ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Revisiting the progesterone to oocyte ratio

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Objective: To critically evaluate the P to oocyte (O) ratio (P/O) in the prediction of live birth in assisted reproductive technology (ART) cycles.
Design: Retrospective cohort study.
Setting: Not applicable.
Patient(s): A total of 7,608 fresh autologous ART ET cycles.
Intervention(s): None.
Main Outcome Measure(s): Live birth.
Result(s): Generalized estimating equation (GEE) models and receiver operating characteristic curves assessed the ability of P, O, and the P/O ratio to predict live birth. In univariate GEE models, P, O, and P/O were each associated with live birth. However, in multivariate GEE models, the P/O ratio was not associated with live birth, but P alone was. This suggested that converting P and O into a ratio of P/O was not more helpful than the two independent variables themselves. Measures of overall model fit further suggested that P/O did not increase the predictive ability of the model over P and O alone. Receiver operating characteristic curves using incremental predictors further demonstrated that the P/O provided no incremental improvement in predicting live birth over P and O separately.
Conclusion(s): These data suggest that P and O have utility in prediction modeling but demonstrate that additional oocytes were not protective from the negative association of P with live birth. There was no incremental improvement related to the P/O ratio specifically for predicting live birth over each variable independently. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: ART, premature progesterone elevation, progesterone, progesterone to oocyte ratio

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Our understanding of the effect of prematurely elevated P on assisted reproductive technology (ART) outcomes has deepened dramatically in the past 6 years. In 2010, Bosch et al. (1) and Xu et al. (2) separately published data from more than 14,000 ART cycles demonstrating that premature P elevations were negatively associated with the likelihood of pregnancy. This negative association has been confirmed in a meta-analysis of more than 60,000 ART cycles from Venetis et al. (3). Further research has demonstrated that this negative association of P with pregnancy persists across various parameters of ART cycles to include GnRH antagonist cycles, GnRH and hCG trigger cycles, and in patients with young age, good-quality embryos, and blastocysts for transfer (4–7).

Recent studies have proposed that the relationship of P levels to treatment outcomes may vary by ovarian response and suggest the ratio of P to oocytes (P/O) as an alternative and robust predictor of the likelihood of pregnancy. The body of literature demonstrating a relation of increasing P/O with decreasing pregnancies proposes the ratio to be a better predictor of pregnancy than P alone and recommends various thresholds for use of P/O (8–10). These assertions warrant further consideration. Among the questions raised by these studies, foremost is with regard to biologic plausibility. Elevated P is not

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associated with poor oocyte quality, donor–recipient outcomes, or subsequent frozen–thawed cycle outcomes (3). Further, the literature suggests elevated P causes premature advancement of the endometrium in fresh transfer cycles (3). Theoretically it should not matter how many follicles are producing the elevated P level, but rather what the level is. In other words, if sufficient P is produced to advance the endometrium, biologic plausibility suggests that endometrial advancement will occur whether that sufficient P was generated from a few or numerous follicles.

Second, the ratio P/O utilizes two variables independently associated with live birth and raises questions regarding the implications of use of ratios in statistical prediction models. Statistical considerations and potential issues with use of ratios have been described in the biomedical and statistical literature and suggest consideration of alternative approaches for modeling of variables comprising ratios (11, 12). Prior studies have suggested that P/O is superior to P alone, but it is unclear whether the P/O ratio per se provides optimal prediction, compared with alternative approaches for inclusion of O in models. Given that P and O are already demonstrated independent predictors of live birth, the approach for use of P and O to yield optimal predictive ability is not established. Our objective was to assess the ratio of P/O and other methods for inclusion of P and O for predictive probability for live birth.

We tested the hypothesis that the P/O adds incremental predictive probability over P and O as separate variables. The null hypothesis (that the P/O does not add incremental predictive probability) was based on the biologic plausibility that elevated P is likely to advance the endometrium and decrease live birth, regardless of how many follicles generated that elevated P level.

MATERIALS AND METHODS

Study Design

This was a retrospective cohort analysis of fresh ART cycles from 2013–2015. Cycles were included if serum P was obtained on the day of trigger and a fresh embryo transfer occurred. The study was performed at Shady Grove Fertility Reproductive Science Center in Rockville, MD with institutional review board approval.

Patients

All patients who underwent a fresh autologous embryo transfer with known serum P levels measured on the day of trigger were included in the analysis. Exclusion criteria included cycles in which no embryo was transferred, donor oocyte recipients, frozen–thawed embryo transfers, and cycles without P measured on day of trigger.

Stimulation Protocol

Ovarian stimulation protocols included mixed FSH/hMG protocols with either GnRH agonist or GnRH antagonist for pituitary suppression. Oral contraceptive treatment was generally initiated 2 to 3 weeks before stimulation. For GnRH antagonist cycles, the antagonist was started when the lead follicle was 14 mm in size. For GnRH agonist cycles, 20 U of leuprolide acetate was administered SC during the last 3 days of oral contraceptive use. The leuprolide acetate was decreased to 5 U when ovarian suppression was confirmed with ultrasound and serum E_2 <5 pg/mL. Ovarian stimulation was achieved with both FSH and hMG preparations. When the lead follicle was ≥18 mm, 10,000 IU of hCG or 4 mg of GnRH agonist was used for final oocyte maturation. If GnRH agonist was used for trigger, 1,500 IU of hCG was administered after oocyte retrieval when <30 oocytes were obtained. In 2% of the study population, GnRH agonist trigger was used and ≥30 oocytes were obtained, in which case hCG was withheld after oocyte retrieval. In general, patients predicted to be higher responders were placed on an antagonist protocol and were more likely to receive GnRH agonist trigger. Serum P levels were obtained on the day of trigger. Oocyte retrieval was performed 36 hours after the trigger injection. Fertilization was achieved with either conventional IVF or intracytoplasmic sperm injection as clinically indicated. After retrieval, the majority of patients received vaginal P daily for luteal support. All patients received 2 mg estrace twice daily starting the evening of oocyte retrieval.

Ultrasound-guided ET was performed on day 3 or on day 5 if an adequate number of high-quality embryos were available. Embryos were graded as good, fair, or poor according to the simplified Society for Assisted Reproductive Technology scoring system (13). Serum hCG levels were assessed at 4 weeks' gestational age, followed by ultrasonography confirmation of a intrauterine pregnancy in all pregnant patients.

Serum P levels were measured using a solid-phase, competitive chemiluminescent enzyme immunoassay (Immunolyte 2000 Progesterone assay; Siemens Medical Solutions Diagnostic). The lower limit of detection for the assay was 0.2 ng/mL, and the analytical sensitivity of the assay was 0.1 ng/mL. Intra-assay and interassay coefficients of variation were 6.7% and 7.2%, respectively.

Outcome

The primary outcome was live birth, defined as a live-born infant after the 23rd week of pregnancy.

Statistics

To evaluate approaches for inclusion of P and O in prediction models, values of P and O were used to create the P/O ratio. In addition, because the P/O ratio effectively utilizes 1 divided by oocytes (1/O) as a predictor, 1/O was evaluated as well. First, generalized estimating equation (GEE) models were utilized to assess relations of the probability of live birth with each of the independent variables in unadjusted and adjusted models, yielding odds ratio estimates. The GEE modeling was used to account for patients with multiple cycles and while allowing adjustment for covariates. Multivariable models were specified using variables significantly associated with live birth in univariate models ($P<.05$) and included the following: age, body mass index, total dose of gonadotropins, E_2 on the day of trigger, P on the day of trigger, oocytes retrieved, P/O, 1/O, embryo stage, embryo quality, the number of embryos