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Pig blastocyst–uterine interactions

Fuller W. Bazer [a,*], Gregory A. Johnson [b]

[a] Departments of Animal Science, Texas A&M University, College Station, TX 77843, United States
[b] Veterinary Integrative Biosciences, Texas A&M University, College Station, TX 77843, United States

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ABSTRACT

The litter-bearing pig is an invaluable model for research in reproductive biology. Spherical pig blastocysts on Day 10 of pregnancy undergo rapid morphological changes to tubular and then filamentous forms by Day 12 and a filamentous conceptus of almost 1 m in length by Day 16 of pregnancy. Thus, trophectoderm of each conceptus achieves intimate contact with luminal uterine epithelium (LE) for exchange of nutrients, gases, hormones, growth factors and other key molecules for survival and development. Estrogens secreted between Days 11 and 13 of pregnancy signals pregnancy recognition to ensure that nutrients and prostaglandin F2-alpha (PGF) are secreted into the uterine lumen (exocrine secretion) rather than into the uterine vein (endocrine secretion) which would lead to regression of the corpora lutea (CL) and failure to maintain pregnancy. Pigs have a true epitheliochorial placenta. The fluid filled amnion bouys the embryo so that it develops symmetrically. The allantois fills with allantoic fluid to expand contact of the chorioallantois with uterine LE, and the allanotois supports the vascular system of the placenta. The chorion/trophectoderm in direct contact with uterine LE exchanges gases and nutrients and forms unique structures call areolae that absorb nutrient-rich secretions from uterine glands and transports them directly into fetal blood. The period from Days 20 to 70 of pregnancy is for placental growth in preparation for rapid fetal growth between Days 70 and 114 (term) of gestation. Maturation of the fetal hypothalamic–pituitary–adrenal axis leads to increases in secretion of cortisol from the fetal adrenal glands. Cortisol sets in motion secretion of estrogens, oxytocin, relaxin and prolactin, as well as increases in their receptors, which are required for delivery of piglets and for initiation of lactation and expression of maternal behavior. This review provides details of gestation in the pig with respect to uterine biology, implantation, placentation, fetal development and parturition.

1. Introduction

Domestic livestock species have provided biomedical research with a number of compelling models that have yielded opportunities to generate insights into physiology and disease. Many unique aspects of pregnancy in farm animals have been exploited by prominent scientists to unravel the mechanisms that underlie fertility and fetal development in mammals, but domestic animal research models remain generally underappreciated by the current scientific community at large (Roberts et al., 2009).

Classical animal models may have limited usefulness in representing the human condition for studies of disease and for testing drugs for treatment of diseases such as cystic fibrosis, spinal muscle atrophy and Parkinson's disease; however, the pig is an excellent model for research on human health and disease due to similarities in anatomy, genetics, cardiovascular and gastrointestinal systems, and pathophysiology (Walters et al., 2012). With improved understanding of their genome, the pig is expected to be an even more important animal model for research on the genetic basis for human diseases. Another advantage of pigs is that females reach puberty at about 7 months of age, have a gestation period of only 114 days and produce a litter that averages 9 to 10 piglets. Pregnant pigs have been an exceptional resource for studies to understand all aspects of reproductive biology, including embryonic/fetal survival, growth and development, placentation, nutrient and gas exchange between the maternal and conceptus systems, the endocrinology of pregnancy, mammogenesis and lactogenesis.

In gilts (nulliparous females) and sows (parous females), mating occurs during estrus, which lasts 24 to 72 h, and ovulation occurs approximately 44 h after onset of estrus. After fertilization, embryos are near the ampullary-isthmic junction of the oviduct before entering the uterus (Bazer and First, 1983). Embryos fail to develop beyond the early blastocyst stage if confined to the oviduct in pigs due to either the absence of essential factors for embryonic development or the presence of embryotoxic or embryostatic factors. If the female becomes pregnant, a gestation period of 114 days follows. Gestation in pigs varies greatly from that of rodents and primates, and therefore provides an excellent platform for the generation of knowledge to inform, challenge and

* Corresponding author. Tel.: +1 979 862 2659; fax: +1 979 862 3399.
E-mail address: fbazer@cvm.tamu.edu (F.W. Bazer).

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corroborate studies from traditional laboratory animals. The following review highlights unique events of pig pregnancy including: (1) elongation of the blastocyst into a filamentous conceptus (embryo and associated placental membranes) (Figs. 1 and 2); (2) the protracted peri-implantation period of pregnancy when the conceptus is free within the uterine lumen requiring extensive paracrine signaling between conceptus and endometrium, as well as nutritional support provided by uterine secretions (Fig. 3); (3) a protracted and incremental attachment cascade of trophectoderm to uterine luminal epithelium (LE) during implantation (Fig. 3); (4) development of a true epitheliochorial placenta that utilizes extensive uterine and placental vasculatures for hematotrophic, and placental areolae for histotrophic support of the developing fetuses (Fig. 4); and parturition (Fig. 5).

1.1. *Elongation of the blastocyst into a filamentous conceptus (Fig. 1)*

The following section is based on published research by Bazer and First (1983), Geisert et al. (1982a, 1982b). The pig embryo moves from the oviduct into the uterus at about the four-cell stage, i.e., 60 to 72 h after onset of estrus and reaches the blastocyst stage by Day 5. The spherical blastocyst (0.5–1 mm diameter) sheds the zona pellucida between Days 6 and 7 and expands to 2 to 6 mm diameter on Day 10. At this stage development of pig embryos diverges dramatically from rodents or primates, and the presumptive placental membranes (trophectoderm and endoderm) elongate rapidly to a filamentous form by Day 16. These dramatic changes in morphology occur just before initial attachment of trophectoderm to uterine LE for what is defined as non-invasive "central-type" implantation (Steven, 1975). The presence of spherical, tubular and filamentous conceptuses within the same uterine horn of pigs suggests a rapid transition from late spherical to filamentous forms (Fig. 1). It is during this period of morphological and functional transition that 30 to 40% of the conceptuses die perhaps due to failure to elongate and achieve extensive contact of trophectoderm with uterine LE which is essential for uptake of secretions from uterine glandular epithelium (GE) for survival of the conceptus.

Blastocysts undergo a rapid initial transition from spherical to tubular and elongated filamentous forms between Days 10 and 12 of pregnancy (Fig. 1). Blastocysts expand at about 0.25 mm/h between the early spherical blastocyst stage to the 4 to 9 mm diameter spherical blastocyst stage. Then, there is a remarkable increase in the rate of elongation to 30 to 45 mm/h from the 10 mm blastocyst to the 150 to 200 mm long filamentous conceptus that occurs within a few hours due primarily to an increase in cellular hypertrophy. Total DNA is not different between spherical (9 to 10 mm), tubular (11 to 50 mm) and early filamentous (> 100 mm) blastocysts (Day 12). The mitotic index of tubular blastocysts is less than for spherical blastocysts indicating that cellular hyperplasia does not account for initial elongation of pig blastocysts. The rapid morphological changes in both trophectoderm and extra-embryonic endoderm occur when blastocysts are 10 mm in diameter. At this stage, a dense band of cells (the elongation zone) composed of both endoderm and trophectoderm extends from the inner cell mass (ICM) to the tip of the ovoid blastocyst. After formation of the elongation zone, there is further rapid elongation of the 100 to 200 mm long pig conceptus to a conceptus of 800 to 1000 mm in length by Day 16 of pregnancy mediated through alterations in microfilaments and junctional complexes of trophectoderm cells and formation of filapodia by endodermal cells. This last period of elongation involves cellular hyperplasia and each conceptus within the litter achieves maximum surface area for contact between trophectoderm and uterine LE to facilitate uptake of nutrients from uterine LE and uterine glandular epithelium (GE) which increase coincidentally with elongation of the conceptuses (Fig. 1).

Total DNA and RNA in porcine conceptuses increase exponentially from Days 10 through 16. Spherical blastocysts with diameters of 5.0, 5–7, 8–9 and 10–20 mm have mitotic indices (Mean ± SEM) of 1.8 ± 0.04, 1.6 ± 0.06, 1.8 ± 0.04 and 1.0 ± 0.03, respectively. The mitotic index then decreases almost 40% at the onset of blastocyst elongation (10 to 20 mm tubular blastocysts).

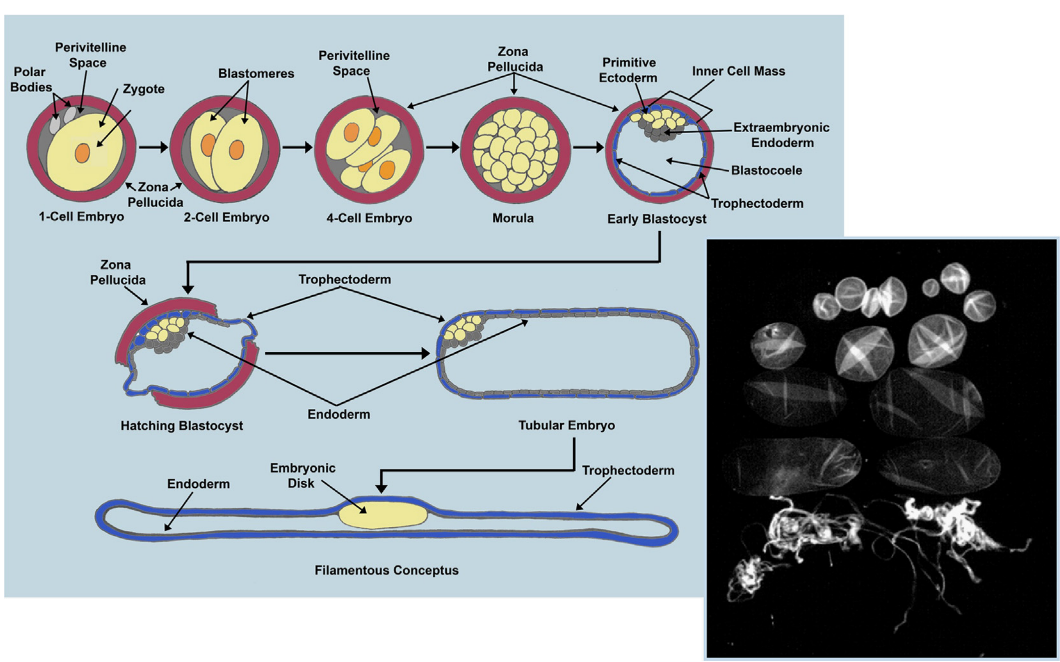

Fig. 1. Pig embryos enter the uterus at about the 4-cell stage, hatch from the zona pellucida around Day 7, reach the expanded blastocyst stage around Day 10 and then change rapidly in morphology from spherical to tubular to filamentous forms to achieve maximum area of surface contact between the trophectoderm and uterine luminal epithelium. The inset shows photos of pig conceptuses, from top to bottom, from Days 10 (small spherical), 10.5 (large spherical), 11 (tubular) and 12 (filamentous) of gestation.

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