Impact of diet reduction in obese ewes during early pregnancy on placentomal type and cotyledonary vascularity in the ewe

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Introduction

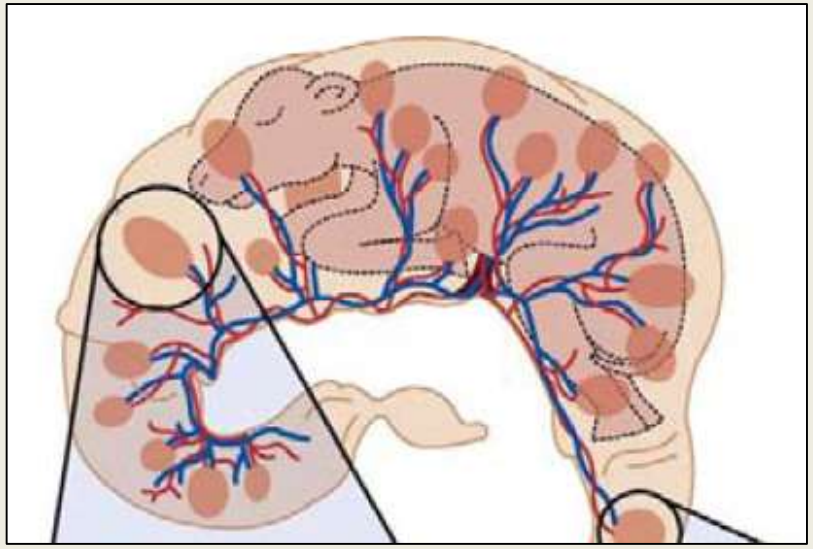
- Obesity in the United States:
  - Greater than 50% of women of childbearing age (20-39 years) are either overweight or obese
  - Neonates of obese mothers show increased fat deposition and high insulin at birth, resulting in increased adiposity and insulin resistance in early childhood, adolescence, and maturity.
- Current epidemiological data suggests a cycle of obesity that is epigenetically fixed within the generations of a population.

Obesogenic Ovine Model

- Development of an ovine pregnancy model of overnutrition during gestation has led to significant understanding of physiological programming which occurs in offspring of obese mothers leading to a predisposition to obesity.
  - Changes in pancreatic and skeletal muscle composition.
  - Alteration of neurological pathways that predispose offspring to orexigenic behaviors.

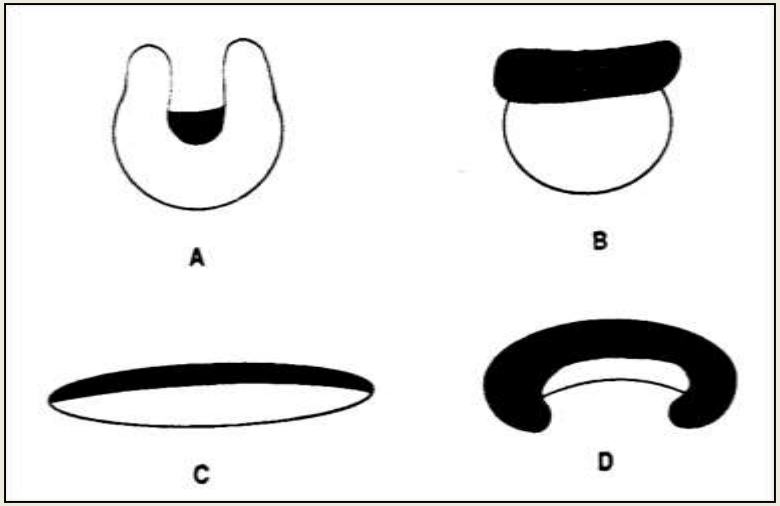
Placental Morphology

- The extent of vascularization in the placenta determines fetal blood flow, which in turn determines nutrient transfer.
- Ovine placenta contains 75-120 placentomes spread over the uterine surface that provide maternal-fetal nutrient and waste exchange.



Ovine Placentomes

- Each placentome is comprised of a placental cotyledonary (COT) and an endometrial caruncular (CAR) portion.
- Classsified into four types according to morphology: A, B, C, and D
  - Placentomes increase in size and blood flow efficiency as they change from type A to type D in response to increased fetal nutritional demands after mid-gestation.
- Studies suggest that that obesity reduces placental vascularization by midgestation, slowing fetal growth rate.
  - Decreased angiogenic factor after mid-gestation in order to prevent dystocia at term.



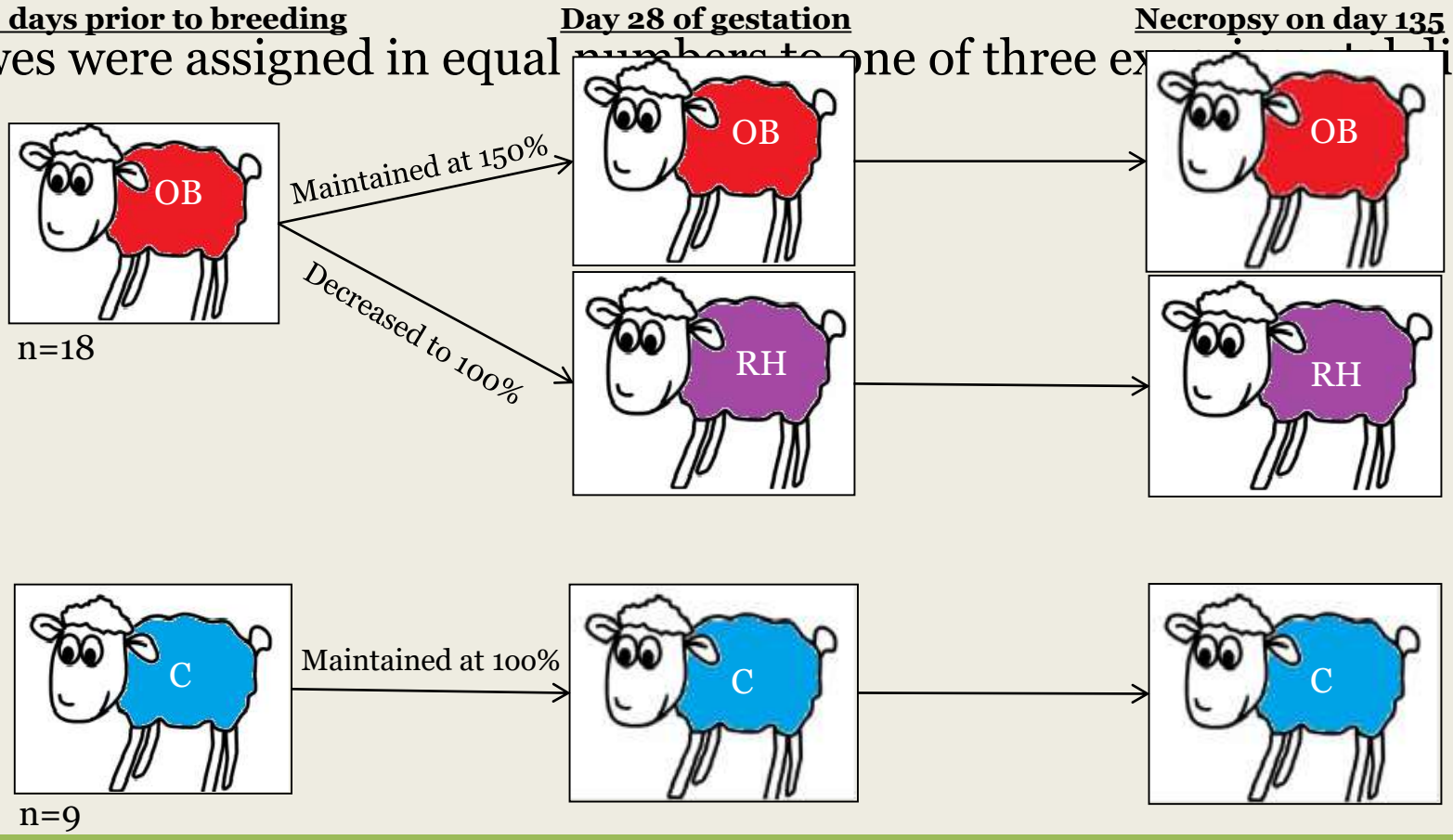
Objective of this study

- Evaluate the impact of reducing the diet of pregnant obese ewes to that of control ewes during early gestation on placentomal type and vascularity.

Methods

- Twenty seven well-nourished 3 to 4 year old multiparous Rambouillet/Columbia cross ewes, each having had 2 to 3 successful deliveries.
- Similar body weight and body condition scores (BCS) for each dietary group when selected in September 2011.

Methods Continued



Note: Data for the Rehabilitation Group was not complete at this time, only Obese and Control data will be presented.

Methods Continued

- Experimental diets were initiated 60 days prior to breeding on October 1, 2011 and were maintained until day 135 of pregnancy when the ewes were necropsied.
  - Diets were provided on a metabolic body weight and gestational age basis and were adjusted for weight gain and Body Mass Index (BMI) on a weekly basis.
- Pregnancies were confirmed on day 45 of gestation by ultrasonography.

Tissue Collection

- On day 135 of gestation the ewes were weighed, sedated with ketamine, maintained under isofluorane inhalation anesthesia and euthanized via exsanguination.
- Gravid uterus from each ewe was removed and weighed and selected fetal organs were removed and weighed, then frozen for later analysis.

Tissue Preparation

- A single type A placentome was removed from the placenta of each conceptus for perfusion.
  - Placentomes are perfused with Biodur ™ latex casting material (consists of base, a hardener, a coloring agent, and thinned with methyl ethyl ketone).
  - Perfusion via a blunt needle inserted into the COT vasculature
  - Casts are dissected free from surrounding tissue, dried overnight, and then placed in a 5% KOH solution for four to six weeks to remove any remaining tissue.

Tissue Collection and Preparation

- After the period of tissue digestion the casts are then rinsed with distilled water and dried.
- Casts are then visualized with a TM-1000 scanning electron microscope and four representative images are taken of each placentome at 200x and 250x magnifications.

Assessment of Capillary Density and Diameter

- Each 200x image is analyzed for capillary area density (CAD) and each 250x image for capillary diameter (CD) using ImageJ software.
  - Foreground capillaries are separated from the inter-capillary space (background) using automatic image threshold adjuster, converting the image into a black and white contrast photo.
  - Lines are then drawn around each capillary cluster to obtain capillary density value (black to white area ratios).

Assessment of Capillary Density and Diameter Continued

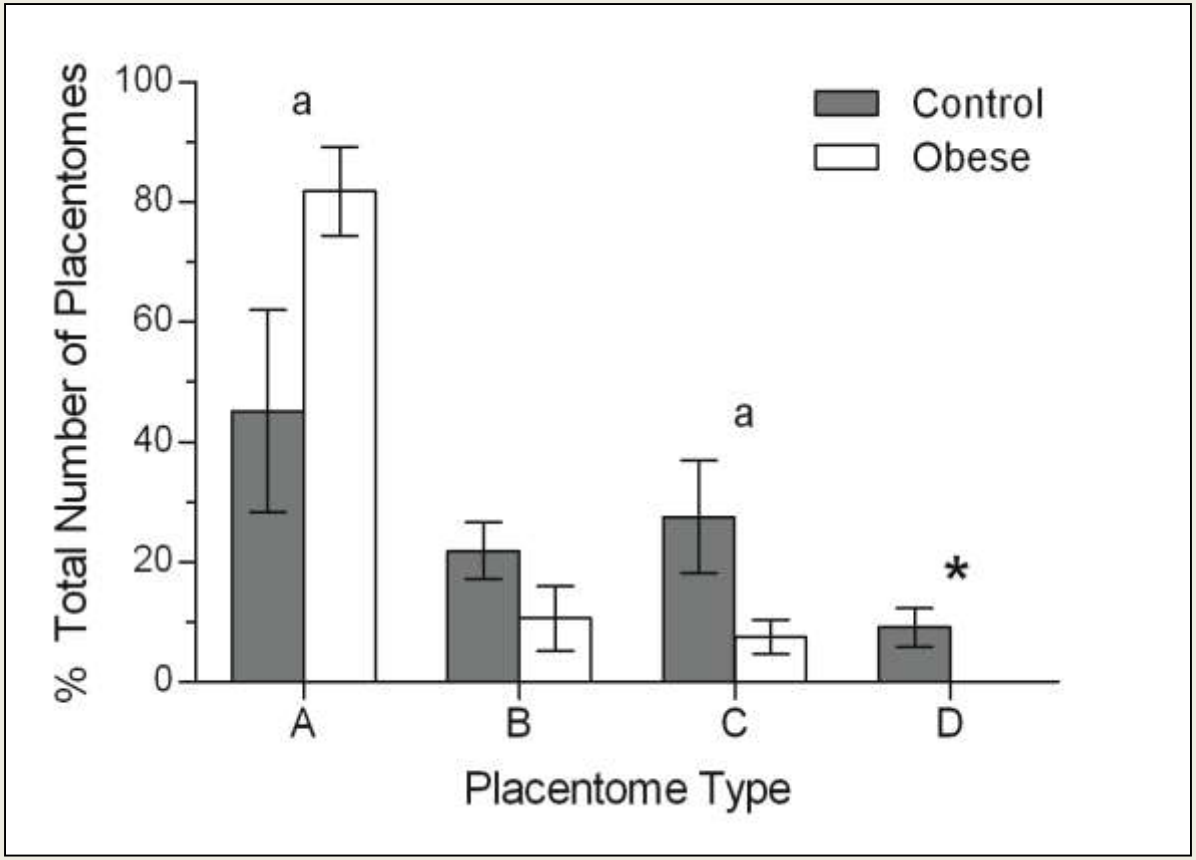
- All CAD measurements are added together and divided by the total CAD area of the capillary bed.
  - Measures the density of the capillary bed while avoiding the gaps between the villous fingers.
- These values from all four pictures will then be averaged to obtain a total CAD from each placentome.
- For the CD analysis the 250x images are utilized. Ten capillary diameters are randomly measured per image and measurements are averaged to obtain a value for each placentome.

Results

- Similar fetal weights and measurements in the Obese and Control groups.
- Total placental weight was decreased in Obese fetuses.

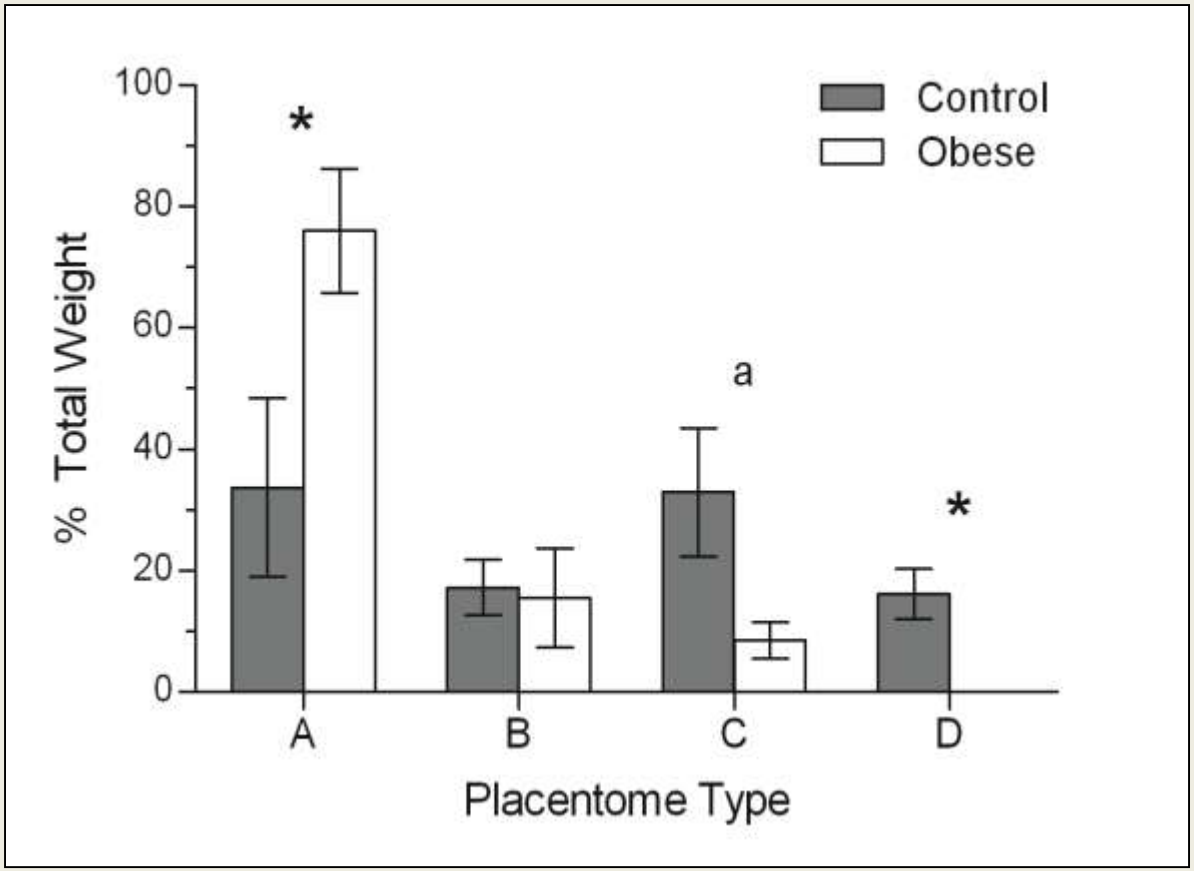
Results Continued

- Altered placentomal type in Obese ewes



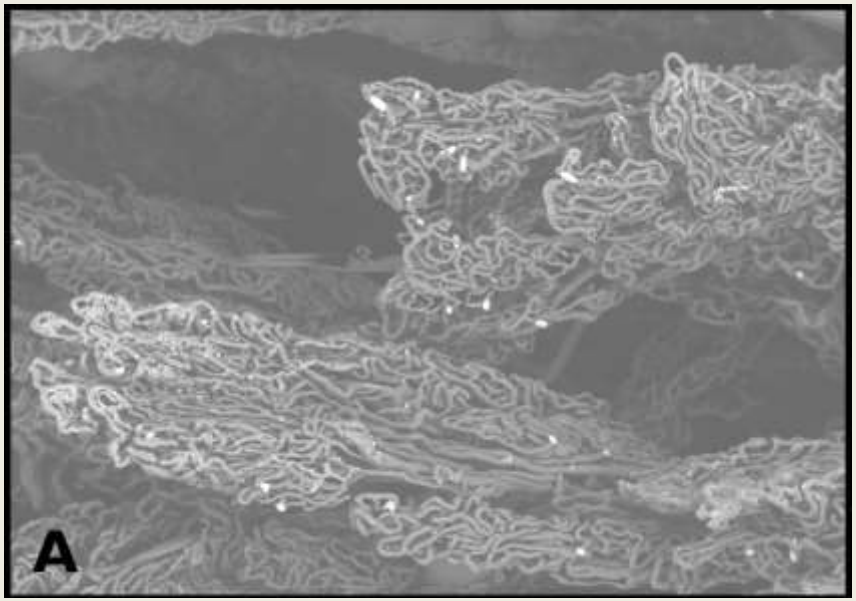
Results Continued

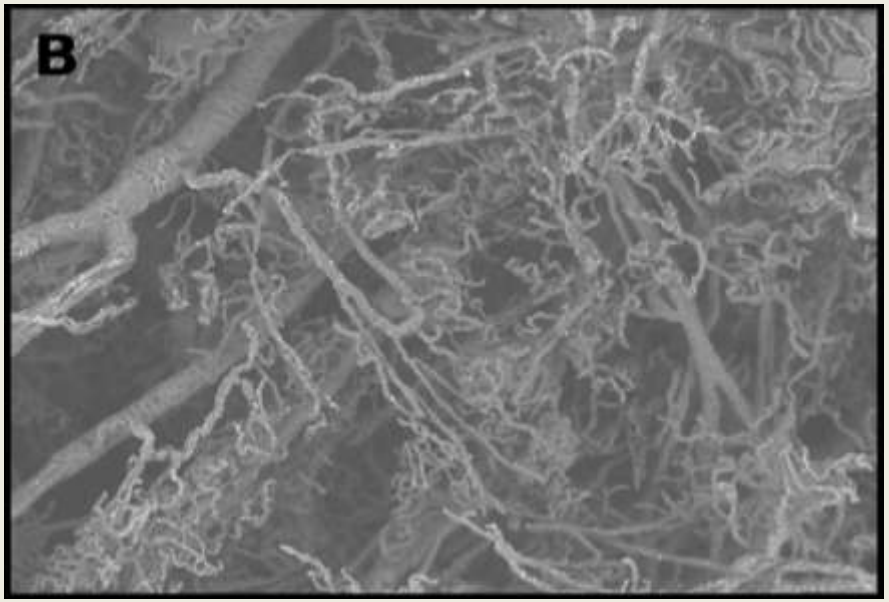
- Altered total placentomal weights in Obese ewes



Results Continued

- Vascular Casts
  - Capillary Area Density (CAD) of the COT vasculature of Obese ewes (B) was less than those of Control ewes (A), 42.4±1.0% and 58.2±1.6% respectively.
  - Capillary Diameter (CD) was reduced in the COT vasculature of Obese versus Control ewes at 5.99±1.31 µm and 8.18±0.08 µm respectively.





Discussion of Results

- Obese ewe placental efficiency was higher than Control ewes according to fetal weight/placental weight.
- Yet a number of factors suggest that the Obese placenta is actually physiologically less efficient than the Control.
  - The obese group showed increased type A placentomes and decreased type D placentomes compared to control ewes.
  - Qualitative visual analysis of vascular casts utilizing the scanning electron microscope revealed gross morphological differences in COT vasculature.
  - Analysis of casts revealed decreased COT CAD and CD of the type A placentomes of the obese group fetuses compared to control fetuses.

Conclusion

- Decreased placental efficiency in obese ewes, suggesting an unknown mechanism by which the placenta or the fetus is able to downregulate vascularity as a protective mechanism against overgrowth due to the effects of maternal obesity.
- The rehabilitation group study was not completed this semester, but the obeseogenic model will continue to explore this mechanism of placental differentiation and protection.

Thank you:

Dr. Stephen Ford
Center for the Study of Fetal Programming

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