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Original Research

The Role of Equine Chorionic Gonadotropin in the Stimulation of Luteal Steroidogenesis in Mares Carrying Horse or Mule Pregnancies

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ABSTRACT

The stimulatory role of equine Chorionic Gonadotropin (eCG) in the production of steroid hormones was evaluated during the first 4 months of pregnancy in mares impregnated by either stallions or jack donkeys. Twenty mares were divided in two groups: Mares in the first group were inseminated with stallion semen (horse pregnancies), and those in the second group were inseminated with donkey semen (mule pregnancies). Blood samples were collected twice weekly from day 30 to day 120 of pregnancy to determine the concentrations of eCG, progesterone, androstenedione, and testosterone. Analysis of variance for repeated measures was used to compare the concentrations of each hormone between groups. Linear regression models that considered the linear and quadratic effects of week of gestation as well as the linear and quadratic effects of the concentrations of eCG on the production of each steroid hormone were carried out. Concentrations of eCG, progesterone, and androstenedione were higher in horse than in mule pregnancies ($P < .01$ for eCG and $P < .05$ for progesterone and androstenedione). Testosterone concentrations were also higher in horse pregnancies than in mule pregnancies at weeks 7, 9, and 10 ($P < .05$). Regression analysis indicated that eCG had considerable stimulatory effects on the secretion of progesterone and androstenedione and weaker effects on the secretion of testosterone. The results suggest that eCG stimulates luteal production of progesterone, androstenedione, and testosterone in horse and mule pregnancies, these effects being more evident in horse pregnancies than in mule pregnancies due to the higher concentrations of eCG in horse pregnancies.

1. Introduction

Ovarian follicular waves associated with periodic increases in follicle-stimulating hormone (FSH) secretion occur every 10 to 12 days in cyclic mares [1,2]. These follicular waves also occur during the first months of pregnancy [3]. Before day 30 of pregnancy, the dominant follicles growing during each follicular wave do not ovulate due to the inhibitory effect of high progesterone levels on LH secretion [4]. In contrast, ovulation of large dominant follicles can occur after day 35 of pregnancy due to the presence of high levels of eCG [5–7]. In the mare, this hormone has LH-like activity because it binds to LH receptors in ovarian cells [8,9]. The ovulations induced by equine Chorionic Gonadotropin (eCG) during pregnancy result in the formation of secondary corpora lutea (CL) [7,10,11]. Additionally, accessory CL can form as a result of anovulatory follicles being stimulated to luteinize by eCG during the first months of pregnancy [7,12,13].

Through its luteogenic and luteotropic effects, eCG stimulates the production of progesterone by both the

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primary CL and the supplementary ones formed during pregnancy [7,14], The stimulatory effects of eCG on steroidogenesis might not be limited to progesterone synthesis but also seems to affect the production of androgens [15,16] and estrogens [17–19]. Several *in vitro* studies have demonstrated diverse effects of eCG on luteal steroidogenesis [15,19–22], whereas *in vivo* studies have confirmed that the steroidogenic effects of eCG on pregnant mares are dependent on the presence of a functional CL because the increase failed to occur if the CL of pregnancy was induced to regress by the administration of PGF2α, and pregnancy was maintained by the administration of altrenogest [14,16,18].

The mare carrying a mule pregnancy is a naturally eCG-deficient model [7,23–25]. Lower concentrations of eCG in mule pregnancies than in horse pregnancies result from a narrower chorionic girdle, smaller endometrial cups, and an earlier immunological rejection by the mother [25,26]. It is known that the low eCG concentrations in mule pregnancies are associated with lower progesterone concentrations than those found in horse pregnancies [7,23,24]. However, there is no published information regarding the effect of the natural eCG deficiency occurring during mule pregnancies on androgen production.

The objective of the present study was to evaluate if the low production of eCG that occurs during mule pregnancies is associated with lower progesterone, testosterone, and/or androstenedione concentrations than those found during horse pregnancies, and if eCG concentrations are related to the concentrations of those steroid hormones within each type of pregnancy.

2. Materials and Methods

2.1. Animals

The study was carried out at an experimental farm located close to Mexico City, at 19°09′20″ north. The experimental protocol was approved by the Institutional Committee for the Use and Care of Experimental Animals according to international standards and to Mexican Official Norms relative to experimentation on animals. Twenty adult pregnant mares were used. They were fed according to requirements with oat hay, alfalfa hay, and commercial concentrate. Ten of the mares had been inseminated with donkey semen and therefore carried mule conceptuses. The other 10 had been inseminated with horse semen and thus carried horse conceptuses.

2.2. Ultrasonographic Examinations and Blood Samples

Starting on day 30–33 of pregnancy, ultrasonographic examinations were carried out twice a week to confirm embryo/fetal viability. A 5-MHz linear transducer was used for examinations between day 30 and day 60 of pregnancy, and a 3.5-MHz transducer was used after day 60. On each occasion embryo/fetal viability was assessed through the presence of heart beat, fetal movement, and/or confirmation of umbilical cord pulse [23].

Blood samples were collected twice a week by jugular puncture using vacuum tubes containing serum activating gel (BD Diagnostic systems, Mexico City). The samples were centrifuged 1 hour after collection, and serum was separated and kept frozen at −20°C until assayed for hormonal quantification.

2.3. Hormone Determinations

Concentrations of eCG were determined by an enzyme-linked immunoassay (PMSG-ELISA, DRG Instruments, Marburg, Germany). The assay sensitivity was 3 IU/mL, with 7.1% intraassay coefficient of variation for low values and 11.6% for high values. The eCG interassay coefficient of variation was 10%. The specificity of the assay is such that a concentration of 50 ng/mL of FSH produces a reaction equivalent to 0.9 IU/mL of eCG, and 50 ng/mL of LH produces a reaction equivalent to 0.5 IU/mL of eCG. Progesterone was quantified by solid-phase RIA, (Coat-A-Count Progesterone, Siemmens Healthcare Diagnostics, Los Angeles, CA). The progesterone assay sensitivity was 0.1 ng/mL, the intraassay and interassay coefficients of variation were 4.12 and 7.3%, respectively. The progesterone assay cross-reacts with 5α-Pregnan-3,20-dione. (9%), 5β-Pregnan-3,20-dione (3.2%) and 17α-Hydroxiprogesterone (3.4%). Cross reaction with other steroid hormones is less than 1%. Testosterone levels were quantified by ELISA, using a polyclonal antiserum (Testosterone, R156/7) kindly provided by Dr Coralie Munro, University of California, Davis. The testosterone ELISA had intraassay and interassay coefficients of variation of 1.2 and 3.8%, respectively. The sensitivity was 10 pg/mL. The testosterone antibody cross-reacts with 5α-dihydrotestosterone (57.4%) and androstenedione (0.27%). Androstenedione was measured using a RIA commercial kit (Coat-A-Count Androstenedione, Siemmens Healthcare Diagnostics, Los Angeles, CA, USA), with a sensitivity of 40 pg/mL, intra assay coefficient of variation of 2.2%, and inter assay variation coefficient of 1.8%. The androstenedione assay cross-reacts with testosterone (1.49%) and 5α-dihydrotestosterone (0.21%). Androstenedione concentrations were not measured during weeks 16 and 17 due to accidental spillage of the samples.

2.4. Data Analysis

Data were subjected to analysis of variance for repeated measures to compare the concentrations of eCG, progesterone, androstenedione and testosterone between groups in different weeks of pregnancy. Data from the two samples obtained from each mare during each week were entered individually in the database to increase the number of observations within each group-week interaction. To further test for the steroidogenic actions of eCG, the concentrations of each steroid hormone were analyzed within each type of gestation by a regression model that considered the linear and quadratic effects of the week of gestation as well as the linear and quadratic effects of eCG concentrations as independent variables. The fitted lines resulting from those equations and the lines fitted when the effects of eCG were removed were compared against the actual observed concentrations of each steroid hormone.