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Gonadotropin-Releasing Hormone Agonist Suppression of Ovarian Tumorigenesis in Mice of the W^x/W^v Genotype[1]

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ABSTRACT

Although many investigations have shown a correlation between elevated gonadotropin levels and ovarian tumors (the gonadotropin theory), the ovarian response to a specific suppression of the gonadotropins has not been elucidated.

The ovaries of (C57BL/6J × C3H/HeJ) F_1-W^x/W^v mice, which contain 1% of the normal oocyte count at birth, rapidly lose the follicular apparatus and develop a 100% incidence of bilateral complex tubular adenomas from the surface germinal epithelium, which is also the origin of 90% of human ovarian carcinomas. Plasma levels of LH and FSH are known to rise fourfold during the period of tumorigenesis. We compared tumor development in W^x/W^v mice after either injecting a GnRH agonist (3.6 mg slow-release goserelin depot, Zoladex Depot) or administering a sham injection every 28 days from the age of 7 days up to 245 days.

All 15 W^x/W^v mice that received sham injections developed bilateral ovarian tubular adenomas from the surface germinal epithelium. In none of the 11 mice receiving the GnRH agonist was any tumor found ($p < 0.00005$), and a significant suppression of the gonadotropins was demonstrated ($p < 0.00005$).

INTRODUCTION

The gonadotropin theory as stated by Biskind and Biskind [1] suggests that elevated gonadotropin concentrations may contribute to the development of ovarian tumors. A number of animal studies have supported the theory of a causal relationship between high levels of gonadotropins and the development of ovarian tumors [2–5], but the suppression of ovarian tumorigenesis by a selective suppression of the gonadotropins has not been performed.

In order to elucidate the possible significance of gonadotropins for the development of ovarian tumors, we used the female mouse B6C3F_1-W^x/W^v. This strain was described by Murphy and Beamer [3] and is characterized by the development of complex tubular adenomas in 100% of female mice at 4–5 mo of age [6, 7]. The tumor is derived from the germinal epithelium, which is also the origin of 90% of human ovarian carcinomas [8].

At birth the W^x/W^v mice have less than 1% of the normal number of oocytes in the ovary, and their oocytes disappear completely during the first 13 wk of life in parallel with the development of the ovarian tumor [7] and a fourfold rise of the normal values of serum levels of LH and FSH [3]. The premature loss of oocytes is attributable to a genetic interference with the migration and proliferation of primordial germ cells [6].

We suppressed the pituitary secretion of LH and FSH with a GnRH agonist and compared the occurrence of ovarian tumors at the age of 8 mo in treated and untreated experimental animals of the W^x/W^v strain.

MATERIALS AND METHODS

Mice

Congenic B6C3F_1-W^x/W^v females were produced by matings of C57BL/6J $W^x/+$ females with C3H/HeJ $W^v/+$ males. For simplicity, W^x/W^v will be used to denote these mice. The same matings produced B6C3F_1-/+, B6C3F_1-$W^x/+$, and B6C3F_1-$W^v/+$ offspring.

Female mice were weaned between 3 and 4 wk of age and placed in groups of animals born on the same day in polycarbonate cages (14 × 20 × 26 cm) in germ-free incubators at +23°C with water and food continually available. A photoperiod of 12L:12D with electric lights-on between 0700 h and 1900 h was maintained.

GnRH Agonist and Sham Injections

The GnRH agonist goserelin, prepared in a 3.6-mg slow-release depot (Zoladex Depot; Zeneca, Macclefield, Manchester, UK), was injected every 28 days from the age of 7 days up to 245 days, for a total of nine depots injected into each mouse. The dosage of goserelin corresponds to approximately 125 mg/day, which we estimated as necessary in view of the finding by Thau et al. [9] that mice are more resistant to GnRH analogues than are rats.

The depot was delivered in a disposable syringe with a 1-mm needle from the Zeneca factories. The sham injections

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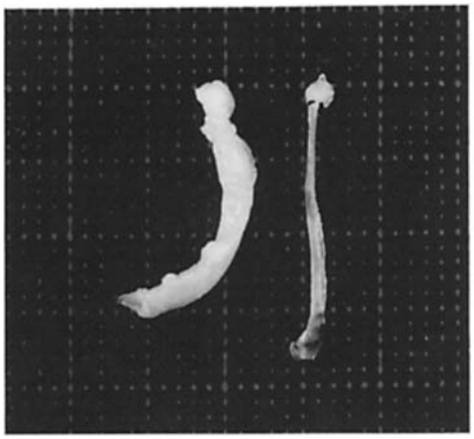

FIG. 1. The macroscopic difference in size of the uterus and ovary from untreated (left) and treated (right) mice. The ovary is on top of the uterine horn. The specimens were placed on graph paper.

were prepared in a similar syringe and needle. The goserelin depot and sham injections were administered in the loose tissue in the neck region. After injection, the needle perforation was closed with Histoacryl (Braun, Melsungen AG, Germany) tissue adhesive.

Two weeks after the last injections (goserelin and sham), the mice were anesthetized with carbon dioxide; following laparotomy, truncal (heart) blood samples were drawn for analyses of LH and FSH.

Histology

Ovaries and uterine horns were dissected in toto, fixed in 4% phosphate-buffered formaldehyde, embedded in paraffin, cut in 10-μm serial sections, and stained with hematoxylin and eosin before light microscopy.

Ovarian and uterine volumes were too small for accurate weights to be obtained (see *Results*). However, the volume was estimated as hypoplastic (< 50% of the $B6C3F_1$ volume) or normal (= $B6C3F_1$ volume). Liver and uterine horn sections were prepared from all mice.

Hormone Analyses

After clotting, the blood samples were centrifuged, and serum was stored at −20°C until analyzed. Materials for RIA of rat FSH and LH were supplied by NIH, NIDDK, and NHPP (Baltimore, MD). All values for serum hormones are expressed as ng per ml serum of mouse reference standards from Dr. A.F. Parlow (AFP-515–3MP for FSH and AFP-5306 A for LH). The sensitivity of RIA was about 0.05 ng/ml and 30 ng/ml for LH and FSH, respectively.

Statistics

The Mann-Whitney rank sum test and the chi-square test were used for statistical evaluation. A nonparametric statistical method was used to avoid assuming normal distribution. The level of significance was defined as $p < 0.05$.

RESULTS

Results are expressed as the median value and 95% confidence limits.

A total of 15 female W^x/W^v mice received injections of the goserelin depot, and 15 female W^x/W^v mice, serving as controls, received sham injections according to the schedule described above.

One goserelin-treated mouse died and 3 goserelin-treated mice were excluded due to violation of the protocol (date of second Zoladex Depot injection was missed). Therefore, 11 goserelin-treated and 15 sham-treated mice were evaluable.

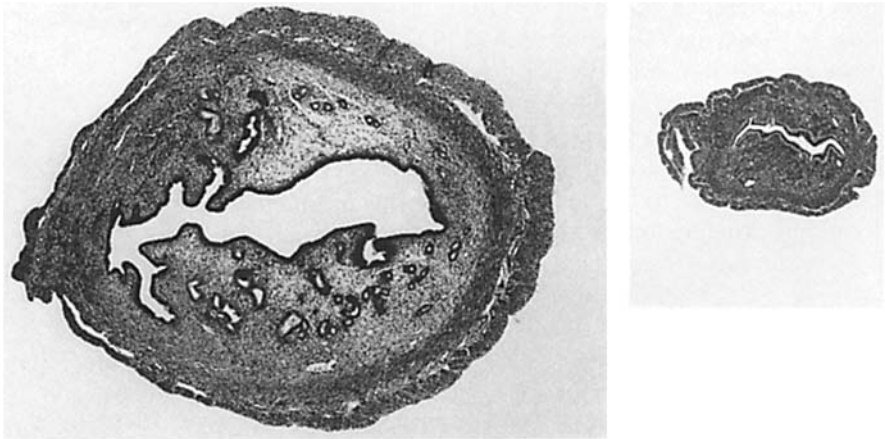

FIG. 2. Cross sections of uterus from untreated (left) and treated (right) mice. The difference in size can be seen from these cross sections, shown in the same magnification (×30).

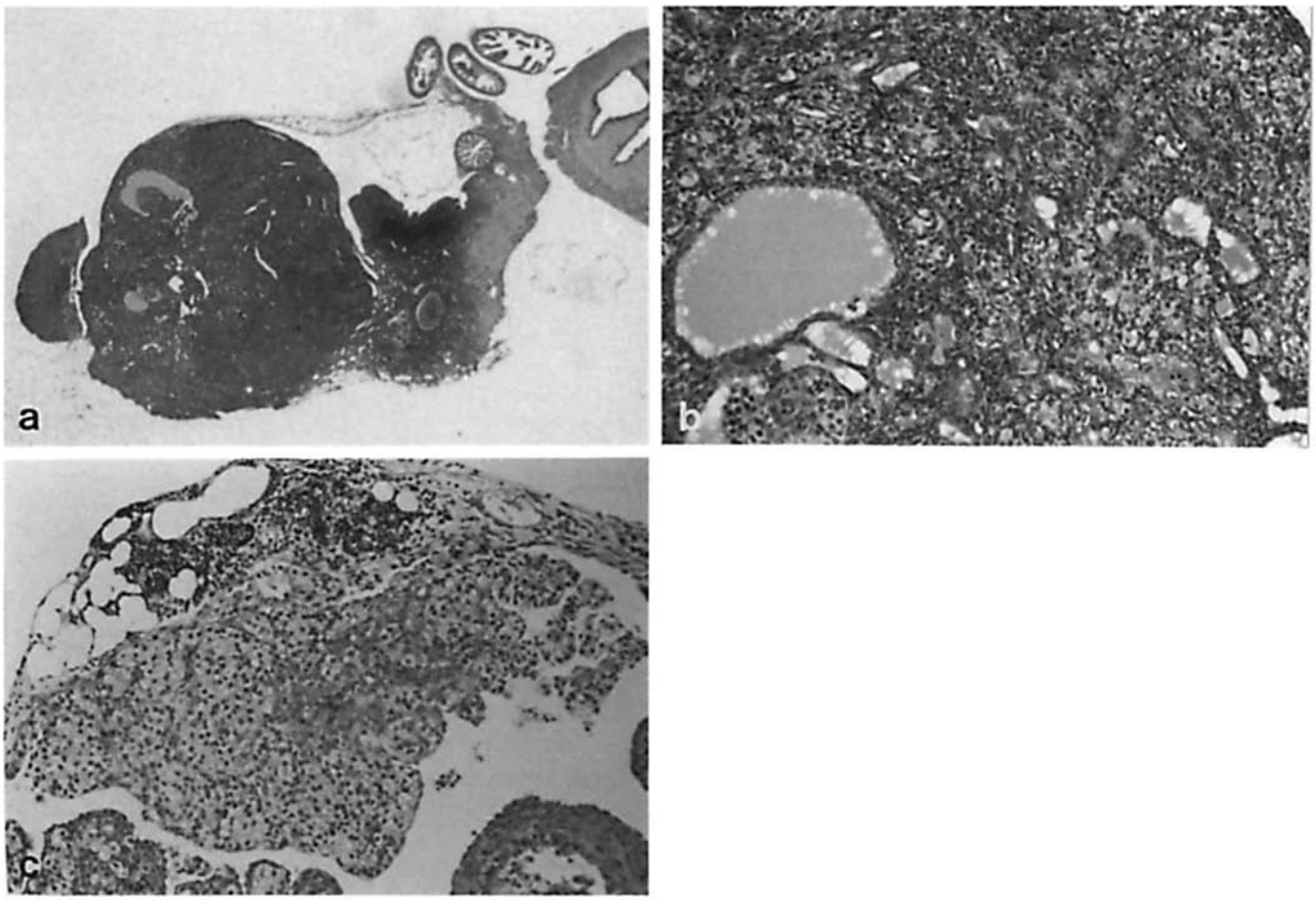

FIG. 3. Ovary of untreated W^x/W^v mouse with complex adenoma formation (×15) (**a**). High-power (×125) image of the same adenoma (**b**) showing solid and cystic components. Predominantly clear cell adenoma (**c**) from another untreated W^x/W^v mouse (×125).

Ovarian and Uterine Volumes

All ovarian and uterine volumes from goserelin-treated mice were evaluated as hypoplastic, while all sham-treated mice had normal uterine and ovarian volumes compared to the $B6C3F_1$ mice (Fig. 1). The difference in size is best demonstrated on cross sections from the uterine horn (Fig. 2).

Histology

In all control animals (untreated), complex tubular adenomas of the ovaries were found. The histologic appearance differed from area to area in individual mice and between mice. The adenomas were mainly solid, made up of small polygonal cells with relatively large, dark, dense nuclei. In some tumors/areas, clear cells dominated and occasional tubular or cystic structures could be seen. Mitotic activity was low. No abnormal mitotic figures were observed. Tumors were restricted to the ovaries: no local invasion, carcinosis of the peritoneum, or distant metastases were found (Fig. 3).

In none of the goserelin-treated mice was any tumor found ($p < 0.00005$). In all treated animals there were remnants of the ovaries consisting of few stromal cells and several normally structured small blood vessels (Fig. 4).

No differences in morphology were found in the liver and uterine horn sections from the two groups.

Hormone Analyses

In all animals that received GnRH agonist injections, a marked suppression of both FSH and LH was demonstrated. The FSH level in the treated W^x/W^v mice was 80.2 (73.2–118.1) ng/ml compared to 562.7 (533.6–642.9) ng/ml in the control (untreated) animals ($p < 0.00005$). Correspondingly, the LH level was 0.05 (0.05–0.12) ng/ml in the treated W^x/W^v mice and 3.30 (2.30–4.50) ng/ml in the controls ($p < 0.00005$).

DISCUSSION

Investigating the W^x/W^v ovary may be useful in answering theoretical questions of epithelial ovarian tumori-

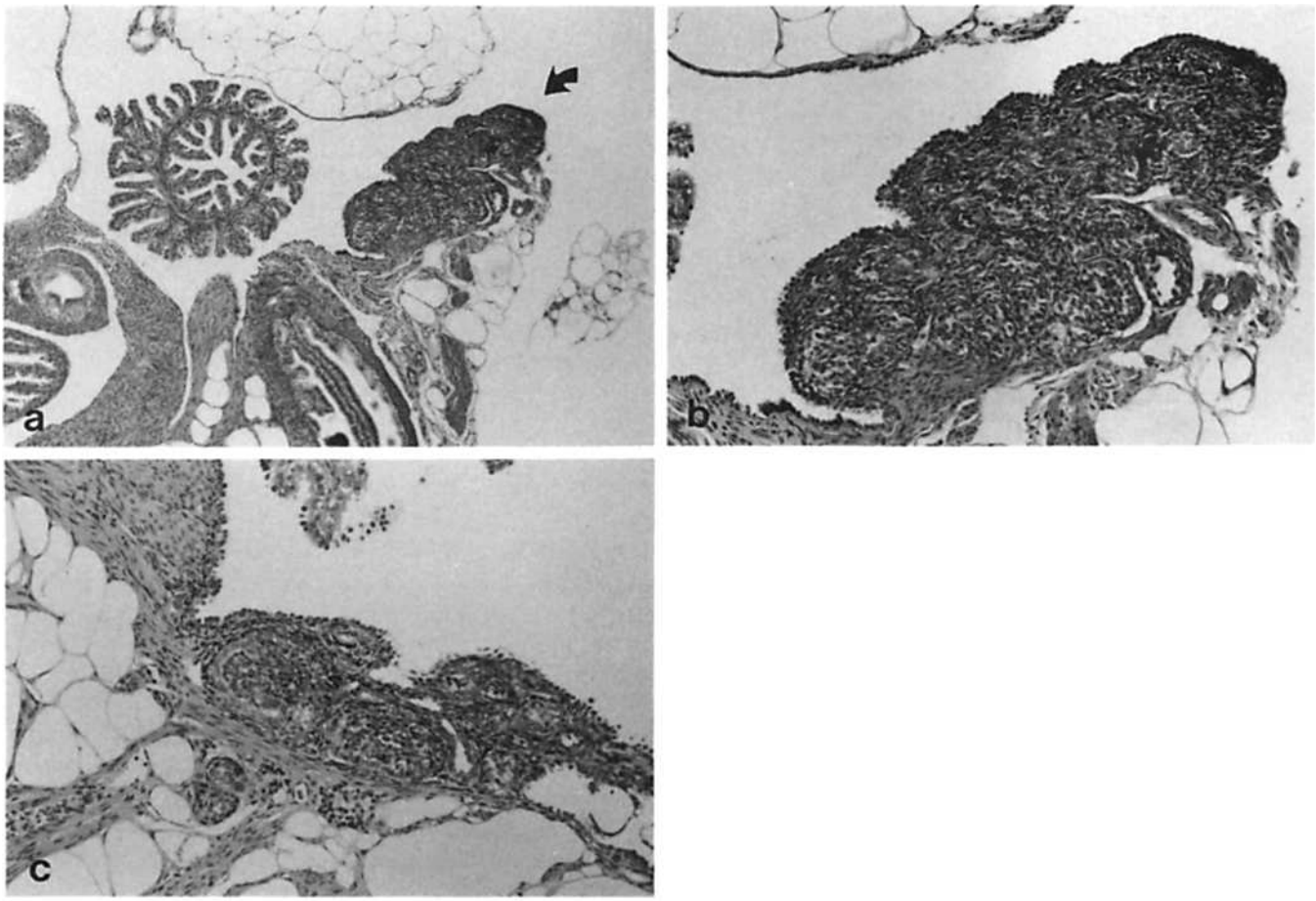

FIG. 4. Ovarian remnant (arrow) from GnRH agonist-treated W^x/W^v mouse (×50) (a) and higher magnification (×125) of the same (b) showing stromal and vascular components. Similar ovarian remnant (c) from another agonist-treated mouse (×125).

genesis. Mutant alleles at the W locus are known to produce well-defined effects upon viability, erythrocyte size and number, coat pigmentation, and fertility [10]. Characteristically, tubular invaginations from the surface germinal epithelium form at 4–6 mo of age and penetrate the underlying ovarian stroma to form complex tubular adenomas showing invasion of fat and of thin-walled blood vessels and lymphatics, whereas metastasis is not seen [7, 10].

In other known models used in the study of ovarian tumorigenesis, the mouse ovary has been severely manipulated. Most models involve application of either carcinogens [11, 12] or X-irradiation [13], affecting the ovary and making it difficult to examine endogenous etiological factors.

A previous finding of undetectable uptake of gonadotropins at 6 mo of age by the W^x/W^v ovaries [14] was contradictory to the gonadotropin theory. However, in another experiment Tennent and Beamer [15] found that oocyte death (gamma irradiation) in hypogonadal mice deficient in GnRH and in gonadotropins did not stimulate tubular adenoma formation. They also found that oocyte destruction plus gonadotropins was necessary for disorganization of ovarian follicles prior to ovarian tumor formation [15] and that exogenous administration of gonadotropins after irradiation was insufficient to stimulate tumorigenesis in hypogonadal mice. In the latter experiment, gonadotropins were injected three times weekly; this might be an unphysiological stimulation in an animal with a 4- to 5-day estrous cycle. The aforementioned observed loss of uptake of gonadotropins [14] might have resulted from normal receptor regulation in the presence of chronically elevated levels of gonadotropins.

Biskind and Biskind [1] demonstrated that oophorectomy and transplantation of one ovary into the splenic pulp of adult rats led to the development of ovarian tumors. The estrogen secreted in the portal circulation was inactivated in the liver, and the tumorigenesis was ascribed to the elevated gonadotropin levels occurring in these animals as a consequence of the lack of negative steroid feedback on the pituitary [1]. Murphy and Beamer [3] contributed to this assumption through demonstration of inhibition of the ovarian tumorigenesis by repeated s.c. implantation of normal ovaries at 6-mo intervals in the W^x/W^v genotype. However, the

possibility of other mechanisms such as steroid hormone actions on ovarian epithelial receptors cannot be excluded.

In human epithelial ovarian cancer it has been demonstrated that the growth of cell lines from advanced tumors of epithelial ovarian origin in vitro was stimulated by human FSH and LH [16]. A number of studies have reported gonadotropin binding sites in benign and malignant tumors of the human ovary, and have concluded that the presence of these receptors might be a sign of gonadotropic control of ovarian tumors [17–20]. In contrast, Graves et al. [21] and Stouffer et al. [22] were not able to demonstrate significant gonadotropin binding sites in human epithelial ovarian malignancies.

Recently, the demonstration of GnRH agonist inhibition of the growth of a human epithelial ovarian carcinoma heterotransplanted in the nude mouse [23] gave rise to current interest in further research on the gonadotropins in ovarian tumorigenesis.

In our research model we have demonstrated the ability to completely suppress the spontaneous development of epithelial ovarian tumors in intact research animals by significantly suppressing the normal hypophyseal secretion of gonadotropins using a GnRH agonist. Our recent finding [24] that immunoreactive inhibin production suppresses FSH and is possibly a defense mechanism in these patients against an elevated gonadotropin level, as well as our demonstration of low gonadotropin levels in postmenopausal women who have epithelial ovarian carcinomas [25] and show no signs of a central depression of gonadotropin release mediated by the dopaminergic system or GnRH release [25], makes further investigations of the gonadotropin theory in ovarian tumorigenesis important.

The W^x/W^v mice may help to clarify the apparent role of gonadotropins in ovarian tumorigenesis. Of great interest is the question whether the ovarian germinal epithelium contains GnRH binding sites (receptors) as has been demonstrated in human epithelial ovarian cancer [26]. If receptors are present, the mechanism might be an action of the agonist itself as indicated by the findings of Thompson et al. [27]. The possibility of this intrinsic action could be demonstrated by restoring the gonadotropins to determine whether the tumorigenesis is attributable to high levels of gonadotropin and GnRH acting by inhibiting their release. Other important questions are the crucial age of the W^x/W^v mice at which to start injections, the number of injections, and the dosage of the agonist. All these questions are at the moment under investigation in our laboratories.

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