

Serum luteal phase progesterone in women undergoing frozen embryo transfer in assisted conception

Melo, Pedro; Chung, Yealin; Pickering, Oonagh; Price, Malcolm; Fishel, Simon; Khairy, Mohammed; Kingsland, Charles ; Lowe, Philip; Petsas, Georgios ; Rajkhowa, Madhurima; Sephton, Victoria ; Tozer, Amanda ; Wood, Simon ; Labarta, Elena ; Wilcox, Mark; Devall, Adam; Gallos, Ioannis; Coomarasamy, Arri

DOI:

[10.1016/j.fertnstert.2021.07.002](https://doi.org/10.1016/j.fertnstert.2021.07.002)

License:

Creative Commons: Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)

Document Version

Peer reviewed version

Citation for published version (Harvard):

Melo, P, Chung, Y, Pickering, O, Price, M, Fishel, S, Khairy, M, Kingsland, C, Lowe, P, Petsas, G, Rajkhowa, M, Sephton, V, Tozer, A, Wood, S, Labarta, E, Wilcox, M, Devall, A, Gallos, I & Coomarasamy, A 2021, 'Serum luteal phase progesterone in women undergoing frozen embryo transfer in assisted conception: a systematic review and meta-analysis', *Fertility and Sterility*, vol. 116, no. 6, pp. 1534-1556.
<https://doi.org/10.1016/j.fertnstert.2021.07.002>

[Link to publication on Research at Birmingham portal](#)

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

1 **Serum luteal phase progesterone in women undergoing frozen embryo**
2 **transfer (FET) in assisted conception: a systematic review and meta-analysis**

3 **Running title: Serum progesterone in FET cycles**

4 Pedro Melo, M.D., M.R.C.O.G.,^{a,b*} Yealin Chung, M.B.B.S., M.R.C.O.G.,^{a,b} Oonagh Pickering, B.Sc.,^a Malcolm J
5 Price, Ph.D.,^{c,d} Simon Fishel, Ph.D., F.R.S.B.,^{e,f} Mohammed Khairy, M.D., M.R.C.O.G.,^b Charles Kingsland, M.D.,
6 F.R.C.O.G.,^g Philip Lowe, M.B.Ch.B., F.R.C.O.G.,^h Georgios Petsas, M.D., Ph.D.,ⁱ Madhurima Rajkhowa, M.D.,
7 F.R.C.O.G.,^b Victoria Sephton, M.B.Ch.B., M.R.C.O.G.,^j Amanda Tozer, M.B.Ch.B., F.R.C.O.G.,^k Simon Wood, M.D.,
8 F.R.C.O.G.,^j Elena Labarta, M.D., Ph.D.,^l Mark Wilcox, D.M., F.R.C.O.G.,^e Adam Devall, Ph.D.,^a Ioannis Gallos, M.D.,
9 M.R.C.O.G.,^a Arri Coomarasamy, M.D., F.R.C.O.G.^{a,b}

10 ^aTommy's National Centre for Miscarriage Research, Institute of Metabolism and Systems Research, College of
11 Medical and Dental Sciences, University of Birmingham, Edgbaston, B15 2TT, United Kingdom

12 ^bCARE Fertility Birmingham, Edgbaston, B15 3DP, United Kingdom

13 ^cInstitute of Applied Health Research, University of Birmingham, Edgbaston, B15 2TT, United Kingdom

14 ^dNIHR Birmingham Biomedical Research Centre, University Hospitals Birmingham NHS Foundation Trust and
15 University of Birmingham, United Kingdom

16 ^eCARE Fertility Nottingham, NG8 6PZ, United Kingdom

17 ^fSchool of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool, L3
18 3AF, United Kingdom

19 ^gCARE Fertility Liverpool, L3 1DL, United Kingdom

20 ^hCARE Fertility Manchester, M14 5QH, United Kingdom

21 ⁱCARE Fertility Sheffield, S7 1RA, United Kingdom

22 ^jCARE Fertility Chester, CH2 1UL, United Kingdom

23 ^kCARE Fertility London, NW8 7JL, United Kingdom

24 ^lHuman Reproduction Department, IVI-RMA Valencia, Valencia, 46015, Spain

25 ***Corresponding author:** Dr. Pedro Melo

26 **Address:** Tommy's National Centre for Miscarriage Research, Institute of Metabolism and Systems Research,
27 College of Medical and Dental Sciences, University of Birmingham, Edgbaston, B15 277, United Kingdom.

28 **E-mail:** pedro.melo1@nhs.net

29 **Telephone:** +44(0)121 371 8202

30 **Capsule:** For thresholds below 10 ng/ml, women with lower progesterone levels experience fewer live births
31 and increased risk of miscarriage compared to women with higher serum progesterone.

32 Abstract

33 Objective

34 To investigate the association between luteal serum progesterone levels and frozen embryo transfer (FET)
35 outcomes.

36 Design

37 Systematic review and meta-analysis.

38 Setting

39 Not applicable.

40 Patients

41 Women undergoing FET.

42 Interventions

43 We conducted electronic searches of MEDLINE, PubMed, CINAHL, EMBASE, the Cochrane Database of
44 Systematic Reviews, CENTRAL, Web of Science, ClinicalTrials.gov and grey literature (not widely available) from
45 inception to March 2021 to identify cohort studies where serum luteal progesterone was measured around the
46 time of FET.

47 Main Outcome Measures

48 Ongoing pregnancy or live birth rate, clinical pregnancy rate and miscarriage rate.

49 Results

50 Among studies analyzing serum progesterone thresholds below 10 ng/ml, higher serum progesterone was
51 associated with increased rates of ongoing pregnancy or live birth (relative risk [RR] 1.47, 95% confidence
52 interval [CI] 1.28 to 1.70), higher chance of clinical pregnancy (RR 1.31, 95% CI 1.16 to 1.49) and lower risk of
53 miscarriage (RR 0.62, 95% CI 0.50 to 0.77) in cycles using exclusively vaginal progesterone and blastocyst
54 embryos. There was uncertainty about whether progesterone thresholds higher than 10 ng/ml were associated
55 with FET outcomes in sensitivity analyses including all studies, owing to high inter-study heterogeneity and wide
56 confidence intervals.

57 Conclusion

58 Our findings indicate that there may be a minimum clinically important luteal serum concentration of
59 progesterone required to ensure an optimal endocrine milieu during embryo implantation and early pregnancy
60 following FET treatment. Future clinical trials are required to assess whether administering higher-dose luteal
61 phase support improves outcomes in women with low serum progesterone at the time of FET.

62 PROSPERO Number

63 CRD42019157071

64

65 Key Words

66 Progesterone, endometrial receptivity, frozen embryo transfer, luteal phase support, live birth, miscarriage

67

68

69

70

71

72

73

74

75

76

77

78

79

80

81

82

83

84

85

86

87

88

89

90

91

92

93

94

95

96

97

98

99

100

101

102

103

104

105

106

107

108

109

110 Introduction

111 The use of freeze-thaw embryo transfer (FET) treatment is an important option in assisted conception practice.
112 FET allows clinicians to comply with single embryo transfer (SET) policies while significantly reducing the chance
113 of ovarian hyperstimulation syndrome (OHSS) associated with controlled ovarian stimulation (COS) (1).
114 Furthermore, it is thought that FET mitigates the risk of endometrial asynchrony by avoiding the need for
115 treatment with gonadotropins and by allowing greater flexibility on the timing of embryo transfer (2). For these
116 reasons, some clinics have started to offer a universal freeze-all policy (3). According to the Human Fertilisation
117 and Embryology Authority (HFEA), the number of FET undertaken in the UK nearly doubled between 2013 and
118 2018, now accounting for 38% of all in vitro fertilization (IVF) cycles. Conversely, the number of fresh embryo
119 transfers decreased by 11% in the same time period (4). This mirrors similar trends in continental Europe (5),
120 the USA (6) and across the world (7). The success rate of FET cycles remains relatively low nonetheless, with
121 fewer than one in three women achieving a live birth following FET treatment worldwide (7).

122 There has been a growing body of research aimed at identifying ways to predict and improve FET outcomes in
123 the past decade. It is widely accepted that treatment success depends on a plethora of factors including embryo
124 ploidy status and endometrial receptivity (8). Endometrial thickness as measured by ultrasound has been
125 historically used as a marker of receptivity, while recent studies have focussed on identifying receptive molecular
126 patterns through histological and transcriptomic analyses of endometrial tissue (9, 10). Despite these advances,
127 there remains a significant level of uncertainty about strategies to optimize day-to-day clinical practice and
128 increase success rates in FET. For example, there is no consensus on how to best prepare the endometrium for
129 embryo implantation in freeze-thaw treatment (2). Some authors advocate that in ovulatory women it is optimal
130 to transfer the embryo in the mid-luteal phase of a natural cycle without administering exogenous hormones
131 (NC-FET), whereas others support the artificial preparation of the endometrium with hormone replacement
132 therapy (HRT-FET). To date, however, both approaches have been shown to be largely equivalent in
133 effectiveness and safety (11-14).

134 There is some evidence that circulating progesterone may be associated with treatment success in FET (15-17).
135 Progesterone is a steroid hormone produced by the corpus luteum, shortly after ovulation in a natural menstrual
136 cycle. It is through the regulatory effect of progesterone that the endometrium becomes secretory and receptive
137 to the implanting embryo, during a four-day interval in the luteal phase of the menstrual cycle termed “window
138 of implantation” (WOI) (18). Before or after this strictly timed interval, implanting embryos face an asynchronous
139 endometrium where the immune-endocrine milieu may impede rather than facilitate implantation (19). Serum
140 progesterone levels peak during the WOI, and have been used as a marker of endometrial receptivity in natural
141 conception and assisted reproductive technology (ART) treatment (20). During the follicular phase, high
142 circulating progesterone identifies premature luteinization and endometrial asynchrony (21-23). By contrast, in
143 the luteal phase, it has been suggested that single progesterone measurements lower than 10 ng/ml indicate
144 luteal phase deficiency (LPD), which in turn has been associated with infertility and recurrent pregnancy loss
145 (24). Further, fresh IVF cycles have long been known to lack endogenous progesterone support in the luteal

146 phase, mainly due to the inhibition of luteinizing hormone (LH) secretion. The administration of exogenous
147 hormones such as progesterone remains therefore critical to achieve treatment success in IVF (25).

148 The hypothesis that serum progesterone levels may be used as a predictor of FET success stems from data linking
149 higher circulating progesterone with increased live birth rates and reduced risk of miscarriage in freeze-thaw
150 cycles (26-28). Some researchers also postulate that women with low serum progesterone may benefit from
151 additional progesterone supplementation to achieve optimal luteal phase support (LPS), although good-quality
152 interventional evidence remains scarce (29, 30). Existing data are conflicting, however, with other studies
153 suggesting that excessive progesterone may be equally detrimental to treatment outcomes as low progesterone
154 (15, 31, 32). It remains therefore unclear whether serum progesterone levels may constitute a reliable predictor
155 of FET outcomes, and whether additional progesterone supplementation in women with low circulating levels
156 may lead to luteal rescue and improved treatment success.

157 The aim of this systematic review and meta-analysis was to summarize and appraise published data on the
158 association between circulating progesterone in the luteal phase and treatment outcomes in FET cycles.

159

160

161

162

163

164

165

166

167

168

169

170

171

172

173

174

175 Methods

176 Registration

177 This review was registered with PROSPERO (CRD42019157071) before commencement.

178 Search strategy

179 We performed extensive bibliographic searches according to the Preferred Reporting Items for Systematic
180 Reviews and Meta-Analysis (PRISMA) guidelines (33). The following computerized databases were searched
181 from their inception until March 15, 2021: MEDLINE, PubMed, CINAHL, EMBASE, the Cochrane Database of
182 Systematic Reviews, the Cochrane Central Register of Controlled Trials (CENTRAL), Web of Science,
183 ClinicalTrials.gov and grey literature (not widely available). The key MeSH/Emtree expressions and search terms
184 (and respective word variants) used to identify studies investigating serum progesterone levels in frozen embryo
185 transfer cycles were the following: progesterone AND (in vitro fertilization OR intracytoplasmic sperm injection,
186 frozen embryo, embryo transfer, assisted reproduction techniques). The search strategy was reviewed by two
187 medical sciences librarians. Additional studies were identified from the reference lists of selected abstracts and
188 manuscripts to avoid missing relevant data. We applied no language restrictions to any of our searches.

189 Study selection and quality assessment

190 We selected studies in a two-step process using Covidence (34). Two independent reviewers (P.M. and Y.C., or
191 P.M. and O.P.) carried out the study selection, first by assessing titles and then by screening the abstracts of
192 selected citations. The full manuscripts of studies that were deemed likely to meet our eligibility criteria were
193 obtained. Any disagreements about inclusion were resolved by consensus or arbitration by a third author (A.C.).

194 We included observational studies investigating the relationship between serum progesterone levels around the
195 time of FET and treatment outcomes. In natural FET cycles, we included publications where serum progesterone
196 was measured at any time in the luteal phase of participants' menstrual cycles. In HRT-FET cycles, due to
197 variation in existing HRT regimens for endometrial preparation and differences in study protocols, we included
198 manuscripts where serum progesterone levels were measured at any time from the onset of exogenous
199 progesterone supplementation (by any route available) to at least the first pregnancy test following embryo
200 transfer.

201 We excluded studies performed solely in fresh cycles, or where serum progesterone measurement was
202 undertaken as part of a trial (for example, comparing different routes of progesterone administration) but not
203 directly correlated with treatment outcomes. We also excluded interventional studies where women with low
204 serum progesterone around the time of FET received additional LPS, either by increasing the progesterone
205 dosage or by adding a progestogen with a different route of administration.

206 All articles meeting the selection criteria underwent quality assessment using the Newcastle-Ottawa scale for
207 quality assessment of observational studies by two independent reviewers (P.M. and Y.C.), as recommended by
208 the Cochrane Collaboration (35, 36).

209 Study outcomes

210 The primary outcome of interest was the composite rate of ongoing pregnancy or live birth (OPR or LBR) per
211 cycle. Ongoing pregnancy was defined as a viable intrauterine pregnancy of at least 12 weeks' gestation
212 confirmed on ultrasound, while live birth was defined as the delivery of a live fetus after 22 completed weeks of
213 gestational age (37). Secondary outcomes included the clinical pregnancy rate (CPR) per cycle, defined as the
214 presence of one or more gestational sacs on ultrasound; the miscarriage rate (MR) per pregnancy, defined as
215 the spontaneous loss of an intrauterine pregnancy before 22 completed weeks of gestation (37); and the rate
216 of adverse events attributable to exogenous progesterone supplementation (e.g. abdominal pain, bloating,
217 nausea, depression and headache).

218 Data extraction process

219 Two authors (P.M. and Y.C.) independently extracted data from the eligible studies, including publication date,
220 country of origin, study objective, participants (number and demographic characteristics), methods and
221 statistical analysis, study findings and study conclusions. Disagreements were resolved by consensus or
222 arbitration by a third reviewer (A.C.).

223 Data synthesis and analysis

224 All data analyses were performed using Stata Statistical Software (Release 16, TX, USA). We described effect
225 sizes using relative risk (RR) and 95% confidence intervals (CI). We assessed statistical heterogeneity by measure
226 of the I^2 statistic and set the acceptability of heterogeneity at $I^2 < 50\%$, in line with the recommendations from
227 the Cochrane handbook (36). We performed meta-analyses of dichotomous outcomes using a random effects
228 model.

229 Where studies reported progesterone concentrations in nmol/l, we converted them to ng/ml (conversion factor
230 3.18), as this is how serum progesterone was expressed in most of the included papers. For the purposes of
231 meta-analysis, we grouped studies according to the serum progesterone thresholds they reported (<10 ng/ml,
232 10-20 ng/ml, >20-30 ng/ml and >30 ng/ml). We dichotomized outcome data according to whether they
233 pertained to "high" or "low" serum progesterone as defined by each study (i.e., respectively above or below the
234 threshold specified for serum progesterone in each individual study).

235 Where authors reported adjusted effect estimates to evaluate the impact of confounders on their crude analyses
236 (e.g. adjusted odds ratios [aOR]), we conducted meta-analyses to investigate the pooled adjusted effect size of
237 "low" serum progesterone versus "high" serum progesterone according to the specified cut-off.

238 Subgroup analyses and sensitivity analyses

239 The primary analysis included studies where only blastocysts were used in FET treatment. In addition, we
240 planned to analyze data according to the route of progesterone administration where a sufficient number of
241 studies were available (intramuscular [IM], oral [PO], rectal [PR], subcutaneous [SC], or vaginal [PV]). We
242 performed sensitivity analyses for all outcomes of interest (OPR or LBR, CPR and MR) to determine whether our

243 conclusions were robust to arbitrary decisions regarding eligibility and analysis. The sensitivity analyses aimed
244 to assess whether our conclusions would have differed if (i) eligibility for the meta-analyses had been restricted
245 to studies at low risk of bias and (ii) all studies had been included in the meta-analysis, regardless of cycle type
246 (NC-FET and HRT-FET), progesterone route (IM, PO, PV, PR and PV), stage of embryo development (cleavage and
247 blastocyst) or risk of bias.

248 Results

249 Search results

250 The PRISMA Flow Diagram (Supplemental Fig. 1) shows details of the study selection process. The systematic
251 search on March 15, 2021 identified 4974 articles which were imported into Covidence. Following the removal
252 of duplicates, 3639 titles and abstracts were screened, of which 3586 were excluded as they were clearly not
253 relevant. One study was ongoing (38). The remaining fifty-two articles underwent full-text screening, and thirty-
254 one were excluded for the following reasons: seven studies were interventional; seven did not correlate
255 progesterone levels with treatment outcomes; four were review or commentary papers; two measured serum
256 progesterone before the luteal phase (Supplemental Table 1); and eleven studies are awaiting classification due
257 to unanswered or unsuccessful correspondence with the authors (Supplemental Table 2). We included twenty-
258 one studies in the narrative synthesis, sixteen of which were suitable for meta-analysis.

259 We contacted the authors of thirty-two manuscripts to obtain additional study details and data (Supplemental
260 Table 2). To date, we have received responses for ten publications (15, 26-28, 39-44).

261 Included studies

262 Study characteristics

263 Table 1 contains a detailed description of the twenty-one included studies. All included manuscripts were cohort
264 studies, of which eight were prospective (26, 28, 43, 45-49) and thirteen were retrospective (15, 17, 27, 31, 32,
265 40-42, 44, 50-53). Twenty studies have been published as full articles (15, 17, 26-28, 31, 32, 40-45, 47-53) and
266 one as a conference abstract (46). Five of the included studies were conducted in Spain (26-28, 50, 52); three in
267 Japan (32, 44, 47); two each in Australia (15, 51), Denmark (17, 45), France (40, 41) and Turkey (42, 43); and one
268 each in China (53), India (46), Iran (49), Iraq (48) and the USA (31). Eighteen studies focused solely on HRT-FET
269 cycles; two included HRT-FET and fresh donor recipient cycles (26, 28); and one study included only NC-FET
270 cycles (52). Finally, although the largest publication featured results from both HRT-FET and NC-FET cycles, only
271 the HRT-FET data were suitable for meta-analysis (51).

272 In aggregate, the quantitative synthesis included 6175 FET cycles, of which 5881 were HRT-FET and 294 were
273 NC-FET. Participants were aged between 18 and 50 years, and the body mass index (BMI) of included women
274 ranged from 18 to 37 kg/m². Furthermore, eleven studies analyzed autologous cycles only (15, 17, 27, 31, 41,
275 42, 45, 49, 50, 52, 53), while one publication focused exclusively on donor cycles (26) and two included both
276 autologous and donor cycles (28, 51). Oocyte source was not specified in the remainder of included manuscripts.

277 Most of the included studies (n = 15) analyzed cycles including only day 5/6 blastocysts; four studies included
278 both cleavage-stage and blastocyst embryos (32, 40, 47, 51); and two publications analyzed exclusively cleavage-
279 stage embryos (48, 53). Three of the included studies analyzed FET cycles using euploid embryos only (31, 43,
280 50), while in the remaining studies the ploidy status of the embryos was not specified.

281 Serum progesterone measurement

282 The timing of serum progesterone measurement varied across all included studies. Ten studies measured
283 progesterone on the day of FET (26, 28, 31, 32, 42, 43, 46-49), three measured serum progesterone on the day
284 before FET (27, 50, 52), and one study one or two days before transfer (41). The remaining studies tested
285 progesterone levels later on in the luteal phase: one study two to three days after FET (15); one sixteen days
286 after the onset of progesterone supplementation (51); three studies measured progesterone on the day of
287 pregnancy test (17, 45, 53); and in one study progesterone was tested on the day of pregnancy test, and then
288 again 46h and 96h later (40). Finally, one study measured serum progesterone every five days from the day after
289 FET until 9w 1d gestation (44).

290 Twelve studies reported on the time interval between the last dose of progesterone and venepuncture in HRT-
291 FET cycles (15, 17, 27, 28, 31, 42, 45, 47-50, 54). Of these, ten studies were included in the quantitative synthesis,
292 with dose-to-measurement intervals ranging between one and twelve hours (15, 17, 26, 28, 31, 42, 45, 47, 49,
293 50).

294 There was also variation in the progesterone cut-off levels reported, ranging between 5 ng/ml (32) and 53.2
295 ng/ml (43). The most common threshold was 8.8 ng/ml, used in two studies (28, 45). Eight studies reported on
296 treatment outcomes according to serum progesterone percentile analyses (42, 43, 45, 47, 49-51, 53); three
297 studies reported on progesterone thresholds using a “high” versus “low” dichotomy (26, 28, 51); three
298 publications reported thresholds according to previously published literature or what the authors perceived to
299 be accepted standard practice (17, 31, 52); one study reported outcomes according to a receiver operating
300 characteristic curve (ROC) analysis of serum progesterone levels (46); and in two manuscripts no rationale was
301 given for the reported progesterone thresholds (15, 32). Finally, two publications identified a linear positive
302 correlation between serum progesterone and live birth, without a significant cut-off point associated with
303 treatment success (27, 44).

304 Ten of the included studies reported on intra- and inter-assay coefficients of variation associated with serum
305 progesterone measurements, ranging between 1.2% and 23.1% (15, 17, 26, 28, 43, 45, 49, 55-57). In the
306 remaining studies this was not stated.

307 Outcomes

308 Twelve publications reported on live birth (15, 27, 28, 31, 44, 46, 47, 49-53). However, the gestational age
309 threshold to define this outcome varied between >20 weeks in two studies (15, 51), >22 weeks in another two
310 manuscripts (44, 52), and >28 weeks in the study by Liu and Wu (53), while the remaining six publications did
311 not specify a gestational age for live birth (27, 28, 31, 46, 47, 49, 50).

312 Eleven studies reported on ongoing pregnancy, defined by most as the presence of fetal heart activity at ≥ 12
313 weeks' gestation (17, 26, 28, 40-42, 45, 46, 48), with one study using the threshold of 16 weeks (43) and another
314 leaving the threshold unspecified (31).

315 Seventeen studies reported on clinical pregnancy, defined as the presence of a gestational sac on ultrasound in
316 ten studies (15, 26, 28, 32, 42, 43, 47, 51-53), while the remaining seven publications defined clinical pregnancy
317 as the presence of an intra-uterine gestational sac with visible fetal heart activity (17, 31, 40, 41, 45, 48, 49).

318 Finally, seventeen studies reported on miscarriage, of which eleven used thresholds between 6-12 weeks'
319 gestation (17, 26, 28, 31, 40, 42-45, 48, 51); one used a cut-off of 20 weeks (49); another study used a threshold
320 of 22 weeks (52); and four studies did not specify gestational ages for miscarriage (41, 46, 47, 50).

321 No studies reported on adverse events attributable to progesterone.

322 Quantitative synthesis of serum progesterone effects

323 We performed meta-analyses of studies reporting on OPR or LBR and CPR per cycle, and MR per pregnancy. We
324 grouped study results according to different threshold categories used by authors to present outcome data.
325 Within each threshold category (<10 ng/ml, 10-20 ng/ml, >20-30 ng/ml and >30 ng/ml), outcome data were
326 presented for women with "high" (i.e., above that particular cut-off) and "low" (i.e., below the cut-off)
327 progesterone. While it was possible to conduct meta-analyses of studies using PV-only progesterone and
328 blastocyst embryos, there were an insufficient number of studies to perform meta-analyses for cleavage-stage
329 embryos and for the IM, PO, PR and SC routes of progesterone administration.

330 Progesterone threshold category <10 ng/ml

331 Among studies using solely PV progesterone and blastocyst embryos, meta-analysis of outcome data for cut-off
332 values lower than 10 ng/ml showed that serum progesterone higher than the specified threshold was associated
333 with increased OPR or LBR (RR 1.47, 95% CI 1.28 to 1.70; five studies; n = 1990; $I^2 = 0\%$) (Fig. 1), increased CPR
334 (RR 1.31, 95% CI 1.16 to 1.49; three studies; n = 1603; $I^2 = 0\%$) (Fig. 2) and reduced MR (RR 0.62, 95% CI 0.50 to
335 0.77; five studies; n = 1990; $I^2 = 0\%$) (Fig. 3). The aforementioned sensitivity analyses did not change the
336 conclusions (Supplemental Fig. 2, Supplemental Fig. 3 and Supplemental Fig. 4).

337 Pooled aOR for studies using PV-only progesterone showed that lower serum progesterone was associated with
338 a reduction in OPR or LBR (aOR 0.48, 95% CI 0.35 to 0.62; two studies; n = 1074; $I^2 = 30.9\%$) (Supplemental Fig.
339 5). Meta-analysis of adjusted effect estimates was not possible for CPR and MR within this threshold category
340 due to insufficient data.

341 Progesterone threshold category 10-20 ng/ml

342 For studies using PV-only progesterone and blastocyst embryos where serum progesterone cut-off values
343 ranged between 10 and 20 ng/ml, the primary analysis showed that higher progesterone was associated with
344 increased OPR or LBR (RR 1.27, 95% CI 1.09 to 1.48; three studies; n = 916; $I^2 = 0\%$) (Fig. 1) and improved CPR
345 (RR 1.28, 95% CI 1.10 to 1.50; two studies; n = 773; $I^2 = 0\%$). However, sensitivity analyses including all studies,
346 regardless of progesterone route, type of FET or risk of bias, showed uncertainty about whether higher
347 progesterone was associated with increased OPR or LBR (RR 1.19, 95% CI 0.98 to 1.45; eleven studies; n = 4436;

348 $I^2 = 77.6\%$) (Supplemental Fig. 2) and higher CPR (RR 1.21, 95% CI 0.99 to 1.47; eleven studies; $n = 4252$; $I^2 =$
349 77.5%) (Supplemental Fig. 3).

350 The evidence was also uncertain about whether women with progesterone levels above the specified threshold
351 within this category experienced fewer miscarriages than those with lower progesterone (RR 0.76, 95% CI 0.47
352 to 1.22; four studies; $n = 1160$; $I^2 = 60.2\%$) (Fig. 3). Sensitivity analyses for MR did not change the conclusions
353 (Supplemental Fig. 4).

354 Pooled aOR for studies using PV-only progesterone showed that lower serum progesterone was associated with
355 a reduction in OPR or LBR (aOR 0.37, 95% CI 0.27 to 0.47; three studies; $n = 2068$; $I^2 = 43.6\%$) (Supplemental Fig.
356 5) and CPR (aOR 0.45, 95% CI 0.32 to 0.57; two studies; $n = 1824$; $I^2 = 0\%$) (Supplemental Fig. 6), while there was
357 uncertainty about whether lower progesterone was associated with increased risk of miscarriage (aOR 1.64, 95%
358 CI 0.73 to 2.56; three studies; $n = 2068$; $I^2 = 36.9\%$) (Supplemental Fig. 7).

359 Progesterone threshold category >20-30 ng/ml

360 When pooling data from studies using exclusively PV progesterone and blastocysts where results were
361 presented for progesterone thresholds between >20 and 30 ng/ml, serum progesterone measurements did not
362 confidently correlate with OPR or LBR (RR 1.27, 95% CI 0.92 to 1.76; two studies; $n = 672$; $I^2 = 55.9\%$) (Fig. 1) and
363 MR (RR 1.05, 95% CI 0.70 to 1.57; two studies; $n = 672$; $I^2 = 0\%$) (Fig. 3). Only one study in the PV progesterone
364 group reported within this threshold for CPR, and its findings were uncertain (RR 1.15, 95% CI 0.97 to 1.36; $n =$
365 529). Sensitivity analyses did not materially change the conclusions (Supplemental Fig. 2, Supplemental Fig. 3
366 and Supplemental Fig. 4).

367 Meta-analysis of adjusted effect estimates was not possible for OPR or LBR, CPR and MR within this threshold
368 category due to insufficient data.

369 Progesterone threshold category >30 ng/ml

370 For progesterone thresholds above 30 ng/ml, only one study using blastocysts and the PV route of
371 administration reported on OPR or LBR, CPR and MR, with uncertain findings for all three outcomes (Fig. 1, Fig.
372 2 and Fig. 3). The aforementioned sensitivity analyses did not change the effect size of progesterone levels within
373 this category (Supplemental Fig. 2, Supplemental Fig. 3 and Supplemental Fig. 4).

374 Meta-analysis of adjusted effect estimates was not possible for OPR or LBR, CPR and MR within this threshold
375 category due to insufficient data.

376 Additional analyses

377 There were an insufficient number of studies administering IM, PO, PR or SC progesterone for additional
378 analyses according to administration route. Furthermore, only three studies analyzed cycles using exclusively
379 euploid embryos, thus precluding sensitivity analyses according to embryo ploidy status.

380 Risk of bias across studies

381 Most of the included studies were deemed to be at a low risk of bias (15, 17, 26-28, 31, 40, 42, 43, 45-49, 51-
382 53), while four publications were judged to be at a moderate risk of bias (32, 41, 44, 50) due to a lack of data on

383 whether women within different serum progesterone ranges were comparable in baseline demographic
384 characteristics such as age and BMI (Supplemental Table 3).

385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423

424 Discussion

425 Summary of evidence

426 In this systematic review and meta-analysis of twenty-one cohort studies, we aimed to investigate how serum
427 progesterone around the time of FET correlates with treatment outcomes. The primary analysis showed that for
428 thresholds below 10 ng/ml, women with higher progesterone levels experienced more ongoing pregnancies or
429 live births, more clinical pregnancies, and fewer miscarriages than those with serum progesterone lower than
430 the specified cut-off. This was corroborated by our sensitivity analyses. For progesterone thresholds equal or
431 above 10 ng/ml, however, there was uncertainty in whether higher progesterone was associated with better
432 treatment outcomes, owing to significant inter-study heterogeneity, a paucity of prospective data and wide
433 confidence intervals around pooled effect estimates.

434 Among the included studies, progesterone was administered via different routes and in varying doses. To date,
435 there has been no consensus on the best form of LPS in frozen embryo cycles, due to a lack of robustly designed
436 randomized trials. In a seminal study by Miles et al. (58) including twenty ovulatory women, the authors
437 compared the pharmacokinetic profile of micronized progesterone administered vaginally versus
438 intramuscularly. Although serum concentrations of progesterone were more than twice as high following IM
439 administration, women assigned to the vaginal route had a mean endometrial progesterone concentration
440 nearly eight times higher than the IM group. Importantly, however, the study participants did not have a
441 diagnosis of infertility, and reproductive outcomes were not assessed. In a subsequent randomized trial,
442 Lightman et al. (59) compared micronized progesterone PV 200 mg three times daily with IM progesterone 100
443 mg once daily in 354 women undergoing HRT-FET cycles. Serum progesterone on the day of pregnancy test was
444 more than double in the IM progesterone group, yet crucially did not result in higher clinical pregnancy rates.
445 These findings support the hypothesis that endometrial rather than serum progesterone is more important in
446 determining treatment success in FET cycles. This may additionally reflect the phenomenon of first uterine pass,
447 whereby a targeted delivery of vaginal progesterone to the uterus enhances the local endocrine milieu and
448 hence facilitates implantation and pregnancy success (60). Overall, however, studies comparing different routes
449 and regimens of LPS in FET have failed to categorically identify a formulation with superior results (42, 47, 61-
450 65).

451 Despite the lack of evidence attesting to superior efficacy, the use of micronized vaginal progesterone for LPS in
452 ART has become widespread in recent decades. There were originally concerns with reduced bioavailability of
453 natural progesterone due to its large molecular size, but in its micronized form progesterone has since been
454 shown to be easily absorbed by the vaginal and intestinal mucosa (66, 67). Furthermore, gel-based formulations
455 containing micronized progesterone exhibit a sustained-release mechanism which leads to steady levels of
456 absorption over time and prevents excessive variation in serum progesterone levels (63, 64). The vaginal
457 formulation is most commonly used in ART treatment across the world, and the preferred route of
458 administration for up to one in four ART practitioners (68). In this review, most studies used PV progesterone,
459 IM progesterone, or a combination of both routes for LPS. While it is difficult to link the route of administration

460 with progesterone measurements across different studies, the analysis by Polat et al. (42) identified higher
461 progesterone levels in the group receiving PV plus IM progesterone in comparison to those assigned to PV
462 progesterone only. However, there was no evidence that treatment outcomes differed between the two
463 cohorts. In addition to PV-only progesterone, we aimed to conduct meta-analyses evaluating other routes of
464 administration. Although the number of studies using IM, PO, PR and SC progesterone were insufficient to
465 perform grouped analyses, we carried out sensitivity analyses including all studies, regardless of route of
466 administration and stage of embryo development. The primary analysis of studies using PV-only progesterone
467 showed that higher circulating levels were associated with increased OPR or LBR for the threshold categories of
468 <10 ng/ml and 10-20 ng/ml, yet our sensitivity analyses including all studies showed that this was no longer the
469 case for progesterone thresholds ranging between 10 and 20 ng/ml. These data strengthen the argument that
470 although systemic routes of administration such as SC or IM may result in higher circulating progesterone, this
471 does not necessarily reflect the amount that actually reaches the endometrium, which may be lower, thus
472 explaining the dilution in effect size observed in the sensitivity analysis for the outcome of OPR or LBR.

473 There was variation across the studies in the timing of progesterone measurement, although those assessing
474 blastocyst transfers undertook venepuncture at least 4 days after the onset of LPS. The pharmacokinetics of
475 different progesterone formulations have been the subject of much research. For example, there have been
476 concerns regarding low bioavailability of progesterone when administered orally due to the effect of food on
477 intestinal absorption and the phenomenon of first hepatic pass leading to the rapid metabolization and excretion
478 of progesterone metabolites (69). In the past decade, however, dydrogesterone, a synthetic progestogen, has
479 been increasingly utilized in ART due to its improved bioavailability, safety and convenience of use (70). When
480 progesterone is administered vaginally or intramuscularly, a steady state in serum concentration is usually
481 achieved within 24-48 hours (66, 71, 72). In the case of subcutaneous progesterone, time to steady state
482 concentrations ranges between 48 to 72 hours (73). Therefore, in this review, it is reasonable to postulate that
483 serum progesterone measurements in all studies evaluating HRT-FET provided a reliable estimate of steady state
484 concentrations, and were thus reflective of circulating progesterone at the time of embryo transfer. In addition,
485 existing evidence also suggests that serum progesterone levels remain above 10 ng/ml for over eighteen hours
486 following vaginal administration (27). Our primary analysis included only studies whose dose-to-measurement
487 interval ranged between one and six hours, and are therefore likely to accurately reflect the correlation between
488 serum progesterone and FET treatment outcomes in this cohort of patients.

489 Our results confidently identified that for cut-offs lower than 10 ng/ml, higher progesterone levels were on
490 average associated with better treatment outcomes. However, as the threshold values increased, there was an
491 overall shift towards the line of no effect. For the highest cut-off levels (>30 ng/ml), it was in fact unclear whether
492 progesterone might even be detrimental to ongoing pregnancy and live birth rates (Fig. 1, Supplemental Fig. 2).
493 It is therefore possible that a minimally important level of serum progesterone is required to ensure sufficient
494 binding to endometrial progesterone receptors and facilitate their secretory activity. Above this minimum
495 threshold, however, the data suggest that treatment outcomes may not be optimized when higher circulating
496 levels of progesterone are identified. While mechanistic studies investigating the pharmacodynamics of

497 progesterone receptors in the uterus of women undergoing ART are scarce, existing evidence from humans and
498 other mammals indicates that endometrial progesterone receptors undergo saturation at or below physiological
499 concentrations of endogenous progesterone (74, 75). Furthermore, adding progesterone to endometrial
500 samples has shown that specific binding was saturable after a threshold of approximately 3.14 ng/ml (74, 76),
501 suggesting that beyond that level further progesterone may not enhance the secretory activity of the
502 endometrium.

503 While it may be difficult to extrapolate in vitro data to clinical practice, a recent study by Deng et al. (77) has
504 added strength to the hypothesis of progesterone receptor saturation. The authors demonstrated that in 726
505 women with threatened miscarriage in the first trimester of naturally conceived pregnancies, the risk of
506 pregnancy loss decreased incrementally as serum progesterone levels rose, but only up to a concentration of
507 28.5 ng/ml. Above this value, the risk of miscarriage was no longer associated with circulating progesterone
508 levels (77). Although progesterone in its natural and micronized forms has a proven safety record in pregnancy
509 (78, 79), studies in mammals have indicated that excessive progesterone may impair implantation and
510 decidualization (80). There is hence a need for additional data investigating the effect of higher levels of
511 circulating progesterone upon FET outcomes.

512 Crucially, it remains uncertain whether providing additional LPS to women with low serum progesterone
513 improves outcomes in FET. Cédric-Durnerin et al. (81) conducted a retrospective analysis of 227 FET cycles
514 where women received a total daily dose of 600 mg of PV micronized progesterone for endometrial preparation.
515 Although the dose of progesterone was increased to 800 mg daily in participants whose serum progesterone
516 was lower than 10 ng/ml on the day of FET, these women still experienced fewer live births than those with
517 progesterone ≥ 10 ng/ml. In a subsequent retrospective study, Volovsky et al. (82) analyzed 2010 FET cycles
518 where women whose serum progesterone on the day of transfer was < 8.0 ng/ml received additional
519 supplementation. In contrast to Cédric-Durnerin et al. (81), Volovsky et al. (82) observed no differences in CPR
520 and LBR between women with serum progesterone < 10 ng/ml and those where progesterone was ≥ 10 ng/ml,
521 suggesting that additional LPS may have led to luteal rescue. More recently, Álvarez et al. (30) published a non-
522 randomized prospective study where women with serum progesterone lower than 10.6 ng/ml on the day before
523 FET received additional SC progesterone supplementation and experienced similar outcomes to participants
524 whose serum progesterone was > 10.6 ng/ml. Importantly, this study was restricted to euploid-only cycles, thus
525 eliminating the contribution of aneuploidy to miscarriage rates in this cohort. High-quality randomized
526 interventional data are needed to further inform the above findings by ascertaining whether women with low
527 progesterone (< 10 ng/ml) who receive additional LPS (for example, by adding a second route of administration)
528 experience non-inferior outcomes to those with higher progesterone levels.

529 Most studies included in this review stated that LPS was continued at least until the day of pregnancy test, with
530 the exception of four publications where the duration of LPS was not stated (17, 43, 46, 50), and one study
531 where women underwent NC-FET without LPS (27). In natural conception, the corpus luteum is the main source
532 of progesterone from ovulation until approximately 7-8 weeks of gestation, when the luteoplacental shift occurs
533 and endogenous steroid synthesis continues predominantly in the placenta (83). Due to the LPD phenomenon

534 in fresh IVF cycles, it has been recommended that exogenous LPS should be undertaken until at least the day of
535 pregnancy test (84), although an international survey of over a thousand clinicians has shown that worldwide
536 most practitioners continue LPS until twelve weeks (68). However, a recent systematic review identified no
537 difference in live birth, ongoing pregnancy or miscarriage rates between women who ceased progesterone early
538 (weeks four to seven) and those who discontinued it later on in the first trimester (up to twelve weeks) (85).
539 Fresh cycle data are nonetheless difficult to extrapolate to HRT-FET treatment, because the lack of a corpus
540 luteum in the latter results in diminished levels of endogenous progesterone. It is possible that this may affect
541 implantation and impact upon LPS requirements in FET (86). Furthermore, it has recently been suggested that
542 vasoactive molecules produced by the corpus luteum, such as relaxin and vascular endothelial growth factor
543 (VEGF), play a key role in facilitating initial placentation, and that their absence may contribute to higher rates
544 of hypertensive disorders observed in pregnancies resulting from HRT-FET treatment (87, 88).

545 [Strengths and limitations](#)

546 The findings of this systematic review are strengthened by the large number of studies analyzing progesterone
547 levels within different threshold categories, which allowed for meta-analyses for all outcomes of interest. We
548 conducted our primary analyses according to progesterone administration route and embryo development
549 stage, therefore significantly reducing clinical heterogeneity. There were an insufficient number of studies
550 evaluating euploid-only FET cycles, however, and we were therefore unable to conduct sensitivity analyses
551 adjusting for ploidy status, a crucial determinant of pregnancy success. There is a need for additional prospective
552 data evaluating how serum progesterone correlates with FET outcomes in the absence of embryonic aneuploidy.

553 In addition to the retrospective nature of most of the included studies, there was a significant degree of variation
554 in vaginal progesterone formulations, doses, timing of administration and serum levels found to be associated
555 with treatment outcomes. Due to the wide range of serum progesterone measurements reported in relation to
556 pregnancy outcomes, we opted to group the various cut-offs by regular 10 ng/ml intervals. This classification,
557 while arbitrary, allowed for meta-analyses of studies reporting on similar ranges of serum progesterone. For the
558 primary analyses, most of the cut-offs in each threshold category were in fact within 5 ng/ml intervals, hence
559 reducing heterogeneity.

560 There were multiple coefficients of variation reported for different study assays in this review, ranging between
561 1.2% and 23.1%. This may have affected the precision and reproducibility of the included manuscripts, and
562 crucially it may have skewed the cut-off levels reported as significant by different studies, thus potentially
563 impacting on the real-life applicability of our findings. Further, it was not possible to conduct separate meta-
564 analyses evaluating non-PV administration routes (IM, PO, PR and SC), or for natural cycle FET, although these
565 were included in the sensitivity analyses which corroborated our primary findings for serum progesterone levels
566 <10 ng/ml. For serum levels ≥ 10 ng/ml, however, the sensitivity analyses featured high statistical and clinical
567 heterogeneity, thus precluding generalizable recommendations.

568 Finally, some of the included studies did not find a linear dichotomy whereby “high” and “low” progesterone
569 levels inversely correlated with treatment outcomes, and reported instead on serum measurement ranges that
570 were associated with improved treatment success. This was further compounded by the use of mid-range

571 progesterone levels as the reference group where studies calculated adjusted effect estimates, therefore
572 preventing their inclusion in the supplemental adjusted analyses evaluating “high” versus “low” progesterone.
573 We conducted exploratory analyses by comparing “high-” versus “mid-” versus “low-level” progesterone for
574 publications that analyzed interval data, although the high degree of variation in mid-level progesterone
575 measurements between studies represented a significant challenge and precluded informative conclusions.
576 Future cohort studies should focus on a percentile evaluation that goes beyond the dichotomous approach.
577 Furthermore, a collaborative effort between study authors to undertake an individual participant data meta-
578 analysis may help clarify the trends of effect of serum progesterone upon FET outcomes.

579 Acknowledgements

580 We thank Lynne Harris and James Barnett for their help in performing the literature searches. We would also
581 like to acknowledge Cristina Rodriguez for her assistance in providing additional data for two studies by Labarta
582 et al. (26, 28); and Kevin Keane for his help with providing additional data for the study by Yovich et al. (15).
583 Finally, we wish to thank the study authors who contributed to this review by supplying additional information
584 about their research, including Kubra Boynukalin, Marine Commissaire, Iñaki Gonzalez-Foruria, Satoshi
585 Kawachiya, Maëliiss Peigné, Paul Pirtea, Mehtap Polat, Olivier Pouget and John Yovich.

586 Authors’ roles

587 P.M. and A.C. designed the study and wrote the protocol. P.M., Y.C. and O.P. performed the searches, abstract
588 screening and full-text selection. P.M. and Y.C. undertook the data extraction, synthesis and quality assessment.
589 P.M. and M.J.P. carried out the data analyses. P.M., A.C., I.G. and M.J.P. wrote the final draft of the manuscript.
590 Y.C., O.P., S.F., M.K., C.K., P.L., G.P., M.R., V.S., A.T., S.W., E.L., M.W. and A.D. provided critical input into the final
591 manuscript. All authors approved the final version and accept responsibility for the paper as published.

592 Funding

593 This work has been supported by a doctoral research fellowship awarded to P.M. by the Tommy’s Charity and
594 the University of Birmingham. M.J.P. is supported by the NIHR Birmingham Biomedical Research Centre.

595

596 Conflicts of Interest

597 S.F. is a minor shareholder of CARE Fertility, but has no financial or other interest with progesterone testing or
598 manufacturing companies.

599 P.L. reports personal fees from Pharmasure, outside the submitted work.

600 G.P. reports personal fees from Besins Healthcare, outside the submitted work.

601 E.L. was the lead author in two of the manuscripts in this review (Labarta et al., 2017; and Labarta et al., 2021).
602 She has additionally received a grant from Ferring in 2020, has provided consultancy services for MSD and
603 Ferring Pharmaceuticals and is part of the Ferring Pharmaceuticals LIFE program and Merck Global program for
604 Fertility Innovation Leaders. During the past 12 months, E.L. has received honoraria from Angelini/IBSA, Gedeon
605 Richter and Ferring Pharmaceuticals for lecturing.

606 M.W. reports personal fees from Ferring Pharmaceuticals, outside the submitted work.

607 A.C. has conducted two RCTs evaluating the role of progesterone in miscarriage prevention (the PROMISE trial,
608 published in 2015 (89); and the PRISM trial, published in 2019 (79)). However, he has no financial or other
609 interest with progesterone testing or manufacturing companies.

610 The views expressed are those of the authors and not necessarily those of the NHS, NIHR or the Department of
611 Health and Social Care.

612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649

650

651 References

- 652 1. Wong KM, van Wely M, Mol F, Repping S, Mastenbroek S. Fresh versus frozen embryo transfers in
653 assisted reproduction. *Cochrane Database Syst Rev* 2017;3:Cd011184.
- 654 2. Mackens S, Santos-Ribeiro S, van de Vijver A, Racca A, Van Landuyt L, Tournaye H et al. Frozen
655 embryo transfer: a review on the optimal endometrial preparation and timing. *Hum Reprod* 2017;32:2234-42.
- 656 3. Roque M, Valle M, Guimarães F, Sampaio M, Geber S. Freeze-all policy: fresh vs. frozen-thawed
657 embryo transfer. *Fertil Steril* 2015;103:1190-3.
- 658 4. Human Fertilisation and Embryology Authority (HFEA). Fertility treatment 2018: trends and figures.
659 2020.
- 660 5. European Society of Human Reproduction and Embryology. ART fact sheet. In, 2020.
- 661 6. Centers for Disease Control and Prevention, Society for Assisted Reproductive Technology. 2016
662 Assisted Reproductive Technology National Summary Report. Atlanta (GA): US Dept of Health and Human
663 Services, 2018.
- 664 7. Kushnir VA, Barad DH, Albertini DF, Darmon SK, Gleicher N. Systematic review of worldwide trends in
665 assisted reproductive technology 2004-2013. *Reprod Biol Endocrinol* 2017;15:6.
- 666 8. Casper RF. Frozen embryo transfer: evidence-based markers for successful endometrial preparation.
667 *Fertil Steril* 2020;113:248-51.
- 668 9. Díaz-Gimeno P, Horcajadas JA, Martínez-Conejero JA, Esteban FJ, Alamá P, Pellicer A et al. A genomic
669 diagnostic tool for human endometrial receptivity based on the transcriptomic signature. *Fertil Steril*
670 2011;95:50-60, .e1-15.
- 671 10. Díaz-Gimeno P, Ruiz-Alonso M, Blesa D, Bosch N, Martínez-Conejero JA, Alamá P et al. The accuracy
672 and reproducibility of the endometrial receptivity array is superior to histology as a diagnostic method for
673 endometrial receptivity. *Fertil Steril* 2013;99:508-17.
- 674 11. Groenewoud ER, Cohlen BJ, Al-Oraiby A, Brinkhuis EA, Broekmans FJ, de Bruin JP et al. A randomized
675 controlled, non-inferiority trial of modified natural versus artificial cycle for cryo-thawed embryo transfer. *Hum*
676 *Reprod* 2016;31:1483-92.
- 677 12. Mounce G, McVeigh E, Turner K, Child TJ. Randomized, controlled pilot trial of natural versus
678 hormone replacement therapy cycles in frozen embryo replacement in vitro fertilization. *Fertil Steril*
679 2015;104:915-20 e1.

- 680 13. Yarali H, Polat M, Mumusoglu S, Yarali I, Bozdog G. Preparation of endometrium for frozen embryo
681 replacement cycles: a systematic review and meta-analysis. *J Assist Reprod Genet* 2016;33:1287-304.
- 682 14. Casper RF, Yanushpolsky EH. Optimal endometrial preparation for frozen embryo transfer cycles:
683 window of implantation and progesterone support. *Fertil Steril* 2016;105:867-72.
- 684 15. Yovich JL, Conceicao JL, Stanger JD, Hinchliffe PM, Keane KN. Mid-luteal serum progesterone
685 concentrations govern implantation rates for cryopreserved embryo transfers conducted under hormone
686 replacement. *Reprod Biomed Online* 2015;31:180-91.
- 687 16. Labarta E, Rodríguez C. Progesterone use in assisted reproductive technology. *Best Pract Res Clin*
688 *Obstet Gynaecol* 2020;69:74-84.
- 689 17. Alsbjerg B, Thomsen L, Elbaek HO, Laursen R, Povlsen BB, Haahr T et al. Progesterone levels on
690 pregnancy test day after hormone replacement therapy-cryopreserved embryo transfer cycles and related
691 reproductive outcomes. *Reprod Biomed Online* 2018;37:641-7.
- 692 18. Lessey BA. Assessment of endometrial receptivity. *Fertil Steril* 2011;96:522-9.
- 693 19. Craciunas L, Gallos I, Chu J, Bourne T, Quenby S, Brosens JJ et al. Conventional and modern markers of
694 endometrial receptivity: a systematic review and meta-analysis. *Hum Reprod Update* 2019;25:202-23.
- 695 20. Harper MJ. The implantation window. *Baillieres Clin Obstet Gynaecol* 1992;6:351-71.
- 696 21. Lawrenz B, Melado L, Fatemi H. Premature progesterone rise in ART-cycles. *Reprod Biol* 2018;18:1-4.
- 697 22. Labarta E, Martínez-Conejero JA, Alamá P, Horcajadas JA, Pellicer A, Simón C et al. Endometrial
698 receptivity is affected in women with high circulating progesterone levels at the end of the follicular phase: a
699 functional genomics analysis. *Hum Reprod* 2011;26:1813-25.
- 700 23. Van Vaerenbergh I, Fatemi HM, Blockeel C, Van Lommel L, In't Veld P, Schuit F et al. Progesterone rise
701 on HCG day in GnRH antagonist/rFSH stimulated cycles affects endometrial gene expression. *Reprod Biomed*
702 *Online* 2011;22:263-71.
- 703 24. Jordan J, Craig K, Clifton DK, Soules MR. Luteal phase defect: the sensitivity and specificity of
704 diagnostic methods in common clinical use. *Fertil Steril* 1994;62:54-62.
- 705 25. van der Linden M, Buckingham K, Farquhar C, Kremer JA, Metwally M. Luteal phase support for
706 assisted reproduction cycles. *Cochrane Database Syst Rev* 2015;2015:Cd009154.

- 707 26. Labarta E, Mariani G, Holtmann N, Celada P, Remohí J, Bosch E. Low serum progesterone on the day
708 of embryo transfer is associated with a diminished ongoing pregnancy rate in oocyte donation cycles after
709 artificial endometrial preparation: a prospective study. *Hum Reprod* 2017;32:2437-42.
- 710 27. González-Foruria I, Gaggiotti-Marre S, Álvarez M, Martínez F, García S, Rodríguez I et al. Factors
711 associated with serum progesterone concentrations the day before cryopreserved embryo transfer in artificial
712 cycles. *Reprod Biomed Online* 2020;40:797-804.
- 713 28. Labarta E, Mariani G, Paoletti S, Rodriguez-Varela C, Vidal C, Giles J et al. Impact of low serum
714 progesterone levels on the day of embryo transfer on pregnancy outcome: a prospective cohort study in
715 artificial cycles with vaginal progesterone. *Hum Reprod* 2021;36:683-92.
- 716 29. Alsbjerg B, Labarta E, Humaidan P. Serum progesterone levels on day of embryo transfer in frozen
717 embryo transfer cycles-the truth lies in the detail. *J Assist Reprod Genet* 2020;37:2045-6.
- 718 30. Álvarez M, Gaggiotti-Marre S, Martínez F, Coll L, García S, González-Foruria I et al. Individualised luteal
719 phase support in artificially prepared frozen embryo transfer cycles based on serum progesterone levels: a
720 prospective cohort study. *Hum Reprod* 2021;36:1552-60.
- 721 31. Kofinas JD, Blakemore J, McCulloh DH, Grifo J. Serum progesterone levels greater than 20 ng/dl on
722 day of embryo transfer are associated with lower live birth and higher pregnancy loss rates. *J Assist Reprod*
723 *Genet* 2015;32:1395-9.
- 724 32. Akaeda S, Kobayashi D, Shioda K, Momoeda M. Relationship between serum progesterone
725 concentrations and pregnancy rates in hormone replacement treatment-frozen embryo transfer using
726 progesterone vaginal tablets. *Clin Exp Obstet Gynecol* 2019;46:695-8.
- 727 33. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M et al. Preferred reporting items for
728 systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev* 2015;4:1.
- 729 34. Covidence systematic review software VHI, Melbourne, Australia. Covidence systematic review
730 software, Veritas Health Innovation, Melbourne, Australia. 2020.
- 731 35. Wells G, Shea B, O'Connell J. The Newcastle-Ottawa Scale (NOS) for Assessing The Quality of
732 Nonrandomised Studies in Meta-analyses. Ottawa Health Research Institute Website 2014;7.
- 733 36. Higgins JPT TJ, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors) *Cochrane Handbook for*
734 *Systematic Reviews of Interventions* version 6.1. 2020.
- 735 37. Zegers-Hochschild F, Adamson GD, Dyer S, Racowsky C, de Mouzon J, Sokol R et al. The International
736 Glossary on Infertility and Fertility Care, 2017. *Fertil Steril* 2017;108:393-406.

- 737 38. NCT04278508. Serum Progesterone on the Day of Thawed Embryo Transfer and Pregnancy Rate After
738 an Artificial Endometrial Preparation 2020. Available on: <https://clinicaltrials.gov/ct2/show/NCT04278508>
- 739 39. Pouget O, Alsawaf M, Zuna I, Bonneau M, Tailland ML, Ripart S et al. Serum progesterone level on
740 frozen embryo transfer day is lower with hormonal therapy than with a natural cycle, a retrospective single
741 university centre study. *Hum Reprod* 2020;35.
- 742 40. Commissaire M, Epelboin S, Vigan M, Tubiana S, Llabador MA, Gauché-Cazalis C et al. Serum
743 progesterone level and ongoing pregnancy rate following frozen-thawed embryo transfer after artificial
744 endometrial preparation: a monocentric retrospective study. *J Gynecol Obstet Hum Reprod* 2020:101828.
- 745 41. Ramos NN, Pirtea P, Benammar A, Ziegler Dd, Jolly E, Frydman R et al. Is there a link between plasma
746 progesterone 1–2 days before frozen embryo transfers (FET) and ART outcomes in frozen blastocyst transfers?
747 *Gynecol Endocrinol* 2020:1-4.
- 748 42. Polat M, Mumusoglu S, Bozdog G, Ozbek IY, Humaidan P, Yarali H. Addition of intramuscular
749 progesterone to vaginal progesterone in hormone replacement therapy in vitrified–warmed blastocyst
750 transfer cycles. *Reprod Biomed Online* 2020;40:812-8.
- 751 43. Boynukalin FK, Gultomruk M, Turgut E, Demir B, Findikli N, Serdarogullari M et al. Measuring the
752 serum progesterone level on the day of transfer can be an additional tool to maximize ongoing pregnancies in
753 single euploid frozen blastocyst transfers. *Reprod Biol Endocrinol* 2019;17:102.
- 754 44. Kawachiya S, Bodri D, Hirosawa T, Yao Serna J, Kuwahara A, Irahara M. Endogenous progesterone
755 levels could predict reproductive outcome in frozen embryo replacement cycles supplemented with synthetic
756 progestogens: A retrospective cohort study. *Reprod Med Biol* 2019;18:91-6.
- 757 45. Alsbjerg B, Thomsen L, Elbaek HO, Laursen R, Povlsen BB, Haahr T et al. Can combining vaginal and
758 rectal progesterone achieve the optimum progesterone range required for implantation in the HRT-FET
759 model? *Reprod Biomed Online* 2020;40:805-11.
- 760 46. Kakkad VP, Reddy NS, Pandurangi M, Vembu R, Nagireddy S, Soni Ak et al. Serum progesterone level:
761 a predictor of pregnancy in vitrified-warmed blastocyst transfer. *Fertil Steril* 2019;112:e200-e1.
- 762 47. Shiba R, Kinutani M, Okano S, Kawano R, Kikkawa Y. Efficacy of four vaginal progesterones for luteal
763 phase support in frozen-thawed embryo transfer cycles: A randomized clinical trial. *Reprod Med Biol*
764 2020;19:42-9.
- 765 48. Al Jarrah DM, Al Obaidi MT, Al Asadi IJ. Endometrial compaction and serum progesterone
766 measurements at the day of embryo transfer cannot predict pregnancy outcomes in frozen-thaw embryo
767 transfer cycles. *Int J Res Pharm Sci* 2021;12:407-15.

- 768 49. Alyasin A, Agha-Hosseini M, Kabirinasab M, Saeidi H, Nashtaei MS. Serum progesterone levels greater
769 than 32.5 ng/ml on the day of embryo transfer are associated with lower live birth rate after artificial
770 endometrial preparation: a prospective study. *Reprod Biol Endocrinol* 2021;19.
- 771 50. Gaggiotti-Marre S, Martinez F, Coll L, Garcia S, Álvarez M, Parriego M et al. Low serum progesterone
772 the day prior to frozen embryo transfer of euploid embryos is associated with significant reduction in live birth
773 rates. *Gynecol Endocrinol* 2019;35:439-42.
- 774 51. Basnayake SK, Volovsky M, Rombauts L, Osianlis T, Vollenhoven B, Healey M. Progesterone
775 concentrations and dosage with frozen embryo transfers - What's best? *Aust N Z J Obstet Gynaecol*
776 2018;58:533-8.
- 777 52. Gaggiotti-Marre S, Álvarez M, González-Foruria I, Parriego M, Garcia S, Martínez F et al. Low
778 progesterone levels on the day before natural cycle frozen embryo transfer are negatively associated with live
779 birth rates. *Hum Reprod* 2020;35:1623-9.
- 780 53. Liu Y, Wu Y. Progesterone Intramuscularly or Vaginally Administration May Not Change Live Birth Rate
781 or Neonatal Outcomes in Artificial Frozen-Thawed Embryo Transfer Cycles. *Front Endocrinol (Lausanne)*
782 2020;11:539427.
- 783 54. Labarta E, Mariani G, Holtmann N, Celada P, Remohi J, Bosch E. Low serum progesterone on the day
784 of embryo transfer is associated with a diminished ongoing pregnancy rate in oocyte donation cycles after
785 artificial endometrial preparation: A prospective study. *Hum Reprod* 2017;32:2437-42.
- 786 55. Polat M, Mumusoglu S, Bozdogan G, Ozbek IY, Humaidan P, Yarali H. Addition of intramuscular
787 progesterone to vaginal progesterone in hormone replacement therapy in vitrified-warmed blastocyst transfer
788 cycles. *Reprod Biomed Online* 2020;40:812-8.
- 789 56. Gonzalez-Foruria I, Gaggiotti-Marre S, Alvarez M, Martinez F, Garcia S, Rodriguez I et al. Factors
790 associated with serum progesterone concentrations the day before cryopreserved embryo transfer in artificial
791 cycles. *Reprod Biomed Online* 2020;40:797-804.
- 792 57. Gaggiotti-Marre S, Álvarez M, González-Foruria I, Parriego M, Garcia S, Martínez F et al. Low
793 progesterone levels on the day before natural cycle frozen embryo transfer are negatively associated with live
794 birth rates. *Hum Reprod* 2020;35:1623-9.
- 795 58. Miles RA, Paulson RJ, Lobo RA, Press MF, Dahmouh L, Sauer MV. Pharmacokinetics and endometrial
796 tissue levels of progesterone after administration by intramuscular and vaginal routes: a comparative
797 study**Presented at the 40th Annual Meeting of the Pacific Coast Fertility Society, Indian Wells, California,
798 April 8 to 12, 1992. *Fertil Steril* 1994;62:485-90.

- 799 59. Lightman A, Kol S, Itskovitz-Eldor J. A prospective randomized study comparing intramuscular with
800 intravaginal natural progesterone in programmed thaw cycles. *Hum Reprod* 1999;14:2596-9.
- 801 60. De Ziegler D, Bulletti C, De Monstier B, Jääskeläinen AS. The first uterine pass effect. *Ann N Y Acad Sci*
802 1997;828:291-9.
- 803 61. Wang Y, He Y, Zhao X, Ji X, Hong Y, Wang Y et al. Crinone Gel for Luteal Phase Support in Frozen-
804 Thawed Embryo Transfer Cycles: A Prospective Randomized Clinical Trial in the Chinese Population. *PLoS One*
805 2015;10:e0133027.
- 806 62. Zarei A, Sohail P, Parsanezhad ME, Alborzi S, Samsami A, Azizi M. Comparison of four protocols for
807 luteal phase support in frozen-thawed Embryo transfer cycles: a randomized clinical trial. *Arch Gynecol Obstet*
808 2017;295:239-46.
- 809 63. Prato LD, Bianchi L, Cattoli M, Tarozzi N, Flamigni C, Borini A. Vaginal gel versus intramuscular
810 progesterone for luteal phase supplementation: a prospective randomized trial. *Reprod Biomed Online*
811 2008;16:361-7.
- 812 64. Asoglu MR, Celik C, Karakis LS, Findikli N, Gultomruk M, Bahceci M. Comparison of daily vaginal
813 progesterone gel plus weekly intramuscular progesterone with daily intramuscular progesterone for luteal
814 phase support in single, autologous euploid frozen-thawed embryo transfers. *J Assist Reprod Genet*
815 2019;36:1481-7.
- 816 65. Devine K, Richter KS, Widra EA, McKeeby JL. Vitrified blastocyst transfer cycles with the use of only
817 vaginal progesterone replacement with Endometrin have inferior ongoing pregnancy rates: results from the
818 planned interim analysis of a three-arm randomized controlled noninferiority trial. *Fertil Steril* 2018;109:266-
819 75.
- 820 66. Corleta H, Capp E, Ferreira M. Pharmacokinetics of Natural Progesterone Vaginal Suppository.
821 *Gynecol Obstet Invest* 2004;58:105-8.
- 822 67. Levy T, Yairi Y, Bar-Hava I, Shalev J, Orvieto R, Ben-Rafael Z. Pharmacokinetics of the progesterone-
823 containing vaginal tablet and its use in assisted reproduction. *Steroids* 2000;65:645-9.
- 824 68. Di Guardo F, Midassi H, Racca A, Tournaye H, De Vos M, Blockeel C. Luteal Phase Support in IVF:
825 Comparison Between Evidence-Based Medicine and Real-Life Practices. *Front Endocrinol* 2020;11.
- 826 69. Tavaniotou A, Smitz J, Bourgain C, Devroey P. Comparison between different routes of progesterone
827 administration as luteal phase support in infertility treatments. *Hum Reprod Update* 2000;6:139-48.

- 828 70. Griesinger G, Tournaye H, Macklon N, Petraglia F, Arck P, Blockeel C et al. Dydrogesterone:
829 pharmacological profile and mechanism of action as luteal phase support in assisted reproduction. *Reprod*
830 *Biomed Online* 2019;38:249-59.
- 831 71. Paulson RJ, Collins MG, Yankov VI. Progesterone pharmacokinetics and pharmacodynamics with 3
832 dosages and 2 regimens of an effervescent micronized progesterone vaginal insert. *J Clin Endocrinol Metab*
833 2014;99:4241-9.
- 834 72. Stanczyk FZ. Pharmacokinetics of progesterone administered by the oral and parenteral routes. *J*
835 *Reprod Med* 1999;44:141-7.
- 836 73. Cometti B. Pharmaceutical and clinical development of a novel progesterone formulation. *Acta Obstet*
837 *Gynecol Scand* 2015;94:28-37.
- 838 74. MacLaughlin DT, Richardson GS. Progesterone binding by normal and abnormal human endometrium.
839 *J Clin Endocrinol Metab* 1976;42:667-78.
- 840 75. Levy C, Robel P, Gautray JP, De Brux J, Verma U, Descomps B et al. Estradiol and progesterone
841 receptors in human endometrium: normal and abnormal menstrual cycles and early pregnancy. *Am J Obstet*
842 *Gynecol* 1980;136:646-51.
- 843 76. Bayard F, Damilano S, Robel P, Baulieu EE. Cytoplasmic and nuclear estradiol and progesterone
844 receptors in human endometrium. *J Clin Endocrinol Metab* 1978;46:635-48.
- 845 77. Deng Y, Chen C, Chen S, Mai G, Liao X, Tian H et al. Baseline Levels of Serum Progesterone and the
846 First Trimester Pregnancy Outcome in Women with Threatened Abortion: A Retrospective Cohort Study.
847 *Biomed Res Int* 2020;2020:8780253.
- 848 78. Coomarasamy A, Devall AJ, Brosens JJ, Quenby S, Stephenson MD, Sierra S et al. Micronized vaginal
849 progesterone to prevent miscarriage: a critical evaluation of randomized evidence. *Am J Obstet Gynecol*
850 2020;223:167-76.
- 851 79. Coomarasamy A, Devall AJ, Cheed V, Harb H, Middleton LJ, Gallos ID et al. A Randomized Trial of
852 Progesterone in Women with Bleeding in Early Pregnancy. *New Engl J Med* 2019;380:1815-24.
- 853 80. Liang Y-X, Liu L, Jin Z-Y, Liang X-H, Fu Y-S, Gu X-W et al. The high concentration of progesterone is
854 harmful for endometrial receptivity and decidualization. *Sci Rep* 2018;8:712-.
- 855 81. Cédric-Durnerin I, Isnard T, Mahdjoub S, Sonigo C, Seroka A, Comtet M et al. Serum progesterone
856 concentration and live birth rate in frozen-thawed embryo transfers with hormonally prepared endometrium.
857 *Reprod Biomed Online* 2019;38:472-80.

858 82. Volovsky M, Pakes C, Rozen G, Polyakov A. Do serum progesterone levels on day of embryo transfer
859 influence pregnancy outcomes in artificial frozen-thaw cycles? *J Assist Reprod Genet* 2020;37:1129-35.

860 83. Csapo A. The luteo-placental shift, the guardian of pre-natal life. *Postgrad Med J* 1969;45:57-64.

861 84. Bosch E, Broer S, Griesinger G, Grynberg M, Humaidan P, Kolibianakis E et al. ESHRE guideline: ovarian
862 stimulation for IVF/ICSI†. *Hum Reprod Open* 2020;2020.

863 85. Watters M, Noble M, Child T, Nelson S. Short versus extended progesterone supplementation for
864 luteal phase support in fresh IVF cycles: a systematic review and meta-analysis. *Reprod Biomed Online*
865 2020;40:143-50.

866 86. Pabuçcu E, Pabuçcu R, Gürgan T, Tavmergen E. Luteal phase support in fresh and frozen embryo
867 transfer cycles. *J Gynecol Obstet Hum Reprod* 2020;101838.

868 87. Singh B, Reschke L, Segars J, Baker VL. Frozen-thawed embryo transfer: the potential importance of
869 the corpus luteum in preventing obstetrical complications. *Fertil Steril* 2020;113:252-7.

870 88. Maheshwari A, Pandey S, Amalraj Raja E, Shetty A, Hamilton M, Bhattacharya S. Is frozen embryo
871 transfer better for mothers and babies? Can cumulative meta-analysis provide a definitive answer? *Hum*
872 *Reprod Update* 2017;24:35-58.

873 89. Coomarasamy A, Williams H, Truchanowicz E, Seed PT, Small R, Quenby S et al. A Randomized Trial of
874 Progesterone in Women with Recurrent Miscarriages. *New Engl J Med* 2015;373:2141-8.

875

876

877

878

879

880

881

882

883

884

885 Figure Legends

886

887 **Figure 1. Forest plot describing the association between different threshold categories of**
888 **serum progesterone and ongoing pregnancy or live birth rates for women undergoing**
889 **frozen embryo transfer cycles using blastocyst embryos and PV progesterone only. CI,**
890 **confidence interval; PV, vaginal route.**

891

892 **Figure 2. Forest plot describing the association between different threshold categories of**
893 **serum progesterone and clinical pregnancy rates for women undergoing frozen embryo**
894 **transfer cycles using blastocyst embryos and PV progesterone only. CI, confidence interval;**
895 **PV, vaginal route.**

896

897 **Figure 3. Forest plot describing the association between different threshold categories of**
898 **serum progesterone and miscarriage rates for women undergoing frozen embryo transfer**
899 **cycles using blastocyst embryos and PV progesterone only. CI, confidence interval; PV,**
900 **vaginal route.**

901

902 **Supplemental Figure 1. Flow diagram of study selection process.**

903

904 **Supplemental Figure 2. Forest plot describing the association between different threshold**
905 **categories of serum progesterone and ongoing pregnancy or live birth rates for women**
906 **undergoing frozen embryo transfer cycles. Sensitivity analysis including all studies suitable**
907 **for meta-analysis. CI, confidence interval; IM, intramuscular route; NA, not applicable**
908 **(natural cycle); PR, rectal route; PV, vaginal route; SC, subcutaneous route.**

909

910 **Supplemental Figure 3. Sensitivity analysis describing the association between different**
911 **threshold categories of serum progesterone and clinical pregnancy rates for women**
912 **undergoing frozen embryo transfer cycles. Sensitivity analysis including all studies suitable**
913 **for meta-analysis. CI, confidence interval; IM, intramuscular route; NA, not applicable**
914 **(natural cycle); PR, rectal route; PV, vaginal route; SC, subcutaneous route.**

915

916 **Supplemental Figure 4. Sensitivity analysis describing the association between different**
917 **threshold categories of serum progesterone and miscarriage rates for women undergoing**
918 **frozen embryo transfer cycles. Sensitivity analysis including all studies suitable for meta-**
919 **analysis. CI, confidence interval; IM, intramuscular route; NA, not applicable (natural cycle);**
920 **PR, rectal route; PV, vaginal route; SC, subcutaneous route.**

921

922 **Supplemental Figure 5. Meta-analysis describing the pooled adjusted effect of low serum**
923 **progesterone (below specified cut-off) versus high serum progesterone (above specified**
924 **cut-off) on ongoing pregnancy or live birth rates in women undergoing FET. aOR, adjusted**
925 **odds ratios; CI, confidence interval; FET, frozen embryo transfer; IV, inverse variance; PV,**
926 **vaginal route.**

927

928 **Supplemental Figure 6. Meta-analysis describing the pooled adjusted effect of low serum**
929 **progesterone (below specified cut-off) versus high serum progesterone (above specified**
930 **cut-off) on clinical pregnancy rates in women undergoing FET. aOR, adjusted odds ratios; CI,**
931 **confidence interval; FET, frozen embryo transfer; IV, inverse variance; PV, vaginal route.**

932 **Supplemental Figure 7. Meta-analysis describing the pooled adjusted effect of low serum**
933 **progesterone (below specified cut-off) versus high serum progesterone (above specified**
934 **cut-off) on miscarriage rates in women undergoing FET. aOR, adjusted odds ratios; CI,**
935 **confidence interval; FET, frozen embryo transfer; IV, inverse variance; PV, vaginal route.**
936

937 [Table Legends](#)

938

939 **Table 1. Characteristics of included studies.**

940

941 **Supplemental Table 1. Characteristics of excluded studies.**

942

943 **Supplemental Table 2. Correspondence with study authors.**

944

945 **Supplemental Table 3. Quality of included studies according to the Newcastle-Ottawa**
946 **Scale for the assessment of cohort studies.**