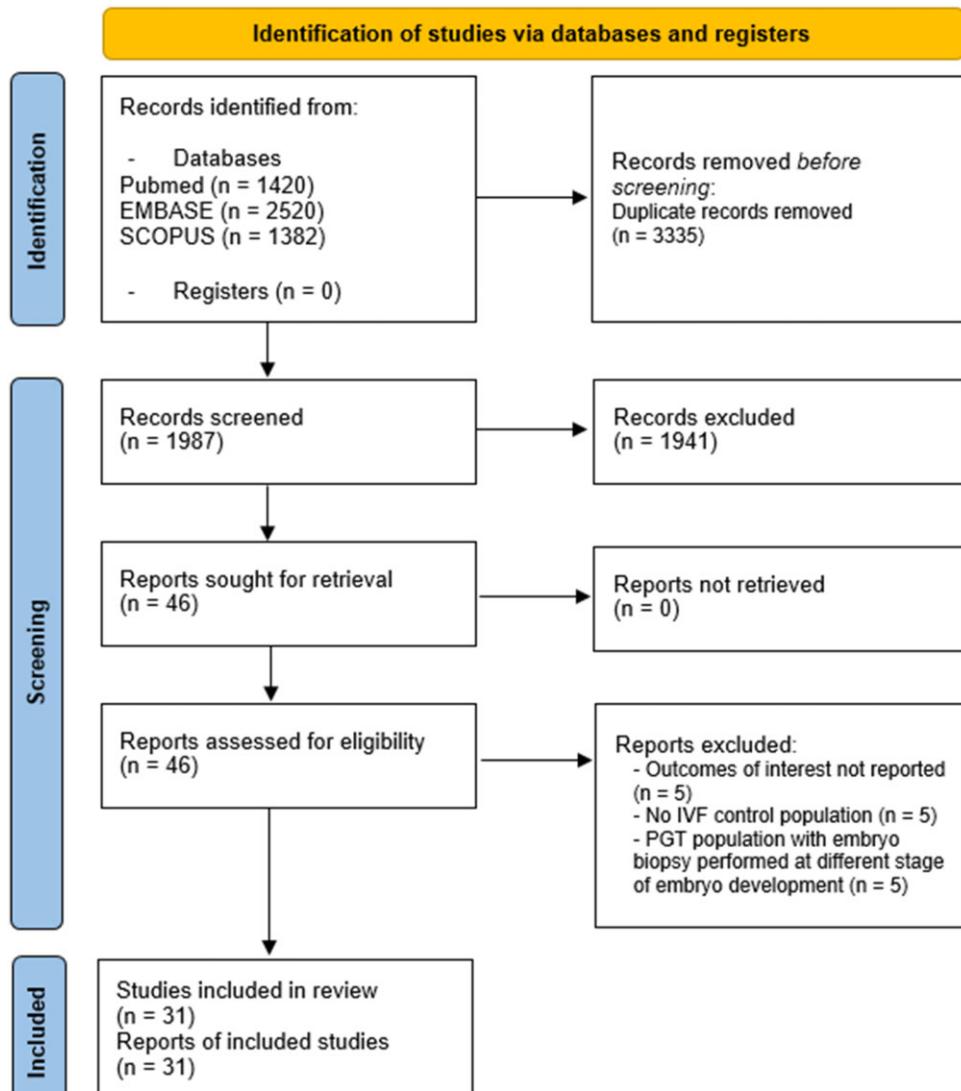
Figure 1. PRISMA flow diagram of article screening for the scoping review. PGT, preimplantation genetic testing; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-analysis.

haematological abnormalities or foetal growth restriction (Brown et al., 2018). Pre-eclampsia is defined as *de novo* hypertension after 20 weeks of gestation associated with proteinuria and/or evidence of maternal organ dysfunction or foetal growth restriction (Brown et al., 2018). Gestational diabetes is defined as glucose intolerance with hyperglycaemia that begins or is first diagnosed in pregnancy (Saravanan, 2020). Abnormal placentation is defined as a composite outcome measure including several placental disorders, such as placental abruption, placenta accreta spectrum, and placenta or vasa previa. Since heterogeneity was observed among different studies, further details about specific placental abnormalities may be found in each individual paper (Jauniaux et al., 2019). LBW is defined as weight at birth <2500 g, and VLBW is defined as weight at birth <1500 g (World Health Organization, 2014). SGA is defined as an estimated foetal weight or birthweight below the 10th percentile, and LGA is defined as foetal weight or birthweight above the 90th percentile, according to the standard population reference (Nicolaides et al., 2018). Birth defects can be defined as structural or functional abnormalities that are present from birth. Long-term outcomes were defined according to heterogeneous definitions found in each individual study and were related mainly to arterial blood pressure, anthropometric measures, and neurological, psychomotor, and cognitive development.

Data items

First, the authors collected a combination of qualitative or quantitative data from the final list of publications in a 'data-charting form' (Arksey and O'Malley, 2005), reporting: study characteristics, including study

design and sample size of study groups; embryo biopsy methods; and reported outcome data, including definition of outcomes, key findings, and statistical analysis.

Synthesis of results

To synthesize the range of evidence, the results were classified under three main conceptual categories, defined as 'obstetric outcomes', 'neonatal outcomes', and 'long-term outcomes'.

Results

A total of 5322 records were retrieved from the search. After the removal of duplicate records, 1987 records were screened, and 46 were assessed for eligibility, leading to 31 articles being included in the review after exclusions for specific reasons. Figure 1 shows the detailed PRISMA flow diagram.

Obstetric outcomes

Table I describes the main characteristics and findings of studies investigating obstetric complications of PGT pregnancies. As shown, no data are available for these outcomes following PB biopsy.

Preterm delivery
Polar body (PB) biopsy. No outcome data were available following PB biopsy.

Blastomere biopsy. Three cohort studies described the incidence of PTD defined as a delivery before 37 weeks of gestation (Bay *et al.*, 2016; Hasson *et al.*, 2017; Feldman *et al.*, 2020) and VPTD defined as a delivery before 32 (Feldman *et al.*, 2020) and/or 34 weeks (Hasson *et al.*, 2017; Feldman *et al.*, 2020) in MAR pregnancies with and without a blastomere biopsy, reporting no significant differences between the two groups.

Trophectoderm (TE) biopsy. Nine of the studies investigated the rate of PTD and/or VPTD in pregnancies that occurred following TE biopsy (Forman *et al.*, 2014; He *et al.*, 2019; Sacchi *et al.*, 2019; Zhang *et al.*, 2019; Lu *et al.*, 2020; Awadalla *et al.*, 2021; Li *et al.*, 2021; Makhijani *et al.*, 2021; Sites *et al.*, 2021). In none of these studies, except those by Forman *et al.* (2014) and Li *et al.* (2021), a significant difference was observed between the biopsy and non-biopsy groups.

In the RCT on PGT-A by Forman *et al.* (2014), the PTD risk was significantly higher after the transfer of two untested embryos compared to that after the transfer of a single biopsied euploid embryo (relative risk 2.21, 95% CI 1.04–4.70, $P = 0.03$). As stated by the authors, differences between groups could likely be attributed to the difference in the risk of a twin pregnancy. Indeed, although not presented, obstetrical and neonatal outcomes between singleton deliveries in the biopsy and non-biopsy groups were reported to be similar. This study will thus not be commented on further in the following paragraphs, as the negative outcomes reported could all be attributed to multiple gestations and not to the technique.

Recently, a large retrospective cohort study based on the Society for Assisted Reproductive Technology Clinical Outcomes Reporting System (SART-CORS) database showed that singletons from the transfer of frozen biopsied embryos ($n = 6244$) had a slightly increased

likelihood of PTD [adjusted odds ratio (aOR) 1.20, 95% CI 1.09–1.33] compared to babies derived from non-biopsied blastocysts ($n = 10002$) (Li *et al.*, 2021). A sub-analysis comparing cycles with or without biopsy in an infertile population confirmed these findings (aOR 1.23, 95% CI 1.10–1.37) (Li *et al.*, 2021).

Hypertensive disorders of pregnancy
Polar body (PB) biopsy. No outcome data were available following PB biopsy.

Blastomere biopsy. Feldman and coworkers showed an increased risk of HDP, ranging from gestational hypertension to eclampsia, in association with biopsy for PGT-M. This difference persisted even after stratifying for singleton pregnancies ($n = 345$ in the group after biopsy and $n = 422$ in the non-biopsy group) (aOR 5.9, 95% CI 1.9–18.2) or twin pregnancies ($n = 76$ in the group after biopsy and $n = 101$ in the non-biopsy group) (aOR 3.7, 95% CI 1.1–12.8) (Feldman *et al.*, 2020). A Danish nationwide study failed to show different rates of pre-eclampsia in $n = 126$ pregnancies after biopsy for PGT-M/PGT-SR compared to $n = 30418$ pregnancies derived from non-biopsied embryos (Bay *et al.*, 2016). We observed that both studies combined both fresh embryo transfers and FETs for analysis, providing unclear information on the real impact of the biopsy (Sacha *et al.*, 2020).

Trophectoderm (TE) biopsy. Five of the studies assessed the potential association between TE biopsy and HDP (Zhang *et al.*, 2019; Lu *et al.*, 2020; Makhijani *et al.*, 2021; Sites *et al.*, 2021; Swanson *et al.*, 2021), reporting conflicting data. In all the studies, analyses were adjusted for confounding factors such as maternal age and BMI. Zhang *et al.* (2019) found a significantly (3-fold) increased risk of pre-eclampsia following fresh embryo transfers or FETs ($n = 177$) of biopsied embryos compared to those of non-biopsied embryos ($n = 180$) (aOR 3.02, 95% CI 1.10–8.29, $P = 0.02$). However, the statistical significance was lost when the analysis was limited to FETs, likely because of the small sample size ($n = 134$ in the biopsy group vs $n = 124$ in the non-biopsy group). On the other hand, no difference in gestational hypertension was found between the two groups considered (Zhang *et al.*, 2019).

Similarly, a recent retrospective cohort study comparing $n = 241$ FETs with TE biopsy and $n = 515$ cycles without biopsy showed a significantly higher risk of HDP in association with biopsy (aOR 1.94, 95% CI 1.07–3.52) (Makhijani *et al.*, 2021). Stratifying for the severity of hypertensive disorders, the statistical significance was maintained only for non-severe forms. The opposite conclusions were drawn by three studies based on FETs, in which no significant difference in the rate of HDP following the transfer of biopsied or non-biopsied blastocysts was found (Lu *et al.*, 2020; Sites *et al.*, 2021; Swanson *et al.*, 2021).

Abnormal placentation
Polar body (PB) biopsy. No outcome data were available following PB biopsy.

Blastomere biopsy. Only one of the studies assessed the presence of abnormal placentation between pregnancies conceived after blastomere biopsy and those without biopsy, considering both fresh embryo transfers and FETs (Bay *et al.*, 2016). No correlation was observed between the placenta previa rate and blastomere biopsy.

Table I Main characteristics and findings of studies investigating the obstetric and perinatal complications of pregnancies derived from biopsied human embryos, according to the biopsy method.

Author, year	Study design	Number of children (PGT vs control group)	Study population	Biopsy method	Outcomes
Montag et al., 2004	Retrospective cohort study	Singletons: N = 20 vs N = 30 Multiples: N = 4 vs N = 19	Biopsied (PGT-A) vs non-biopsied embryos	PB	Birthweight (−)
Liebaers et al., 2010	Prospective observational cohort study	Singletons: N = 377 vs N = 1479 Multiples: N = 176 vs N = 1323	Biopsied (PGT-M + PGT-SR + PGT-A) vs non-biopsied embryos	BL	Birth weight (−) Low birth weight (+ in multiples pregnancies) Very low birth weight (−) Birth defects (−)
Desmyttere et al., 2012	Prospective comparative follow-up study	Singletons: N = 670 vs N = 1059 Multiples: N = 325 vs N = 448	Biopsied (PGT-M + PGT-A) vs non-biopsied embryos	BL	Birth weight (−) Low birth weight (+ in multiples pregnancies) Very low birth weight (−) NICU admission (−) Birth defects (-)
Hasson et al., 2017	Retrospective cohort study	Singletons: N = 51 vs N = 83 Twins: N = 32 vs N = 70	Biopsied (PGT-M + PGT-SR) vs non-biopsied embryos	BL	Preterm delivery (−) Birth weight (−) Very low birth weight (−) NICU admission (−) Birth defects (−)
Bay et al., 2016	Retrospective cohort study	Singletons: N = 104 vs N = 24 533 Twins: N = 45 vs N = 11 582	Biopsied (PGT-M + PGT-SR) vs non-biopsied embryos	BL	Preterm delivery (−) Hypertensive disorders of pregnancy (−) Abnormal placentation (−) Birth weight (−) Low birth weight (+ in PGT-M sub-analysis) Apgar scores (−) NICU admission (−) Birth defects (−)
Feldman et al., 2020	Retrospective cohort study	Singletons: N = 345 vs N = 422 Twins: N = 152 vs N = 202	Biopsied (PGT-M) vs non-biopsied embryos	BL	Preterm delivery (−) Hypertensive disorders of pregnancy (+) Gestational diabetes mellitus (+) Birth weight (−) Small for gestational age (+) NICU admission (−)
Forman et al., 2012	Retrospective study	Singletons: N = 140 vs N = 182 Twins: N = 0 vs N = 2	Biopsied (PGT-A) vs non-biopsied embryos	TE	Birth weight (−)
Forman et al., 2014	RCT	Singletons: N = 60 vs N = 32 Twins: N = 1 vs N = 29	Biopsied (PGT-A) vs non-biopsied embryos	TE	Preterm delivery (+)
Sacchi et al., 2019	Prospective observational cohort study	Singletons: N = 81 vs N = 264 Twins: N = 0 vs N = 4	Biopsied (PGT-A) vs non-biopsied embryos	TE	Preterm delivery (−) Birth weight (−) Low birth weight (−)
Zhang et al., 2019	Prospective observational cohort	Singletons: N = 155 vs N = 150 Twins: N = 30 vs N = 22	Biopsied (PGT-SR + PGT-A) vs non-biopsied embryos	TE	Preterm delivery (−) Hypertensive disorders of pregnancy (+) Abnormal placentation (−) Gestational diabetes mellitus (−) Birth weight (−) Low birth weight (−) Very low birth weight (−) Apgar scores (−) NICU admission (−) Birth defects (+ in frozen embryo transfer sub-analysis)

(continued)

Table I Continued

Author, year	Study design	Number of children (PGT vs control group)	Study population	Biopsy method	Outcomes
He *et al.*, 2019	Retrospective cohort study	Singletons: N = 646 vs N = 612 Twins: N = 242 vs N = 221	Biopsied (PGT) vs non-biopsied embryos	TE	Preterm delivery ($-$) Birth weight ($-$) Low birth weight ($-$) Very low birth weight ($-$) Birth defects ($-$)
Lu *et al.*, 2020	Retrospective cohort study	Singletons: N = 305 vs N = 328	Biopsied (PGT) vs non-biopsied embryos	TE	Preterm delivery ($-$) Hypertensive disorders of pregnancy ($-$) Gestational diabetes mellitus ($-$) Birth weight ($-$) Low birth weight ($-$) Very low birth weight ($-$) Small for gestational age and large for gestational age ($-$)
Swanson *et al.*, 2021	Retrospective cohort study	Singletons: N = 158 vs N = 153	Biopsied (PGT) vs non-biopsied embryos	TE	Hypertensive disorders of pregnancy ($-$) Abnormal placentation (-)
Awadalla *et al.*, 2021	Retrospective cohort study	Singletons: N = 67 vs N = 78	Biopsied (PGT) vs non-biopsied embryos	TE	Preterm delivery ($-$) Birth weight ($-$) Low birth weight ($-$) Very low birth weight ($-$)
Sites *et al.*, 2021	Retrospective cohort study	Singletons: N = 585 vs N = 2191	Biopsied (PGT-A) vs non-biopsied embryos	TE	Preterm delivery ($-$) Hypertensive disorders of pregnancy ($-$) Abnormal placentation ($-$) Gestational diabetes mellitus ($-$) Birth weight ($-$) Low birth weight ($-$)
Makhijani *et al.*, 2021	Retrospective cohort study	Singletons: N = 241 vs N = 515	Biopsied (PGT-M + PGT-SR + PGT-A) vs non-biopsied embryos	TE	Preterm delivery ($-$) Hypertensive disorders of pregnancy ($+$) Abnormal placentation ($-$) Gestational diabetes mellitus ($-$) Birth weight ($-$) Low birth weight ($-$) Very low birth weight ($-$) NICU admission ($-$) Birth defects ($-$)
Riestenberg *et al.*, 2021	Retrospective cohort study	Singletons: N = 475 vs N = 237	Biopsied (PGT-A) vs non-biopsied embryos	TE	Abnormal placentation ($-$)
Li *et al.*, 2021	Retrospective cohort study	Singletons: N = 6244 vs N = 10 002	Biopsied (PGT-M + PGT-A) vs non-biopsied embryos	TE	Preterm delivery ($+$) Birth weight ($-$) Small for gestational age and large for gestational age ($-$)

"$+$", significant difference between the two groups; "$-$", no significant difference between the two groups.

BL, blastomere; NICU, neonatal intensive care unit; PB, polar body; PGT, preimplantation genetic testing; PGT-A, preimplantation genetic testing for aneuploidies; PGT-M, preimplantation genetic testing for monogenic disorders; PGT-SR, preimplantation genetic testing for structural rearrangements; RCT, randomized controlled trial; TE, trophectoderm.

Trophectoderm (TE) biopsy. Four studies found no significant difference in the rate of placental anomalies, such as placenta previa and placenta accreta, between pregnancies derived from biopsied and non-biopsied blastocysts (Zhang *et al.*, 2019; Makhijani *et al.*, 2021; Sites *et al.*, 2021; Swanson *et al.*, 2021). In line with these findings, a recent study did not show significant differences in the rates of foetal and placental anomalies in MAR pregnancies that occurred after biopsy for PGT-A or not (Riestenberg *et al.*, 2021).

Gestational diabetes mellitus

Polar body (PB) biopsy. No outcome data were available following PB biopsy.

Blastomere biopsy. Feldman *et al.* (2020) found that the rate of gestational diabetes mellitus (GDM) was lower in singleton pregnancies (9.2% vs 15.6%, aOR 0.55, 95% CI 0.3–0.8) associated with embryo biopsy for PGT-M compared to the non-biopsy group. A plausible explanation might be that patients who undergo PGT for monogenic diseases do not suffer from infertility and may have, *per se*, a lower GDM risk.

Trophectoderm (TE) biopsy. On the other hand, in the four studies in which TE biopsy was performed for PGT cycles without distinction among indications, no significant difference in GDM risk between MAR pregnancies with and without blastocyst biopsy was found (Zhang *et al.*, 2019; Lu *et al.*, 2020; Makhijani *et al.*, 2021; Sites *et al.*, 2021), suggesting that embryo biopsy does not represent a predictor of GDM in pregnancies conceived with MAR.

Neonatal outcomes

The main characteristics and findings of studies investigating the neonatal complications of PGT pregnancies are described in Table I.

Birthweight
Polar body (PB) biopsy. Only a single retrospective study was conducted on this topic (Montag *et al.*, 2004), comparing n = 106 women subjected to embryo biopsy for PGT-A and n = 220 patients whose embryos were not subjected to biopsy in the same period of time. The median birthweight of children in the PB biopsy group was similar to that of children in the control group. No other information was available on neonatal outcomes following PB biopsy.

Blastomere biopsy. Five cohort studies showed that the birthweight of neonates born following an embryo biopsy was comparable to that of neonates conceived without biopsy in both singleton and twin pregnancies (Liebaers *et al.*, 2010; Desmyttere *et al.*, 2012; Bay *et al.*, 2016; Hasson *et al.*, 2017; Feldman *et al.*, 2020). In a sub-analysis of children conceived after biopsy for PGT owing to parental monogenetic disorders, Bay *et al.* (2016) found that children born after biopsy (n = 58) had a significantly increased risk of LBW (aOR 2.0; 95% CI 1.1–3.9) compared to children conceived without embryo manipulation (n = 14782). Similarly, in multiple pregnancies, the risk of LBW seemed to be higher in the biopsy group (n = 176) than in the non-biopsy group (n = 1323) (OR 1.71; 95% CI 1.21-2.39) (Liebaers *et al.*, 2010). Conversely, according to Desmyttere *et al.* (2012), fewer multiple births following biopsy for PGT presented an LBW compared to non-biopsy-derived multiple births (16.2% vs 17.8%, respectively, P = 0.005).

Finally, no difference in singletons' and twins' VLBW between PGT newborns and an ICSI group was found overall (Liebaers *et al.*, 2010; Desmyttere *et al.*, 2012; Hasson *et al.*, 2017).

Trophectoderm (TE) biopsy. In eight of the studies, the birthweight, and the rates of LBW, and VLBW of singleton infants did not differ between those derived from biopsied or unbiopsied blastocysts, also adjusting for confounding factors (Forman *et al.*, 2012; He *et al.*, 2019; Sacchi *et al.*, 2019; Zhang *et al.*, 2019; Lu *et al.*, 2020; Awadalla *et al.*, 2021; Makhijani *et al.*, 2021; Sites *et al.*, 2021). No statistically significant differences in birthweight were observed between multiple pregnancies with or without TE biopsy (He *et al.*, 2019; Zhang *et al.*, 2019). These data were confirmed by a multivariate analysis of n = 6244 cycles with biopsies for PGT and n = 10002 non-biopsy

cycles in the SART-CORS database showing similar birthweight Z scores in the two groups (Li *et al.*, 2021).

Small and large for gestational age
Polar body (PB) biopsy. No outcome data were available following PB biopsy.

Blastomere biopsy. A retrospective cohort study showed that the SGA rate was higher in the PGT-M singleton group than in the non-biopsy group (12.4% vs 4.5%, respectively, aOR 2.5, 95% CI 1.2–5.0) after adjusting for female age, BMI, and parity (Feldman *et al.*, 2020).

This finding might be correlated with an increased risk of HDP after biopsy for PGT-M compared with pregnancies derived from non-biopsied embryos observed in the same study.

Trophectoderm (TE) biopsy. Two retrospective studies, one of which was based on the SART-CORS database, reported the odds for SGA and LGA newborns and found no difference for FET cycles with or without blastocyst biopsy (Lu *et al.*, 2020; Li *et al.*, 2021).

Neonatal intensive care unit admission
Polar body (PB) biopsy. No outcome data were available following PB biopsy.

Blastomere biopsy. Children born after blastomere biopsy did not seem to have been hospitalized more frequently in their neonatal phase than in controls (Desmyttere *et al.*, 2012; Bay *et al.*, 2016; Hasson *et al.*, 2017; Feldman *et al.*, 2020). In a sub-analysis of a single study, children conceived after embryo biopsy for PGT-M (n = 58) had significantly longer hospital admission (mean difference 30; 95% CI 22–39) than children born from non-biopsied embryos (n = 14782) (Bay *et al.*, 2016).

Trophectoderm (TE) biopsy. Analysis of the admission rate to the neonatal intensive care unit (NICU) after delivery in two studies indicated that children born after blastocyst biopsy were not hospitalized more frequently in their neonatal phase than in controls (Zhang *et al.*, 2019; Makhijani *et al.*, 2021).

Birth defects
Polar body (PB) biopsy. No outcome data were available following PB biopsy.

Blastomere biopsy. Four retrospective studies investigated the major malformation rate of singleton infants following cleavage stage embryo biopsy (Liebaers *et al.*, 2010; Desmyttere *et al.*, 2012; Bay *et al.*, 2016; Hasson *et al.*, 2017). Liebaers *et al.* (2010) reported a malformation rate of 2.13% after PGT for various indications and of 3.38% for children born from non-biopsied embryos. According to Desmyttere *et al.* (2012) and Hasson *et al.* (2017), birth defects were present, respectively, in 2.3% and 3.5% of children born from a biopsied embryo compared to 2.6% and 1.9% of control children derived from a non-biopsied embryo. Bay *et al.* (2016) described n = 19 children with major malformations derived from n = 149 (12.7%) PGT-M/PGT-SR procedures (Bay *et al.*, 2016). None of these studies showed a significant difference in the percentage of birth defects in children born after embryo biopsy or not.

It is, however, difficult to compare the malformation rates owing to significant heterogeneity in disease definitions and examination methods among the studies.

Trophectoderm (TE) biopsy. A similar birth defect rate was also found between children born after a TE biopsy and controls in three studies (He *et al.*, 2019; Zhang *et al.*, 2019; Makhijani *et al.*, 2021). All three studies included subjects with several indications for PGT (aneuploidy screening, detection of monogenic gene conditions, or structural rearrangements). While two studies considered only vitrified-warmed transfer cycles (He *et al.*, 2019; Makhijani *et al.*, 2021), Zhang *et al.* (2019) included both fresh and frozen blastocyst transfers in the main analysis. In the Zhang *et al.* (2019) study, while no statistically significant difference in the overall rate of birth defects was observed, the sub-analysis including only FETs showed a significantly increased likelihood of birth defects in neonates born after biopsy (n = 134) vs no biopsy (n = 124) (aOR 11.90; 95% CI 1.40-100.87). Another study identified a few specific birth defects in children born after embryo biopsy for PGT, including arachnoid cysts, ventricular septal defects, auditory canal malformations, Marfan syndrome, renal cysts, and pyelektasis (Zhang *et al.*, 2019). However, this finding should be interpreted very cautiously owing to the small sample size, wide confidence intervals, and paucity of studies reproducing these results.

Long-term outcomes

Table II shows the main characteristics and findings of studies investigating the long-term outcomes of children born after an embryo biopsy.

No data are available for the long-term outcome following PB or TE biopsy. All studies examining the long-term outcomes of children born after PGT focused on the effects of biopsy of cleavage stage embryos.

Neurological outcomes

Neurological outcomes have been evaluated in children aged 18 months to 9 years. From the paper by Mastenbroek *et al.* (2007), four prospective, assessor-blinded follow-up studies were based on children born from women who had or had not undergone embryo biopsy for PGT-A to evaluate neurodevelopmental outcomes at 18 months and 2, 4, and 9 years (Middelburg *et al.*, 2010, 2011; Schendelaar *et al.*, 2013; Kuiper *et al.*, 2018). Dysfunctions in fine motor skills and mildly dysfunctional posture and muscle tone tended to be present significantly more frequently in children up to the age of 18 months after PGT-A, but because of the very small sample size (n = 25 children in the embryo biopsy group compared to n = 31 children in the control group), the results were not conclusive for unfavourable neurological outcomes in association with embryo biopsy for PGT-A (Middelburg *et al.*, 2010). Subsequently, the same authors found a significantly lower neurological optimality score (NOS), a sensitive measure of minor neurological dysfunction, in PGT-A children aged 2 years compared to control children (Middelburg *et al.*, 2011). The same children were evaluated at 4 years of age, and lower fluency scores and NOS scores were found in PGT-A twins but not in singletons (Schendelaar *et al.*, 2013). Since singletons and twins were analysed together in the first 2 years of follow-up (Middelburg *et al.*, 2011), we cannot exclude an important role of twinning in the observation. The negative association between embryo biopsy for PGT-A and the neurological conditions mentioned here did not seem to persist to 9 years. Indeed, at 9 years of age, the same children showed similar NOS scores whether from the group with embryo biopsy for

PGT-A or the control group (Kuiper *et al.*, 2018). The population considered was composed only of n = 43 children in the biopsy group and n = 56 children in the control group.

Blood pressure and anthropometric outcomes

As already mentioned, prospective follow-up studies following the RCT performed by Mastenbroek *et al.* (2007) on PGT-A efficacy focused on the anthropometrics and auxological outcomes of 2-, 4-, and 9-year-old children (Beukers *et al.*, 2013; Seggers *et al.*, 2013; Kuiper *et al.*, 2018). No differences in terms of major and minor anomalies per child were described in 2-year-old children born following or not an embryo biopsy for PGT-A (Beukers *et al.*, 2013). Other studies comparing 2-year-old children born after biopsy for PGT for various indications with matched control children found no differences in major and minor malformation rates, hospital admissions, or surgical interventions (Desmyttere *et al.*, 2009a,b). Children born after biopsy for PGT underwent significantly more complementary investigations (e.g. radiological examinations, measure of pH, isotopic investigations) $[\chi^2(I) = 4.24]$, but the number of abnormal results was not significantly different between the two groups (Desmyttere *et al.*, 2009a).

In addition, at 4 and 9 years of age, the embryo biopsy did not affect systolic blood pressure, diastolic blood pressure, and anthropometric values as well as weight, height, BMI, total skinfold thickness, and head/waist circumference (Seggers *et al.*, 2013; Kuiper *et al.*, 2018). These findings are in line with those from Belva *et al.* (2018) and Heijligers *et al.* (2019), who did not find any difference in anthropometric measures and blood pressure in 6-year-old singletons born after embryo biopsy for PGT-M/PGT-SR and in 5-year-old children born after a biopsy for PGT-M compared to the group without biopsy.

Mental, psychomotor, and cognitive development

Four studies analysed the mental and psychomotor development of children born after embryo biopsy (Nekkebroeck *et al.*, 2008; Middelburg *et al.*, 2011; Beukers *et al.*, 2012; Winter *et al.*, 2014). At 2 years, the mental developmental index and the psychomotor developmental index of MAR children were not associated with biopsy for PGT-A or PGT-M, even after adjusting for sociodemographic confounders (Nekkebroeck *et al.*, 2008; Middelburg *et al.*, 2011; Beukers *et al.*, 2012). These data were also confirmed in 5- to 6-year-old singletons born after biopsy for PGT-M, although the sample size of this latter study was small (n = 47 children in the PGT-M group compared to n = 49 children in the control group) (Winter *et al.*, 2014).

The overall cognitive development of children after embryo biopsy for PGT did not differ from the controls at 2 years of age, from 4 to 6 years of age, and at 9 years of age (Middelburg *et al.*, 2011; Schendelaar *et al.*, 2013; Winter *et al.*, 2014; Kuiper *et al.*, 2018). Although these studies used different tests and some of them included twins and triplets, delayed development was not observed in children born after biopsy for PGT-M or PGT-A.

Synthesis of results

The data presented are shown in a graphical summary to best convey the findings revealed in this scoping review and to highlight the gaps in

Table II Main characteristics and findings of studies investigating the long-term outcomes of children born after embryo biopsy for PGT cycles.

Author, year[i]	Study design	Number of children (PGT vs control group)	Follow-up period	Study population	Biopsy method	Outcomes
Nekkebroeck et al., 2008	Prospective follow-up study	Singletons: N = 70 vs N = 70	2 years	Biopsied (PGT-M + PGT-A) vs non-biopsied embryos	BL	Mental and psychomotor development (−)
Desmyttere et al., 2009a	Prospective follow-up study	Singletons: N = 70 vs N = 70	2 years	Biopsied (PGT) vs non-biopsied embryos	BL	Anthropometric outcomes (−)
Desmyttere et al., 2009b	Prospective follow-up study	Singletons + twins: N = 102 vs N = 102	2 years	Biopsied (PGT) vs non-biopsied embryos	BL	Anthropometric outcomes (−)
Middelburg et al., 2010	Prospective multicenter follow-up of an RCT	Singletons: N = 15 vs N = 21 Twins: N = 10 vs N = 10	18 months	Biopsied (PGT-A) vs non-biopsied embryos	BL	Neurological outcomes (+)
Middelburg et al., 2011	Prospective multicenter follow-up of an RCT	Singletons: N = 36 vs N = 49 Twins: N = 18 vs N = 28	2 years	Biopsied (PGT-A) vs non-biopsied embryos	BL	Neurological outcomes (+) Mental, psychomotor and cognitive development (−)
Schendelaar et al., 2013	Prospective multicenter follow-up of an RCT	Singletons: N = 31 vs N = 42 Twins: N = 18 vs N = 22	4 years	Biopsied (PGT-A) vs non-biopsied embryos	BL	Neurological outcomes (+ only in twins) Cognitive development (−)
Beukers et al., 2012	Prospective multicenter follow-up of an RCT	Singletons: N = 33 vs N = 46 Twins: N = 18 vs N = 26	2 years	Biopsied (PGT-A) vs non-biopsied embryos	BL	Mental and psychomotor development (−)
Beukers et al., 2013	Prospective multicenter follow-up of an RCT	Singletons: N = 32 vs N = 46 Twins: N = 18 vs N = 26	2 years	Biopsied (PGT-A) vs non-biopsied embryos	BL	Anthropometric outcomes (−)
Seggers et al., 2013	Prospective multicenter follow-up of an RCT	Singletons: N = 31 vs N = 42 Twins: N = 18 vs N = 22	4 years	Biopsied (PGT-A) vs non-biopsied embryos	BL	Blood pressure and anthropometric outcomes (−)
Winter et al., 2014	Prospective observational cohort study	Singletons: N = 47 vs N = 49	5-6 years	Biopsied (PGT-M) vs non-biopsied embryos	BL	Mental, psychomotor and cognitive development (−)
Belva et al., 2018	Prospective single-center matched-pair cohort study	Singletons: N = 87 vs N = 87	6 years	Biopsied (PGT-M + PGT-SR) vs non-biopsied embryos	BL	Blood pressure and anthropometric outcomes (−)
Kuiper et al., 2018	Prospective multicenter follow-up of an RCT	Singletons: N = 29 vs N = 38 Twins: N = 14 vs N = 18	9 years	Biopsied (PGT-A) vs non-biopsied embryos	BL	Neurological outcomes (−) Blood pressure and anthropometric outcomes (−) Cognitive development (−)
Heijligers et al., 2019	Prospective observational cohort study	Singletons: N = 85 vs N = 86 Twins: N = 18 vs N = 4	5 years	Biopsied (PGT-M) vs non-biopsied embryos	BL	Blood pressure and anthropometric outcomes (−)

"+", significant difference between the two groups; "−", no significant difference between the two groups.
BL, blastomere; PGT, preimplantation genetic testing; PGT-A, preimplantation genetic testing for aneuploidies; PGT-M, preimplantation genetic testing for monogenic disorders; PGT-SR, preimplantation genetic testing for structural rearrangements; RCT, randomized controlled trial.

knowledge (Fig. 2). The amber symbol represents weak and/or controversial evidence. The red symbol highlights the absence of safety-related evidence. In summary, there are no safety data for PB biopsy and for the long-term outcomes of TE biopsies, while evidence is controversial for all other groups and outcomes.

Discussion

Adaptive responses to environmental stimuli during developmental periods in utero or in early life may strongly influence postnatal life (Barker, 1990). Thus, the possibility that embryo biopsy may be associated with
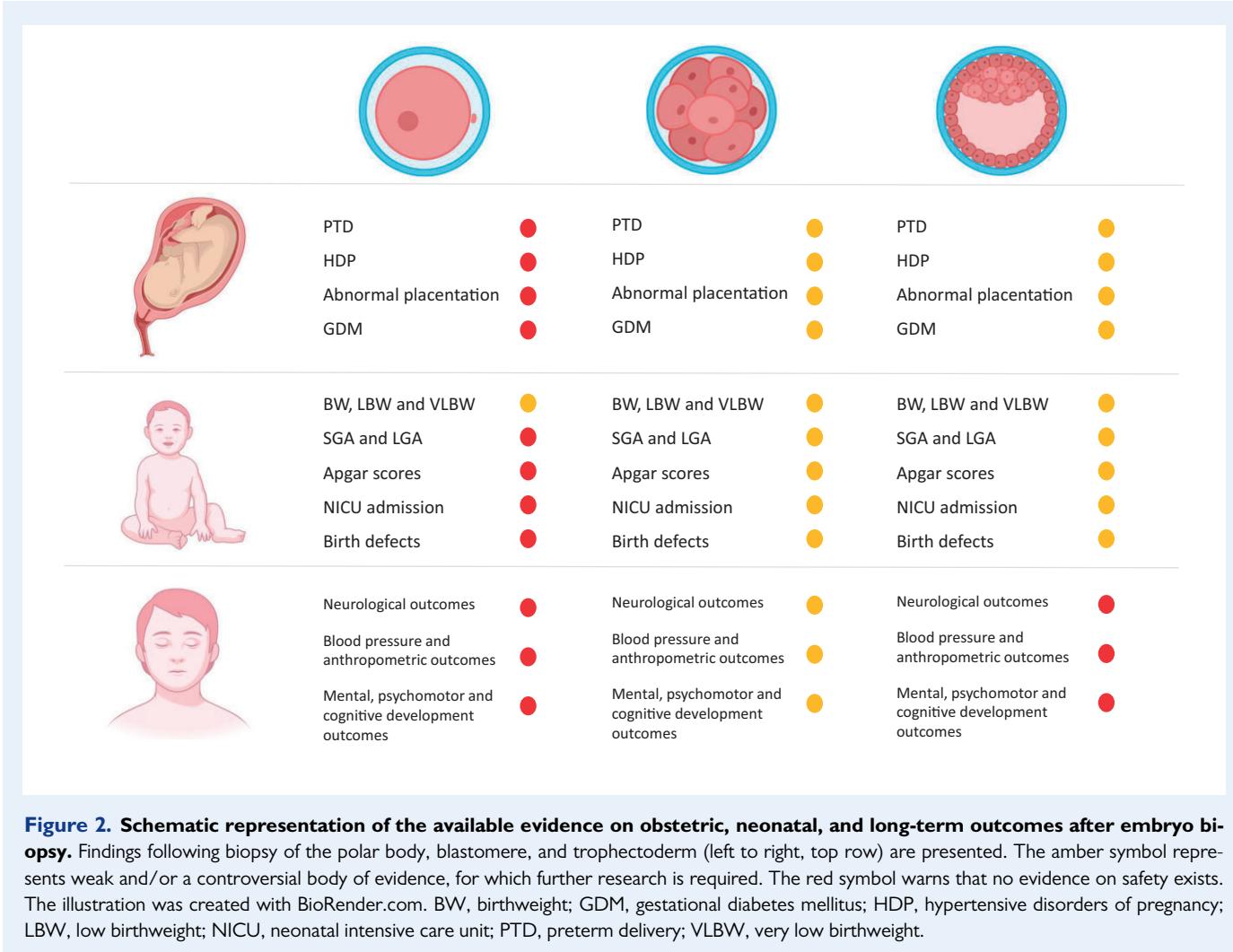
Figure 2. Schematic representation of the available evidence on obstetric, neonatal, and long-term outcomes after embryo biopsy. Findings following biopsy of the polar body, blastomere, and trophectoderm (left to right, top row) are presented. The amber symbol represents weak and/or a controversial body of evidence, for which further research is required. The red symbol warns that no evidence on safety exists. The illustration was created with BioRender.com. BW, birthweight; GDM, gestational diabetes mellitus; HDP, hypertensive disorders of pregnancy; LBW, low birthweight; NICU, neonatal intensive care unit; PTD, preterm delivery; VLBW, very low birthweight.

potential risks for subsequent offspring development is a concern. In line with this observation, evaluation of the potential adverse maternal and offspring outcomes after biopsy treatment was the main aim of this scoping review. To summarize our findings, this study showed a lack of conclusive evidence in support of increased adverse obstetric, neonatal, and long-term outcomes in conceptions after biopsy for PGT.

In more detail, some findings suggest a possible association between embryo biopsy and an increased risk of LBW and SGA in the case of blastomere biopsy and an increase in PTD and birth defects in the case of TE biopsy. For both biopsy methods (at the cleavage and blastocyst stages), an increased risk for pre-eclampsia and, more generally, to HDP was found. Conversely, no association between embryo biopsy at either the cleavage or blastocyst stage and abnormal placentation, GDM or LGA was detected. For PB biopsy, the literature on this topic is too scant to draw definitive conclusions on a link with adverse maternal and neonatal outcomes (Fig. 2). Concerning long-term outcomes, emerging evidence suggests that children are not negatively affected after embryo biopsy for PGT treatment, but these data were derived from a few studies focusing only on blastomere biopsy. No evidence is available regarding the safety of both PB and TE biopsy in terms of postnatal health (Fig. 2).

Correct interpretation of the available evidence is limited by the extreme heterogeneity in the conditions for embryo freezing and infertility status of the populations evaluated in the various studies. Moreover, some adverse outcomes, such as LBW and adverse neurological outcomes, are associated with multiple pregnancies (Middelburg et al., 2011; Desmyttere et al., 2012). In general, the data regarding potential adverse outcomes associated with invasive PGT are controversial, scarce, and weak.

Similarly, it is difficult to interpret the data derived from the two recently published systematic reviews and meta-analyses on this topic. As already mentioned, in these analyses, the data were pooled without distinguishing the different biopsy techniques (Hou et al., 2021; Zheng et al., 2021). Even if subgroup analyses were performed to reduce heterogeneity among studies and the impact of confounding factors, few studies were included, decreasing the strength and reliability of the evaluation. Zheng and coworkers assessed LBW, PTD, VPTD, VLBW, NICU admission, congenital malformations, gestational age, HDP, placenta previa, and GDM in pregnancies following PGT. The subgroups analysed included PGT-M, PGT-A, fresh transfer, FET, cleavage stage biopsy, and blastocyst biopsy. While the overall meta-analysis found that the risk of some neonatal and maternal

complications was modified following PGT, no significant differences could be obtained for any of the outcomes considered in the subgroup analyses (Zheng et al., 2021). Hou et al. (2021) analysed similar outcomes, but in contrast to Zheng et al. (2021), their subgroup analysis comparing FET pregnancies after embryo biopsy or not found some differences between the two groups. A higher rate of intrauterine growth retardation and a lower risk of caesarean section were found to be associated with pregnancies derived from embryo biopsy compared to gestations without biopsy. Overall, because of these relevant limitations and the controversy in the findings, systematic reviews and meta-analyses (Hou et al., 2021; Zheng et al., 2021) on this topic might at present lead to misleading conclusions. A scoping review represents a better strategy when the topic is of a complex or heterogeneous nature and when it is still unclear what other, more specific, questions should be posed and solved to clarify the issue (Munn et al., 2018).

Limitations

Some limitations of the studies reviewed here should be considered. First, the methodology of the invasive biopsy techniques may not be standardized across studies. Thus, an unknown source of heterogeneity is the operator dependency of these procedures. Second, correction for confounders potentially affecting the outcomes (such as maternal age, BMI, comorbidities, parity, presence of infertility and its aetiology, MAR protocols, and use of cryopreservation) was not performed to a large extent. Finally, the majority of the studies were not solid enough in terms of sample size to detect subtle differences in the outcome rates analysed.

Overall, we mapped the body of literature on the topic, but pooling of available evidence was significantly limited owing to the difficulties in removing heterogeneity, biases, confounders, and issues inherent to the study design, potentially leading to unreliable conclusions. Based on the aims of a scoping review, we provided an opportunity to identify key concepts, knowledge gaps to be investigated with future research, and types and sources of evidence to inform practice and research.

Future developments in embryo biopsy

The new challenge in PGT development is the non-invasive approach (niPGT) involving analysis of cell-free DNA obtained from the spent culture media to limit all possible impairments associated with embryo biopsy. The potential advantages of this strategy are wide ranging: the risk of embryo damage is completely averted (Pagliardini et al., 2020); it represents a promising possibility for countries where embryo biopsy is not permitted by law (Feichtinger et al., 2017); and it requires a lower level of expertise of embryologists. While PGT-M from cell-free DNA in spent culture medium does not seem to be sufficiently informative in detecting single-gene disorders, the screening data for aneuploidy are more encouraging (Cimadomo et al., 2020). Not surprisingly, no data on infants born following niPGT are presently available. It is plausible that such a non-invasive approach, with a lower risk of damage to the embryos, could limit potential obstetric, neonatal, and long-term complications in children born after embryo biopsy for PGT.

Conclusion

PGT remains one of the most intensely contested procedures in reproductive medicine. This scoping review has, first, described the confounding factors that limit the reliability of the studies addressing this topic, such as other embryo manipulation procedures or patient characteristics, and, second, suggested a possible association of embryo biopsy at both the cleavage and blastocyst stages with an increased risk of HDP and adverse perinatal outcomes. Long-term adverse effects may be rare but are possible, at least for blastomere biopsy. Third, this study identified gaps in knowledge, including the absence of data on long-term outcomes following TE biopsy or the lack of sufficiently reliable data to be pooled according to the different genetic procedures. Overall, the short- and long-term risks of PGT on prenatal or postnatal health remain uncertain but cannot be excluded.

Data availability

No new data were generated in support of this research.

Authors' roles

A.A. and G.C.C. performed the literature search; A.A., G.C.C., M.P., and G.G. extrapolated the data; A.A., G.C.C., M.P., and G.G. wrote the initial article; P.V. revised the draft; and P.I.C. and P.V. contributed to critical revision. All authors approved the final version of the article.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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