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Placental development during early pregnancy in sheep: estrogen and progesterone receptor messenger RNA expression in pregnancies derived from in vivo–produced and in vitro–produced embryos

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ABSTRACT

Sex steroids are important regulators of angiogenesis and growth in reproductive tissues, including the placenta. In experiment (exp.) 1, to examine the expression of a suite of sex steroid receptors throughout early pregnancy, maternal (caruncular [CAR]) and fetal (fetal membranes [FM]) placental tissues were collected on days 14 to 30 after mating and on day 10 after estrus (nonpregnant controls). In exp. 2, to examine the hypothesis that assisted reproductive technology would affect the expression of the same suite of sex steroid receptors, pregnancies were achieved through natural mating (NAT) or transfer of embryos from natural mating (NAT-ET), in vitro fertilization (IVF), or in vitro activation (IVA), and CAR and FM were collected on day 22. In exp. 1, for CAR messenger RNA (mRNA) expression of estrogen receptors (*ESR*) *1* and *2*, nuclear (*n*) progesterone receptors (*PGR*) and membrane (*m*) *PGRα*, *β*, and *γ* were affected ($P < 0.02$) by pregnancy stage, as were *ESR1*, *nPGR*, and *mPGRα*, *β*, and *γ* for FM ($P < 0.03$). In exp. 2, for CAR, mRNA expression of *ESR1* and *nPGR* was decreased ($P < 0.001$) in NAT-ET, IVF, and IVA groups compared with NAT. For FM, mRNA expression of *ESR1* tended to be greater ($P = 0.10$) in the IVA group compared with NAT and NAT-ET, and *GPER1* was greater ($P < 0.05$) in NAT-ET and IVF compared with NAT. These data establish the normal pattern of sex steroid receptor mRNA expression in maternal and fetal placenta during early pregnancy in sheep, and in addition, suggest that altered expression of placental sex steroid receptors may be an early event leading to poor placental vascularization and growth after assisted reproductive technology.

1. Introduction

The critical events that occur during early pregnancy include dramatic growth and development of the embryo/fetus as well as growth and vascularization of the placenta [1–4]. It is not surprising, therefore, that most embryonic loss, amounting to 30% to 50% of fertilized oocytes, occurs during early pregnancy in all mammals studied, including humans [1–4].

Sex steroids are important regulators of uterine blood flow. For example, intravenous administration of estradiol (E2) to chronically ovariectomized ewes results in a 6- to 10-fold increase in uterine blood flow within 90 min [5,6]. In addition, chronic (14 d) administration of E2 to ovariectomized ewes mimics many of the cardiovascular and hemodynamic changes seen during pregnancy, including

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increased cardiac output, blood volume, and uterine blood flow and decreased systemic vascular resistance and mean arterial pressure [7]. In addition, progesterone (P4) moderates the vasoactivity of estrogens [8–14].

Estrogens also regulate endometrial (maternal placental) angiogenesis, angiogenic factor expression, and nitric oxide synthase expression in several species [6,11,12,15–18]. For example, administration of physiological levels of E2 to ovariectomized ewes increases vascular endothelial growth factor expression by 30-fold within 4 h and size of the endometrial capillary bed by 2-fold within 24 h [15,16].

Progesterone also is critical for maintenance of pregnancy, in part because of its effects on uterine function, and thereby, along with interferon tau, on embryonic survival and growth [19–25].

Recently, we have shown a dramatic increase in maternal placental and fetal placental angiogenesis during early pregnancy [26,27]. Moreover, we have shown that assisted reproductive techniques (ART), including in vitro fertilization (IVF) and embryo transfer (ET), affect vascularization of the uteroplacenta (defined as uterine endometrium plus fetal chorioallantois, which together form the functional placenta [1–4,28–31]) and fetal and placental size as early as day 22 of pregnancy [32,33].

Despite their potential importance, only a few studies have evaluated sex steroid receptor expression in early uteroplacenta. During early pregnancy in sheep, expression of the classical nuclear estrogen and P4 receptors (*ESR* and *PGR*, respectively) decreases in several uterine compartments except the vasculature, where it remains elevated and undoubtedly drives both blood flow and angiogenesis in the uteroplacenta [5,7,34–38]. The decrease in sex steroid receptors in endometrial epithelium also occurs in humans and seems to be critical for successful pregnancy establishment [39]. However, for placenta during early pregnancy, the pattern of change in messenger RNA (mRNA) expression of the nuclear *ESR* and *PGR* has not been examined, and in addition, little is known about membrane *ESR* and *PGR*.

The purpose of these experiments, therefore, was to examine uteroplacental mRNA expression of a suite of sex steroid receptors during early pregnancy. We hypothesized that placental (endometrial caruncular [CAR; maternal placental] and fetal membrane [FM; chorioallantois or fetal placental]) mRNA expression of sex steroid receptors would: (1) change throughout the first 30 d of early pregnancy and (2) be affected by ART. Therefore, to better understand their physiological role(s), we quantified the expression of mRNAs for nuclear (n) and membrane (m) receptors for estrogens (*ESR1*, *ESR2*, and *GPER1*) and P4 (*nPGR* and *mPGR*α, β, and γ) in the placenta throughout early pregnancy (experiment [exp.] 1), and also in pregnancies resulting from ART (exp. 2).

2. Materials and methods

2.1. Animals

The Institutional Animal Care and Use Committee at North Dakota State University approved all animal procedures in this study.

For exp. 1 (n = 73 ewes) and 2 (n = 67 ewes), estrus was synchronized during the breeding season in naturally bred donor and recipient ewes (primarily Rambouillet, Targhee, and Columbia crossbred, sometimes referred to as Western Range ewes) using a controlled internal drug release (CIDR) device (MWI, Boise, ID) implanted for 14 d [26,32].

2.2. Experiment 1

This experiment has been described in detail by Grazul-Bilska et al [26,27]. Briefly, 24 h after CIDR removal, ewes were exposed to a fertile ram and allowed to breed. To establish the day of breeding (day 0), the rams were outfitted with a marking harness with colored crayons, and breeding marks were recorded twice daily. Gravid uteri were obtained from the ewes (n = 6–8/d) on days 14, 16, 18, 20, 22, 24, 26, 28, and 30 after mating and also from midluteal, nonpregnant (NP; day 10 after estrus; n = 8) control ewes. Maternal (CAR) and fetal (FM [chorion on day 16 and chorioallantois on days 18–30; FM was not evaluated for day 14 because of the small amount of tissue present]) placental tissues were dissected from the area of the uterus closest to the embryo (ie, from the external intercornual bifurcation to the uterine body, within approximately 2–4 cm of the embryo), snap-frozen, and stored at −70°C for extraction of total cellular RNA.

2.3. Experiment 2

In this experiment, which has been described in detail by Grazul-Bilska et al [32,33], in addition to the control group which was naturally bred (NAT), we chose 3 ART methods to establish pregnancies as follows (in each, we induced superovulation by multiple injections of FSH, as described in the following): (1) natural breeding, embryo flushing from donors and transfer to recipients (NAT-ET), (2) transfer of embryos obtained through IVF, and (3) transfer of embryos obtained through in vitro activation (IVA; ie, parthenotes, which are clones containing maternal genes only) of oocytes.

Briefly, 24 h after CIDR removal, NAT ewes were exposed to a fertile ram and allowed to breed. The day of breeding (day 0) was established by using breeding harnesses as described for exp. 1. For donor ewes from NAT-ET, IVF and IVA groups, after CIDR removal estrus was checked twice daily using a vasectomized ram outfitted with a breeding harness. Beginning on day 13 of the estrous cycle, donor ewes from the NAT-ET group were treated twice daily with FSH for 3 d, whereas donor ewes from IVF and IVA groups were treated with FSH for 2 d, as we have described [32,33,40]. On day 15 of the estrous cycle, donor ewes from the NAT-ET group were exposed for 24 to 48 h to a fertile ram outfitted with a breeding harness, and breeding marks were recorded twice daily. For donor ewes from the IVF and IVA groups, ovaries were collected on day 15, and cumulus oocyte complexes were isolated from follicles >3 mm, matured, and then fertilized or activated in vitro as described in detail previously [32,33].

Briefly, for IVF and IVA procedures, up to 30 cumulus oocyte complexes/0.5 mL in a 4-well Nunc culture dish were incubated overnight in maturation medium (TCM199;