Mid-pregnancy, perinatal, and neonatal reproductive endocrinology: a prospective cohort study in twins and singleton control subjects

Esther A. M. Kuijper, M.D., Ph.D.,[a] Jos W. R. Twisk, Ph.D.,[b] Ted Korsen,[a] Mirte R. Caanen, M.D.,[a] Mark M. Kushnir, Ph.D.,[c,d] Alan L. Rockwood, Ph.D.,[c,d] A. Wayne Meikle, M.D.,[c,d] Peter G. Hompes, M.D., Ph.D.,[a] Jan M. Wit, M.D., Ph.D.,[e] and Cornelis B. Lambalk, M.D., Ph.D.[a]

[a] Division of Reproductive Medicine, Department of Obstetrics and Gynecology, [b] Department of Epidemiology and Biostatistics, VU University Medical Center, Amsterdam, the Netherlands; [c] ARUP Institute for Clinical and Experimental Pathology; [d] Department of Pathology, University of Utah School of Medicine, Salt Lake City, Utah; and [e] Department of Pediatrics, Leiden University Medical Center, Leiden, the Netherlands

Objective: To answer the questions: Are perinatal reproductive hormone profiles different in case of a twin compared with a singleton pregnancy? Are reproductive endocrine profiles of twin girls influenced by their male co-twin and vice versa?
Design: Prospective cohort study from January 2004 to October 2009.
Setting: Not applicable.
Patient(s): A total of 204 mothers of twins and 248 singleton control subjects, aged >18 years, pregnant with a twin or singleton and no endocrine disease or malignancy.
Intervention(s): Blood samples were collected at mid-gestation from the mother and at delivery from the mothers and the umbilical cords. Estrogens, androgens, sex hormone-binding globulin, progesterone, and gonadotropins were measured.
Main Outcome Measure(s): Hormonal profiles were compared between singletons and twins, different types of twins, and opposite-sex and same-sex twins.
Result(s): Estrogen and progesterone concentrations were higher in mothers of twins compared with singletons, but twin babies had lower estrogen and progesterone concentrations at birth. Opposite-sex twin girls did not have higher androgens in cord blood compared with same-sex twin girls. Boys of an opposite-sex twin had lower luteinizing hormone concentrations compared with dizygotic twin boys with a brother as a co-twin.
Conclusion(s): Children from a twin are not overexposed to sex steroids at the time of birth, despite higher concentrations in their mothers, and girls from opposite sex twins do not show androgenic influences from their male co-twin. The female co-twin may influence the hypothalamic-pituitary-testicular axis of her brother via central inhibition. (Fertil Steril® 2015;104:1527–34. ©2015 by American Society for Reproductive Medicine.)
Key Words: Estrogens, androgens, twins, pregnancy

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/kuijpere-neonatal-twin-reproductive-endocrinology/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Received June 11, 2015; revised and accepted August 12, 2015; published online September 9, 2015.
E.A.M.K. has nothing to disclose. J.W.R.T. has nothing to disclose. T.K. has nothing to disclose. M.R.C. has nothing to disclose. M.M.K. has nothing to disclose. A.L.R. has nothing to disclose. A.W.M. has nothing to disclose. P.G.H. has nothing to disclose. J.M.W. has nothing to disclose. C.B.L. has received grants and personal fees from Ferring, personal fees from MSD, grants and personal fees from Auxogyn, and grants from Merck Serono.
Reprint requests: Esther A. M. Kuijper, M.D., Ph.D., VU University Medical Center, de Boelelaan 1117, 1081 HV Amsterdam, the Netherlands (E-mail: e.kuijper@vumc.nl).

Fertility and Sterility® Vol. 104, No. 6, December 2015 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2015.08.016

The intrauterine environment is considered to be a possible starting point for development of diseases later in life (1). For example, undernutrition during gestation, depending on the trimester of exposure, may result in a higher prevalence of coronary heart disease, type 2 diabetes, bronchitis, and altered lipid profile in the offspring (2). Hormones larger

than 7–12 kDa are not able to pass the placenta, therefore making the fetal endocrine component largely independent from maternal influences (3). Although in general much thought has been given to intrauterine hormone exposure, little is known in the complicated situation of multiple pregnancies, because circulating hormones both influence and are influenced by at least two fetuses and concentrations may differ from those found in singleton pregnancies.

A postulated increased risk of developing breast or testicular cancer in dizygotic (DZ) twins, is often related to intrauterine exposure to high estrogen concentrations (4–6). Proxy indicators of prenatal estrogens such as high birth weight (>4 kg), advanced maternal age, and severe nausea in the first trimester have been used to evaluate these associations (4, 5, 7–9). Being part of a twin is considered to be such a risk factor as well, because estrogen concentrations are consequently reported higher in serum and urine in mothers of twins (10–12), but there are almost no data on fetal exposure (13, 14). A study analyzing data from Finland before the age of contraception reported a reduced fecundity in women born as part of an opposite-sex twin. Overexposure to androgens produced by their male co-twin was suggested to be the cause of this loss of reproductive capacity (15). Suggested effects of prenatal exposure to androgens have been reported in childhood as changes in gender-related play behavior (16) and in adults as the trait of sensation seeking being more prone in women with a male co-twin (17). So, although there is no direct evidence, twin babies may influence their co-twin, which emphasizes the importance to obtain adequate information on actual reproductive hormone concentrations within the fetal surroundings. To fill this gap of knowledge we conducted a prospective study in which we collected maternal serum around mid-pregnancy and again at delivery, plus umbilical cord blood from a large cohort of women carrying twins and singleton control subjects. We measured estrogens, progesterone (P), androgens, sex hormone-binding globulin (SHBG), and gonadotropins.

We aimed to answer the following main, so far unanswered, questions:

1) Do reproductive hormonal profiles, particularly estrogens and P, in mothers and babies differ in case of a twin pregnancy compared with a singleton pregnancy?
2) Are reproductive endocrine profiles at birth of a newborn female twin influenced by a male co-twin and vice versa?

SUBJECTS AND METHODS

Subjects

A prospective cohort of pregnant women was recruited between January 2004 and October 2009 from the VU University Medical Center (VUmc) outpatient clinic, VUmc IVF center, a local midwife practice, and other teaching hospitals throughout the Netherlands. To participate, women had to be above the age of 18 years, be pregnant with a singleton or twin, and have no apparent endocrine disease (hypothyroidism, diabetes) or malignancy. Women who gave birth before 32 weeks of gestation were excluded. We collected maternal venous serum samples between 18 and 22 weeks of gestation and during labor. After birth, umbilical cord blood was sampled. From twin neonates of the same sex, buccal swabs were taken to determine zygosity. The study was approved by the Institutional Review Board and the Medical Ethical committee of the VUmc and all participating parents signed informed consents.

Methods

Blood samples. Venous maternal serum and mixed venous and arterial umbilical cord blood samples were centrifuged for 10 minutes (3,000 rpm) and stored at −20°C. Samples were transported between the participating centers on dry ice.

Hormonal measurements. We report only unconjugated steroid concentrations since they represent the biologically active fraction of steroids present in the blood.

Estrogens and androgens. Samples were analyzed for estrogens (estrone = E_1; estradiol = E_2; and estriol = E_3) and androgens (testosterone = T; androstenedione = A; and dehydroepiandrostenedione = DHEA) with the use of liquid chromatography–tandem mass spectrometry (LC-MS/MS) methods at ARUP Laboratories, Salt Lake City, Utah. T, E_1, E_2, E_3, hydroxylamine, formic acid, trifluoroacetic acid, dansyl chloride, and sodium carbonate were purchased from Sigma Aldrich. A and DHEA were purchased from Steraloids. The internal standards were deuterium-labeled analogues of the steroids: d_3-T was purchased from Cambridge Isotope Laboratories; d_4-E_1, d_3-E_2, d_3-E_3, d_7-A, and d_2-DHEA were purchased from CDN Isotopes. All other chemicals were of the highest purity commercially available. Samples were analyzed with the use of LC-MS/MS as previously described (18–20). Briefly, steroids were extracted from the samples. DHEA, A, and T were derivatized with the use of hydroxylamine to form oxime derivatives and E_1, E_2, and E_3 were derivatized with the use of dansyl chloride to form dansyl derivatives (18, 19). The lower limit of quantification for estrogens (E_1, E_2, and E_3) was 1 pg/mL, for DHEA 0.05 ng/mL, and for T and A 0.01 ng/mL (19, 20). The intra-assay and interassay coefficients of variation (CVs) were <8% and <11%, respectively (19, 20). All steroids were analyzed in positive ion mode with the use of an electrospray ion source on a triple quadruple mass spectrometer (API4000; AB Sciex). The high-performance liquid chromatography (HPLC) system consisted of Series 1260 HPLC pumps (Agilent Technologies) and an HTC PAL autosampler (LEAP Technologies) equipped with a fast wash station. The quadrupoles Q1 and Q3 were tuned to unit resolution, and the mass spectrometer conditions were optimized for maximum signal intensity of each steroid. Two mass transitions were monitored for each steroid and its internal standard. Quantitative data analysis was performed with the use of Analyst 1.5.2 software. Calibration curves were generated with every set of samples with the use of six calibrators, and three quality control samples were included with every set of samples. Specificity of the analysis for each steroid in every sample was evaluated by comparing concentrations determined with the use of the primary and the secondary mass transitions of each steroid and its internal standard (18, 21).