Effect of Estradiol-17β on Placental Size

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Summary and Implications

Meishan embryos transferred to recipient females on day 2.5 are larger, contain greater numbers of trophectoderm cells, and secrete greater amounts of estradiol-17β when gestated in a Yorkshire compared with Meishan uterus to day 12. Additionally, placentae of Meishan conceptuses are larger when gestated in a Yorkshire compared with Meishan uterus throughout gestation. Embryonic estradiol-17β secretion during elongation on day 12 to 13 of gestation is temporally associated with endometrial secretion of growth factors, including IGF-I, which has been shown to increase mitotic rate in the trophectoderm of pig embryos. This experiment was conducted to determine if estradiol-17β administration to Meishan females at the time of conceptus elongation would increase placental size at term. Meishan females (n=12) were checked twice daily for estrus (0700 and 1900), and each bred to a Meishan boar at 0 and 24 h after the onset of estrus (day 0). Females were randomly assigned in equal numbers to receive injections of sesame oil (VEH) starting on day 12 (CONTROL), 1 mg of estradiol-17β in VEH starting on day 12 ($E_2$12), or 1 mg of estradiol-17β in VEH starting day 13 ($E_2$13). The injections were initiated at 0700 or 1900 (corresponding to the time of day they first exhibited estrus) and continued at 6-hour intervals for 48 hours, resulting in 8 mg of estradiol-17β given in eight injections. Pregnant females were killed on day 112 of gestation and ovulation rate, litter size, implantation site length, fetal weight, crown-rump length, placental weight, and placental surface area were quantified. There were no differences among $E_2$12, $E_2$13, and CONTROL females in ovulation rate or litter size, which averaged 16.3 ± .7 and 11.8 ± .7, respectively. Fetal weight and crown-rump length were not different (P>.10) among $E_2$12, $E_2$13, and CONTROL females, averaging 802 ± 26 g and 24.3 ± .3 cm. Placentae were markedly heavier (176 ± 14 and 174 ± 16 g vs. 134 ± 10 g, P<.05) and larger (1337 ± 97 and 1520 ± 70 cm^2 vs. 978 ± 29 cm^2, P<.001) for $E_2$12 and $E_2$13 vs. CONTROL females, respectively. Placental efficiency (estimated as fetal weight:placental weight) was greater (P<.05) in the CONTROL compared with $E_2$12 and $E_2$13 females (5.8 ± .2 vs. 4.8 ± .2 and 5.1 ± .4). These data demonstrate that the amount of estradiol-17β exposure around the time of elongation affects placental size at term. Additionally, the difference in placental efficiency between CONTROL and estradiol-17β groups indicate that estradiol-17β-induced increases in placental size led to a reduced placental efficiency.

Introduction

Meishan gilts (three to seven postpubertal estrous cycles) exhibit the same ovulation rate and uterine size as domestic Yorkshire gilts, while farrowing three to four more piglets/litter. Meishan and Yorkshire conceptuses initiate estradiol-17β secretion and elongation synchronously on day 12 to 13 after mating, although Meishan embryos are smaller and contain fewer cells when they initiate steroidogenesis and elongate. The reduced size of the Meishan conceptus results from a reduced trophectoderm mitotic rate from day 6 to day 12 compared with similar stage Yorkshire conceptuses. Meishan conceptuses secrete less estradiol-17β into uterine luminal fluid and elongate to a reduced length than similar stage Yorkshire conceptuses. The decreased uterine luminal estradiol-17β concentrations in Meishan vs. Yorkshire females on day 11 to 12 of gestation are associated with the lower concentrations of IGF-I in the same flushings. Further, IGF-I has been shown to increase trophectoderm mitotic rate in pig embryos.

Meishan conceptuses exhibit a reduced placental size and weight on day 30, 70, 90, and 110 of gestation and at term than do Yorkshire conceptuses. We hypothesized that the smaller size and length of the Meishan vs. the Yorkshire embryo at elongation may result from the Meishan's decreased secretion of estradiol-17β. The decreased size of the Meishan conceptus at elongation may then lead to the reduced size of the Meishan compared with Yorkshire conceptus throughout gestation. The objective of this experiment was to determine if injections of estradiol-17β around the time of conceptus elongation would result in an increased placental size at term compared with controls.

Materials and Methods

All procedures and protocols involving the use of animals were approved by the Iowa State University Committee on Animal Care. Meishan gilts (n=12) were checked twice daily for estrous behavior (0700 and 1900) with a mature Meishan boar. Gilts were bred at the onset of estrus (day 0) and again 24 hours later. Pregnant gilts were then randomly assigned to receive 1-ml injections (im), of sesame oil (VEH) starting on day 12 (CONTROL), 1 mg of estradiol-17β in VEH starting on day 12 ($E_2$12), or 1 mg of estradiol-17β in VEH starting day 13 ($E_2$13). The injections were administered at 6-hour intervals for 48 hours starting at 0700 or 1900 on day 12 (CONTROL and $E_2$12) or day 13 ($E_2$13) corresponding to the time of day they first exhibited estrus, resulting in a total of 8 mg of estradiol-17β administered in eight injections. Gilts were killed on day 112 of gestation and their reproductive tract removed and transported to the laboratory. The mesometrium was trimmed from the uterus and the base of each horn ligated before separating the right and left horns to determine uterine horn weight (kg), length (cm; measured along the antimesometrial border) and volume (L; by measuring the volume of water displaced by submersion of the horn into a full bucket of water).

Each fetus was then exteriorized through an ~5-cm incision in the antimesometrial aspect of the uterus and the associated chorioallantoic fluid was collected and its volume determined. Each umbilical cord was then double ligated with numbered tags and cut between the ligations. The sex, crown-rump length, and weight of each fetus were determined. Individual placentae were then manually separated from the endometrium, placing a dissecting pin in the endometrium at each end of a placenta to mark its location. The placentae were weighed, and the relative location and distance between each pin determined. This allowed us to determine the length of each implantation site, as well as the distances between implantation sites when they existed. Placentae were then spread out on plastic coated paper ("Butcher's paper") and traced for later determination of placental length and surface area using a planimeter (Compensating Polar Planimeter, Keufel and Esser Co., New York) as previously described.

Results and Discussion

There was no difference in ovulation rate, litter size or percentage of fetal survival among the three treatment groups that averaged 16.3 ± .7, 11.8 ± .7 and 73.1 ± 3.8%, respectively. There were no differences among treatment groups in uterine length, weight or volume which averaged 503 ± 23 cm, 15.8 ± .9 kg and 14.4 ± .8 liters, respectively. There were also no differences in weight or crown-rump length of fetuses among the three treatment groups (Figure 1).

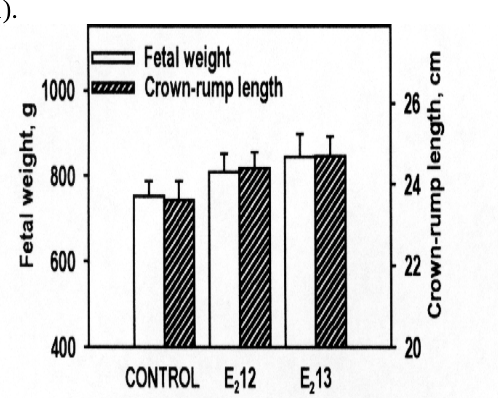

Figure 1. Fetal weight and crown-rump length for conceptuses from CONTROL, $E_2$12 and $E_2$13 treated Meishan females.

There was a marked difference, however, in placental size among the three treatment groups whether measured by weight, surface area, or length. Figure 2 depicts placental weight and surface area. Placental weight was 30% greater in the $E_2$12 and $E_2$13 gilts compared with CONTROL gilts (Figure 2). Placental surface area was 37% greater in $E_2$12 gilts and 55% greater in $E_2$13 gilts compared with CONTROL gilts (Figure 2). Placental length (not depicted) was 22% greater in $E_2$12 gilts and 32% greater in $E_2$13 gilts

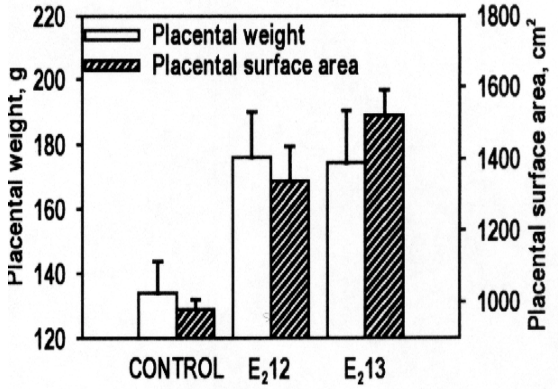

Figure 2. Placental weight and placental surface area for conceptuses from CONTROL, $E_2$12 and $E_2$13 treated Meishan females.

compared with CONTROL gilts. There was a significant increase in implantation site length for $E_2$13 gilts compared with CONTROL gilts, with $E_2$12 gilts intermediate between the two. The lack of difference in fetal weight and marked difference in placental weight resulted in a marked difference in the ratio of fetal weight to placental weight between estradiol-17β treated gilts and CONTROL gilts (4.75 ± .16 and 5.07 ± .35 vs. 5.78 ± .23, $P<.05$).

There was a positive correlation between fetal weight and placental weight ($r=.82$, $P<.001$) for all gilts in this study. Furthermore, whereas there was a negative correlation between placental weight and the fetal weight to placental weight ratio ($r=-.84$, $P<.001$), no association was found ($P>.15$) between fetal weight and the fetal weight to placental weight ratio.

It has been hypothesized that the length a preimplantation embryo attains at elongation will influence, if not determine, placental size later in gestation. Furthermore, the amount of estradiol-17β synthesized and secreted by the preimplantation embryo has been positively associated with the endometrial secretion of growth factors, including IGF-I. These growth factors are thought to stimulate the growth and cellular proliferation of the preimplantation conceptus, and IGF-I has been shown to stimulate trophectoderm mitotic rate. Therefore, the amount of estradiol-17β secreted by the conceptus may promote conceptus growth through modulation of endometrial secretory products. Although we did not measure uterine luminal growth factor concentrations, we were clearly able to increase placental size through the administration of estradiol-17β around the time of conceptus elongation. This increase in placental growth is evident whether measured as increases in weight, length, or surface area. Whereas the placentae from CONTROL gilts in this study were similar in size to those previously reported for untreated Meishan gilts at a similar stage of gestation (978 ± 29 vs. 729 ± 35 cm^2, respectively), the placentae from $E_2$12 and $E_2$13 gilts were as large as those collected from untreated Yorkshire gilts (1337 ± 96 and 1520 ± 70 vs. 1639 ± 72 cm^2, respectively).

The assertion that uterine capacity is the major limitation to increasing litter size in the pig is derived from experiments in which females were subjected to an increase in the number of embryos contained in a uterine horn which was well beyond the normal litter size, by superovulation, superinduction, and unilateral hysterectomy-ovariectomy (UHO). Superovulation and superinduction increase the number of embryos present in a normal uterus, whereas UHO involves the removal of one ovary and the ipsalateral uterine horn, which results in the same number of ovulations in approximately one-half the uterine space. It is widely believed that between d 12 and 18 of a normal gestation ~30% of embryos are lost in U.S. and European pig breeds. Although sustaining the same percentage of embryo loss as untreated control pigs, superovulation and superinduction reliably increase the number of embryos that survive beyond day 30. UHO females, which have a normal number of embryos on day 12, also sustain the normal 30% embryo loss to day 30, but have only one-half the uterine space. Consistent with the concept of a limited uterine capacity, superovulated and superinduced females do not farrow larger litters than controls whereas UHO females farrow litters approximately one-half the size of controls.

We have recently extended our view of uterine capacity as simply a result of uterine size, to include conceptus differences in the mass of placental tissue required for normal fetal growth and development. Using this expanded definition, we hypothesized that an important component responsible for limiting increases in litter size might be the amount of placental tissue required by each conceptus for normal fetal growth and development to term. The average placental efficiency (the ratio of a piglet's weight to that of its placenta) calculated over seven Yorkshires litters was 4.2 ± .2, with individual efficiencies ranging from 2.7 to 7.4. More importantly, differences within a single litter of Yorkshire piglets ranged from 3.8 to 7.4. At farrowing, gilts selected for high placental efficiency gave birth to piglets with markedly smaller placentae (>39% smaller) than gilts selected for low placental efficiency. More importantly, gilts selected for high placental efficiency farrowed three more live pigs per litter than gilts with low placental efficiency (12.8 ± .7 vs 9.5 ± .6, respectively).

In agreement with previous data, we observed a positive correlation between fetal weight and placental weight in this study. This indicates that in general, larger fetuses are attached to larger placentae and smaller fetuses are attached to smaller placentae. However, when we examined the ratio of fetal weight to placental weight, or placental efficiency, we find that the variation in efficiency results primarily from variations in placental weight. Another way to envision placental efficiency's lack of association with fetal weight and negative association with placental weight, is that both large and small piglets can develop on relatively efficient or relatively inefficient placentae, but that in general relatively efficient placentae are small and relatively inefficient placentae are large.

Of interest is the lack of difference in litter size and uterine length in the face of marked differences in placental size in this study. The lack of difference in uterine length among treatments is probably due to the marked variation among females in this measurement (503 ± 23, range 376 to 605 cm). This marked variation in uterine size would contribute to the lack of an observed difference in litter size, especially as the largest and smallest uterine lengths and litter sizes are in the $E_2$13 group. These data indicate that litter size is, as might seem obvious, affected by a number of physiologic inputs. This is important as selection for a smaller, more efficient placenta can markedly increase the potential for a larger litter size, but this must be considered within the constraints of the other physiologic limitations of litter size (i.e. ovulation rate, fertilization rate, uterine length, uterine function, etc.). Therefore, if other physiologic parameters influencing litter size are within a normal range, then smaller, more efficient placentae can conceivably result in an increased litter size.