Use of Asynchronous Embryo Transfer to Investigate the Role of Uterine-embryo Timing on Placental Size

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Summary and Implications

The ability of the uterus to accommodate a finite amount of placental tissue appears to be a major limitation to litter size. Meishan preimplantation conceptuses contain fewer cells, produce less estradiol-17β, elongate to a shorter length, and exhibit a reduced placental size throughout gestation than Yorkshire conceptuses. Uterine luminal embryonic estradiol-17β and growth factor content are positively associated at elongation. Based on these data, we have argued that growth factor quantity regulates the length an embryo attains at elongation, and ultimately limits placental size. Recently, we injected Meishan gilts every 6 hours with estradiol-17β on day 12 and 13 of gestation, resulting in a 40% increase in placental size at term compared with vehicle-injected Meishan gilts. This study was conducted to determine if transfer of embryos into the oviducts of asynchronous females (more or less advanced uterine environments) would alter fetal and/or placental size at term. Embryos (1 to 4 cells) were flushed from the oviducts of each donor gilt on day 2.5 of gestation and transferred in equal numbers to the oviducts of a recipient gilt on day 1.5, 2.5, or 3.5 of their estrous cycle. Gilts were slaughtered on day 112 of gestation and fetal and placental weight, placental surface area, and implantation site lengths were determined. Although litter sizes were similar (8.4 ± 1.1), conceptuses transferred to day 3.5 recipients had heavier fetuses (1.57 ± .09 vs. 1.23 ± .04 kg, P<.001), larger placental surface area (1812 ± 106 vs. 1458 ± 43 cm^2, P<.01) and occupied longer implantation site length (34 ± 3 vs. 25 ± 1 cm, P<.001) than those transferred to recipients on day 1.5 or 2.5. These data demonstrate that oviductal transfer of embryos to a reproductive tract as little as 24 hours more advanced can result in dramatic alterations in placental growth and function during gestation.

Introduction

Litter size is widely thought to be the most important component of sow productivity. Currently, U.S. pig breeds average 8.8 pigs per litter, which is far from optimal when one considers that ovulation rate is 14 to 16, of which 95% are fertilized and sows have between 12 and 14 functional teats. Litter size can be impacted by a number of different physiologic factors, including ovulation rate, fertilization rate, and embryo mortality. However, when sufficient numbers of embryos survive periods of early loss (i.e., 12 to 18 days of gestation), placental size and uterine size can effect the number of fetuses that survive to term.

We use the piglet weight-to-placental weight ratio (i.e., the number of grams of placenta required to support a gram of fetus) as an indication of placental efficiency. Recently, we have demonstrated that selection of Yorkshire gilts and boars for small, efficient placentae resulted in a significant increase in litter size compared with those selected for large, inefficient placentae. Around day 12 of gestation the embryo begins to elongate and secrete estradiol-17β which stimulates marked changes in the secretion of endometrial components, including growth factors. We have shown that estradiol-17β administration around the time of elongation (likely increasing endometrial growth factor secretion) results in markedly increased placental size at term. These data suggest that the uterine environment an embryo is exposed to prior to or during elongation can impact conceptus growth and survival. Our hypothesis was that as conceptus growth is a consequence of estradiol-17β stimulated endometrial secretion of growth factors, a narrow window of time for this endometrial-conceptus interaction may exist. If so, inducing an asynchrony between conceptuses and the oviductal-uterine environment ought to effect placental size. The objective of this experiment was to determine the impact of embryo-uterine asynchrony on conceptus growth and development.

Materials and Methods

Yorkshire gilts (n=24) were checked twice daily for estrous behavior (0700 and 1900) with a mature Yorkshire boar. Twelve gilts served as embryo recipients and 12 served as embryo donors. Recipient gilts fell into one of three categories: 1) those that first exhibited estrus 24 hours after a donor had first exhibited estrus (24 hours less advanced oviductal-uterine environment), 2) those that first exhibited estrus synchronously with a donor (synchronous oviductal-uterine environment), or 3) those that first exhibited estrus 24 hours after a donor had first exhibited estrus (24 hours more advanced oviductal-uterine environment). On day 2.5 of gestation donor gilts were anesthetized, laparotomized, and embryos recovered from the oviducts. Recipient gilts (either day1.5, 2.5, or 3.5 of their estrous cycle) were then anesthetized, laparotomized, and had one-half of the embryos transferred to each oviduct.

Recipient gilts were then slaughtered on day 112 of gestation. Gravid uteri were collected and transported back to the laboratory on ice. Ovulation rate, litter size, and intact uterine horn length were then determined. Each fetus was then exteriorized and its sex, weight and crown-rump length determined. A block of placental-endometrial tissue was excised for each conceptus for histologic determination of vascular density. Dissecting pins were placed at either end of each placenta, and each placenta was then separated from the endometrium and its weight determined. Implantation site length was determined by measuring the distance between dissecting pins. Placentae were then spread out on plastic coated ‘Butcher’s paper’ and their perimeter traced for determination of placental surface area.

Results and Discussion

There were no differences in combined uterine horn length or litter size among the three recipient groups; those receiving day 2.5 embryos on day 1.5, those receiving embryos synchronously on day 2.5, or those receiving day 2.5 embryos on day 3.5 (Table 1). However, there was a positive correlation between combined uterine horn length and litter size across all three groups (Figure 1).

Table 1. Combined uterine horn length and litter size for all three recipient groups.

Recipient Group	Combined Uterine Horn Length	Litter Size
Day 1.5 (n=4)	472 ± 39	9.0 ± 1.4
Day 2.5 (n=3)	546 ± 84	9.7 ± 1.2
Day 3.5 (n=3)	586 ± 125	8.7 ± 2.3

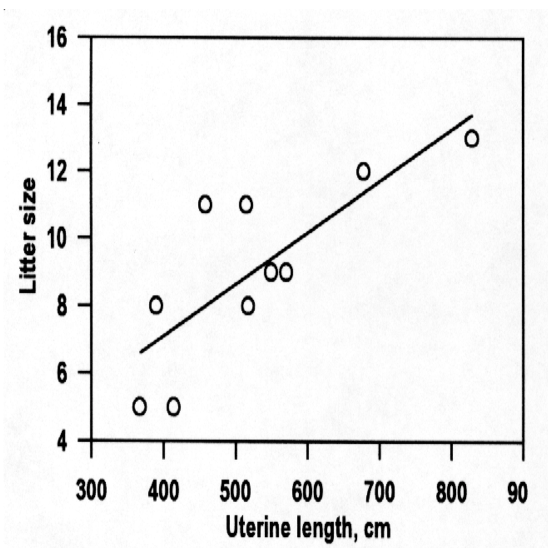

Figure 1. Relationship between litter size and uterine length across all three recipient groups.

Both fetal weight and crown-rump length were markedly greater for recipient females receiving embryos on day 3.5 of their estrous cycle compared with the other two recipient groups (Figure 2). There were no differences in placental weight among the three recipient groups (Figure 3). Implantation site length on day 112 of gestation was markedly greater for conceptuses gestated by day 3.5 recipients and was slightly less for conceptuses gestated by day 1.5 recipient females, compared with those receiving synchronously transferred embryos (Figure 3). Both placental surface area and placental efficiency were markedly greater for conceptuses gestated by day 3.5 recipient females compared with the other two recipient groups (Figure 4).

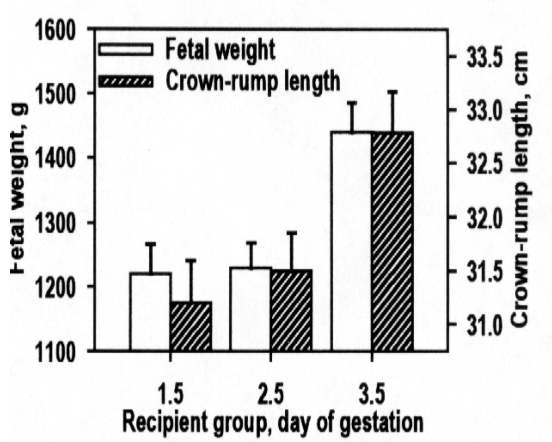

Figure 2. Weight and crown-rump length of fetuses gestated by each of the three recipient groups.

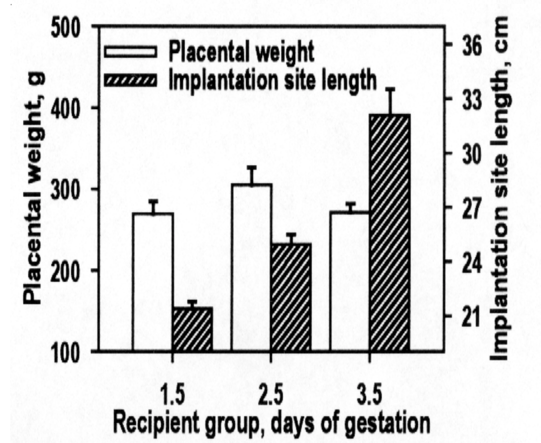

Figure 3. Placental weights and implantation site lengths for conceptuses gestated by each of the three recipient groups.

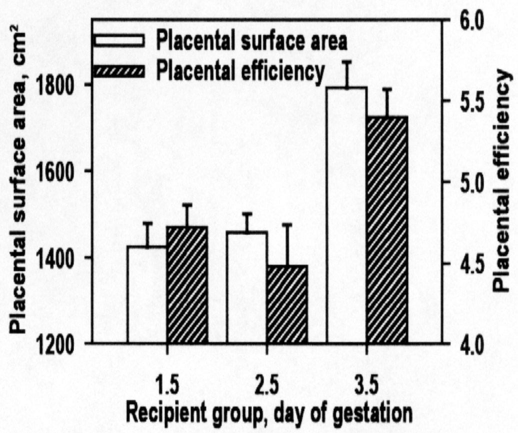

Figure 4. Placental surface area and efficiency for conceptuses gestated in each of the three recipient groups.

As we have previously reported, there was no association between placental efficiency and fetal weight across the three recipient groups. However, there was a significant negative correlation between placental efficiency and placental weight across the three recipient groups ($r = -.74$, $P < .01$). There were no differences in placental or endometrial vascular density among the conceptuses of the three recipient groups. There was, however, a positive correlation between placental and the adjacent endometrial vascular density across the three recipient groups ($r = .68$, $P = .03$) Interestingly, there were also positive correlations between placental efficiency and both placental and endometrial vascular densities ($r = .70$, $P = .02$ and $r = .26$, $P = .07$, respectively).

These data demonstrate that events during very early embryo development can lead to long-term differences in fetal and placental growth and development. Asynchrony between the conceptus and maternal system very early in development can have a tremendous affect on placental growth, suggesting that an optimal window of time for conceptus-uterine interaction may exist. Although these data do not eliminate the potential of differing oviductal environments impacting later conceptus growth, comparisons of pig breeds exhibiting marked differences in early embryonic development (i.e., Meishan vs. Yorkshire), demonstrated no breed differences between oviductal embryo development, but breed differences become evident by day 5.5 to 6 of gestation. Previously, we have shown that the modulation of the uterine environment, with exogenous estradiol-17β treatment, around the time of conceptus elongation can have a marked impact on placental size and weight. Potentially, as a result of transferring day 2.5 embryos into the oviducts of a day 3.5 recipient female, we may have allowed for a more uniform or robust endometrial growth factor response to conceptus estradiol-17β, thus growing a larger conceptus. Although we found that transfer of embryos to day 1.5 recipients resulted in a slight decrease in implantation site length, there were no differences in placental surface area and placental weight compared with those transferred to day 2.5 recipients. The difference in the length of occupied horn must be the result of slight increases in placental circumference or placental interdigitation with the endometrium.

As reported previously, we find no association between the weight of a fetus and placental efficiency; however, a strong negative correlation exists between placental weight and placental efficiency. Thus, both large and small fetuses can grow on either relatively efficient or inefficient placentae, but those placentae that are most efficient are small and those that are least efficient are large. There was a marked positive correlation between placental and the adjacent endometrial vascular density across the three groups. This association would imply that communication between the mother and conceptus exists to optimize nutrient transfer. This is consistent with data in the ewe suggesting that placental tissue (i.e. cotyledon) produces angiogenic factors during late gestation at a time when placentomal vascularity is increasing.

We have used the piglet weight-to-placental weight ratio for some time as an indicator of placental function, often referring to it as placental efficiency. These are the first data to demonstrate an association between our functional measure of placental efficiency and the vascularity of the placental membranes. Further, these data demonstrate that the level of placental efficiency is associated with the vascularity of the adjacent endometrium.

Therefore, attempts to optimize reproductive efficiency need to consider a number of aspects of conceptus growth and development as well as uterine growth and function. Placental size can markedly influence litter size and events that occur very early in embryo development can tremendously influence placental size. However, factors such as insufficient ovulation rate, insufficient fertilization rate, poor uterine capacity (either as a result of diminished endometrial function or less than optimal uterine length), etc. can all impact the ultimate litter size realized at term.