## Evidence that undernutrition in early gestation reduces maternal leptin and impairs AMH in juvenile offspring in dairy cattle

Adele Frau<sup>1</sup>, David Edache<sup>2</sup>, Roberta Cresci<sup>2</sup>, Sebastiano Sale<sup>3</sup>, Antonio Gallo<sup>4</sup>, Federica Franciosi<sup>5</sup>, Vincenzo Miragliotta<sup>6</sup>, Sara Succu<sup>1</sup>, Daniela Bebbere<sup>1</sup>, Olimpia Barbato<sup>7</sup>, Giovanni Ricci<sup>7</sup>, Alberto Stanislao Atzori<sup>2</sup>, Francesca Mossa<sup>1</sup> <sup>1</sup>Department of Veterinary Medicine, University of Sassari, Sassari, Italy; <sup>2</sup>Department of Agriculture, University of Sassari, Sassari, Italy; <sup>3</sup>Self-employed Veterinarian, Dorgali, Nuoro, Italy; <sup>4</sup>Department of Animal Science, University Cattolica del Sacro Cuore, Piacenza, Italy; <sup>5</sup>Department of Veterinary Medicine and Animal Sciences, University of Milan, Italy; <sup>6</sup>Department of Veterinary Medicine, University of Pisa, Italy; <sup>7</sup>Department of Veterinary Medicine, University of Perugia, Italy; a.frau4@studenti.uniss.it

In beef heifers, undernutrition up to 110 days of gestation (DG) increased maternal testosterone (T) peripheral concentration and diminished the number of healthy growing ovarian follicles in female offspring, as assessed by reduced total number of antral follicles and peripheral concentration of anti-Mullerian hormone (AMH). Leptin (L) production by adipose tissue decreases during weight loss and L can alter androgen production. We hypothesized that maternal nutritional restriction from shortly before conception to two different stages of early gestation would cause 1) an increase in maternal L and T peripheral concentrations and 2) a decrease in AMH circulating concentration in dairy female calves.

Holstein-Friesian heifers (n=42) homogenous for age (14-17mo) and weight (366±41kg) were randomly assigned to three experimental groups and, starting 10d before artificial insemination (AI), were individually fed at: (i) 0.6 of their maintenance energy requirements (M) up to 80DG (Nutrient Restricted, NR80; n=16) or (ii) 120DG (NR120, n=16), and (iii) 1.8M until 120DG (Control, C; n=10). Estrous cycles were synchronized, and heifers were inseminated with sex-sorted semen from a single sire. Pregnancy was diagnosed and confirmed via ultrasound 28 and 55DG, respectively. After the end of the differential diet, heifers were group fed ad libitum until calving. Peripheral maternal L and T concentrations were measured in heifers pregnant with a single female calf (NR80, n=8; NR120, n=9; C, n=5). Twenty-two single female calves were born (NR80, n=8; NR120, n=9; C, n=5) and peripheral AMH concentrations were measured regularly from birth to 120 days of age (d). Data normality was tested with the Shapiro-Wilk test.Hormonal concentrations were analyzed as repeated measures within treatments using the multivariable linear regression model.

Maternal L concentration was influenced by diet (p<0.001) and DG (p<0.05), but their interaction tended to be significant (p=0.075). Leptin concentration was similar among groups before the start of the differential diet, it was lower in both NR80 and NR120 vs C from 30 to 120DG (p<0.05) and was similar among groups from 150DG to calving. Peripheral T concentration in pregnant dams increased as gestation progressed (p<0.001) but was not affected by diet. Circulating AMH concentration in female calves was influenced by maternal diet (p<0.001) and decreased as calves grew older (p<0.001) but was not conditioned by the interaction of maternal diet and age. NR80 and NR120 calves had lower AMH than C from birth to 60d, whereas no difference was detected among groups when calves were 90 and 120d.

In conclusion, maternal undernutrition from preconception to either 80 or 120DG reduced peripheral AMH in female progeny in dairy calves, indicating a potential impairment of ovarian reserve. In the dams, nutritional restriction reduced peripheral L, yet did not influence T concentrations.

Acknowledgements: DESTINE, MIUR-PRIN 2017