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The effect of maternal body condition on *in vivo* production of zygotes and behavior of delivered offspring in mice

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ABSTRACT

This study investigated the effects of maternal body condition on oocyte quality and zygote production. Additionally, we examined the possible consequences on somatic parameters and behavior of naturally delivered offspring. We used an experimental model based on overfeeding of outbred mice during intrauterine and early postnatal development to produce the following four types of females: physiological (7%–8%), slightly increased (8%–11%), highly increased (>11%), and low (<7%) body fat content (Echo Magnetic Resonance Imaging). The fertilized females with slightly increased body fat showed increased numbers of spontaneously ovulated oocytes and an increased fertilization index compared with control animals. On the contrary, mice with slightly and highly increased body fat showed increased numbers of isolated immature oocytes and degenerates. Furthermore, animals with increased body fat had significantly decreased deposits of neutral lipids in the cytoplasm of mature oocytes (Nile red staining) and showed lower reduction in DNA cytosine methylation signal in parental pronuclei (5-methylcytosine immunohistochemistry). The highly increased amount of body fat in mothers was accompanied with lower weights in newborn pups and 5-week-old offspring. We also observed several deviations from normal behavior (open-field test and forced swimming test). The females with low body fat displayed a lower fertilization index, a lower percentage of zygotes at pronuclear stage 4 with demethylated DNA cytosine in parental pronuclei, and lower newborn weights. Although delivered offspring were able to gain normal weight by the fifth week of life, there were several deviations from normal behavior observed. Our results show that periconceptional status of maternal body condition adversely affects the quality of oocytes and might be correlated with significant changes during postnatal offspring development. The data documenting later onset of DNA demethylation in zygotes and decreased amounts of neutral lipids in oocytes suggest that the observed alterations in offspring might originate in modifications established at the earliest stages of conceptus development.

1. Introduction

It is known that an adequate body fat mass is important for the onset of reproductive function, particularly in females. Furthermore, physical status is one of the major factors determining reproductive success in productive animals [1].

Numerous human clinical studies have reported the following findings in obese women: frequent cycle cancellations, increased gonadotropin requirement during ovarian stimulation, fewer collected oocytes, reduced oocyte quality, lower pregnancy rate after IVF, reduced function of the corpus

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luteum, and increased risk of miscarriage. However, other studies did not find any disorders in ovarian stimulation or any differences in either the oocyte fertilization rate or the numbers of successful pregnancies when comparing obese and nonobese patients (reviewed in [2,3]). Experimental studies on rodents have proven defective ovarian function, increased follicular apoptosis, poor oocyte quality, decreased oocyte size, oocyte meiotic aneuploidy, impaired oocyte mitochondrial function, and significant delays after *in vitro* cleavage in animals with diet-induced obesity [4–8]. Furthermore, during later development, decreased expression of embryonic Insulin-like growth factor I receptor, increased glucose consumption, and growth retardation of fetuses and pups have been documented in mouse dams fed a high-fat diet [5–7]. The effect of maternal leanness or undernutrition on the *in vivo* production of oocytes has not been studied so profoundly. However, there is a negative effect on the postpartum fertilization rate in cows with excess body fat. Additionally, impaired oocyte competence and oocyte quality have also been documented [1]. However, in most epidemiologic studies and animal models, it is difficult to determine which outcomes are because of factors associated with maternal metabolic profile and which are because of the consumption of a specific diet.

A two-generation model based on overnutrition of experimental animals during intrauterine and early postnatal development to produce adult female mice with four different body types has been developed in our laboratory. The body types include normal controls with physiological body weight and amount of body fat, mice with slightly increased body fat, mice with highly increased body fat and weight (obesity-like phenotype), and lean mice with decreased body fat and weight [9,10]. Using this model, we demonstrated the effect of altered maternal body condition on the development of *in vivo*–derived preimplantation embryos. The embryos isolated from dams with highly increased and highly decreased maternal body fat had slowed development and an increased incidence of apoptosis. Consistent with previous reports, our model possesses several important advantages. Specifically, our model simulates population heterogeneity in a manner analogous to naturally reproducing mammalian subjects (it uses outbred mice). Our model also allows for the study of maternal body condition and at the same time minimizing the impact of the composition of actual nutrition (during reproductive process, all animals are fed standard diet only), the impact of maternal aging (all females are at the early adulthood at the age standard used for reproductive studies), and the impact of hormonal treatment (only spontaneously ovulating donors of oocytes and pups are used) [10].

The aim of present study was to evaluate the relationship between maternal periconceptional body condition and *in vivo* production of oocytes in mice using a standardized experimental model. The model simulates natural variability in monogastric mammals including the human population and minimizes confounding adverse effects that may mask changes induced by maternal condition. In addition to assessing basic reproductive parameters (ovulation rate and fertilization index), we evaluated specific and novel features of oocyte quality, including the accumulation of lipid deposits in oocytes and the process of active DNA demethylation of the zygotic genome. Furthermore, we investigated the possible consequences of the observed changes on the somatic development and behavior of offspring naturally delivered from dams grouped according to body fat percentage.

2. Materials and methods

2.1. Animals and experimental design

All experiments were performed on mice of the outbred ICR (CD-1 IGS) strain (Velaz, Prague, Czech Republic) and the entire experimental design was repeated four times.

A two-generation dietary model based on overfeeding of experimental mice during prenatal and early postnatal development was used to produce females with various body conditions (Fig. 1) [10]. Adult female mice (30–35 day's old) of the parental generation (P) underwent hormonal synchronization (pregnant female's serum gonadotropin [eCG 5 IU intraperitoneally, Folligon; Intervet International, Boxmeer, Holland], followed 47 hours later by the administration of hCG [4 IU intraperitoneally, Pregnyl; Organon, Oss, Holland]). These females were mated with males of the same strain overnight. The fertilized mice were randomly divided into the control and experimental groups and were individually housed in plexiglass cages under standard conditions (temperature 22 ± 2 °C, humidity 55 ± 5%, 12:12-hour light-dark cycle with lights on at 6:00 AM with free access to food and water). During the gestation period (21 days) and the lactation period (from birth to weaning: 21 days) dams in the control group (C) were fed standard pellet diet (M1, 3.2 cal/g; Ricmanice, Czech Republic). The dams in the experimental group were fed standard diet M1 with the addition of high-energy liquid product Ensure Plus (1.5 cal/mL; Abbott Laboratories, Hoofddorp, The Netherlands [11]) *ad libitum*. To

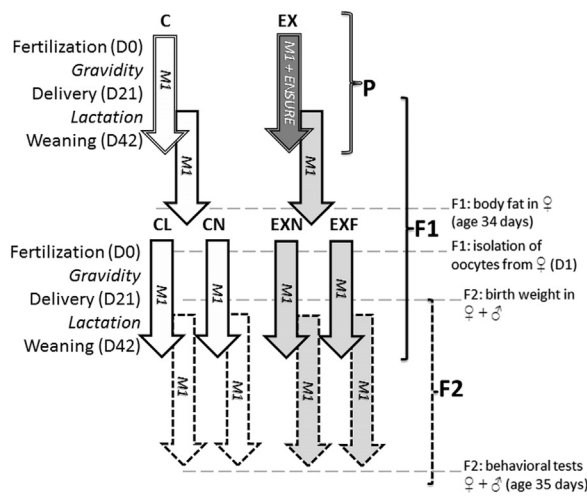

Fig. 1. Experimental design. Diagram shows dietary regime of three generations of mice used in the study (P, parental [♀]; F1, the first filial [♀]; F2, the second filial generation [♀ + ♂)]). M1, standard diet; ENSURE, diet supplement with Ensure PLUS; C, control mice; EX, experimental mice; CN, dams with the physiological amount of body fat; CL, lean control females with decreased body fat; EXN, experimental mice with slightly increased body fat; EXF, experimental mice with highly increased body fat.