

1 **Risk of miscarriage following amniocentesis and chorionic villus sampling –**
2 **systematic review of the literature and updated meta-analysis**

3

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33

34 **ABSTRACT**

35

36 **Objectives:** To estimate the procedure-related risks of miscarriage after amniocentesis
37 and trans-abdominal chorionic villus sampling (CVS) based on a systematic review of
38 the literature and an updated meta-analysis.

39 **Methods:** A search of MEDLINE, EMBASE, and The Cochrane Library was carried out
40 to identify studies reporting complications following CVS or amniocentesis. The inclusion
41 criteria for the systematic review were studies reporting results from large controlled
42 studies and those reporting data for pregnancy loss prior to 24 weeks' gestation. Study
43 authors were contacted when required to identify additional necessary data. Data for
44 cases that had invasive procedure and controls groups were inputted in contingency
45 tables and risk of miscarriage was estimated for each study. Summary statistics based
46 on a fixed and random effects model were calculated after taking into account the
47 weighting for each study included in the systematic review. Procedure-related risk of
48 miscarriage was estimated as a weighted risk difference from the summary statistics for
49 cases and controls. A subgroup analyses according to the similarity risk levels in the
50 invasive testing and control groups was performed. Heterogeneity was assessed using
51 Cochrane's Q and I^2 statistic. Egger Bias was estimated to assess reporting bias in
52 published studies. Summary statistics for procedure-related risk of miscarriage were
53 graphically represented in Forest plots.

54 **Results:** The electronic search from the databases yielded 2,943 potential citations, from
55 which, we selected 20 controlled studies for inclusion in the systematic review to estimate
56 the procedure-related risk of miscarriage from invasive procedures. There were a total
57 of 580 miscarriages from 63,273 amniocentesis procedures with a weighted risk of
58 pregnancy loss of 0.91% (95%CI: 0.73 to 1.09). In the control group, there were 1,726
59 miscarriages in 330,469 pregnancies with a loss rate of 0.58% (95CI%: 0.47 to 0.70).
60 The weighted procedure-related risk of miscarriage was 0.30% (95%CI: 0.11 to 0.49,
61 $I^2=70.1\%$). There were a total of 163 miscarriages from 13,011 CVS procedures with a
62 risk of pregnancy loss of 1.39% (95%CI: 0.76 to 2.02). In the control group, there were
63 1,946 miscarriages in 232,680 pregnancies with a loss rate of 1.23% (95CI%: 0.86 to
64 1.59). The weighted procedure-related risk of miscarriage following CVS was 0.20%
65 (95%CI: -0.12 to 0.52, $I^2=51.9\%$). However, when only studies with similar risk profiles
66 between the intervention and control groups were considered, the procedure related risk
67 for amniocentesis became 0.03% (95%CI -0.08 to 0.14, $I^2=0\%$) and for CVS -0.38 (95%
68 CI -1.12 to 0.36, $I^2=0\%$).

69 **Conclusion:** The procedure-related risks of miscarriage following amniocentesis and
70 CVS are lower than currently quoted to women. The risk appears to be negligible when
71 these interventions are compared to control groups of the same risk profile.
72

73 **Introduction**

74

75 There is considerable evidence suggesting that the procedure-related risks of
76 miscarriage following amniocentesis and chorionic villus sampling (CVS) are much lower
77 than are currently quoted by professional bodies.¹⁻³ The pooled summary statistics of
78 these procedure-related risks based on data reported in large controlled cohort studies
79 published until January 2014 were reported in a systematic review and meta-analysis.^{4,5}
80 There have been further large studies published in the last few years reporting the
81 procedure-related risks of miscarriage following invasive procedures from large cohort,
82 population and randomised controlled studies with data from more than 20,000
83 procedures.⁶⁻¹¹ We aimed to derive updated procedure-related risks of miscarriage after
84 including data from all studies published till 31st January 2019 in order to provide
85 clinicians with most recent estimates which can be used to counsel women.

86

87

88 **Methods**

89

90 Eligibility criteria

91 The articles eligible for inclusion in our study were randomised controlled trials,
92 prospective or retrospective cohort or case-control studies reporting on the pregnancy
93 outcomes in women who had invasive prenatal testing and that of control pregnancies
94 that did not have an invasive procedure. In view of the improvements and advances in
95 ultrasound resolution and the subsequent improvements in the techniques of performing
96 CVS/amniocentesis over the last couple of decades, we only included studies published
97 from year 2000 onwards to ensure uniformity in comparing results of studies using similar
98 equipment and techniques. In case of studies reporting data spanning years before and
99 after 2000, we only included cases that underwent invasive testing from 2000 onwards.
100 We only included studies reporting their data in English language.

101 *Types of participants:* We included studies reporting their data on invasive procedures
102 carried out in singleton pregnancies. Studies reporting results from both singleton and
103 multiple pregnancies were deemed eligible if data from multiple pregnancies were <5%
104 of the total sample size.

105 *Types of interventions:* We compared women who underwent invasive prenatal testing
106 (CVS or amniocentesis) to those that did not have any invasive procedure. When

107 separate data were reported for transabdominal and transcervical CVS, only the former
108 group was entered in the analyses.

109 *Outcome measures:* Miscarriage, defined as fetal loss before 24 or 22 weeks¹². In
110 studies reporting data for miscarriage due to various causes, we only included
111 procedure-related losses, i.e. those not associated with structural anomalies or other
112 factors likely to cause miscarriage independently from invasive testing.

113

114 Data sources and search strategy

115 This systematic review and meta-analysis was undertaken based on an *a-priori* designed
116 study protocol and was registered in advance with the PROSPERO International
117 Prospective Register of Systematic Reviews (Registration number: CRD42019130495).
118 An electronic search of MEDLINE, EMBASE, and The Cochrane Library was carried out
119 on 31st January 2019 utilising combinations of the relevant Medical Subject Heading
120 (MeSH) terms, key words, and word variants for “Amniocentesis”, “Chorionic Villus
121 Sampling (CVS)”, “miscarriage”, “pregnancy loss” and “procedure-related risk”. The
122 search and selection criteria were restricted to studies reported in English language. The
123 citations retrieved following this search strategy were examined for relevance to this
124 study based on the type of invasive prenatal procedure, study design, sample size of the
125 study, study period and gestational age at assessing pregnancy outcome. We
126 complemented the searches by perusing the references of retrieved articles and the
127 studies included in previous systematic reviews on the topic.

128

129 Study selection and data extraction

130 Search results were screened by two of the authors (RA and LJS) and the full text of all
131 relevant studies was reviewed. The citations were examined to produce a list of relevant
132 studies after excluding studies that were duplicates, those that did not fit selection criteria
133 after review of title and abstract and those that were case-reports, letters, or review
134 articles. These two authors independently assessed for inclusion all the potential studies
135 identified from the search strategy. Data were extracted using a pre-specified form. We
136 resolved any disagreement through discussion or, if required, we consulted a third author
137 (AS).

138

139 For each study, we recorded information about the authors, country of origin, years of
140 enrolment for the intervention and control groups, indications for invasive testing,
141 technique of CVS/amniocentesis, experience of the operators, characteristics of control

142 women and risk level for the two groups. We recorded the number of terminations of
143 pregnancy and we subtracted these cases from the denominator. We contacted the
144 authors of primary study if further details or clarifications were required.

145

146 Risk of bias of individual studies

147 The methodological quality of studies included in the systematic review was assessed
148 using the Newcastle-Ottawa Scale (NOS)¹³. Briefly, NOS assess the quality of cohort or
149 case-control studies across three domains, i.e. selection (including representativeness
150 of the exposed cohort, selection of the non-exposed cohort, ascertainment of exposure
151 and demonstration that the outcome of interest was not already present at the start of
152 the study), comparability and outcome (including assessment of outcome and adequacy
153 of follow-up length). The assessment of the domains is performed based on a
154 standardized checklist and indicators of high quality are awarded a star; the number and
155 combination of stars expresses the overall quality of a study in an AHRQ-compliant way
156 (good, fair or poor).

157

158 We reported our study as per PRISMA (Preferred Reporting Items for Systematic reviews
159 and Meta-Analyses) (Supplementary Table 1).¹⁴

160

161 Summary measures and synthesis of the results

162 The data from each study was extracted to note the type of procedure, study design,
163 sample size, and miscarriage rate in each study group. Study authors were contacted
164 when required to identify additional necessary data. Data were entered in contingency
165 tables and miscarriage rate (95% confidence interval [CI]) was estimated in the invasive
166 procedure and the control group, for each study as well as a pooled estimate, weighted
167 by sample size of each study. The procedure-related risk for amniocentesis and CVS for
168 each study were estimated as a risk difference (RD) based on the miscarriage rates in
169 the invasive and control group, which were then used to calculate the weighted pooled
170 summary estimate (95% CI). Given the non-randomized design and the anticipated
171 heterogeneity of the studies, we calculated the summary effect sizes using random
172 effects models. The random effects model assumes that the true effect size varies
173 between the studies, and that included studies represent a random sample of effect sizes
174 that could have been observed. Therefore, we opted to use this model as it allows for
175 not just for variation within studies but also variation between studies, thus providing a
176 conservative estimate of the summary statistics with wider CIs.¹⁵ The procedure-related

177 risks for amniocentesis and CVS were graphically expressed in Forest plots. Pooled
178 proportions were calculated using the *metaprop* command. The heterogeneity between
179 studies was assessed by estimation of Cochrane's Q and I² statistic; Egger's meta-
180 regression test was used to assess reporting bias in studies when 10 or more studies
181 were available.¹⁶⁻¹⁸

182

183 Subgroup analyses

184 We planned subgroup analyses according to the similarity risk levels in the invasive
185 testing and control groups, as extracted by their description in the primary studies (similar
186 or dissimilar risk), as this could be related to confounders that can affect the procedure-
187 related risk, and therefore the risk difference (RD).

188

189 We carried out statistical analyses in Stata 14.0 software (StataCorp College Station,
190 TX) using the *metan* and *metaprop* commands.

191

192

193 **Results**

194

195 Data search results

196 The electronic search from the databases yielded 2,943 potential citations of which 2,907
197 were excluded as they were duplicates, or a review of the title or abstract did not meet
198 the inclusion criteria, leaving 36 studies for full-text review. After the full manuscript
199 review, we finally considered 12 studies for amniocentesis^{6,7,9-11,19-25} and 8 studies for
200 CVS^{6-8,10,11,26-28}. Of those, one study (Lau *et al.*, 2005) was eventually excluded²⁶, as it
201 gave cumulative data for miscarriage and stillbirth, leaving 12 studies for amniocentesis
202 and 7 for CVS. The raw data for the Wulff *et al.*, 2016⁶ study were calculated from the
203 published adjusted estimates and were complemented with additional information
204 provided by the authors. Similarly, the authors of Malan *et al.*, 2018 were contacted to
205 obtain data additional data for amniocentesis and CVS.¹⁰ The characteristics of the
206 included studies are shown in Supplementary Table 2.

207

208 Assessment of the quality of included studies

209 The methodological quality of studies was assessed using the NOS. The rating of the
210 included studies according to the NOS based on study type, selection, comparability and
211 outcome are shown in Supplementary Table 3.

212

213 Amniocentesis group

214 There were a total of 580 miscarriages from 63,723 amniocentesis procedures with a
215 pooled risk of pregnancy loss of 0.91% (95%CI 0.73 to 1.09, $I^2=88.2\%$). In the control
216 group, there were 1726 miscarriages in 330469 pregnancies with a pooled loss rate of
217 0.58% (95%CI 0.47 to 0.70, $I^2=96.1\%$). The pooled procedure-related risk of miscarriage
218 was 0.30% (95%CI: 0.11 to 0.49), $I^2=70.1\%$ (Figure 2).

219

220 Chorionic villus sampling group

221 There were a total of 163 miscarriages from 13,011 CVS procedures, with a pooled risk
222 of pregnancy loss of 1.39% (95%CI 0.76 to 2.02, $I^2=89.1\%$). In the control group, there
223 were 1,946 miscarriages in 232,680 pregnancies with a pooled loss rate of 1.23%
224 (95%CI 0.86 to 1.59, $I^2=98.1\%$). The pooled procedure-related risk of miscarriage
225 following CVS was non-significant (0.20, 95%CI: -0.12 to 0.52, $I^2=51.9\%$) (Figure 3).

226

227 Subgroup analyses

228 For each invasive test (amniocentesis or CVS) we examined the procedure-related risk
229 of miscarriage separately for studies with similar or dissimilar risk level for chromosomal
230 abnormalities. The pooled risk RD (i.e. procedure related risk) for amniocentesis was
231 0.46% (95%CI 0.25 to 0.67, $I^2=38.1\%$) when studies with dissimilar risk level for the two
232 groups were synthesized. When studies with similar risk levels for the amniocentesis and
233 control groups were synthesized, there was no significant procedure-related risk (RD
234 0.12 95%CI -0.05 to 0.30%, $I^2=44.1\%$) (Figure 4).

235

236 The procedure-related risk for CVS was non-significant when studies with similar level
237 of risk between the two groups were compared (pooled RD -0.011, 95%CI -0.29 to 0.08,
238 $I^2=0\%$). In contrast, CVS was associated with a pooled procedure-related risk of 0.48%
239 (95%CI 0.17 to 0.78, $I^2=0\%$) when studies with dissimilar level of risk between the
240 intervention and control group were synthesized (Figure 5).

241

242 Publication bias

243 We were able to assess the potential for publication bias for amniocentesis only, as the
244 number of CVS studies was less than 10. The Egger's meta-regression test did not
245 demonstrate the presence of small-study effects ($p=0.179$) (Supplemental figure 1.)

246

247

248 **Discussion**

249

250 Main findings

251 The results of our study demonstrate the following facts: first, the procedure-related risk
252 of miscarriage is considerably lower than is currently quoted in guidelines from
253 professional bodies and is 0.30% following amniocentesis, whereas there is no
254 significant procedure-related risk associated with CVS, which may be a safer procedure
255 than amniocentesis;; second, our results highlight that the point-estimates for
256 miscarriage are even substantially lower with no significant increase in risk of miscarriage
257 for both, amniocentesis and CVS, when the analysis is restricted to studies in which the
258 control population has a similar risk profile for chromosomal abnormalities as the women
259 who underwent invasive prenatal testing.

260

261 Strengths and limitations

262 This is an updated version of our previous meta-analyses, adding the only new
263 randomized trial¹⁰ in three decades and following a new approach to address the issue
264 of heterogeneity between reported studies.

265

266 The published studies have used different indications for invasive testing and different
267 selection criteria for the control women, culminating in different background risk levels
268 for the compared groups both within and across studies. The resulting heterogeneity has
269 been the major argument against quantitative synthesis of such studies. To this end, we
270 have taken the following measures. First, we excluded terminations of pregnancy from
271 both the intervention and control groups. Second, we excluded cases of miscarriage
272 directly attributable to structural defects or obstetric complications unrelated to invasive
273 testing, if data were available. Third, we only analysed invasive procedures performed
274 from 2000 onwards, if such data were available, to account for the progress in ultrasound
275 resolution and sampling techniques. Fourth, we stratified the intervention and control
276 groups according to their risk profiles, as extracted from their inclusion criteria, and we
277 separately analysed studies where the two groups had similar risk profiles. This analysis
278 highlighted the impact of dissimilar background risks as a source of risk inflation and
279 statistical heterogeneity. Fifth, anticipating that the studies do not represent random
280 samples from the same population, we used the random effects model, which does not
281 assume a common underlying effect size and produces more conservative estimates. In

282 any case, as the control groups have usually more favourable risk profile, any bias would
283 be against invasive procedures, which means that the latter may be even safer than they
284 appear in an aggregate analysis.

285

286 There are some limitations to our study which could not be overcome despite our robust
287 methodology of well-defined eligibility criteria, inclusion of controlled studies with large
288 sample size, as well as the strict approach to minimising heterogeneity. A limitation of
289 our study is that when we compared the risks of miscarriage in the intervention and
290 control pregnancies with similar risk profile and background risks, these were mainly in
291 high-risk populations and the results of our study therefore cannot comment on risk
292 estimates of miscarriage, if these were performed in large low-risk populations. In our
293 study, we did not examine other pregnancy complications such as preterm birth and
294 stillbirth nor did we examine the serious but rare outcomes such as maternal septicaemia
295 or amniotic fluid embolism as they were not consistently reported by included studies.
296 Another limitation refers to lack of analyses of data with regard to operator experience
297 as it is potentially an important factor associated with procedure-related loss.
298 Unfortunately, with the exception of two recent studies with conflicting results (Akolekar
299 *et al.*, 2011 and Bakker *et al.*, 2017)^{7,27}, the rest of the studies do not provide data about
300 the effect of operator experience on the risk of miscarriage, and we were unable to
301 account for this in our analyses.

302

303 Interpretation of the findings

304 The first question we aimed to address is whether invasive prenatal diagnosis is a safe
305 procedure. Our results suggest that amniocentesis is associated with a procedure-
306 related risk of 1:300 at most, or more likely, no significant increase in risk if we considered
307 the results from our analysis which only included studies with comparable risk profile in
308 the intervention and control groups. With regard to CVS, our results demonstrate that,
309 there is no significant procedure-related risk associated with undertaking this procedure.

310

311 A second, related question is whether, which is a safer procedure to undertake, CVS or
312 amniocentesis. There is no statistically appropriate way to answer this through either a
313 direct or network meta-analysis, as the two methods do not have a common comparator.
314 The closest approximation to a valid answer to this question is to estimate a pooled
315 procedure-related risk from those studies which reported results for both amniocentesis
316 and CVS by comparison with a control group. There were four such studies (Wulff *et al.*,

317 2016, Bakker *et al.*,2017, Malan *et al.*,2018 and Beta *et al.*, 2019)^{6,7,10,11} comparing both
318 CVS and amniocentesis to their corresponding control groups; their pooled procedure-
319 related risk was 0.11% (95%CI -0.28 to 0.50, I²=42.1%) for CVS and 0.55% (95%CI 0.29
320 to 0.81, I²=0%) for amniocentesis. There are a few hypotheses than can potentially
321 explain the reasons for these differences and the apparent greater safety of CVS
322 compared to amniocentesis. Firstly, CVS is usually performed by specialist and
323 experienced Fetal Medicine operators compared to amniocentesis; secondly, performing
324 a CVS involves introduction of the needle into the placental tissue, which is highly
325 vascular tissue with blood flow of about 90-100 mL/minute/kg at 12 weeks' gestation^{29,30},
326 as opposed to introduction of a needle into the amniotic sac, which is a closed cavity and
327 therefore has a higher chance of a potential infection introduced into a confined
328 intraamniotic space and lastly, the option to reschedule the procedure and undertake
329 amniocentesis if there are technical limitations.

330

331 The quest to accurately quantify procedure-related risks for invasive procedures may
332 seem trivial. However, we consider it to be quite important and relevant to current clinical
333 practice. There is a recent systematic review in 2017 attempting to quantify procedure-
334 related risks of miscarriage from published RCTs with the conclusion that second
335 trimester amniocentesis increases the risk of procedure-related loss but it is not possible
336 to quantify this increase precisely from one study.³¹ Such conclusions are unhelpful and
337 do not provide the women nor their clinicians any clear evidence-based estimates of risks
338 for decision making. These invasive procedures are still routinely carried out for prenatal
339 diagnosis but instead of accurate and recent estimates of risks from expert operators,
340 these are rather based on historical and inflated estimates of risks. There are significant
341 advances in cytogenetics analysis and genomic sequencing which are progressing at a
342 rapid pace, and pregnant women must receive appropriate counselling to enable them
343 to make informed choices about their options for prenatal testing, without being deterred
344 by falsely exaggerated rates of procedure-related risks of miscarriage. Until such a time
345 that non-invasive testing is not diagnostic and as comprehensive as cytogenetics
346 techniques, the questions about the safety of invasive procedures and the factors
347 affecting it remain topical.³²

348

349 Comparison with previous studies

350 In comparison to our previous meta-analyses^{4,5}, this update includes the first RCT
351 published in the last three decades and it addresses the issue of heterogeneity by

352 carefully excluding cases potentially affected by confounders and by accounting for the
353 effect of different background risks. In terms of numerical estimates, the procedure
354 related risk of amniocentesis seems to stabilize around 1:300, whereas the risk
355 difference between CVS and controls still fails to reach significance. The most important
356 novelty is the synthesis of studies in which the intervention and control groups had similar
357 background risks for chromosomal abnormalities, as derived from their description. It
358 appears that, when comparing women at a similar risk level (be it high, intermediate or
359 low, Table 1), the procedure-related risk for amniocentesis also fails to reach
360 significance, whereas the statistical heterogeneity substantially decreases. This is a
361 significant finding, supporting the concept that, all other things being equal, an invasive
362 procedure is not associated with a significant increase in the rate of miscarriage. This
363 does not imply that a miscarriage following an invasive procedure cannot occur, but this
364 is more likely to be related to either operator-independent maternal factors (Akolekar *et*
365 *al.*, 2011)²⁷, or to the experience and technique of the operator, rather than the procedure
366 itself. In a large UK study, the authors reported that there was no significant increase in
367 overall procedural risk of miscarriage, regardless of whether the procedure was carried
368 out by fetal medicine experts or trainees under direct supervision of an expert (Akolekar
369 *et al.*, 2011), whereas the point estimates of procedure-related miscarriage appeared to
370 decrease with operators' experience, though in a non-significant way in a Dutch study
371 (Bakker *et al.*, 2017).^{7,27} As neither of these studies were randomised, there is no reliable
372 way to ascertain how experience may impact on outcomes or the effects are subject to
373 modifications by confounders like negative selection bias.

374

375 In terms of raw numbers and sample size of studies, the evidence is dominated by a
376 large Danish registry study, which shows that invasive procedures themselves do not
377 carry a significant miscarriage risk (Wulff *et al.*, 2017).⁶ The random effects model we
378 used reduces the weight (and therefore the dominance) of this single trial, which in any
379 case is in line with the aggregate findings. Similarly, the French multicentre RCT, which
380 aimed to show that there would be a reduction in the risk of miscarriage in the group that
381 was offered invasive testing only for positive cfDNA results (n=1,034) as opposed to
382 those with direct invasive testing (n=1,017), also failed to show a significant difference
383 (RD -0.03%).¹⁰

384

385 Conclusion

386 The results of this systematic review and meta-analysis demonstrate that the procedure-
387 related risks of miscarriage from invasive procedures are low, or negligible if groups with
388 similar background risks are compared. In terms of safety of a prenatal diagnostic
389 procedure, it appears that CVS is potentially safer compared to amniocentesis. Women
390 should be reassured that invasive procedures carried out by experienced operators in
391 specialist centres are not associated with a significant increase in miscarriage rate as
392 compared to those women not undergoing these procedures.

393 **References**

394

- 395 1. Ghi T, Sotiriadis A, Calda P, Da Silva Costa F, Raine-Fenning N, Alfirevic Z,
396 McGillivray G; International Society of Ultrasound in Obstetrics and Gynecology
397 (ISUOG). ISUOG Practice Guidelines: invasive procedures for prenatal diagnosis.
398 *Ultrasound Obstet Gynecol* 2016;48:256-268.
- 399 2. Royal College of Obstetricians and Gynaecologists. Amniocentesis and Chorionic
400 Villus Sampling. Green Top Guideline No.8. London: RCOG, 2010.
- 401 3. Invasive prenatal testing for aneuploidy. ACOG Practice Bulletin No. 88, December
402 2007. American College of Obstetricians and Gynecologists. *Obstet Gynecol* 2007;
403 110: 1459-1467.
- 404 4. Akolekar R, Beta J, Picciarelli G, Ogilvie C, D'Antonio F. Procedure-related risk of
405 miscarriage following amniocentesis and chorionic villus sampling: a systematic
406 review and meta-analysis. *Ultrasound Obstet Gynecol.* 2015;45:16-26.
- 407 5. Beta J, Lesmes-Heredia C, Bedetti C, Akolekar R. Risk of miscarriage following
408 amniocentesis and chorionic villus sampling: a systematic review of the literature.
409 *Minerva Ginecol* 2018;70:215-219.
- 410 6. Wulff CB, Gerds TA, Rode L, Ekelund CK, Petersen OB, Tabor A; Danish Fetal
411 Medicine Study Group. Risk of fetal loss associated with invasive testing following
412 combined first-trimester screening for Down syndrome: a national cohort of 147,987
413 singleton pregnancies. *Ultrasound Obstet Gynecol.* 2016;47: 38-44.
- 414 7. Bakker M, Birnie E, Robles de Medina P, Sollie KM, Pajkrt E, Bilardo CM. Total
415 pregnancy loss after chorionic villus sampling and amniocentesis: a cohort study.
416 *Ultrasound Obstet Gynecol.* 2017;49:599-606.
- 417 8. Wah YM, Leung TY, Cheng YKY, Sahota DS. Procedure-Related Fetal Loss
418 following Chorionic Villus Sampling after First-Trimester Aneuploidy Screening. *Fetal*
419 *Diagn Ther.* 2017;41: 184-190.
- 420 9. Theodora M, Antsaklis A, Blanas K, Antsaklis P, Daskalakis G, Sindos M, Mesogitis
421 S, Papantoniou N. Risk for fetal loss and prematurity after 12,413 second trimester
422 amniocenteses in a single center. *J Perinat Med.* 2015;43:347-351.
- 423 10. Malan V, Bussi eres L, Winer N, Jais JP, Baptiste A, Le Lorc'h M, Elie C, O'Gorman
424 N, Fries N, Houfflin-Debarge V, Sentilhes L, Vekemans M, Ville Y, Salomon LJ;
425 SAFE 21 Study Group. Effect of Cell-Free DNA Screening vs Direct Invasive
426 Diagnosis on Miscarriage Rates in Women With Pregnancies at High Risk of Trisomy
427 21: A Randomized Clinical Trial. *JAMA* 2018;320:557-565.

- 428 11. Beta J, Zhang W, Geris S, Kostiv V, Akolekar R. Procedure-related risk of
429 miscarriage from chorionic villus and amniocentesis. *Ultrasound in Obstet Gynaecol*
430 (manuscript submitted).
- 431 12. Survival of extremely preterm infants: Joint statement from the British Medical
432 Association, the British Association of Perinatal Medicine, the Faculty of Sexual and
433 Reproductive Healthcare, the Royal College of Nursing and the Royal College of
434 Obstetricians and Gynaecologists. [http://www.rcog.org.uk/files/rcog-corp/uploaded-](http://www.rcog.org.uk/files/rcog-corp/uploaded-files/JointStatementBMABAPMFSRHRCNRCOG24wkMay08.pdf)
435 [files/JointStatementBMABAPMFSRHRCNRCOG24wkMay08.pdf](http://www.rcog.org.uk/files/rcog-corp/uploaded-files/JointStatementBMABAPMFSRHRCNRCOG24wkMay08.pdf). [Accessed 31st
436 January 2019].
- 437 13. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The
438 Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomized studies
439 in metaanalysis. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.
- 440 14. Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group.
441 Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The
442 PRISMA Statement. *BMJ* 2009; 39:b2535.
- 443 15. DerSimonian R, Laird N: Meta-analysis in clinical trials. *Control Clin Trials* 1986; 7:
444 177-188.
- 445 16. Cochran WG. The combination of estimates from different experiments. *Biometrics*
446 1954; 10: 101-129
- 447 17. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-
448 analyses. *BMJ* 2003; 327: 557-560.
- 449 18. Egger M, Davey Smith G, Schneider M, Minder C: Bias in meta-analysis detected by
450 a simple, graphical test. *BMJ* 1997; 315: 629-634.
- 451 19. Muller F, Thibaud D, Poloce F, Gelineau MC, Bernard M, Brochet C, Millet C, Réal
452 JY, Dommergues M. Risk of amniocentesis in women screened positive for Down
453 syndrome with second trimester maternal serum markers. *Prenat Diagn* 2002; 22:
454 1036-1039.
- 455 20. Eddleman KA, Malone FD, Sullivan L, Dukes K, Berkowitz RL, Kharbutli Y, Porter
456 TF, Luthy DA, Comstock CH, Saade GR, Klugman S, Dugoff L, Craigo SD, Timor-
457 Tritsch IE, Carr SR, Wolfe HM, D'Alton ME. Pregnancy loss rates after
458 midtrimester amniocentesis. *Obstet Gynecol* 2006; 108: 1067-1072.
- 459 21. Kong CW, Leung TN, Leung TY, Chan LW, Sahota DS, Fung TY, Lau TK. Risk
460 factors for procedure-related fetal losses after mid-trimester genetic amniocentesis.
461 *Prenat Diagn* 2006; 26: 925-30.

- 462 22. Towner D, Currier RJ, Lorey FW, Cunningham GC, Greve LC. Miscarriage risk
463 from amniocentesis performed for abnormal maternal serum screening. *Am J Obstet*
464 *Gynecol* 2007;196: 608.e1-5.
- 465 23. Odibo AO, Gray DL, Dicke JM, Stamilio DM, Macones GA, Crane JP. Revisiting the
466 fetal loss rate after second-trimester genetic amniocentesis: a single center's 16-
467 year experience. *Obstet Gynecol* 2008;111: 589-595.
- 468 24. Pitukkijronnakorn S1, Promsonthi P, Panburana P, Udomsubpayakul
469 U, Chittacharoen A. Fetal loss associated with second trimester amniocentesis. *Arch*
470 *Gynecol Obstet* 2011;284: 793-797.
- 471 25. Corrado F, Cannata ML, La Galia T, Magliarditi M, Imbruglia L, D'anna R, Carlo
472 Stella N. Pregnancy outcome following mid-trimester amniocentesis. *J Obstet*
473 *Gynaecol* 2012;32: 117-119.
- 474 26. Lau KT, Leung YT, Fung YT, Chan LW, Sahota DS, Leung NT. Outcome of 1,355
475 consecutive transabdominal chorionic villus samplings in 1,351 patients. *Chin Med*
476 *J (Engl)* 2005; 118: 1675-1681.
- 477 27. Akolekar R, Bower S, Flack N, Bilardo CM, Nicolaides KH. Prediction of miscarriage
478 and stillbirth at 11-13 weeks and the contribution of chorionic villus sampling. *Prenat*
479 *Diagn* 2011;31: 38-45.
- 480 28. Odibo AO, Dicke JM, Gray DL, Oberle B, Stamilio DM, Macones GA, Crane JP.
481 Evaluating the rate and risk factors for fetal loss after chorionic villus sampling.
482 *Obstet Gynecol* 2008; 112: 813-819.
- 483 29. Acharya G, Sonesson SE, Flo K, Räsänen J, Odibo A. Hemodynamic aspects of
484 normal human fetoplacental (umbilical) circulation. *Acta Obstet Gynecol Scand*
485 2016;95:672-682.
- 486 30. Assali, NS, Rauramo, L, Peltonen, T. Measurement of uterine blood flow and
487 uterine metabolism. VIII. Uterine and fetal blood flow and oxygen consumption in
488 early human pregnancy. *Am J Obstet Gynecol* 1960;79:86-98.
- 489 31. Alfirevic Z, Navaratnam K, Mujezinovic F. Amniocentesis and chorionic villus
490 sampling for prenatal diagnosis. *Cochrane Database Syst Rev.* 2017;9:CD003252.
- 491 32. Salomon LJ, Alfirevic Z, Audibert F, Kagan KO, Paladini D, Yeo G, Raine-Fenning
492 N; ISUOG Clinical Standards Committee. ISUOG updated consensus statement on
493 the impact of cfDNA aneuploidy testing on screening policies and prenatal ultrasound
494 practice. *Ultrasound Obstet Gynecol* 2017;49:815-816.
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496 **Figure legends**

497

498 **Figure 1.** Flowchart of study selection of studies included in the meta-analysis.

499 **Figure 2.** Procedure-related risk for miscarriage after amniocentesis, expressed as risk
500 difference (RD, 95% CI) to controls (Random effects model)

501 **Figure 3.** Procedure-related risk for miscarriage after chorionic villus sampling (CVS),
502 expressed as risk difference (RD, 95% CI) to controls (Random effects model)

503 **Figure 4.** Subgroup analysis for the procedure-related risk of miscarriage after
504 amniocentesis, expressed as risk difference (RD, 95% CI) to controls (Random effects
505 model). Subgroup 1 included studies in which the intervention and control groups had
506 similar risk profile (i.e. they were both of high-, intermediate- or low risk). Subgroup 2
507 included studies in which the intervention and control groups had similar risk profile

508 **Figure 5.** Subgroup analysis for the procedure-related risk of miscarriage after
509 amniocentesis, expressed as risk difference (RD, 95% CI) to controls (Random effects
510 model). Subgroup 1 included studies in which the intervention and control groups had
511 similar risk profile. Subgroup 2 included studies in which the intervention and control
512 groups had similar risk profile (i.e. they were both of high-, intermediate- or low risk).

513 **Supplemental figure.** Egger's meta-regression test for the presence of small-study
514 effects.