Romero Roberto (Orcid ID: 0000-0002-4448-5121)
Rode Line (Orcid ID: 0000-0002-9080-3389)
Syngelaki Argyro (Orcid ID: 0000-0001-5856-6072)
Perales-Mar;n Alfredo (Orcid ID: 0000-0002-2221-2560)

Vaginal progesterone for prevention of preterm birth and adverse perinatal outcomes in twin gestation with a short cervix: updated individual patient data meta-analysis

R. Romero[1,2,3,4], A. Conde-Agudelo[1], A. Rehal[5], E. Da Fonseca[6], M. L. Brizot[7], L. Rode[8,9], V. Serra[10,11], E. Cetingoz[12], A. Syngelaki[13], A. Tabor[8,14], A. Perales[11,13], S. S. Hassan[15,16,17], K. H. Nicolaides[18]

[1]Perinatology Research Branch, *Eunice Kennedy Shriver* National Institute for Child Health and Human Development, National Institutes of Health, United States Department of Health and Human Services, Bethesda, Maryland, and Detroit, Michigan, USA; [2]Department of Obstetrics and Gynecology, University of Michigan, Ann Arbor, MI, USA; [3]Department of Epidemiology and Biostatistics, Michigan State University, East Lansing, MI, USA; [4]Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI, USA; and Detroit Medical Center, Detroit, Michigan, USA; [5]Fetal Medicine Research Institute, King's College Hospital, London, UK; [6]Departamento de Obstetrícia e Ginecologia, Hospital do Servidor Publico Estadual 'Francisco Morato de Oliveira' and School of Medicine, University of São Paulo, São Paulo, Brazil; [7]Department of Obstetrics and Gynecology, Pontifical Catholic University of São Paulo School of Medical and Health Sciences, Department of São Paulo, Brazil; [8]Center of Fetal Medicine and Pregnancy, Department of Obstetrics, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark; [9]Juliane Marie Centre, Department of Obstetrics, Kobenhavn, Denmark; [10]Maternal-Fetal Medicine Unit, Instituto Valenciano de Infertilidad, University of Valencia, Valencia, Spain; [11]Department of Pediatrics, Obstetrics and Gynecology, University of Valencia, Valencia, Spain; [12]Department of Obstetrics and Gynecology, Zeynep Kamil Women and Children Diseases Education and Research Hospital, Uskudar, Istanbul, Turkey; [13]Department of Obstetrics and Gynecology, Hospital Universitario La Fe, Valencia, Spain; [14]University of Copenhagen, Faculty of Health Sciences, Copenhagen, Denmark; [15]Office of Women's Health, Integrative Biosciences Center, Wayne State University, Detroit, Michigan, USA; [16]Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, Michigan, USA; [17]Department of Physiology, Wayne State University School of Medicine, Detroit, Michigan, USA; [18]Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, UK

Corresponding author

Dr. Roberto Romero
Perinatology Research Branch,
NICHD/NIH/DHHS
Hutzel Women's Hospital
Box # 4, 3990 John R, Detroit, MI 48201
E-mail: prbchiefstaff@med.wayne.edu

In 2017, we reported the results of an individual patient data (IPD) meta-analysis on the efficacy of vaginal progesterone in asymptomatic women with a twin gestation and a sonographic cervical length (CL) ≤25 mm for the prevention of preterm birth and neonatal morbidity and mortality[1]. The primary outcome was preterm birth <33 weeks of gestation. This meta-analysis included data for 303 women and their 606 fetuses/infants from six randomized controlled trials[2-7] and showed that vaginal progesterone, compared to placebo/no treatment, was associated with a statistically significant reduction in the risk of preterm birth <33 weeks of gestation (relative risk [RR] 0.69, 95% confidence interval [CI] 0.51–0.93). Moreover, vaginal progesterone administration was associated with a significant decrease in the risk of preterm birth <35, <34, <32 and <30 weeks of gestation, neonatal death, respiratory distress syndrome (RDS), composite neonatal morbidity and mortality, use of mechanical ventilation, and birthweight <1500 g.

At present, we are performing a systematic review and meta-analysis on the efficacy and safety of vaginal progesterone for preventing preterm birth and perinatal morbidity and mortality in asymptomatic women with a twin gestation, which was registered with PROSPERO (number CRD42020205184). Recently, one[7] of the studies included in our previous IPD meta-analysis was retracted because, allegedly, "the authors did not obtain approval from a research ethics committee before conducting this interventional randomized control trial and therefore this study is in breach of the Declaration of Helsinki and the editorial policy of the Journal"[8]. Therefore, we have decided to update our IPD meta-analysis by excluding data from the retracted study and including those from eligible studies published since the last literature search date.

We followed the same methodology that was used in our previous IPD meta-analysis[1]. Briefly, a literature search was performed in MEDLINE, EMBASE, CINAHL, LILACS, and the Cochrane Central Register of Controlled Trials for randomized controlled trials published from January 1, 2017, to November 30, 2021, comparing vaginal

progesterone (any dose) vs placebo/no treatment for the prevention of preterm birth and/or adverse perinatal outcomes in women with a twin gestation and a midtrimester sonographic CL ≤25 mm. Trials were eligible if the primary aim of the study was to prevent preterm birth in women with a twin gestation and a short cervix, or to prevent preterm birth in women with an unselected twin gestation but for whom outcomes were available in those with a pre-randomization CL ≤25 mm. The principal investigators of eligible trials were contacted and asked to share their data for this collaborative project. As in the previous IPD meta-analysis, the primary outcome was preterm birth <33 weeks of gestation. Secondary outcomes included preterm birth <37, <36, <35, <34, <32, <30, and <28 weeks of gestation, spontaneous preterm birth <33 and <34 weeks of gestation, and adverse perinatal outcomes. We assessed the risk of bias in each included study, using the tool recommended by the Cochrane Handbook for Systematic Reviews of Interventions[9].

The IPD were combined in a two-stage approach in which outcomes were analyzed in the original trial, and summary statistics (pooled RR with 95% CI) were then generated, using standard summary data meta-analysis techniques[10]. Heterogeneity of treatment effect was assessed with the I^2 statistic, where I^2 ≥30% indicated substantial heterogeneity[11]. We used a fixed-effect model to calculate the pooled RR with 95% CI where it was reasonable to assume that studies were estimating the same underlying treatment effect. We planned to use the random-effects model if there was clinical heterogeneity sufficient to expect that the underlying treatment effects differed between trials or if we found substantial statistical heterogeneity. For perinatal outcomes, we estimated pooled RRs with 95% CIs, assuming independence between fetuses/neonates by using data reported in the studies at the fetal/neonatal level. We also used cluster analysis to estimate pooled adjusted RRs with 95% CIs to take into account non-independence of fetuses/neonates from twin gestations[12]. Adjusted RRs were considered

4

as the main estimates of the vaginal progesterone's effect on perinatal outcomes. The number needed to treat (NNT) for benefit or harm with the 95% CI was calculated for outcomes for which there was a statistically significant reduction or increase in risk difference based on control event rates in the trials. Prespecified sensitivity analyses to explore the impact of risk of bias on results were not performed because all trials were judged to be at low risk of bias. We used the GRADE approach to assess the quality of evidence for the clinically relevant outcomes preterm birth <33 weeks of gestation and composite neonatal morbidity and mortality[13]. The GRADE approach categorizes the quality of the evidence into four levels: high, moderate, low, and very low.

For this update, the search strategy identified three additional studies for possible inclusion[14-16], of which we included one[16] and excluded two[14,15]. The trial by Crowther et al[14], which compared vaginal progesterone 100 mg/day versus placebo from 20 weeks until 34 weeks of gestation in women with a previous spontaneous preterm birth, included 12 women with a twin gestation (8 in the vaginal progesterone group and 4 in the placebo group). This study was excluded because data on cervical length were not collected before randomization. The study by Shabaan et al[15], which compared vaginal progesterone 400 mg/day versus no treatment in 140 women with a twin gestation, was excluded because vaginal progesterone administration was started in the third trimester (mean, 28.9 weeks of gestation). Moreover, this study did not report information about pre-randomization cervical length. The EVENTS trial by Rehal et al[16], which compared vaginal progesterone 600 mg/day to placebo from 11 to 14 weeks until 34 weeks of gestation in 1194 women with a twin gestation, met the inclusion criteria. In this study, all included women underwent cervical length measurement before randomization. A total of 16 women (9 in the vaginal progesterone group and 7 in the placebo group) had a cervical length ≤25 mm (mean gestational age at randomization, 13.2 weeks), and their IPD were provided for this updated meta-analysis.

5

Six double-blind, placebo-controlled trials[2-6,16], which provided IPD for 95 women and their 190 fetuses/infants, met the inclusion criteria for the updated meta-analysis (Supplementary Figure 1). All studies were deemed to be at low risk of bias for all domains of the Cochrane Handbook for Systematic Reviews of Interventions' tool (Supplementary Figure 2). Vaginal progesterone significantly reduced the risk of preterm birth <33 weeks of gestation (38.5% vs 55.8%; RR 0.60; 95% CI 0.38-0.95; P=0.03; I^2 =14%; NNT for benefit 5, 95% CI 3-36) (Figure 1). The frequencies of preterm birth <34, <32, <30, and <28 weeks of gestation and spontaneous preterm birth <33 and <34 weeks of gestation were significantly lower in the vaginal progesterone group than in the placebo group (RRs from 0.41-0.68) (Table 1). There was no evidence of an effect of vaginal progesterone on preterm birth <37, <36, and <35 weeks of gestation. Treatment with vaginal progesterone was also associated with a significant decrease in the risk of composite neonatal morbidity and mortality (RR 0.59; 95% CI 0.33-0.98) and birthweight <1500 g (RR 0.55; 95% CI 0.33-0.94) (Table 2). There were no significant differences between the study groups in the risk of the remaining adverse perinatal outcomes assessed. After applying the GRADE approach, the evidence was judged to be of "moderate quality" for the outcomes preterm birth <33 weeks of gestation and composite neonatal morbidity and mortality (Supplementary Table). We downgraded one level for imprecision due to failure to meet the optimal information size (small total sample size). An assignment of "moderate quality" signifies that we are moderately confident in the effect estimate, and the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

In conclusion, this updated meta-analysis, which excluded data from the retracted study and included information from the EVENTS trial, shows that vaginal progesterone significantly decreases the risk of preterm birth <33 weeks of gestation among women with a twin gestation and a midtrimester CL ≤25 mm. In addition, despite the limited sample

size of the meta-analysis, vaginal progesterone was associated with a significant reduction in the risk of preterm birth <34, <32, <30, and <28 weeks of gestation, spontaneous preterm birth <33 and <34 weeks of gestation, composite neonatal morbidity and mortality, and birthweight <1500 g. Nevertheless, it should be emphasized that evidence from an ongoing randomized controlled trial (PROSPECT study) is needed to establish whether this promising intervention can be recommended to women with a twin gestation and a short cervix. The PROSPECT study (NCT02518594) is a randomized controlled trial of 630 women evaluating the use of vaginal progesterone 200 mg/day or cervical pessary versus control (placebo) to prevent early preterm birth in women carrying twins and with a CL <30 mm between 16 and 23 weeks of gestation[17]. This study began in November 2015, and the estimated completion date is February 2025.

Disclosure: All authors report no conflicts of interest

Reprints will not be available

REFERENCES

1. Romero R, Conde-Agudelo A, El-Refaie W, Rode L, Brizot ML, Cetingoz E, Serra V, Da Fonseca E, Abdelhafez MS, Tabor A, Perales A, Hassan SS, Nicolaides KH. Vaginal progesterone decreases preterm birth and neonatal morbidity and mortality in women with a twin gestation and a short cervix: an updated meta-analysis of individual patient data. *Ultrasound Obstet Gynecol* 2017; **49**: 303-314.

2. Fonseca EB, Celik E, Parra M, Singh M, Nicolaides KH; Fetal Medicine Foundation Second Trimester Screening Group. Progesterone and the risk of preterm birth among women with a short cervix. *N Engl J Med* 2007; **357**: 462–469.

3. Cetingoz E, Cam C, Sakalli M, Karateke A, Celik C, Sancak A. Progesterone effects on preterm birth in high-risk pregnancies: a randomized placebo-controlled trial. *Arch Gynecol Obstet* 2011; **283**: 423–429.

4. Rode L, Klein K, Nicolaides KH, Krampl-Bettelheim E, Tabor A; PREDICT Group. Prevention of preterm delivery in twin gestations (PREDICT): a multicenter, randomized, placebo-controlled trial on the effect of vaginal micronized progesterone. *Ultrasound Obstet Gynecol* 2011; **38**: 272–280.

5. Serra V, Perales A, Meseguer J, Parrilla JJ, Lara C, Bellver J, Grifol R, Alcover I, Sala M, Martínez-Escoriza JC, Pellicer A. Increased doses of vaginal progesterone for the prevention of preterm birth in twin pregnancies: a randomised controlled double-blind multicentre trial. *BJOG* 2013; **120**: 50–57.

6. Brizot ML, Hernandez W, Liao AW, Bittar RE, Francisco RP, Krebs VL, Zugaib M. Vaginal progesterone for the prevention of preterm birth in twin gestations: a randomized placebo-controlled double-blind study. *Am J Obstet Gynecol* 2015; **213**: 82.e1–9.

7. El-Refaie W, Abdelhafez MS, Badawy A. Vaginal progesterone for prevention of preterm labor in asymptomatic twin pregnancies with sonographic short cervix: a randomized clinical trial of efficacy and safety. *Arch Gynecol Obstet* 2016; **293**: 61–67.

8. El-Refaie W, Abdelhafez MS, Badawy A. Retraction Note: Vaginal progesterone for prevention of preterm labor in asymptomatic twin pregnancies with sonographic short cervix: a randomized clinical trial of efficacy and safety. *Arch Gynecol Obstet* 2021 Jul 27. doi: 10.1007/s00404-021-06152-1. Epub ahead of print. PMID: 34313841.

9. Higgins JPT, Savović J, Page MJ, Elbers RG, Sterne JAC. Chapter 8: Assessing risk of bias in a randomized trial. In: Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). *Cochrane Handbook for Systematic Reviews of*

Interventions version 6.2 (updated February 2021). Cochrane, 2021. Available from www.training.cochrane.org/handbook.

10. Tierney JF, Vale C, Riley R, Smith CT, Stewart L, Clarke M, Rovers M. Individual participant data (IPD) meta-analyses of randomised controlled trials: guidance on their use. *PLoS Med* 2015; **12**: e1001855.

11. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; **327**: 557-560.

12. Gates S, Brocklehurst P. How should randomised trials including multiple pregnancies be analysed? *BJOG* 2004; **111**: 213-219.

13. Schünemann H, Brozek J, Guyatt G, Oxman A. GRADE handbook for grading quality of evidence and strength of recommendation. The GRADE Working Group; 2013.

14. Crowther CA, Ashwood P, McPhee AJ, Flenady V, Tran T, Dodd JM, Robinson JS; PROGRESS Study Group. Vaginal progesterone pessaries for pregnant women with a previous preterm birth to prevent neonatal respiratory distress syndrome (the PROGRESS Study): A multicentre, randomised, placebo-controlled trial. *PLoS Med* 2017; **14**: e1002390.

15. Shabaan OM, Hassanin IM, Makhlouf AM, Salem MN, Hussein M, Mohamed M, Abbas AM. Vaginal progesterone for prevention of preterm delivery in women with twin pregnancy: a randomized controlled trial. *Facts Views Vis Obgyn* 2018; **10**: 93-98.

16. Rehal A, Benkő Z, De Paco Matallana C, Syngelaki A, Janga D, Cicero S, Akolekar R, Singh M, Chaveeva P, Burgos J, Molina FS, Savvidou M, De La Calle M, Persico N, Quezada Rojas MS, Sau A, Greco E, O'Gorman N, Plasencia W, Pereira S, Jani JC, Valino N, Del Mar Gil M, Maclagan K, Wright A, Wright D, Nicolaides KH. Early vaginal progesterone versus placebo in twin pregnancies for the prevention of spontaneous preterm birth: a randomized, double-blind trial. *Am J Obstet Gynecol* 2021; **224**: 86.e1-86.e19.

17. A Trial of pessary and progesterone for preterm prevention in twin gestation with a short cervix (PROSPECT). ClinicalTrials.gov Identifier: NCT02518594. Available at: https://clinicaltrials.gov/ct2/show/NCT02518594. Accessed August 13, 2021

FIGURE LEGENDS

Figure 1: Forest plot of the effect of vaginal progesterone on the risk of preterm birth <33 weeks of gestation. CI, confidence interval.

Supplementary Figure 1: Study flow diagram: review update. CL, cervical length.

Supplementary Figure 2: Risk of bias of studies included in the systematic review.