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Substantiation of Ovarian Effects of Leptin by Challenging a Mouse Model of Obesity/ Type 2 Diabetes

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Abstract

The goal of the current was to elucidate if treatment with gonadotrophins and leptin can circumvent infertility in obese mice and to establish whether reproductive effects of leptin are influenced at the hypothalamus-hypophysis or ovarian level by using a leptin deficient mouse model of obesity/type 2 diabetes (ob/ob) treated with leptin. The ovulatory response and the fertilization success were compared with the results obtained in ob/ob dams pretreated with a gonadotrophin-replacement therapy or in two groups (ob/ob and wild-type) of control non-pretreated females. The number of corpora lutea was significantly lower in control ob/ob mice than in wild-type dams. Treatment with gonadotrophin-replacement therapy did not increase significantly the ovulation rate in ob/ob, but the administration of leptin-replacement treatment allowed the authors to obtain a number of corpora lutea and oocytes/zygotes similar to those obtained in wild-type females. Furthermore, the leptin supply succeeded in producing fertilized zygotes, although in a lower number than found in the wild-type control. Thus, the hypogonadotrophic state in obese mice may be circumvented by the administration of a gonadotrophin-replacement therapy combined with a protocol for controlled ovarian stimulation, but fertile ovulations are only obtained after applying leptin-replacement therapy. Current results strongly support the existence of direct local effects of leptin on the ovary.

Keywords: Folliculogenesis; Gonadotrophins; Leptin; Mouse; Ovary

1. Introduction

The changes in circulating levels of glucose and metabolic hormones (insulin, growth hormone, IGF-I and leptin) are thought to be important signaling factors for the female reproductive system as increases in these elements positively correlate with follicle development and ovulation [1]. Several studies that take into consideration the influence of leptin on glucose and insulin homeostasis [2,3] have lead to the hypothesis that this polypeptide has a major role in the regulation of the reproductive function via nutritional contribution [4,5]. Furthermore, blood leptin concentrations vary with changes in nutrition and are correlated with body fatness [6,7], since leptin is produced in the adipose tissue [8].

The obese mouse hereditable syndrome was initially discovered in 1949 [9]; however, leptin was not

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identified until 1994 when cloning of the mouse obese gene was achieved [8]. Obese mice (ob/ob mouse) were characterized as being grossly overweight, due to high food consumption and scarce physical activity, and were established as hyperglycemic, hyperlipidemic, and hyperinsulinemic. Consequently, these mice have been extensively used as a model for diabetes and obesity; however, the obese syndrome also affects immune function, cardiovascular system function, and reproductive function [10], causing both male and female ob/ob mice to be infertile. The ob/ob mice have a mutation in the gene encoding leptin and their characteristics are a direct consequence of leptin deficiency. Thus, the administration of leptin restores weight, metabolic function and fertility [11].

Therefore, ob/ob dams arise as a good model for the evaluation of processes and mechanisms linking leptin and ovarian function. Previous studies have found that female ob/ob mice have a reduced number of antral follicles in the ovary [11] and impaired folliculogenesis with elevated apoptosis in the granulosa cells [12]. Hamm et al. [12] hypothesized that defects in folliculogenesis, and thus in female fertility, may be caused by a hypogonadotrophic-hypogonadal state in the ob/ob mice; gonadotrophins are the primary factors promoting follicular growth and inhibiting granulosa cell apoptosis in ovarian follicles [13,14]. Thus, these data, and data from additional authors [15–17], support the premise that the effects of leptin on ovarian function are mediated through effects on the hypothalamus and pituitary. Alternatively, the existence of leptin receptors within the ovary, the follicle, and the oocyte [18,19], and their variations throughout the reproductive cycle [19,20], support a direct effect of leptin at the ovarian level.

Considering these data, the objective of the current study was to elucidate if treatment with gonadotrophins and leptin can circumvent infertility in obese mice and to establish whether leptin effects are determined at the hypothalamus-hypophysis or ovarian level. The ovulatory response and the fertilization outcome after estrus synchronization, as well as controlled ovarian stimulation and ovulation induction, were determined in ob/ob adult females pretreated with a leptin-replacement therapy. The therapy was based on serial doses of leptin and compared with the results obtained in ob/ob dams pretreated with a gonadotrophin-replacement therapy based on serial low doses of pure FSH and LH (avoiding the hypogonadotrophic state but not mimicking possible local effects of leptin) and two control non-pretreated groups (ob/ob females and wild-type non leptin-deficient mice).

2. Material and Methods

2.1. Animals and husbandry

Ten week-old homozygous female mice maintained at the facilities of the CNIC Animal Laboratory Unit in Madrid, Spain, were used. The lighting cycle was 14 h:10 h light:dark, respectively (07:30 on; 21:30 off). Humidity was maintained around 50% and the temperature was around 21 °C. The CNIC Animal Unit meets the requirements of the European Union for Scientific Procedure Establishments. The experiments were carried out under Project License 176/07 from the CNIC 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 experimentation.

2.2. Experimental design

In a preliminary trial, the efficiency of the combination of a commonly used protocol for controlled ovarian stimulation in mice with a protocol developed in our laboratory for estrus synchronization [21] was tested. Thereafter, this protocol was applied for the proper experimental trial. In brief, as depicted in Figure 1, follicular growth was induced in 18 CD1 females (Crl:CD1[ICR], Charles River Laboratories Inc., Wilmington, MA), by intraperitoneal administration of 5 IU of equine chorionic gonadotrophin (eCG, Folltropin, Intervet International, Boxmeer, The Netherlands) at 13:30 on Day 1. Ovulation was induced by intraperitoneal administration of 5 IU human chorionic gonadotrophin (hCG; Chorulon, Intervet International, Boxmeer, The Netherlands) at 13:30 on Day 3. Just after hCG injection, fertile males were introduced at a rate of 1:1. The appearance of estrus and coital behaviors were determined the following morning by evaluating the presence of a vaginal plug as result of overnight mating.

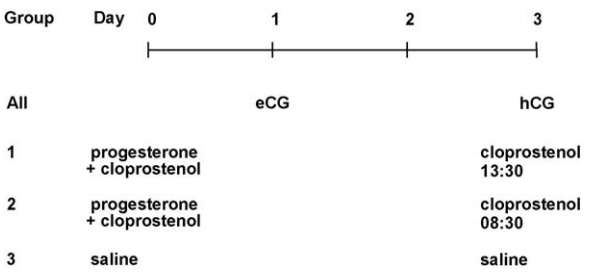

Fig. 1. Schematic representation of the treatment cycle combining estrus synchronization and controlled ovarian stimulation in mice.