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The effect of pre-treatment with a gonadotrophin-releasing hormone agonist on reproductive functions in mature cycling rats

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In order to investigate the performance of follicles in a rat model in which gonadotrophin-releasing hormone agonist (GnRHa) was used for hypothalamic–pituitary–ovarian axis suppression, three groups of mature cycling rats were studied. One group was treated with buserelin followed by pregnant mare's serum gonadotrophin (PMSG), and the second group was treated with PMSG alone. Both these hormonally treated groups received human chorionic gonadotrophin for induction of ovulation. The third group received no hormonal treatment. The average number of ovulated oocytes recovered from rat oviducts pre-treated with GnRHa was significantly higher than that in rats treated with the gonadotrophin alone, in spite of the larger number of pre-ovulatory follicles present in the gonadotrophin-treated group. The morphology of both the pre-ovulatory and the post-ovulatory cumulus–oocyte complexes in the three groups appeared similar. No difference in the capacity of follicles of the three groups to synthesize progesterone *in vitro* in response to luteinizing hormone could be observed. We conclude that ovarian morphology and function are not impaired by pre-treatment with buserelin.

Key words: GnRH agonist/rat follicle biosynthesis/rat oocyte cytology

Introduction

The chronic administration of gonadotrophin-releasing hormone agonists (GnRHa) for suppression of the hypothalamic–pituitary–ovarian axis is widely used in humans (Lunenfeld and Vickery, 1990). Since rat ovarian tissue has been shown to contain high-affinity receptors for GnRH, a direct effect of these agents at the ovarian level also exists (Koves *et al.*, 1989; Dekel *et al.*, 1989).

Our study was designed on the basis of observations made in our in-vitro fertilization (IVF) unit, that the cumulus–oocyte complexes retrieved from follicles exposed to GnRHa are morphologically different from those not exposed to this agent. Since the availability of human follicles and ovulated oocytes for research is quite limited, we used rats pre-treated with buserelin (a GnRHa) to test whether their follicles and oocytes were different to those from spontaneously ovulating rats, or from rats in which ovulation was induced by gonadotrophin treatment.

Materials and methods

Animals

The effect of pre-treatment with a GnRHa was studied on mature (90 day old) cycling female Wistar rats from the colony of the Department of Hormone Research, at the Weizmann Institute of Science, Israel. The animals were kept under a controlled photoperiod (14 h of light/day). The average weight of each rat was ~200 g and the length of their oestrous cycle was 4 days. Starting on the day of oestrus, for the next 3 days, the rats were each given one daily s.c. injection of 2 μg of buserelin [D-Ser (T-Bu)6] des-Gly-10 *N*-ethylamide (Hoechst, Ag, Frankfurt, Germany). According to our previous experience, this protocol of treatment results in suppression of the oestrous cycle. The next pro-oestrus in this animal model is observed on the fourth day after the last buserelin injection (unpublished data). On the third day, in addition to buserelin, the rats received 30 IU of pregnant mare's serum gonadotrophin (PMSG, Gestyl; Organon, Oss, The Netherlands). These rats are referred to as the GnRHa/PMSG group. Two additional groups, meta-oestrous rats injected with 30 IU of PMSG (PMSG group) and untreated spontaneously ovulating rats (spontaneous group), served as controls. In order to obtain mature oocytes, an i.p. injection of 4 IU of human chorionic gonadotrophin (HCG, Pregnyl; Organon) was given to the hormonally treated rats 50 h after PMSG administration.

Morphological examination

For recovery of ovarian follicles, the rats from the hormonally treated groups were killed 48 h after PMSG administration. The untreated rats were killed on pro-oestrus. The ovaries were removed and transferred to Leibovitz-L-15 tissue culture medium. These ovaries consisted mainly of small pre-antral follicles with a small population of large, transparent antral follicles that were easily distinguished. These large antral follicles were dissected under a stereoscopic microscope and counted. Some of the follicles were punctured and their content was morphologically analysed. Another group of antral follicles was further incubated as described in detail in the following section.