Animal Reproduction Science 164 (2016) 14–22

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Uterine and placenta characteristics during early vascular development in the pig from day 22 to 42 of gestation☆

Elane C. Wright*, Jeremy R. Miles, Clay A. Lents, Lea A. Rempel

USDA-ARS U.S. Meat Animal Research Center (USMARC), P.O. Box 166, Clay Center, NE 68933, United States[1]

ARTICLE INFO

Article history:
Received 11 May 2015
Received in revised form 14 October 2015
Accepted 2 November 2015
Available online 10 November 2015

Keywords:
Placenta development
Microfold
Necrotic tip
Pig
Reproduction

ABSTRACT

Insufficient placenta development is one of the primary causes of fetal death and reduced fetal growth after 35 days of gestation. Between day 22 and 42 the placenta consists of a central highly vascular placenta (HVP), adjacent to the fetus, a less vascular placenta (LVP), on either side of the fetus, and necrotic tips (NT). The objective of this study was to comprehensively evaluate uterine–placenta characteristics during early gestation in the gilt and determine time points and physiological changes. Gilts ($n = 25$) were artificially inseminated at first detection of estrus (day 0) and 24 h later, and harvested at 22, 27, 32, 37 or 42 days of gestation. Litter size, 12.1 ± 3.4, was similar for all days of gestation. Fetal and placenta weight increased with day of gestation. The greatest increase in placenta weight occurred between 37 and 42 days of gestation. The LVP zones had no measurable fold formation until day 27. Necrotic tips became apparent after 27 days of gestation. Unoccupied areas of the uterus developed folds with changes in endometrial cell size and morphology from day 32 to 42 of gestation. Limited changes occurred in either fetal growth or placenta weight from day 27 through 32 of gestation; however, significant morphological changes occur at the maternal–fetal interface, demonstrating the dynamic architecture of the developing porcine placenta during early gestation. This work establishes fundamental time points in placenta development corresponding to fetal growth and microfold formation that may influence fetal growth and impact fetal survival.

Published by Elsevier B.V.

☆ Mention of trade names is necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the same by USDA implies no approval of the product to the exclusion of others that may also be suitable.

* Corresponding author.
E-mail address: elane.wright@ars.usda.gov (E.C. Wright).

[1] The U.S. Department of Agriculture (USDA) prohibits discrimination in all its programs and activities on the basis of race, color, national origin, age, disability, and where applicable, sex, marital status, familial status, parental status, religion, sexual orientation, genetic information, political beliefs, reprisal, or because all or part of an individual's income is derived from any public assistance program. (Not all prohibited bases apply to all programs.) Persons with disabilities who require alternative means for communication of program information (Braille, large print, audiotape, etc.) should contact USDA's TARGET Center at (202) 720-2600 (voice and TDD). To file a complaint of discrimination, write to USDA, Director, Office of Civil Rights, 1400 Independence Avenue, S.W., Washington, D.C. 20250-9410, or call (800) 795-3272 (voice) or (202) 720-6382 (TDD). USDA is an equal opportunity provider and employer.

http://dx.doi.org/10.1016/j.anireprosci.2015.11.002
0378-4320/Published by Elsevier B.V.

1. Introduction

The pig, a litter bearing animal, is an excellent model for the study of early fetal development and placentation, because there is a high incidence of prenatal mortality (30–50%) during the first 40 days of gestation which could be due in part to inefficiencies in placenta development (Geisert and Schmitt, 2002; Pope and First, 1985). The epitheliochorial placenta of the pig transports gases, nutrients, and waste between the maternal and fetal systems (Reynolds and Redmer, 1995). The placenta, consisting of a microfold bilayer at the maternal–fetal interface, begins forming during early gestation, and can be used to predict fetal survival in the pig (Dantzer, 1985; Rootwelt et al., 2012, 2013). Placenta development is influenced by litter size, vascular development, stromal depth, and uterine capacity (Freking et al., 2007; Knight et al., 1977). Sows with high ovulation rates have an increased number of embryos at 30 days of gestation however not all embryos will survive day 50 and a high number of embryos has a negative impact on placenta weight (Foxcroft et al., 2009a). Increased litter size causes increased uterine crowding, thus, decreased placental and fetal weights, which is associated with increased preweaning mortality of piglet (Foxcroft et al., 2009b; Mesa et al., 2012; Miles et al., 2008; Rootwelt et al., 2012, 2013; Vallet and Freking, 2007). Placenta development including vascularity and stromal depth occurs once the conceptus has implanted and the interaction between the maternal and fetal tissue has been established. Five placental zones can be distinguished at the end of the first trimester: placental zone, two paraplacental zones and necrotic tips at the terminal ends (Ashdown and Marrable, 1967; Flood, 1973). These early studies observed more blood and vasculature in the placenta zone closest to the fetus compared to the adjacent attached placenta (Flood, 1973). Therefore, the central zone adjacent to the fetus can be referred to as the highly vascular placenta (HVP) and the remaining attached placenta on either side will be the less vascular placenta (LVP). The uterine area between placentas and the unattached placenta will be referred to as the necrotic tip (NT). Development of the microfolds and placenta and uterine vascularity within each zone during early gestation could have an effect on piglet growth and survival throughout gestation. Studying microfold development and the maternal fetal interacts should provide insight to improve fetal development and survival. Fetal losses during the 22–42 day window will likely be due to failure to establish adequate nutritional pathway caused by insufficient maternal fetal attachment or inadequate microfold development. Previous studies showed irregular folds at 26 days of gestation and regular folds by 50 days of gestation and beyond (Miles et al., 2009). However, microfold development has not been closely examined during the latter part of the first trimester of development in the pig. The objective of this study was to characterize morphological changes that occur in the allantochorion and uterine endometrium from day 22 to 42 of gestation, which could impact to utilization of uterine space, placenta and fetal development, and establishment of the maternal fetal interface. We hypothesize that alterations in placenta area or microfold development will be reflected in fetal growth and survival during early gestation.

2. Materials and methods

2.1. Animal care and use

All animal protocols were in accordance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 1999). Twenty-five normally cycling White crossbred gilts (Landrace × Yorkshire × Duroc) were observed for estrus daily. Cyclic gilts were artificially inseminated at the detection of estrus (day 0) and again 24 h later. The same sire was used for all gilts. Gilts were group housed and received the same diet. At the time of AI, gilts were randomly assigned to a day of gestation for slaughter, i.e. 22, 27, 32, 37 or 42 days of gestation. Following slaughter, reproductive tracts were immediately removed and uterine tissues, placenta tissues and fetuses were isolated, evaluated and sampled for future analysis.

2.2. Tissue collection and isolation

The reproductive tract was dissected from the broad ligament membranes. Upon collection of each uterine tract the following measurements were made. The number of corpora lutea per ovary and total uterine weight and length, measured from the anterior of the cervix to the utero-tubular junction of both horns, were recorded. The tract was then opened along the antimesometrial border. Fetuses were removed from the placenta tissues and numbered in sequential order from the tip of the uterine horn to the cervix for each horn to identify their location. Fetuses were individually weighed and the largest, average and smallest fetuses within each litter were identified for additional sample collection of associated placenta and uterine tissues. The placenta was sampled at the HVP and LVP, adjacent uterine samples were also collected as well as the necrotic tips and the uterine area between fetuses with no placenta attachment (Fig. 1). The HVP was located directly adjacent to the fetus and was dark red or purple in color. The length of the HVP region was measured proximal to distal from its medial point. The LVP was measured from the outside border of the HVP to the outer edge of fetal tissue attachment to the uterus. The LVP was pinker in color than the HVP. Unoccupied uterus, where no fetal attachment is observed, on either side of each fetus was measured. Placenta and uterine tissues from the HVP, LVP and unoccupied uterine area on either side of the attached placenta associated with the smallest, average and largest fetuses were sampled from each tract for histological analysis. A 2 cm × 2 cm section of the allantochorion attached to the uterus was dissected from the HVP, LVP and adjacent unoccupied space. Samples were fixed in 10% buffered formalin with rocking overnight at room temperature. Samples were washed twice in PBS for 1 h, followed by incubation in a graded series of ethanol (1 h, 25% ethanol; 1 h, 50% ethanol; 1 h, 70% ethanol; twice each). Samples were then stored in 70% ethanol at 4 °C until embedded in paraffin. Standard paraffin embedding techniques were followed. Paraffin-embedded tissues were sectioned (6 μm),