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The effects of small litter rearing on ovarian function at puberty and adulthood in the rat

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ABSTRACT

Rearing rats in small litters lead to obesity and reproductive dysfunction. We investigated the effects of rearing female rats in small litters on various reproductive parameters during puberty and into adulthood, and examined the possible involvement of local ovarian sympathetic nerve activity. The litter size was adjusted on postnatal day one to four pups per dam for the small litters and 12 pups per dam for the normal litters. Vaginal opening was recorded, and estrous cyclicity was monitored daily immediately post puberty for 14 days and again at 8–9 weeks of age. At the time of puberty and 10 weeks of age, the ovaries were collected. The number of different types of follicles was counted and the thickness of the theca interna of the largest antral follicles was measured. Ovarian sympathetic nerve activity was assessed immunohistochemically by measuring levels of ovarian nerve growth factor receptor (p75NGFR) and tyrosine hydroxylase (TH). In rats reared in small litters, there was a significant advancement of puberty and disruption of estrous cyclicity immediately post puberty. The number of antral follicles increased in the small litter reared rats at puberty compared with their controls. The thickness of the theca interna increased and the expression profiles of ovarian p75NGFR and TH increased in small litter reared rats at puberty, but this did not persist into adulthood. These data suggest that rearing rats in small litters lead to irregular reproductive cycles, which might involve increased local ovarian sympathetic nerve activity.

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1. Introduction

Postnatal overfeeding induced by rearing animals in small litters results in a dramatic increase in body weight gain and programs for overweightness and persistent hyperphagia in adulthood, even though a standard diet was provided after weaning [1]. It is well established that reproductive function is gated by the state of energy reserves of the organism. The timing of puberty in mammals is tightly coupled to the animals' nutritional and metabolic state. Food restriction in female rats delays puberty [2,3], while female rats reared in small litters have been shown to display early puberty onset [1,4]. Obesity also affects female reproductive function by affecting spontaneous ovulation in humans and animals. The relationship between excess body fat and reproductive disorders in women appears to be stronger for early-onset obesity during their life, particularly during adolescence [5]. There is evidence that in adolescent and young women, the age of onset of obesity and of menstrual irregularities and oligo-anovulation are significantly correlated [5,6]. The prevalence of obesity in women with PCOS appears to be much greater than expected in the general population. Studies in the cafeteria diet-overfed [7] and genetically obese Zucker female rats [8] have shown that these animals display a disruption of estrous cyclicity as well as obesity in adulthood. However, the mechanism of how obesity or overweight link to ovarian dysfunction is not established and there is a sparse literature on the effect of rearing in small litters on ovarian activity in the rat.

Rats reared in small litters has been shown to accumulate more noradrenaline in the heart than animals reared in larger litters [9], with cardiac noradrenaline concentrations inversely related to litter size at 40 days of age [10]. It was shown that noradrenaline levels were also increased in hypothalamus [11] and ovary in the rat [12] during puberty. Abnormally increased noradrenaline levels [13] and tyrosine hydroxylase (TH) immuno-staining in ovary has been demonstrated to cause ovarian dysfunction such as early vaginal opening, disrupted estrous cyclicity and appearance of cystic follicle in the polycystic ovary (PCO) rat [14]. Transection of the superior ovarian nerve decreases noradrenergic innervation of the ovary and restores estrous cyclicity and ovulation in this model [15]. Moreover, increased density of catecholamine nerves has been observed in the ovaries of PCO patients [16] and the marked effectiveness of ovarian wedge resection to initiate ovulatory cycles in PCO patients further supports the importance of sympathetic activity on ovarian function [17,18]. However, there is no literature on whether the ovarian sympathetic nerve activity was affected in rats reared in small litters.

The aim of this study is to test the hypothesis that rearing female rats in small litters as a model of postnatal overnutrition, which advances puberty, is associated with ovarian dysfunction at puberty and in adulthood, as manifested by alterations in ovarian cyclicity and morphology and expression of ovarian nerve growth factor receptor (p75NGFR) and TH; markers of sympathetic nerve activity.

2. Materials and methods

2.1. Animal procedure

Pregnant Sprague-Dawley rats (Charles River, Manston, UK) were housed under controlled conditions (12 h of light and 12 h of darkness with lights on at 07:00 h and a controlled ambient temperature of 22 ± 2 °C) and supplied with ad libitum food and water. On postnatal day (pnd) 1 (birth, pnd 0) litter size was adjusted to 4 pups per dam for the small litter reared group ($n = 7$), and to 12 pups per dam for the normal litter reared group ($n = 6$). At least 1 male pup was included in each litter. The dams were singly housed during pregnancy and lactation, with weaning on pnd 21. Post weaning, 4–6 pups were housed in the same cage until they reached 10 weeks of age. They had free access to water and food. All animal procedures were conducted under the British Home Office Animal Scientific Procedure Act 1986 (Project Licence 0671) and in accordance with accepted standards of the local ethical review committee.

2.2. Puberty onset and estrous cyclicity monitoring

Animals were monitored daily for vaginal opening from pnd 28. Once vaginal opening occurred, vaginal smears were taken and monitored daily for 2 consecutive weeks and again at 8–9 weeks of age. The criteria for normal estrous cyclicity were the same as we described previously [19]. Animals were weighed weekly until the end of the experiment.

2.3. Ovarian morphology

Pubertal ovaries were collected on pnd 42 and adult ovaries were collected at diestrus at the age of 10 weeks. The right ovary was cleaned of fat tissue, weighed and fixed in 10% formaldehyde buffer for 20 h at room temperature. Wax-embedded ovaries were sectioned longitudinally at 4 μm and mounted on glass slides. For counting the number of the different types of follicles, every other section was stained with hemotoxylin–eosin and subjected to analysis. Only follicles in which the nucleus of the oocyte was visible were counted [20]. The thickness of the theca interna layer of the largest follicle was determined in every tenth section (six sections per ovary) with a calibrated scale bar in the microscope. The sections were examined under a light microscope (Zeiss Axioskop 2 plus, Oberkochen, Germany) with an image analysis system (Axiovision 2.05; Zeiss) by two independent investigators blind to the treatment group. The follicles were classified as following: primordial-follicles with oocytes surrounded by one layer of flattened pregranulosa cells; primary-follicles with oocytes surrounded by no more than two layers of cuboidal granulosa cells; preantral follicles without any antral cavity and with two or more layers of granulosa cells; antral-follicles with apparent cavity [20].

2.4. Immunohistochemistry

Immunohistochemical staining for p75NGFR and TH were performed on 4 μm paraffin embedded sections from ovaries of 6- and 10-week-old rats. The sections were deparaffinised in