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Review

Intake of high saturated-fat diets disturbs steroidogenesis, lipid metabolism and development of obese-swine conceptuses from early-pregnancy stages

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ABSTRACT

The current study indicates that life-long intake, from early-life, of an obesogenic diet with high saturated-fat (HSF) content induces dyslipidemia (high plasma concentrations of triglycerides in concurrence with low concentrations of HDL-cholesterol) in obese swine with leptin resistance (Iberian sows). In case of pregnancy, ovarian features (ovulatory efficiency and luteal steroidogenesis) of sows fed with HSF are not affected but embryo features are affected at so early stages like 28 days of pregnancy (first quarter), although embryo viability was still not affected. In this way, offspring from HSF sows showed a higher incidence of alterations in their developmental trajectory, mainly due to a higher incidence of growth retardation, in their steroidogenic activity and in their availability of triglycerides and cholesterol. In conclusion, the results obtained in the present study illustrate the deleterious effects of maternal dyslipidemia, induced by the intake of HSF diets, on the oestradiol secretion of the conceptuses at early-pregnancy stages and, thus, on their developmental and metabolic features.

This article is part of a Special Issue entitled 'Pregnancy and steroids'.

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1. Introduction

The interaction between genetic predisposition, maternal nutrition during pregnancy and the determinant role of the exposure to an obesogenic environment later in life (inadequate nutrition, lack of physical activity, stress and even contact with endocrine disruptors) is identified as the main cause for the contemporary epidemics of obesity and metabolic diseases (for review see: [1]). In Western countries and developing countries like China or India, malnutrition is increasingly related to excesses in both quantity (food amount) and quality (mainly, excess of sugar and fat in the diet). Currently, the intake of fat is increasing at a worrying rate; 32.1% of calories in the diet of European countries are derived from fat (35.4% in UK) and, mainly, from saturated fats (13.2% of the total energy of the diet [2]).

In females of childbearing age, obesity and malnutrition are associated with infertility and appearance of early miscarriages [3]. There is increasing epidemiological information, from earlier

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reports of Rogers and Mitchell [4] and Hartz et al. [5], on high rates of subfertility and anovulatory infertility in obese women [6–11]; even if applying assisted reproduction treatments (ART [12–15]). Obesity has also been related to implantation failures and miscarriages in the first trimester of pregnancy [16–19].

If pregnancy is achieved, obesity may be a high-risk condition for the mother and the foetus, since changes in the intrauterine environment may cause aberrant foetal growth and may program the offspring for obesity and metabolic disease in adult life. The main problem, as highlighted by Cochrane in 1965 [20], is the birth of individuals too large for gestational age although, at a lesser extent, obese mothers may also have newborns with reduced body weight due to intrauterine growth retardation (IUGR [21,22]). The offspring from obese mothers may be also normal in size and weight [23], although overnutrition and obesity would induce foetal programming in any case. The concept of prenatal programming implies that conditions during pregnancy determine later life; descendants from obese mothers would have predisposition for developing obesity, atherogenic lipid profiles, insulin resistance, diabetes and cardiovascular disease [24,25].

Thus, from these considerations, pregnant women commonly undergo nutritional control. However, there is compelling evidence that effects of nutritional habits affects conceptus development from early stages of pregnancy, when women often are not still aware of their pregnant condition [26]. Hence, the information acquired by interventional studies is becoming increasingly important. Obviously, interventional research in human beings is limited by ethical issues; investigations need to be therefore conducted on animal models [27].

Most of the studies have been performed in rodents since the most abundant forms of monogenic obesity in humans have been replicated in mice [28]. Main mouse models for obesity studies are based in mutations in genes encoding leptin ($Lep^{ob/ob}$ mouse) and its receptor ($Lepr^{db/db}$ mouse). Such mutations directly affect reproductive features since both male and female ob/ob mice are infertile [29]. Infertility in ob/ob mice is a direct consequence of leptin deficiencies; administration of leptin restores fertility [30]. The use of other rodent strains, without so dramatic fertility problems, has shown that maternal obesity induced by high-fat diets is related to changes in the phenotype of the offspring [31], causing growth retardation of the foetuses and newborns [32,33].

The use of large animals in studies of obesity and pregnancy also offers numerous advantages [34,35]; mainly, body size facilitates application of imaging techniques and sampling of large amounts of blood and tissues. The rabbit is an amenable model for studying the effects of high-fat diets on maternal metabolism and foetal development due to the similarities of its lipid metabolism with that of humans [36]. In rabbits, it has been found that the intake of unbalanced diets, with high-fat contents, also induces foetal growth retardation and, hence, offspring with lower birth-weight; however, adiposity during the juvenile period is increased [36,37]. The pig is also an outstanding model for studies on obesity and malnutrition. The swine model has the advantage of sharing numerous and essential similarities with human beings: omnivorous habits, propensity to sedentary behaviour and obesity, as well as similar metabolic, gastrointestinal and cardiovascular features [35,38,39,40,41]. Serum lipid patterns of obese pigs and humans are similar and earliest studies on obesity and pregnancy have shown that triglycerides concentration and distribution of cholesterol between the lipoprotein fractions was similarly modified in mothers and foetuses [42]. Moreover, there are breeds, like the Iberian pig, with an obese phenotype due to a gene polymorphism in the leptin receptor similar to the syndrome of *leptin resistance* described in human medicine [43]. In previous studies of our group with Iberian pigs, the supply of obesogenic diets was able to trigger obesity and metabolic syndrome in both juvenile and adult individuals [44,45]. This effect was found applying diets with considerably lower fat content and energy than provided for other animal models, which assure the robustness, amenability and reliability of this model. Iberian pigs have also shown to be very sensitive to nutritional changes during pregnancy [46,47] and, in view of all the similarities with obese humans, studies on pregnant Iberian sows may be extrapolated to human medicine.

Thus, the current study, using Iberian sows as a model, aimed to determine the effects of the life-long intake of an obesogenic diet with high saturated-fat content on maternal reproductive features (ovulation rate, implantation success, luteal and placental steroidogenesis and embryo viability) and metabolism and growth patterns of the embryos at early pregnancy stages.

2. Materials and methods

2.1. Animals and experimental procedure

Thirteen Iberian sows with no evidence of health problems and adequate pathogen-monitoring reports were included in this study. All the animals had been genotyped for polymorphism on *LEPR* gene with protocols previously described [43] and found to be homozygous for the allele LEPRc.1987 T. The females were housed in collective pens at the facilities of the INIA Animal Laboratory Unit (Madrid, Spain), which meets the requirements of the European Union for Scientific Procedure Establishments. The experiment was carried out under Project License from the INIA Scientific Ethic Committee. Animal manipulations were performed according to the Spanish Policy for Animal Protection RD1201/05, which meets the European Union Directive 86/609 about the protection of animals used in research.

From four months-old, gilts were randomly divided in two groups that were housed in two different collective pens corresponding to different diets. Six of the gilts acted as controls (control group or group C) and were fed with a standard grain-based diet (89.8% of dry matter, 15.1% of crude protein, 2.8% of polyunsaturated fat and 3.08 Mcal metabolizable energy/kg), whilst the seven remaining gilts were fed with the same diet but enriched with saturated fat (89.8% of dry matter, 15.1% of crude protein, 6.3% of saturated fat and 3.36Mcal metabolizable energy/kg; group HSF). All animals had ad libitum access to food and water; daily food intake was estimated to be around 3 kg/animal/day.

At eleven months-old, all the animals were treated for cycle synchronization. Synchronization consisted of daily administration, for 18 consecutive days, of 20 mg of the progestagen altrenogest (Regumate®, MSD, Boxmeer, The Netherlands), by individually top-dressing over their morning feed; the treatment was initiated irrespective of the stage of the cycle. Oestrus detection was performed twice daily, from 24 h after progestagen removal; both by inspection of the vulva for reddening and swelling (pro-oestrus) and by control of the standing reflex (oestrus) in contact with a mature boar. Sows were inseminated 12 and 24 h after the onset of oestrus with cooled semen doses from the same tested Iberian boar. At day 27 after insemination, pregnancy diagnosis was performed by ultrasonography with a real-time B-mode ultrasound machine (Aloka SSD 500, Ecotron, Madrid, Spain). All the sows were weighed and back-fat depth was ultrasonically determined at 4 cm from the midline, at the level of the head of the last rib, at oestrus synchronization and 28 days of pregnancy.

2.2. Morphometric evaluation and sampling of pregnant genital tracts

At Day 28 of pregnancy, samples from maternal peripheral blood (10 mL) were collected by jugular venopuncture with sterile