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REPRODUCTIVE PHYSIOLOGY AND DISEASE

Role of the superior ovarian nerve in the regulation of follicular development and steroidogenesis in the morning of diestrus 1

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Abstract

Purpose Little is known about the role of the superior ovarian nerve (SON) in follicular development during the estrus cycle. The aim of the present study was to analyze the role of neural signals arriving through the SON at the ovaries in the regulation of follicular development and ovarian steroid secretion in diestrus 1 of cyclic rats.

Methods Cyclic rats were subjected to left, right, or bilateral SON sectioning or to unilateral or bilateral laparotomy at diestrus 1 at 11:00 h. Animals were sacrificed 24 h after surgery.

Results Compared to laparotomized animals, unilateral SON sectioning decreased the number of preovulatory follicles, while bilateral SON sectioning resulted in a decreased number of atretic preantral follicles. An important observation was the presence of invaginations in the follicular wall of large antral and preovulatory follicles in animals with denervation. Furthermore, left SON sectioning increased progesterone levels but decreased testosterone levels, which are effects that were not observed in animals that were subjected to right denervation.

Conclusions At 11:00 h of diestrus 1, the SON was found to stimulate follicle development, possibly via neural signals, such as noradrenaline and/or vasoactive intestinal peptide, and this stimulation induced the formation of follicle-stimulating hormone receptors. The role of the SON in the regulation of ovarian steroid secretion is asymmetric: the left SON inhibits the regulation of progesterone and stimulates testosterone secretion, and the right nerve does not participate in these processes.

Keywords Superior ovarian nerve · Ovarian steroidogenesis · Gonadotropins · Noradrenaline · Follicular development

Introduction

Ovary functions are regulated by hypothalamus-pituitary axis hormones and autonomic nerves [1, 2]. Neural signals reach the ovaries via sympathetic, sensorial, and parasympathetic pathways [3]. Rat ovaries receive sympathetic innervation through two routes: the ovarian nerve plexus, which is distributed along the ovarian artery and innervates the ovarian vasculature, and the superior ovarian nerve (SON), which is located in the suspensory ligament and innervates blood vessels, the interstitial gland, and theca interna cells [3, 4].

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The role of innervation in the regulation of ovarian functions has been assessed by the effects of nerve sectioning using in vivo models [5–12] or their stimulation using in vitro models [13–16].

In the adult rat, bilateral sectioning of the SON was performed at 11:00 h on the day of proestrus, and the results showed a decrease in progesterone and estradiol levels 4 min following the sectioning. At 16:00 h on the day of proestrus, there was a prolonged decline in estradiol and a brief (8 min) decrease in progesterone secretion [6]. The same surgery performed at 11:30–12:30 h on the day of proestrus decreased progesterone levels 30 min post-denervation, but estradiol levels did not change [17]. On the other hand, neither progesterone nor estradiol was altered when bilateral denervation of the SON was performed at 11:00 h on the day of estrus [6]. Taken together, these pieces of evidence show that sympathetic innervation regulates the secretion of ovarian steroids in a stimulatory way depending on the day of the estrus cycle when the surgery is performed.

Flores et al. [18] showed that the right and left ovaries have different capacities to maintain normal hormone levels, and such capacities vary during the estrus cycle and depend on the integrity of the SON. This supports the idea that the ovaries send and receive neural information that is processed in the central nervous system and that this information participates in controlling the secretion of gonadotropins related to the regulation of ovarian functions [9, 19–23].

A few studies of SON sectioning found that the functions of the ovary seem to vary with the time of day [6, 18, 24], the age of the animal [10], and the time elapsed after surgery [20].

Morán et al. [25] analyzed uni- or bilateral sectioning of the SON and found an increased number of atretic follicles in the infant rat and that these effects could be explained by a decrease in the number of follicle-stimulating hormone (FSH) receptors as a result of denervation.

Follicle development was not modified in peripubertal rats (10–11 weeks of age, which is in the estrus stage), as revealed by bilateral sectioning of the SON, but thinning of the theca interna of the antral follicles was observed [2].

Bilateral sectioning of the SON in neonatal rats was shown to inhibit follicle development by suppressing granulosa cell proliferation and enhancing granulosa cell apoptosis, thus promoting follicular atresia [11].

A decrease in ovarian noradrenaline (NA) content was shown to inhibit follicular development and secretion of progesterone and estradiol [26] because the SON is the main source of NA in the ovaries [5]. The function of the hypothalamic-pituitary axis has been previously suggested to be affected in some way, as shown by early ovarian SON sectioning (neonatal rats of 4 days of age) because the circulating levels of FSH have been found to be lower than that of control animals [20].

More studies are necessary to clarify the role of the SON in the regulation of follicular development. Despite the above evidence, little is known about the role of the SON in follicular development during the estrus cycle.

Previously, we showed the effects of the unilateral section of the SON performed on the day of the proestrus on ovulation and follicular ovarian structures, where denervation performed at 11:00 h resulted in different changes to when the surgery was performed at 17:00 h [24]. Thus, in the present study, we aimed to elucidate the role of SON on diestrus 1 at 11:00 h in the regulation of follicular development dynamics, ovarian steroidogenesis, gonadotropin secretion, and ovarian NA secretion in mature female rats. The study was performed on the day of diestrus 1, when the NA is released from nerve endings and recruits the first follicular wave [27].

Material and methods

All experiments were carried out in strict accordance with the Mexican Law of Animal Treatment and Protection Guidelines and the specifications in the Mexican Official Standard NOM-062-ZOO-1999. The Committee of the Facultad de Estudios Superiores Zaragoza approved the experimental protocols.

This study was performed using adult female rats from the CIIZ-V strain; they were from our own breeding stock and were kept under conditions of controlled lighting (lights on from 05:00 to 19:00 h) and temperature (22 ± 2 °C), with free access to food (Purina S. A., Mexico), and tap water. Only rats that exhibited at least two consecutive 4-day cycles were used in the experiment, and estrus cycles were monitored by cytological examination of daily vaginal smears. All surgeries were performed under anesthesia at 11:00 h of diestrus 1 (metestrus). Animals from each experimental group were sacrificed by decapitation 24 h after surgery. All efforts were made to minimize the number of animals used and their suffering ($n = 56$ animals).

Animals were allotted at random to one of the following groups:

A. Control group

There were eight cyclic rats that were subjected to no treatments, and they were sacrificed at diestrus 2.

B. Laparotomy (LAP) group

Eight rats per group were subjected to a dorsolateral incision on the left, right, or both sides. The incision was performed 2 cm below the last rib, affecting the skin, muscle, and peritoneum (Fig. 1a). No organ was affected. The opening was subsequently closed.

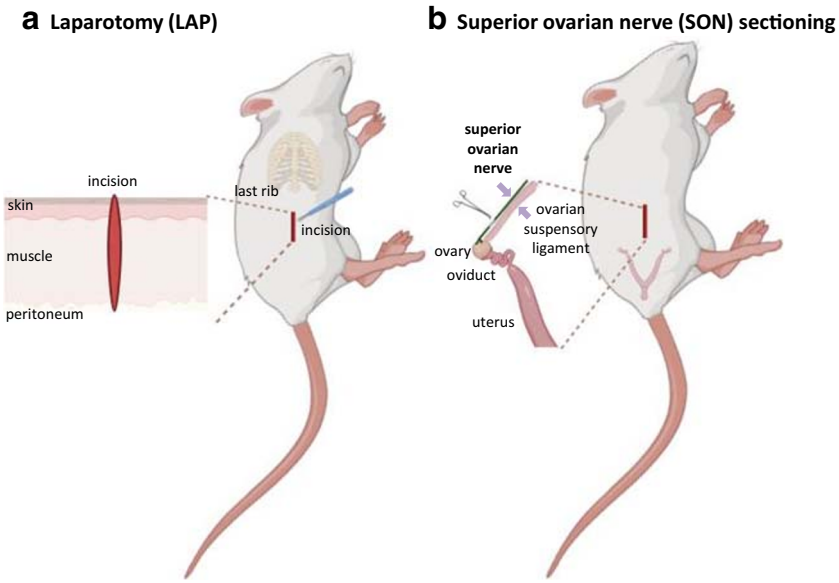

Fig. 1 Schematic representation of **a** laparotomy and **b** superior ovarian nerve sectioning

C. Unilateral or bilateral sectioning of the superior ovarian nerve (SON) group

Groups of eight animals were laparotomized, one or both ovaries were exposed, and the ovarian suspensory ligament (left, right, or both) was identified. With the aid of fine forceps, the ovarian ligament containing the left, right, or both SONs was sectioned at approximately 1 cm from the ovary (Fig. 1b). When a bilateral section was performed, the right nerve was always the first nerve to be sectioned. The ovary was subsequently returned to the abdominal cavity, and the opening was closed [24].

Autopsy procedures

The animals from each experimental group were sacrificed by decapitation. The blood of the trunk was collected from each animal and centrifuged at 3500 RPM for 15 min. The serum was stored at − 20 °C until progesterone, testosterone, estradiol, FSH, and luteinizing hormone (LH) levels were measured by radioimmunoassays (RIA). During the autopsy, we verified the free movement of the ovary in the abdominal cavity to confirm the complete sectioning of the SON. Three ovaries per group were removed and dissected for follicular population assessment, and five ovaries per group were frozen at − 4 °C immediately after autopsy to determine NA levels by high-performance liquid chromatography (HPLC).

Morphometric analysis of the ovaries

The left and right ovaries of all rats used in the study were removed, cleaned of adherent fat tissue, weighed with a precision balance (0.001 mg), and subsequently immersed in Bouin's fixative solution for 24 h, after which they were sequentially placed in ethanol 70%, 96%, 100% and chloroform. The tissues were then embedded in paraffin wax. The left and right ovaries of three randomly selected rats from each group (control, LAP, and SON sectioning) were serially sectioned at 10-μm thick, mounted, and stained with hematoxylin-eosin, and morphometric analysis was performed with the aid of a binocular microscope (Nikon, Model Labophot-2). Using a calibrated ocular micrometer (Nikon), follicle diameter was measured in ovaries where there was a visible nucleus and nucleolus in the oocyte. Measurements were taken from the basement membrane to the basement membrane. The mean diameter was obtained by adding the maximum diameter and the diameter taken at right angles, following methodologies described by Morán et al. [25].

To analyze the follicular population, the average number of follicles in each of the categories was calculated. Primordial follicles were not included in the follicular counting. Follicles were classified into four groups according to mean diameter based on a modification of the classification of Hirshfield: preantral follicles (40–100 μm), small antral follicles (101–350 μm), large antral follicles (351–500 μm), and preovulatory follicles (> 500 μm) [28]. Given that no differences were found in the follicular population between the left and right ovaries of each experimental group, the results were combined.

We also classified the follicles as healthy and atretic (collectively accounting for the total number of follicles) following the parameters described [25, 29]. Follicles were considered atretic when one of the following was observed: ten or more pyknotic granulosa cells in a single section, desquamation of the granulosa cells into the follicular antrum, and oocyte abnormalities or degeneration. All micrographs were taken with a digital camera (Nikon, DS-U2, Japan).

Noradrenaline levels

Levels of NA in the ovary were measured following previously described methodologies [30]. In brief, the ovary was weighed in a precision balance, homogenized in 300 μl of 0.1 N perchloric acid, and centrifuged at 12,500 RPM at 4 °C for 30 min. The supernatant was filtered using 0.2 μm regenerated cellulose filters. Twenty microliters of this extract was injected into the HPLC system (L-250 model; Perkin Elmer Co., Norwalk, CT, USA). The level of NA was quantified electrochemically using an LC-4A amperometric detector and an LC-5A glassy carbon transducer cell at a potential of 850 mV (Bioanalytical Systems Inc.). The mobile phase consisted of 0.1 M citrate buffer (Merck-Mexico, S. A.) at pH 3.0, with 175 mg of 1-octane-sulfonic acid (Sigma Chemical Co., St. Louis, MO, USA); it was filtered and degassed under vacuum.

Immediately after degassing, 20 ml of acetonitrile and 21.5 ml of tetrahydrofuran were added for chromatography (Merck, Darmstadt, Germany) until a total volume of 500 ml was reached. The flow rate of the mobile phase was 1.2 ml/min. Stock standards (Sigma Chemical Co.) were prepared and diluted with 0.1 M perchloric acid on the day of the experiment. The system was calibrated by producing a 0.1–2-ng/ml standard range curve. Monoamine was analyzed as relative retention times compared to those of standards. Using a 1020 Perkin-Elmer Nelson integrator, the level of monoamine was determined by comparing standards with the highest peaks obtained from the samples. The NA levels are expressed as picograms of neurotransmitter/milligram of the ovary (pg/mg). The sensitivity was 0.01 ng.

Hormone measurement

Serum levels of progesterone (ng/ml), testosterone, and estradiol (pg/ml) were measured using solid-phase RIA with kits purchased from Diagnostic Products (Los Angeles, CA, USA). The intra- and inter-assay coefficients of variation were 7.46 and 8.43% for progesterone, 8.75 and 9.48% for testosterone, and 7.83 and 8.74% for estradiol, respectively.

LH and FSH (ng/ml) serum levels were measured with a dual antibody RIA using reagents and protocols kindly supplied by the NIADDK National Pituitary Program (Bethesda, MD, USA). Intra- and inter-assay variations for LH were on the order of 5.1 and 6.5% and 4 and 7.9% for FSH, respectively. The results are expressed in terms of NIADDK standards RP-2 LH and FSH.

Statistical analyses

Statistical analyses were performed using GraphPad InStat 3 Software, Inc. (San Diego, CA, USA). Serum levels of progesterone, testosterone, estradiol, FSH, and LH and ovarian NA levels were analyzed with one-way analyses of variance (ANOVA), which was followed by Tukey's test. The number of total or atretic follicles was analyzed with a Kruskal-Wallis test followed by Dunn's test. Differences between two groups were analyzed with Student's *t* test or a Mann-Whitney *U* test. A *p* value lower than 0.05 was considered statistically significant. Data are expressed as the mean ± standard error of the mean (S.E.M.).

Results

Effects of unilateral or bilateral laparotomy performed at 11:00 h of diestrus 1

Steroid hormones

In comparison with the control group, progesterone levels were lower in the left or bilateral LAP groups, while testosterone levels were lower in animals with a unilateral or bilateral LAP. Left or bilateral laparotomy resulted in higher levels of estradiol than what was observed in the control group (Table 1).

Gonadotropic hormones

FSH levels were higher in animals that received right LAP than they were in the control group, whereas no differences were observed in animals with left or bilateral LAP.

Unilateral or bilateral LAP resulted in lower LH levels than what was observed in control animals (Table 1).

Noradrenaline

NA levels in the right ovary of rats with right LAP were lower than in control animals; the groups with left and bilateral LAP did not show changes in NA levels with respect to the control group. In comparison with the left or bilateral LAP groups, right LAP resulted in lower amine levels in the right ovary (Table 1).

Follicular population analysis

The total number of follicles (healthy + atretic), preantral, small antral, and preovulatory, was not changed by left or bilateral LAP compared to the control group, while right LAP decreased the number of large antral follicles (Fig. 2a). In comparison with control animals, the number of atretic large antral follicles decreased in animals with right LAP, and there were no changes in the groups with left or bilateral LAP (Fig. 2b).

Table 1 Effects of unilateral (left or right) or bilateral laparotomy on hormone levels

Groups	P4 (ng/ml)	T (pg/ml)	E2 (pg/ml)	FSH (ng/ml)	LH (ng/ml)	NA (pg/mg of ovary)	
						Left ovary	Right ovary
Control	21.5 ± 2.9	64.1 ± 4.7	29.6 ± 4.1	0.7 ± 0.06	0.60 ± 0.05	404 ± 37.9	522 ± 21.7
LAP-L	4.7 ± 0.6a	45.3 ± 2.6a	59.0 ± 6.1a	0.8 ± 0.09	0.36 ± 0.04a	413 ± 46.6	523 ± 59.5
LAP-R	24.8 ± 1.8	38.5 ± 4.0a	23.5 ± 2.9	1.8 ± 0.18a	0.35 ± 0.03a	354 ± 34.8	280 ± 33.2ab
LAP-B	7.9 ± 0.9a	38.5 ± 3.3a	62.3 ± 7.6a	1.4 ± 0.35	0.38 ± 0.04a	467 ± 59.5	403 ± 20.1

Media±S.E.M. progesterone (P4), testosterone (T), estradiol (E2), follicle-stimulating hormone (FSH), luteinizing hormone (LH) serum levels, and ovarian noradrenaline (NA) levels in untreated control rats or rats subject to left (LAP-L), right (LAP-R), or bilateral (LAP-B) laparotomy performed at 11.00 h on diestrus 1. Animals were sacrificed 24 h after surgery ($n = 8$ animals per group). a $p < 0.05$ vs. control; b $p < 0.05$ vs. LAP-L and LAP-B (ANOVA test followed by Tukey's test)

Figure 3 shows a representative histological section of the ovary of a control animal in comparison with an animal with right LAP; a greater number of follicles in different stages of development were observed in the control than in the animals with right LAP (Fig. 3a, b). Different classes of follicles were observed by morphometric analysis (preantral, small antral, large antral, and preovulatory follicles (Figure c–f).

Given that the laparotomy modified several of the studied parameters, the effects of the uni- or bilateral sectioning of the SON were compared with those of their respective laparotomy.

Effects of unilateral or bilateral SON sectioning performed at 11:00 h of diestrus 1

Steroid hormones Compared to LAP-treated animals, progesterone levels were higher in animals with left or bilateral SON sectioning. No differences were observed in rats with right SON sectioning (Fig. 4a).

Left SON sectioning resulted in testosterone levels that were lower than those in the LAP group. No differences were observed in rats with right or bilateral SON sectioning (Fig. 4b).

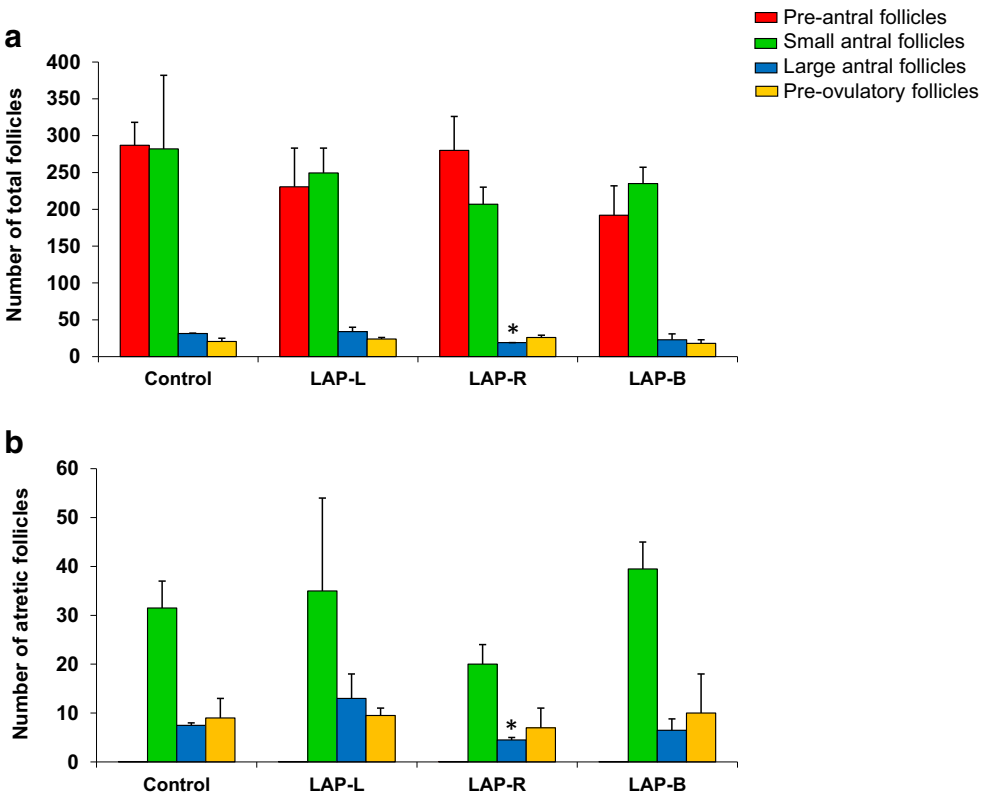

Fig. 2 Effects of unilateral (left or right) or bilateral laparotomy (LAP) on follicular development. Mean ± S.E.M. total number of (healthy+atretic) (**a**) or atretic (**b**) follicles in untreated control rats or rats subjected to left (LAP-L), right (LAP-R), or bilateral (LAP-B) laparotomy performed at 11:00 h of diestrus 1. *$p < 0.05$ vs. control (Kruskal-Wallis followed by Dunn's test)

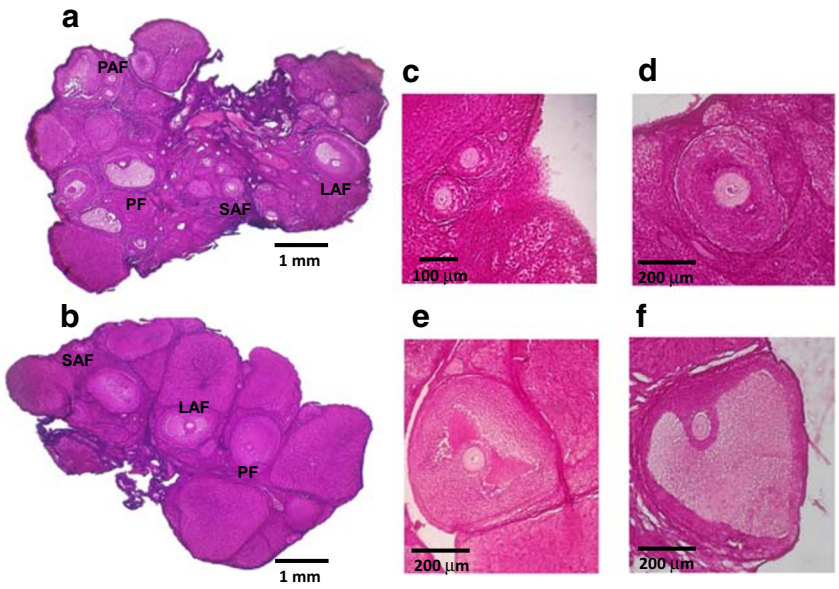

Fig. 3 Micrographs correspond to the largest section of one ovary of animals control (**a**) or with right laparotomy (**b**); images were captured with a × 5 microscopic lens. High magnification of each class of follicles in the ovaries: preantral follicle, PAF (**c**); small antral follicle, SAF (**d**); large antral follicle, LAF (**e**); and preovulatory follicle, PF (**f**). Images were captured with a × 10 microscopic lens

Left, right, or bilateral SON sectioning at 11:00 h of diestrus 1 did not change estradiol levels in comparison with those of the LAP groups (Fig. 4c).

Gonadotropic hormones FSH levels were lower in animals with left or bilateral SON sectioning than they were in their corresponding LAP group. Right SON sectioning resulted in higher FSH levels than what was observed in animals with LAP (Fig. 4d).

Rats with unilateral or bilateral SON sectioning did not show modified LH levels (Fig. 4e).

Noradrenaline Compared with their respective LAP groups, NA levels in both ovaries were lower in animals with left or

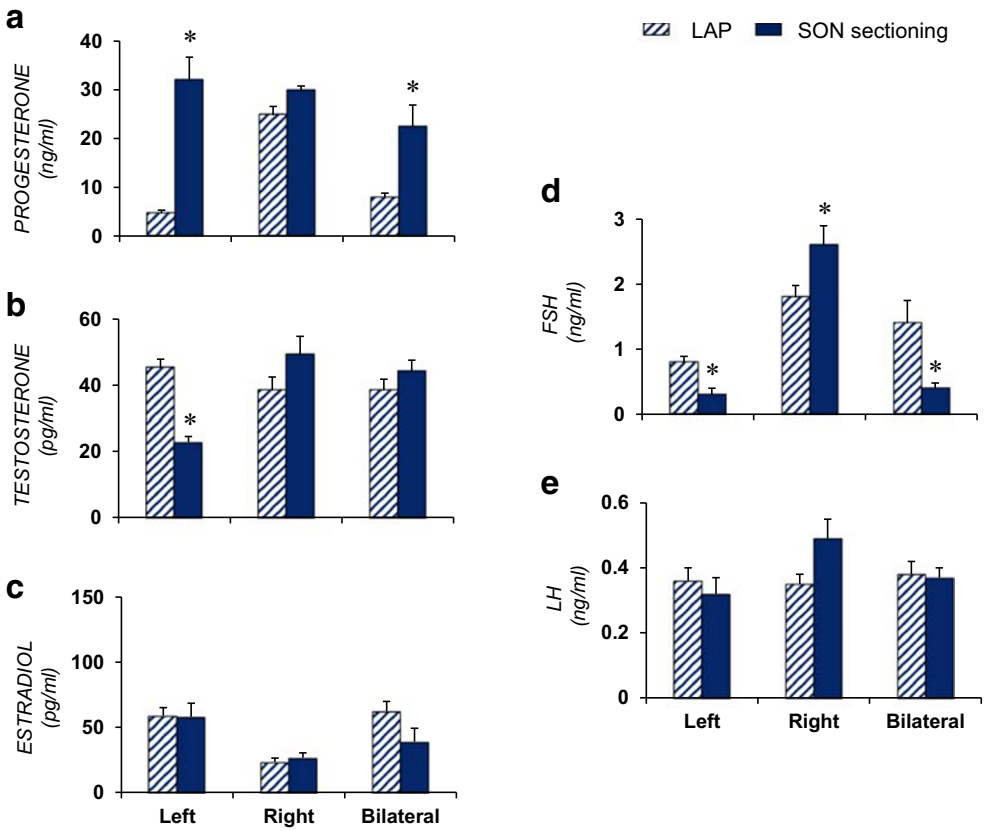

Fig. 4 Effects of unilateral (left or right) or bilateral superior ovarian nerve (SON) sectioning on steroid and protein hormone levels. Mean ± S.E.M. serum progesterone (**a**), testosterone (**b**), estradiol (**c**), follicle-stimulating hormone (FSH) (**d**), and luteinizing hormone (LH) (**e**) levels in rats with left, right, or bilateral laparotomy (LAP) or SON sectioning performed at 11:00 h of diestrus 1. *$p < 0.05$ vs. their corresponding LAP group (Student's *t* test)

bilateral SON sectioning, while NA levels were lower only in the denervated ovary in animals with right SON sectioning (right ovary) (Fig. 5).

Follicular population analysis In comparison with the LAP groups, the number of preovulatory follicles decreased in the ovaries of animals with left or right SON sectioning. No significant changes were observed in the ovaries of animals with bilateral SON sectioning compared to their corresponding LAP group. However, bilateral SON sectioning resulted in a decrease in the number of preantral follicles compared to that of left SON sectioning (Fig. 6a).

The number of atretic small antral follicles decreased with right SON sectioning compared to left SON sectioning, and there were no changes when compared to the LAP group. The number of atretic small antral follicles decreased in the ovaries of rats with bilateral SON sectioning compared to the LAP or left SON sectioning groups (Fig. 6b).

Regardless of denervation type, it should be noted that structural abnormalities in the follicular atresia included pyknosis and desquamation of the granulosa cells in large antral follicles (Fig. 7a, b), as well as oocyte fragmentation observed in small antral follicles (Fig. 7c–e) when the ovaries were subjected to SON sectioning. Furthermore, invagination in the granulosa cell layer was observed more abundantly in large antral and preovulatory follicles and more sparingly in small antral follicles. In Fig. 8, microphotographs of ovarian histological slices show invaginations in the follicular wall of a large antral follicle (Fig. 8a) and loss of structure (disorganization of granulosa cell layer) in a preovulatory follicle (Fig. 8b), which can be compared with the healthy large antral (Fig. 8c) and preovulatory follicles (Fig. 8d) of the ovary of a control animal.

Discussion

The results obtained in the present study show that at 11:00 h of diestrus 1, neural signals arriving at the ovaries through the SON stimulate follicular development and that the role of the SON is asymmetric in the regulation of ovarian steroidogenesis. We suggest that NA differentially stimulates the different types of adrenergic receptors in the ovaries, which would modulate the steroidogenic response. However, these effects depend on the nerve that has been sectioned, since the left SON inhibits progesterone and stimulates testosterone secretion, whereas the right SON does not participate in this process.

Progesterone levels in the serum of animals with unilateral or bilateral laparotomy performed during diestrus 1 at 13:00 h have been found to be similar to those of control animals [31], whereas testosterone and estradiol levels show changes depending on the side of the peritoneum that is perforated and the day of the estrus cycle when the surgery is performed [32, 33]. It has also been found that when left or right laparotomy is performed on diestrus 1 at 10:30 h, there is an increase in progesterone compared to that of a control group, but testosterone and estradiol levels do not depend on the side of where the surgery is performed [12]. Taken together, these results and those of the present study suggest that the mechanism modulating the effects of laparotomy varies depending on the side where the surgery is performed, the type of hormone, and the time of day of the study.

Furthermore, such evidence supports the idea that the effects of laparotomy performed in rats could be the result of an alteration of neural pathways in the abdominal skin and/or peritoneum that send information to the ovary through the SON [12, 34], as suggested by Uchida et al. [35], who observed that the stimulation of the abdominal skin at the left or right side produced an increase in ovarian sympathetic nerve activity.

Morán et al. [25] showed that the number of follicles decreased in the ovaries of infantile rats that underwent laparotomy (left and right) and were killed 4 and 8 days later. In the present study, right laparotomy decreased the number of large antral follicles, and this effect was accompanied by an increase in FSH levels and a decrease in ovarian NA levels, whereas no changes were observed in animals with left or bilateral

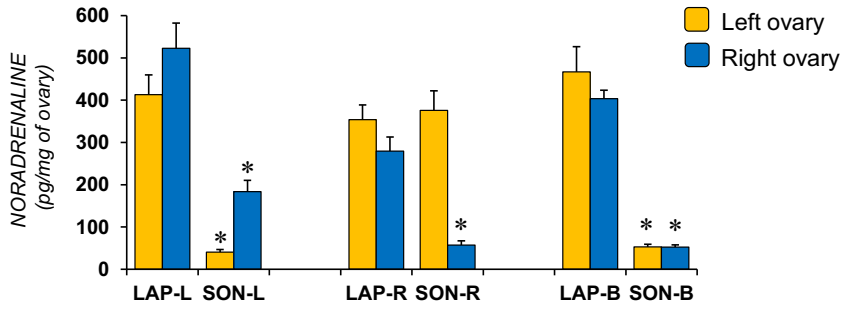

Fig. 5 Effects of unilateral (left or right) or bilateral superior ovarian nerve (SON) sectioning on ovarian noradrenaline levels. Mean ± S.E.M. ovarian noradrenaline levels in rats with left, right, or bilateral laparotomy (LAP) or with left, right, or bilateral SON sectioning performed at 11:00 h of diestrus 1. *$p < 0.05$ vs. their corresponding LAP group (ANOVA followed by Tukey's test)

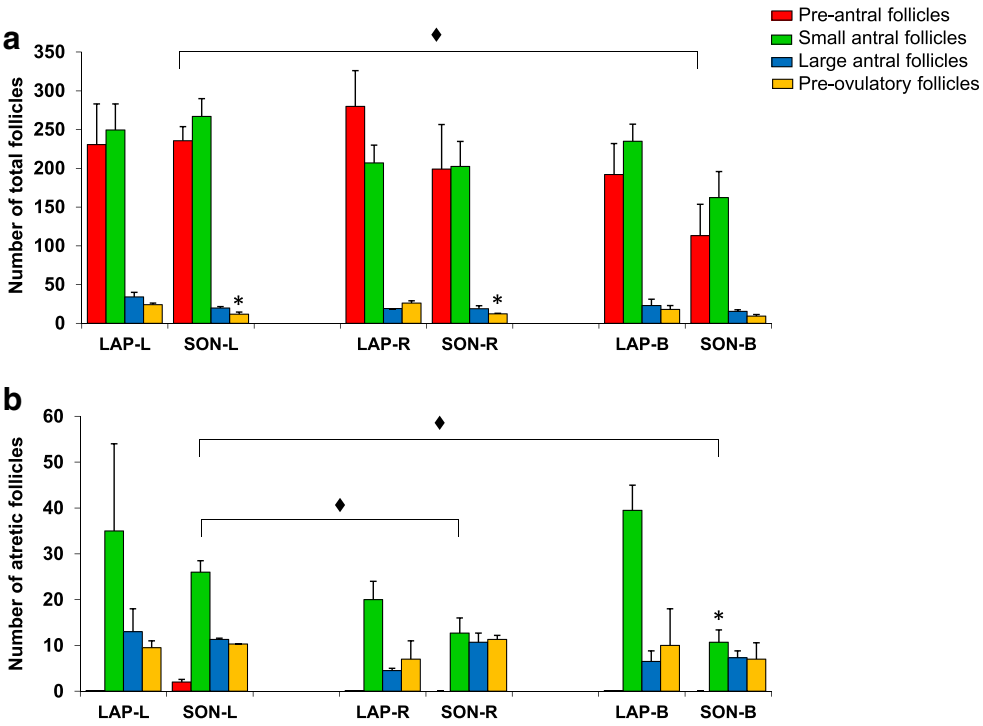

Fig. 6 Effects of unilateral (left or right) or bilateral superior ovarian nerve (SON) sectioning on follicular development. Mean ± S.E.M. total number of healthy+atretic (**a**) or atretic (**b**) follicles in rats with left (LAP-L), right (LAP-R), or bilateral (LAP-B) laparotomy or with left (SON-L), right (SON-R), or bilateral (SON-B) SON sectioning performed at 11:00 h of diestrus 1. *$p < 0.05$ vs. their corresponding LAP group; ♦$p < 0.05$ vs. SON-L (Kruskal-Wallis followed by Dunn's test)

laparotomy. These results suggest an asymmetric effect on the regulation of follicular development, where the information originating from the right side stimulates this process.

There is a proposed hypothesis that laparotomies modulate steroidogenesis through activation of a neural pathway where the two sides of the abdominal wall send different neural

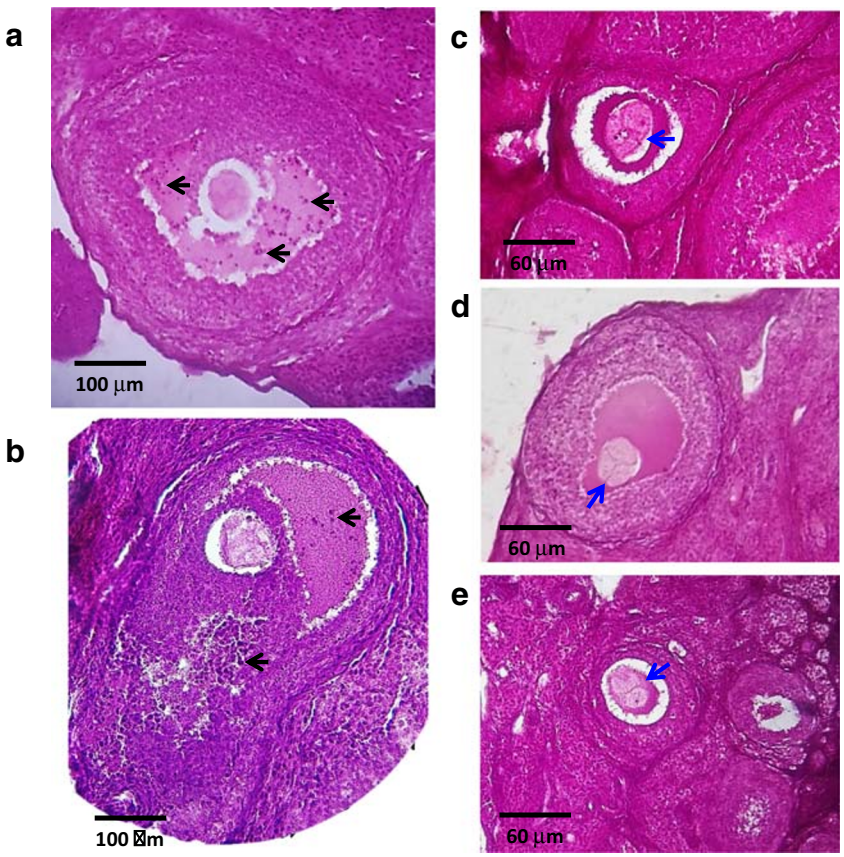

Fig. 7 Micrographs showing atretic follicles from animals with sectioning of the SON. Pyknosis of the granulosa cells and desquamation into the follicular antrum (black arrows) in large antral follicles (**a**, **b**); images were captured with a × 40 microscopic lens. Fragmented oocytes (blue arrows) in small antral follicles (**c**–**e**); images were captured with a × 40 microscopic lens

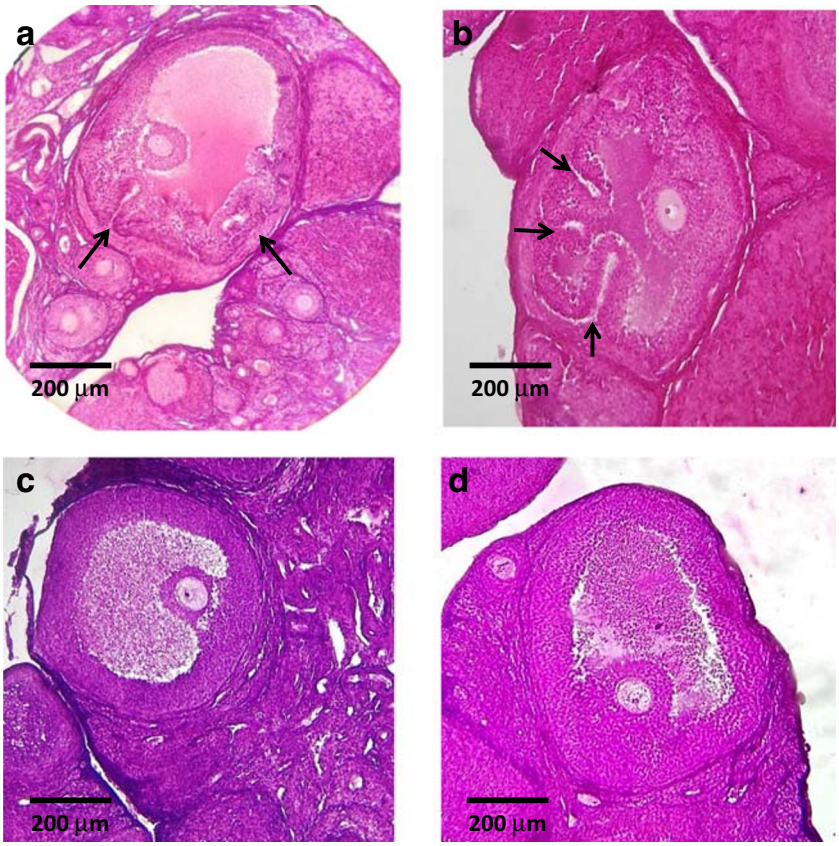

Fig. 8 Ovarian histology of rats with unilateral or bilateral superior ovarian nerve (SON) sectioning. Micrographs of ovaries from a rat with sectioning of the SON performed during diestrus 1 at 11.00 h showing a large antral follicle (351–500 μm) with invaginations in the granulosa cell layer (arrows) (**a**) and a preovulatory follicle (> 500 μm) with loss of structure (disorganization) of the granulosa cell layer (arrows) (**b**). Large antral and preovulatory follicles of the ovaries from control rats (**c**, **d**). Images were captured with a × 10 microscopic lens

information through the SON to the ovaries and the central nervous system, perhaps reaching nuclei related to the vagus nerve [12, 18, 36]. Therefore, it is possible to conclude that the decrease in the synthesis of FSH receptors in large antral follicle populations in animals subjected to a right laparotomy could be due to a decrease in ovarian NA levels, using this suggested pathway, where the proposed mechanism of action for NA in the ovary involves binding to ovarian adrenergic receptors [37] and induction of cAMP, leading to follicular development [38].

In the present study, the role of the left SON on diestrus 1 was inhibitory in terms of the secretion of progesterone and stimulatory in terms of the secretion of testosterone. Even though the right SON does not participate in the regulation of ovarian steroid secretion, it is possible that these results could indicate the interaction or the regulation of one of the neurotransmitters (NA, vasoactive intestinal peptide (VIP), and neuropeptide Y (NPY)) that travel through the SON to the ovaries during steroidogenesis [12, 39].

This idea is supported by a study by Garraza et al. [39], where with an integrated coeliac ganglion-SON-ovary ex vivo system, they show that VIP added to the ganglion compartment has an inhibitory effect on the release of progesterone on diestrus 1, which is amplified by NA. Moreover, using an in vivo study, Rosas et al. [12] proposed that the neural signals arriving at the ovaries through the SON, such as NA and NPY, modulate the effects of VIP on steroid hormone secretion as a result of the action of these neurotransmitters on the steroidogenic enzymes that participate in synthesis [16, 40–42]. However, we cannot rule out a dual effect of the SON on the regulation of steroid secretion; for example, in the case of progesterone, the stimulation of α-adrenergic receptors inhibits the secretion of the hormone, while it is stimulated by the activation of β-adrenergic receptors [43]. Studies in prepubertal rats have shown that bilateral sectioning of the SON produces a depletion of 60% of ovarian NA and a compensatory increase in β-adrenergic receptor number [5].

NA released from nerve endings by electrical stimulation of the SON on the day of estrus reduces estradiol secretion by the ovary [44] via the activation of α2-adrenoceptors [45], and this is independent of the reduction in testosterone, since the SON has an inhibitory role in ovarian testosterone secretion via the activation of α1-adrenoceptors [46].

Several studies have also shown that the neural mechanisms regulating ovarian steroid hormone secretion vary according to the studied hormone [10, 12, 18, 24, 46, 47], where the expression of genes encoding 3β-hydroxysteroid dehydrogenase, 17β-hydroxysteroid dehydrogenase, and aromatase which participate in the steroidogenic pathway, is regulated differently by the innervation of the SON in rats, and these effects depend on the sectioned nerve and the day of the cycle [48].

In the present study, we suggest that unilateral denervation could modify the number, and possibly the affinity, of different types of adrenergic receptors that respond to NA in the ovaries, which would differentially modulate the steroidogenic response and would depend on which nerve has been sectioned. Further studies are necessary to evaluate this conclusion.

In the prepubertal rat, the unilateral sectioning of the SON decreases the number of ova shed by the denervated ovary while increasing the number of ova shed by the innervated ovary, which suggests the existence of neural communication between the ovaries (9). We previously showed that this communication occurs through the intermediolateral column via sympathetic nerves and arrives at the other ovary via the SON [25]. In the present study, we observed similar follicular development between the left or right ovaries in animals with unilateral or bilateral sections of the SON, which supports the idea of communication between the ovaries.

According to Doganay et al. [2], bilateral sectioning of the SON in peripubertal rats does not change follicular development through 10–11 weeks of age, which is in the estrus stage. However, follicular maturation was severely altered by the thinning of the theca interna of antral follicles, which suggests that the removal of VIPergic fibers by sectioning of the SON modified the growth of the follicular wall. Furthermore, Zhang et al. [11] showed that sectioning of the SON in neonatal rats inhibits follicular development by suppressing the proliferation of granulosa cells, and the decrease in ovarian NA enhances cell apoptosis. These pieces of evidence indicate that VIP and NA are two neurotransmitters that arrive through the SON during the modulation of follicular development.

In this study, performed at diestrus 1, we found that the role of the SON in follicular development is stimulatory. A decrease in preovulatory follicle development caused by unilateral SON sectioning cannot be explained by an increase in atresia. Another possibility is that the SON modulates the sensitivity of follicles to gonadotropins in different ways according to their degree of follicular maturity. However, we cannot rule out evidence that granulosa cells are target cells for NA, where it acts as an important mitogenic factor [11]. If NA levels in the ovary decrease due to the denervation, lower follicular growth will be consequently observed.

Unlike unilateral sectioning of the SON, bilateral sectioning decreases the atresia of small antral follicles, suggesting that the integrity of the nervous signal provided by both nerves is necessary to maintain control in the process of follicular atresia.

If the SON is the main source of NA [5] and VIP to the ovary [49], then we suggest that sectioning of the SON reduces levels of NA, and possibly of VIP [50], in the ovary; such effects result in a reduction in the synthesis of FSH receptors [38] and a decrease in the follicular response to FSH, thus causing a reduction in preovulatory follicle development.

In the present study, the effects on follicular development and atresia resulting from sectioning of the SON did not correspond to changes observed in steroid hormone levels. This suggests that although the neuroendocrine signals that regulate both processes are the same, the regulatory neuroendocrine mechanisms are different, as we have previously suggested with the mechanisms of neuroendocrine regulation that modulate ovulation and hormone secretion on the day of proestrus in rats [24].

An important observation in the present study, which was also reported by Escobar-Sánchez et al. [51], was the structural abnormalities in the follicular atresia that included oocyte fragmentation in small antral follicles. Escobar-Sánchez et al. [51] observed that the diestrus phase was characterized by a high frequency of markers of apoptosis and autophagy in the same oocyte, which would explain cellular segmentation, a typical characteristic of apoptosis. This evidence allows us to suggest that the role of the SON is modulatory in the process of apoptosis and autophagy in oocyte fragmentation. Nevertheless, further studies are necessary to confirm this suggestion.

We also observed invaginations in the granulosa cell layer of the large antral and preovulatory atretic follicles in animals with unilateral or bilateral SON sectioning. This response could suggest that a lack of a noradrenergic signal resulting from denervation of the SON reduces the number of FSH receptors, causes the loss of gap junctions in granulosa cells, and, in some cases, causes the loss of follicular structure. This idea is supported by a study by Burghardt and Matheson [52], who showed that FSH increases the number and turnover rate of gap junctions in granulosa cells.

It is also important to note that part of the observed effects in the folded follicular wall could be due to a lack of VIP. Thus, it is necessary to perform further studies to determine whether these follicles can ovulate.

Conclusion

In the present study, we found that the role of SON at 11:00 h of diestrus 1 is stimulatory in follicular development, possibly via NA and/or VIP, which promotes the synthesis of FSH receptors. Our results also support the idea that the SON modulates the secretion of ovarian steroids in an asymmetric way, where the left SON inhibits progesterone and stimulates testosterone secretion, while the right SON does not participate in these processes. Given that NA begins to be released from nerve terminals during diestrus 1, we suggest that it differentially stimulates the different types of adrenergic receptors in the ovaries, which would modulate the steroidogenic response, and the effects depend on which nerve has been sectioned.

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Author contributions DAR, RD, and LM-L designed the experiments. DAR and EV performed the experiments. DAR, EV, GR, RL, JAE, ACHO, RD, and LM-L devised the study, participated in the discussion of the results, and co-wrote the manuscript. All authors read and approved the final version of this manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All experiments were carried out in strict accordance with the Mexican Law of Animal Treatment and Protection Guidelines and followed the Mexican Official Standard NOM-062-ZOO-1999 specifications. All efforts were made to minimize the number of animals used and their suffering.

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