Steven H. Prevatte. EFFECTS OF OVARIAN DENERVATION ON COMPENSATORY OVARIAN HYPERTROPHY IN HYPOPHYSECTOMIZED RATS. (Under the direction of Irvin E. Lawrence, Jr., Ph.D.) Department of Biology, August 1982.

The purpose of this study was to examine the effects of ovarian denervation on compensatory ovarian hypertrophy (COH) in hypophysectomized (HYPOX) rats. Animals were unilaterally ovariectomized (ULO) and denervated two weeks following HYPOX. Denervations were performed by two methods, vagotomy (VAGOT) and chemical sympathectomy using 6-hydroxydopamine (6-HD). Fifteen days after ULO, all animals were sacrificed and statistical differences between ovarian weights were calculated.

The results show that COH did not occur in previously HYPOX rats, either with or without intact ovarian nerves. After fifteen days, the weight of the remaining ovary was not significantly different from the weight of the initial ovary in any of the HYPOX groups. Histologically, all ovaries of HYPOX animals had prominent corpora lutea, while healthy antral follicles were not observed. Because of the luteal tissue present, serum progesterone levels were measured. There were no measurable differences in the levels of progesterone between the HYPOX groups regardless of the type of denervation.

These results suggest that ovarian nerves do not mediate COH in the absence of the pituitary.

EFFECTS OF OVARIAN DENERVATION ON COMPENSATORY OVARIAN HYPERTROPHY IN HYPOPHYSECTOMIZED RATS

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EFFECTS OF OVARIAN DENERVATION

ON COMPENSATORY OVARIAN HYPERTROPHY

IN HYPOPHYSECTOMIZED RATS

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INTRODUCTION

It has been well documented that removal of one ovary results in increase in size and weight of the remaining ovary. Compensatory ovarian hypertrophy is thought to be entirely due to hormonal influences, however, a neural role has recently been suggested.

Both sympathetic and parasympathetic fibers have been shown to innervate the ovary. The role that these nerves play in ovarian function remains to be elucidated. Vagal and sympathetic nerves, however, have been implicated in compensatory ovarian hypertrophy.

Therefore, this study was designed to examine the effects of vagal and sympathetic denervation on compensatory ovarian hypertrophy in previously hypophysectomized rats. This model permitted the examination of the local neural components in ovarian hypertrophy without interference from hypophyseal gonadotropins.

REVIEW OF LITERATURE

The specializations of neurology and endocrinology began as different disciplines; however, in recent years it has become more difficult to form a clear distinction between the two. Because of the complex interrelationships between nerves and endocrines, a new branch of science has evolved called neuroendocrinology. Stimuli received and interpreted by the central nervous system are passed by neurons to neurosecretory cells, which respond by releasing neurohormones in the area of the effector cells or into vascular pathways for more remote action (Turner and Bagnara, 1976). The neurosecretory cells perform the important function of tying together the nervous and endocrine systems. They constitute a final common pathway for conversion of nerve impulses into endocrine messengers. Such may be the case with the mammalian ovary.

Female reproductive physiology is under neuroendocrine control. The hormonal regulation of ovarian physiology works via the hypothalamo-hypophyseal-ovarian axis. At puberty, the anterior pituitary is stimulated by the hypothalamus to produce follicle stimulating hormone (FSH) which activates progressive development of immature follicles. Granulosal cells of activated follicles, under the influence of FSH, proliferate and produce estrogen. The estrogens are the female sex steroids which are responsible for the secondary sex characteristics in the female. When blood titers of estrogen reach a high, FSH is inhibited and the pituitary is stimulated to produce luteinizing hormone (LH). Luteinizing hormone causes the ripe follicle to rupture

(ovulation) and in turn stimulates the formation of the corpus luteum. The corpus luteum under the influence of LH produces progesterone, which prepares the uterus for implantation. High blood titers of progesterone inhibit the production of LH. Without luteotropic support the corpus luteum stops producing progesterone. Falling blood titers of progesterone allows the pituitary to produce more FSH, and thus the cycle begins again. This description of hormonal control of the ovary is classical and very well documented, on the other hand, neural influences are not well understood. Investigations on the physiologic role of neural control of the ovary have been aimed in the following directions: 1) neural influences on cyclic ovarian activity and steroid secretions during pregnancy; 2) neural influences in gonadotropin secretion, follicular development, and steroid secretions; and, 3) neural participation in the ovulatory process (Burden, 1978). Many studies are being performed in order to better understand neural control of the ovary. The present study deals with the specific role nerves play in the development of compensatory ovarian hypertrophy.

Several authors have demonstrated that upon removal of one ovary, the remaining ovary shows compensatory hypertrophy (Hatai, 1913; Arai, 1920). The remaining ovary compensates by an increase in size and weight. After hemispaying, Hatai (1913) found that in albino rats compensatory growth of the surviving ovary is almost perfect, that is, the remaining ovary increased to nearly twice its normal weight. Arai (1920) described the hypertrophy as a greater abundance of well developed normal and degenerative follicles, as well as excess

corpora lutea. It is concluded that these are the changes that take place in compensatory ovarian hypertrophy.

The mechanism for hypertrophy is not fully understood. It is postulated that serum levels of ovarian steroids are decreased due to the loss of one ovary, which reduces negative feedback and causes increased secretions of pituitary gonadotropins (Edgren et al., 1965; Howland and Skinner, 1973; Welschen and Dullaart, 1974; DeGreef et al., 1975). Peppler and Greenwald (1970) found that unilateral ovariectomy on the morning of days 1 to 3 of the cycle (day 1 is estrous) doubled the number of ovulations (complete compensatory ovulation) by the next estrous. Also supporting the theory of increased gonadotropins as the mechanism for compensatory ovarian hypertrophy is a study in which Peppler (1975) found that the number of ova shed is affected by the amount of ovarian tissue present. The amount of ovarian tissue present does not affect, however, the total number of follicles which mature during each estrous cycle. This indicates that the hypothalamo-hypophyseal-ovarian axis is specifically regulated in regards to ovulation number for existing conditions within the rat during each estrous cycle rather than being autonomous. This theory of increased gonadotropins as the mechanism for compensatory ovarian hypertrophy is highly favored, however, recent studies imply that a neural component might be involved (Gerendai and Halasz, 1976; Burden and Lawrence, 1977; Gerendai et al., 1978; Gerendai, 1979).

The mammalian ovary has both sympathetic and parasympathetic innervation. As cited by Burden (1978), some extrinsic sympathetic

innervation comes from the ovarian plexus, which is an extension of the aortic and renal plexuses. The superior ovarian nerve traveling via the suspensory ligament supplies additional sympathetic innervation to the ovary (Lawrence and Burden, 1980). Parasympathetic nerves were thought to be derived from the vagus (tenth cranial nerve) and/or S-2 to S-4 cord levels through branches from the hypogastric plexus. However, Burden and Lawrence (1978) demonstrated that S-2 to S-4 cord levels do not innervate the rat ovary. Rather, their studies demonstrated that the parasympathetic innervation is from the vagus. These findings corroborated previous work by Hill (1962). Intrinsic nerves (cited by Burden, 1978) enter the hilus of the ovary and innervate structures in the parenchyma, either associated with blood vessels or free in the stroma.

The function of nerves in compensatory ovarian hypertrophy (COH) is not completely understood. Burden and Lawrence (1977) implicated the vagus nerve in COH as well as in cyclic activity and gonadotropin secretion. In their study, the effects of pelvic neurectomy, abdominal vagotomy and vagotomy plus chemical sympathectomy [by injection of the drug 6-hydroxydopamine (6-HD) which causes selective degeneration of adrenergic nerve terminals (Malmfors and Thoenen, 1971)] on COH were examined. Vagotomy and vagotomy plus 6-HD interrupted estrous cycles and significantly decreased the degree of COH following unilateral ovariectomy. Vagotomized rats with both ovaries intact had disrupted estrous cycles, but ovarian weights were not affected. These results suggested that

the vagus nerve mediates development of COH, whereas sympathetic nerves and pelvic splanchnic nerves are not involved. To investigate whether abdominal vagotomy results in altered estrous cycles and decreased hypertrophy due to altered gonadotropin secretion, Burden and Lawrence (1977) performed a subsequent study. Rats in estrous were sham-operated, unilaterally ovariectomized, vagotomized, or vagotomized plus unilaterally ovariectomized, and serum levels of LH and FSH were determined at 5 and 24 hours. Unilateral ovariectomy caused a significant ($p < 0.05$) increase in LH and FSH. Vagotomy significantly ($p < 0.05$) depressed LH and FSH in those animals at 5 hours. At 24 hours, LH was still significantly higher in unilaterally ovariectomized controls than in those with vagotomy ($p < 0.01$). Also, vagotomy significantly ($p < 0.01$) depressed serum FSH levels at 24 hours. It would seem that the vagi facilitate FSH and LH release, thus the vagi should be considered components of the gonadotropin control system.

In a related study by Gerendai et al. (1978), a new technique was devised to use a special plastic capsule to chemically treat ovaries locally. Each capsule consisted of two matched hemispheres dug out of a solid plastic bar in order to enclose the ovary completely. A symmetrical hemicircle was cut out of the two hemispheres in order to leave the uterine and ovarian blood supplies intact. The capsule was filled with a neutral cream plus either 6-HD, the experimental group, or dopamine, the control group. The capsule was then surgically placed around the ovary. This was performed on animals

with both ovaries intact, as well as on animals that had undergone unilateral ovariectomies. They found that local treatment of one ovary with 6-HD resulted in a weight increase in the other ovary. Dopamine had no effect. In the unilaterally ovariectomized animals, 6-HD blocked COH of the remaining ovary, but treatment with dopamine permitted its development. These results imply that the mechanism of COH might require an adrenergic component. This appears to differ from Burden and Lawrence (1977) whose techniques were different.

Studies done using hypophysectomized animals (Smith, cited by Papkoff et al., 1977), show that after removal of the pituitary, gross reproductive dysfunction occurs. Immature hypophysectomized rats fail to mature sexually, and sexually functioning rats that underwent hypophysectomy experienced atrophy of the gonads and loss of reproductive function. In the ovaries there were no normal or medium sized follicles present 4 days following hypophysectomy. Primordial follicles continued to develop, but underwent atresia by the time of antral formation. Without the pituitary, the regulatory role of negative feedback, through which the sex steroids operate, can be ruled out.

Gerendai (1979) reported that ovarian weight drops dramatically 2 weeks after hypophysectomy. When these hypophysectomized rats underwent unilateral ovariectomy, the weight of the remaining ovary increased. These results suggest that atrophy is reduced in the remaining ovary when unilateral ovariectomy and hypophysectomy surgeries are done in one session. It was further postulated that removal of

one ovary results in neural stimulation of the central nervous system and that activity of neural efferents to the remaining ovary are responsible for the maintenance of ovarian weight in the hypophysectomized plus unilaterally ovariectomized animals. This gives additional support for a neural mediation in control of ovarian weight.

Since the vagus and sympathetic nerves seem to have a role in development of compensatory ovarian hypertrophy, perhaps their neural role can be more clearly defined. Therefore, the objective of the present study was to examine the effects of vagal and sympathetic denervations on the occurrence of compensatory ovarian hypertrophy in hypophysectomized rats.

MATERIALS AND METHODS

Female nulliparous Sprague-Dawley rats weighing 128-205 grams were used in this study. These animals were purchased from Harlan Sprague-Dawley Laboratories; Madison, Wisconsin, where the operation for hypophysectomy (parapharyngeal approach) had been performed. These rats were housed 3 to 4 per cage in a temperature controlled room with a 14 hour light and a 10 hour dark schedule. They were fed on standard laboratory chow and 5% sucrose ad libitum. To insure clearance of gonadotropins from the bloodstream, the rats were allowed a recovery period of 2 weeks. After this time, the rats were randomly placed into 1 of 5 treatment groups (groups 2-6) with not less than 6 animals per group. A non-treatment group of rats (group 1) which had not been hypophysectomized served as controls.

The treatments were unilateral ovariectomy, vagotomy, and chemical sympathectomy. Unilateral ovariectomy was performed via a dorsolateral incision. The ovary was cleaned of fat and connective tissue and weighed to the nearest 0.1 milligram. Alternating left and right ovaries were removed from the animals in order to obviate differences between their weights. In vagotomy surgery, the liver was reflected, the esophagus exposed, and the anterior and posterior trunks of the vagus avulsed. At autopsy, the presence of an enlarged distended stomach was used as evidence for a complete vagotomy. Only those animals showing complete vagotomy were used. Vagotomy and unilateral ovariectomy surgeries were performed via a single mid-ventral incision under light ether anesthesia. Chemical sympathectomy was induced

by using 6-HD. Animals received injections of 6-HD (100 mg/kg, i.p.) on days 1, 2, and 7 (day 1 being day of unilateral ovariectomy). The drug was dissolved in a solvent of 0.9% sodium chloride containing 0.2% ascorbic acid (an antioxidant) just prior to injection. The experimental design (Table 1) was as follows: Group 1 was designated as controls and were non-hypophysectomized rats that underwent unilateral ovariectomy. The hypophysectomized animals were placed into groups 2-6 and treated in the following manner. Animals in group 2 were sham-unilaterally ovariectomized, vagotomized and drug injected (ovaries were exposed but left intact; vagus nerve was also exposed, but not touched; injections of the solvent were given in a manner identical to that of the drug). In the remaining groups, all animals were unilaterally ovariectomized. Animals in group 3 received no other treatment. Group 4 animals received injections of 6-HD. Animals in group 5 were subjected to abdominal vagotomy. In group 6 the animals underwent vagotomy surgery and also received injections of the drug, 6-HD.

Fifteen days following the surgery, all animals were sacrificed by decapitation. Trunk blood was collected and allowed to clot over night under refrigeration before centrifugation. The serum was decanted and stored at -70°C until assayed for progesterone. Serum progesterone was measured by radioimmunoassay as previously described by Lawrence et al. (1978). The remaining ovaries were excised, cleaned of fat and connective tissue, and weighed to the nearest 0.1 milligram. All ovaries were fixed in Bouin's fixative , embedded

in paraffin, sectioned at 10 microns and stained with hematoxylin and eosin for routine histological examination. The completeness of hypophysectomy was determined by dissecting out the brain and checking for the presence of pituitary tissue. Also, the uterine cornu were removed, cleaned and weighed to the nearest 0.1 milligram as additional evidence for the thoroughness of hypophysectomy. Only those animals exhibiting complete hypophysectomy were used in this study. Body weights were recorded at the time of surgery and at the time of sacrifice. Ovarian weights were calculated as milligrams per 100 grams body weight.

Statistical significance of the differences in the means of the initial versus the remaining ovarian weights within each of the groups was calculated using Student's t-test. One way analysis of variance was used to determine differences between the groups, testing both initial weights and remaining weights. Student-Newman-Keuls (SNK) test was used to find which of the groups differed. Statistical analysis of the data obtained from the progesterone assay was also calculated using a one way analysis of variance and SNK multiple comparison test.

Table I. Experimental Design

	HYPOX	ULO	6-HD	VAGOT
Group 1		*		
Group 2	*			
Group 3	*	*		
Group 4	*	*	*	
Group 5	*	*		*
Group 6	*	*	*	*

HYPOX - Hypophysectomized
ULO - Unilaterally Ovariectomized
6-HD - 6-Hydroxydopamine Injected
VAGOT - Vagotomized

RESULTS

The results of this study are summarized in Table II. The non-hypophysectomized control rats (group 1) which were unilaterally ovariectomized, showed a significant ($p < 0.001$) increase in the weight of the remaining ovary when compared to the one initially removed. In all of the treatment groups that were unilaterally ovariectomized (3-6) the weight of the remaining ovary was not significantly different from the initial one. When comparing the initially removed ovarian weights, the non-hypophysectomized control group was found to differ significantly ($p < 0.01$) from the treatment groups. Also, the remaining ovarian weights of the non-hypophysectomized group differed significantly ($p < 0.001$) from the treatment groups. When comparing ovarian weights within the treatment groups, no significant differences were found between the initially removed ovaries, or between the ovaries remaining at sacrifice (Figure 1).

Histological observations revealed notable differences between the initial and remaining ovaries in the non-hypophysectomized control group. The initially removed ovary showed characteristic ovarian structures including follicles in various stages of development (primordial follicles through mature antral follicles), interstitial gland, and corpora lutea (Figure 2). The main difference in the remaining ovary was the presence of numerous enlarged corpora lutea within a dense stroma (Figure 3). The initially removed ovaries of all hypophysectomized animals had follicles in early stages of

development while healthy antral follicles were not seen. The stroma of these ovaries was loosely packed and there was an abundant amount of interstitial gland. Also, prominent corpora lutea were observed. The histology of the remaining ovaries in all of the hypophysectomized groups revealed no differences.

Because of the luteal tissue present in the hypophysectomized animals, trunk blood was assayed for progesterone levels. The results of this assay are shown in Table III. Again, the non-hypophysectomized control rats showed a significant ($p < 0.001$) difference in progesterone levels when compared to the hypophysectomized groups. There were no measurable differences in the levels of progesterone between the hypophysectomized groups of animals.

Table II. Effects of different experimental denervations on compensatory ovarian hypertrophy in hypophysectomized rats. Values are mean ± SEM and are expressed as mg/100 g b.w.

GROUP	TREATMENT	NUMBER OF ANIMALS	WEIGHT OF INITIAL OVARY	WEIGHT OF REMAINING OVARY
	NON-HYPOX			
1	ULO	10	14.19 ± 1.17[a]	21.82 ± 1.59[b,c]
	HYPOX			
2	SHAM	11		10.82 ± 0.40[1]
3	ULO	11	11.42 ± 0.66	10.96 ± 0.63
4	ULO+6HD	11	11.23 ± 0.68	11.50 ± 0.64
5	ULO+VAGOT	9	10.87 ± 0.71	9.92 ± 0.79
6	ULO+6HD+VAGOT	6	9.73 ± 1.00	9.85 ± 1.05

[a]Indicates the group is significantly ($p < 0.01$) different from the other initially removed ovaries.

[b]Indicates the group is significantly ($p < 0.001$) different from the other ovaries remaining at sacrifice.

[c]Indicates the remaining control ovary is significantly ($p < 0.001$) different from the control ovary initially removed.

[1]Group 2 animals ovarian weight is an average of the two ovaries remaining at sacrifice.

Figure 1. Initial and remaining ovarian weights for animals in groups 1-6. Values (mean ± standard error of the mean) are expressed in mg per 100 grams body weight. Numbers in parentheses represent the number of animals used in each group.

[a]Indicates the group is significantly ($p < 0.01$) different from the other initially removed ovaries.

[b]Indicates the group is significantly ($p < 0.001$) different from the ovaries remaining at the time of killing.

[c]Indicates the remaining control ovary is significantly ($p < 0.001$) different from the initially removed control ovaries.

[1]Sham animals (group 2) ovarian weight is an average of the two ovaries remaining at sacrifice.

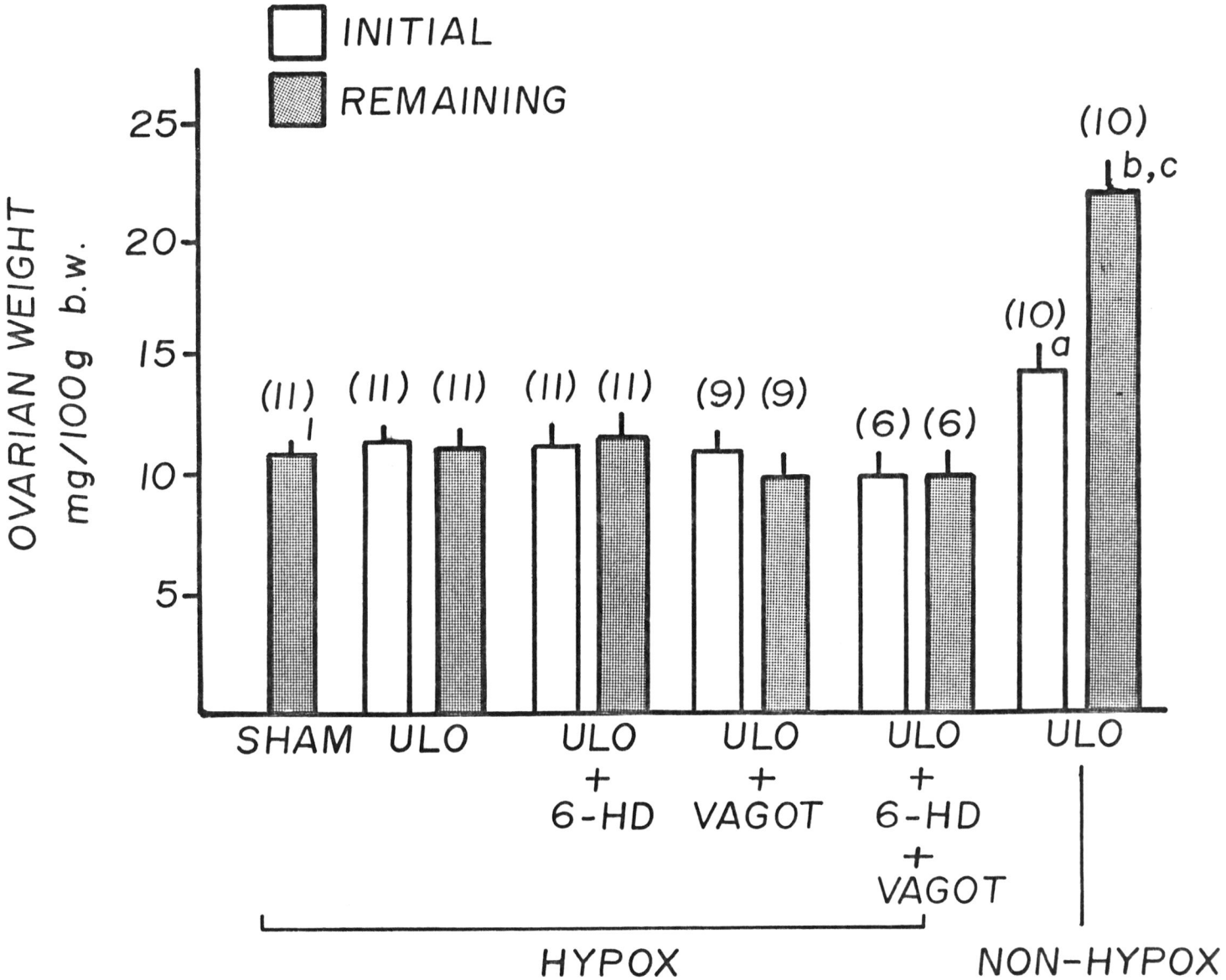
INITIAL
REMAINING
OVARIAN WEIGHT
mg/100g b.w.
25
20
15
10
5
(11)
(11)
(11)
(11)
(11)
(9)
(9)
(6)
(6)
(10)
a
(10)
b,c
SHAM
ULO
ULO + 6-HD
ULO + VAGOT
ULO + 6-HD + VAGOT
ULO
HYPOX
NON-HYPOX

Figure 2. Group 1 (non-hypophysectomized control). Photomicrograph of a section of an initial ovary on day 1 of unilateral ovariectomy. Notice the characteristic ovarian structures including antral follicle (AF), interstitial gland (IG) and corpus luteum (CL). x67.

Figure 3. Group 1 (non-hypophysectomized control). Photomicrograph of a section of a remaining ovary 15 days after unilateral ovariectomy. Note the presence of numerous enlarged corpora lutea (CL) within the densely packed stroma (S). x67.

2

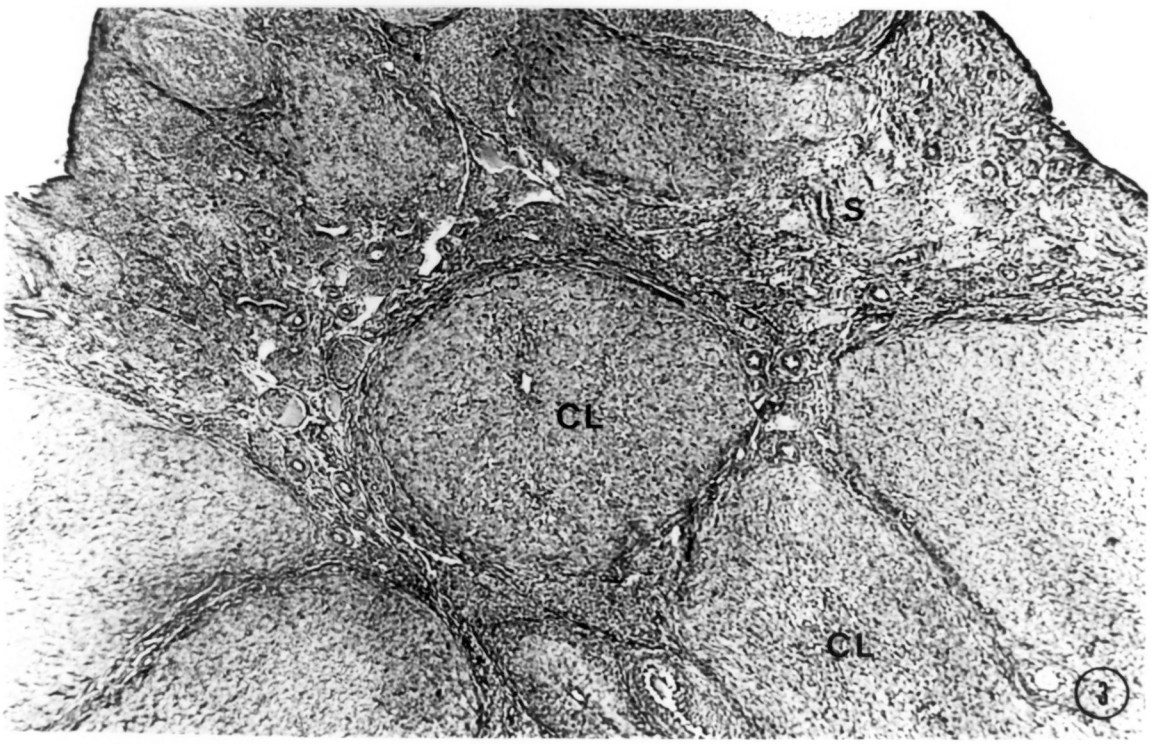

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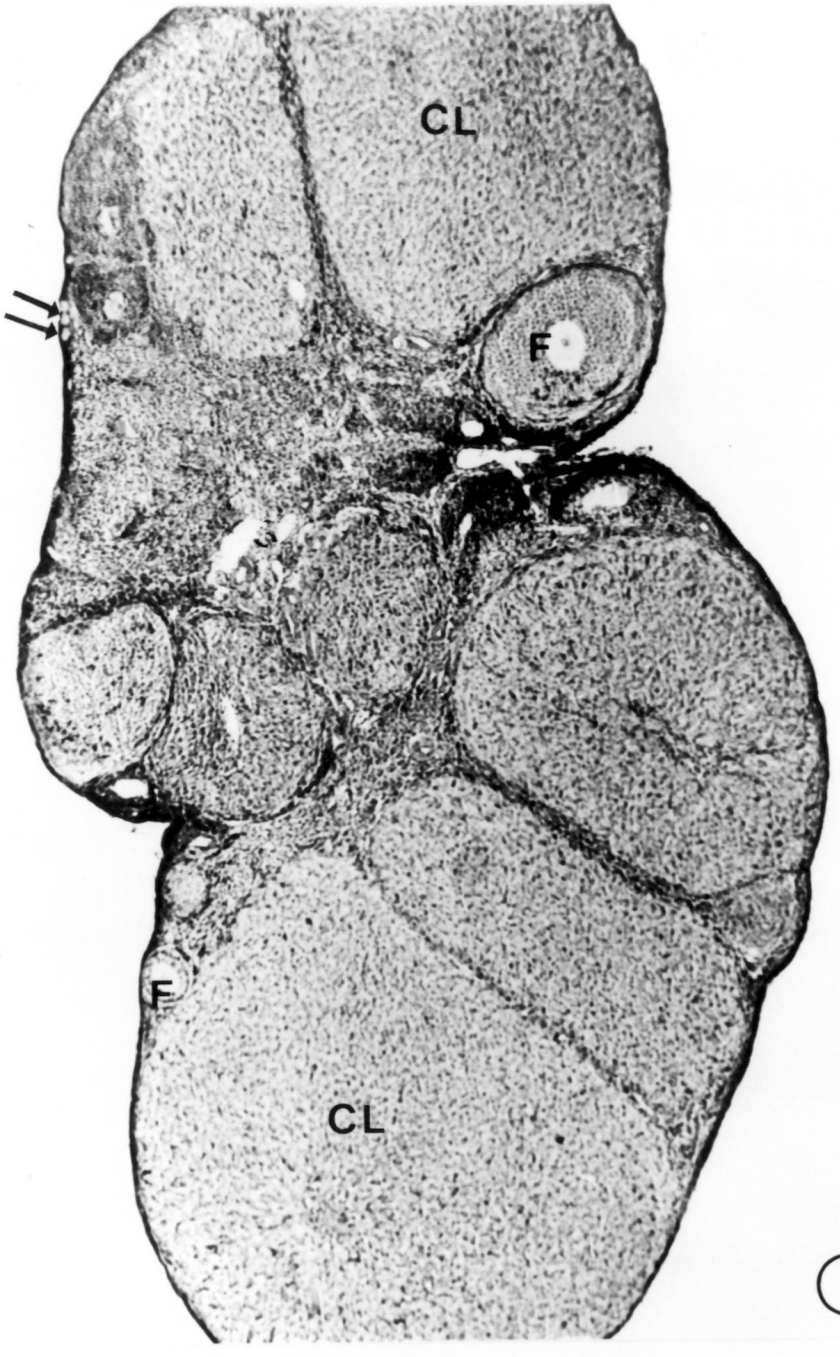
Figure 4. Group 2. Photomicrograph of a section of an ovary 15 days after sham surgery and 30 days after hypophysectomy. Notice the egg nests (arrows) present, the small immature follicles (F) and the luteal tissue (CL). x67.



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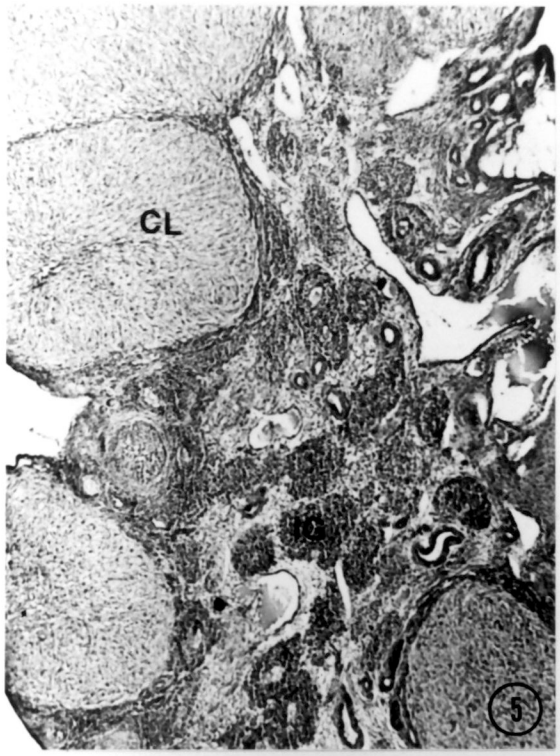
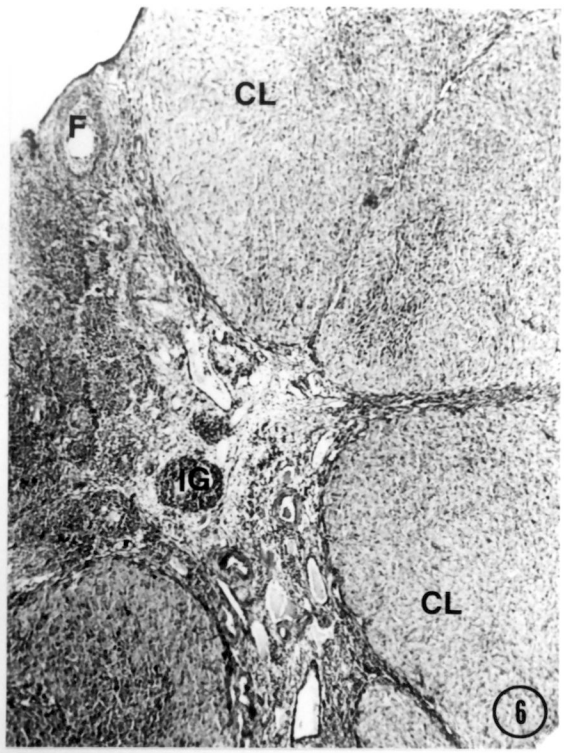

CL
5


CL
F
IG
CL
6


F
IG
CL
7

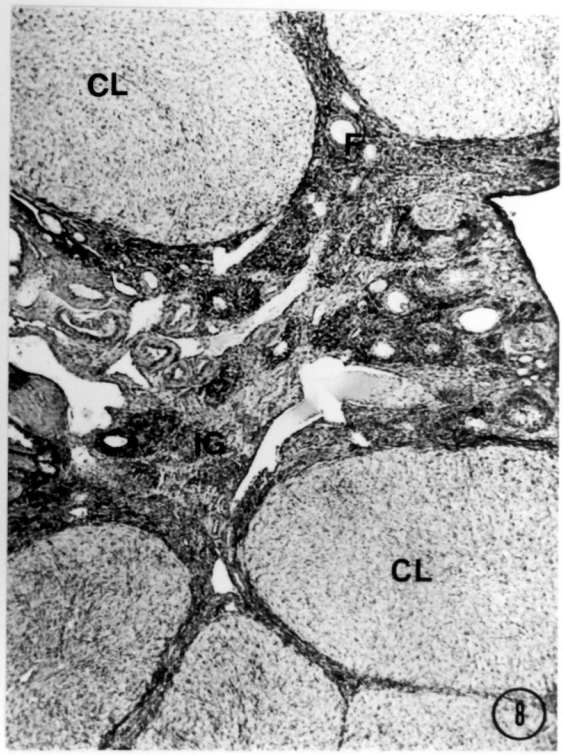

CL
F
IG
CL
8

Figure 5. Group 3. Photomicrograph of a section of a remaining ovary 15 days after ovariectomy and 30 days after hypophysectomy. Note the presence of corpora lutea (CL) and the abundance of interstitial gland (IG). x53.

Figure 6. Group 4. Photomicrograph of a section of a remaining ovary 15 days after ovariectomy and 6-HD (100 mg/kg, i.p., days 1, 2, and 7) and 30 days after hypophysectomy. Note the interstitial gland (IG) and corpora lutea (CL) present. x53.

Figure 7. Group 5. Photomicrograph of a section of a remaining ovary 15 days after ovariectomy and vagotomy and 30 days after hypophysectomy. Note the follicle (F), some interstitial gland (IG) and luteal tissue (CL) present. x53.

Figure 8. Group 6. Photomicrograph of a section of a remaining ovary 15 days after ovariectomy and vagotomy and 6-HD (100 mg/kg, i.p., days 1, 2, and 7) and 30 days after hypophysectomy. A small follicle (F) and much interstitial gland (IG) and luteal tissue (CL). v53.

Table III. Serum progesterone levels for Groups 1-6. Values are ng/ml and are expressed as mean ± SEM.

GROUP	TREATMENT	PROGESTERONE
	NON-HYPOX	
1	ULO	6.64 ± 1.82[a]
	HYPOX	
2	SHAM	0.91 ± 0.12
3	ULO	0.83 ± 0.25
4	ULO+6HD	0.45 ± 0.03
5	ULO+VAGOT	0.94 ± 0.23
6	ULO+6HD+VAGOT	1.52 ± 0.99

[a]Indicates the group is significantly ($p < 0.001$) different from the other groups.

DISCUSSION

It is a well-established observation that compensatory ovarian hypertrophy follows unilateral ovariectomy under normal circumstances (Hatai, 1913; Arai, 1920). Such was the case in the present study when using non-hypophysectomized rats. The hypertrophied ovary had an excess of enlarged corpora lutea which is consistant with the findings that were previously described by Arai (1920). In contrast, compensatory hypertrophy did not occur in any of the ovaries of the hypophysectomized rats. They did possess corpora lutea that appeared well-developed. Earlier, Bunde and Greep (1936) had reported that pre-existing corpora lutea involute slowly following hypophysectomy. Furthermore, in rats hypophysectomized for 40 days, progesterone is present and is reported to be low (Taya and Greenwald, 1982). Therefore, serum progesterone levels were measured in the present study. It was found that progesterone was significantly ($p < 0.001$) lower in the hypophysectomized rats after 30 days, a finding that would suggest the corpora lutea are indeed in a slow state of regression.

Compensatory ovarian hypertrophy is due to increased serum levels of gonadotropins (Edgren *et al.*, 1965; Howland and Skinner, 1973; Welschen and Dullaart, 1974; DeGreef *et al.*, 1975) in response to reduced levels of ovarian steroids following unilateral ovariectomy. More recently, however, a neural component has been implicated in the development of compensatory ovarian hypertrophy (Gerendai and Halasz, 1976; Burden and Lawrence, 1977; Gerendai *et al.*,

1978; Gerendai, 1979). Burden and Lawrence (1977) found that vagotomy and vagotomy plus 6-HD significantly decreased the degree of compensatory ovarian hypertrophy following unilateral ovariectomy. They also found that vagotomy significantly ($p < 0.05$) depressed LH and FSH in unilaterally ovariectomized animals at 5 hours. Furthermore, FSH was significantly ($p < 0.01$) depressed after 24 hours (Burden and Lawrence, 1977). Their data suggest that the vagi facilitate FSH and LH release and they concluded that the vagi should be considered components of the gonadotropin control system. Furthermore, Gerendai et al. (1978) have suggested that the mechanism of compensatory ovarian hypertrophy may require an adrenergic component. Gerendai et al. (1978) found that local treatment of one ovary in an intact control animal with 6-HD caused a weight increase in the remaining ovary, whereas in unilaterally ovariectomized animals 6-HD blocked compensatory ovarian hypertrophy. In another study, Gerendai (1979) found that the remaining ovary showed less atrophy in rats that were hypophysectomized and unilaterally ovariectomized on the same day.

In the present study, vagal and sympathetic effects of compensatory ovarian hypertrophy were examined in previously hypophysectomized rats. This model permitted examination of the local neural component in ovarian hypertrophy without interference from hypophyseal gonadotropins. Rats that were hypophysectomized two weeks prior to unilateral ovariectomy did not show compensatory ovarian hypertrophy, either with or without intact ovarian nerves. After fifteen days,

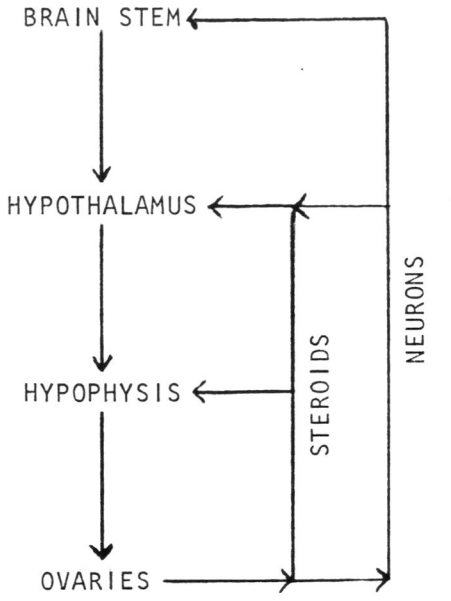
the weight of the remaining ovary was not significantly different from the weight of the initial ovary in any of the hypophysectomized groups. Gerendai (1979) had found ovarian atrophy to be less severe in hypophysectomized rats that had been unilaterally ovariectomized than in hypophysectomized animals having two ovaries. In that case (Gerendai, 1979), both hypophysectomy and unilateral ovariectomy were performed on the same day. In all probability, some residual gonadotropins were still present in the bloodstream. In contrast, unilateral ovariectomy was performed two weeks following hypophysectomy, in the present study, to insure clearance of gonadotropins from the bloodstream. In the latter case, ovarian neural elements were deprived of pituitary influence for an extended time and compensatory ovarian hypertrophy did not occur. These results show that neither vagal nor sympathetic nerves play a role in the development of compensatory ovarian hypertrophy in the absence of the pituitary.

Gerendai (1979) had postulated that neural efferents were responsible for greater weights of the remaining ovary in hypophysectomized-unilaterally ovariectomized rats. On the other hand, Burden and Lawrence (1977) suggested that vagal afferents may monitor gonadotropin release by stimulating the brain stem. In their view (Burden and Lawrence, 1977) nerve impulses originating in the ovary would ascend to the brain stem and, in turn, be relayed to the hypothalamus. Hypothalamic neurons would stimulate the hypophysis to release gonadotropins as needed, leading to the production of the sex steroids. When blood titers reached a high, the hormonal

feedback mechanism would stop production of gonadotropins. The peripheral neural link in the hypothalamo-hypophyseal axis would be an alternate feedback pathway (Figure 9).

Compensatory ovarian hypertrophy clearly results from increased gonadotropins as a consequence of reduced negative feedback of ovarian steroids following unilateral ovariectomy. There may be neural modulation of the gonadotropins. When one ovary is removed, a neural relay to the central nervous system (CNS) would evoke an increase in gonadotropin secretion. When the ovarian nerves are removed through denervation such a relay would not reach the CNS, thus an increase in gonadotropins would not occur. Lastly, in the absence of gonadotropins, also the ovarian nerves would not mediate compensatory ovarian hypertrophy.

Figure 9. Feedback pathway for ovarian control.



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Appendix A. Experimental data of Group 1 (Non-hypophysectomized control).

ANIMAL NUMBER	BODY WEIGHT		OVARIAN WEIGHT		UTERINE WEIGHT	
	INITIAL	REMAINING	INITIAL (WET)/(mg/100 g b.w.)	REMAINING (WET)/(mg/100 g b.w.)	WET	(mg/100 g b.w.)
3254	240	249	33.3 / 13.9	52.4 / 21.0	498.0	200.0
3255	265	264	20.1 / 7.6	36.6 / 13.9	467.2	177.0
3256	220	238	39.0 / 17.7	42.2 / 17.7	670.6	281.8
3257	279	281	32.8 / 11.8	49.8 / 17.7	1554.6	553.2
3258	241	267	49.2 / 20.4	56.1 / 21.0	488.9	183.1
3259	242	250	37.6 / 15.5	70.4 / 28.2	769.4	307.8
3260	224	229	36.3 / 16.2	70.3 / 30.7	361.8	158.0
3261	267	269	42.1 / 15.8	55.0 / 20.4	607.4	225.8
3262	237	247	28.6 / 12.1	60.0 / 24.3	467.6	189.3
3263	264	277	28.8 / 10.9	64.6 / 23.3	651.4	235.2

Appendix B. Experimental data of Group 2 (Hypophysectomized sham).

ANIMAL NUMBER	BODY WEIGHT INITIAL	BODY WEIGHT REMAINING	OVARIAN WEIGHT REMAINING (AVERAGE) (WET)/(mg/100 g b.w.)	UTERINE WEIGHT WET	UTERINE WEIGHT (mg/100 g b.w.)
3196	161	162	16.0 / 9.8	55.6	34.3
3197	172	178	19.0 / 10.7	72.0	40.4
3198	171	172	17.9 / 10.4	88.0	51.2
3199	172	174	16.2 / 9.3	63.0	36.2
3200	164	160	19.3 / 12.1	76.6	47.9
3201	166	164	20.3 / 12.4	77.8	47.4
3202	180	171	15.4 / 9.0	77.0	45.0
3229	132	148	15.9 / 10.7	66.0	44.6
3230	128	139	17.9 / 12.8	76.4	55.0
3231	169	156	18.7 / 12.0	84.0	53.8
3232	167	157	15.5 / 9.8	90.3	57.5

Appendix C. Experimental data of Group 3 (Hypophysectomized ULO).

ANIMAL NUMBER	BODY WEIGHT		OVARIAN WEIGHT		UTERINE WEIGHT	
	INITIAL	REMAINING	INITIAL (WET)/(mg/100 g b.w.)	REMAINING (WET)/(mg/100 g b.w.)	WET	(mg/100 g b.w.)
3142	145	150	17.8 / 12.3	20.1 / 13.4	55.7	37.1
3143	151	149	13.8 / 9.1	22.1 / 14.8	52.1	35.0
3144	140	159	19.6 / 14.0	20.0 / 12.6	47.0	29.6
3146	159	145	13.8 / 8.7	17.7 / 12.2	63.7	43.9
3148	154	151	16.8 / 10.9	16.7 / 11.1	66.0	43.7
3149	160	155	22.0 / 13.8	16.0 / 10.3	62.3	40.2
3150	166	164	18.8 / 11.3	16.0 / 9.8	65.8	40.1
3151	170	164	23.8 / 14.0	16.0 / 9.8	69.0	42.1
3152	150	143	12.0 / 8.0	11.1 / 7.8	60.1	42.0
3153	150	149	19.6 / 13.1	13.3 / 8.9	68.0	45.6
3154	158	161	16.4 / 10.4	15.9 / 9.9	53.8	33.4

Appendix D. Experimental data of Group 4 (Hypophysectomized ULO plus 6-HD).

ANIMAL NUMBER	BODY WEIGHT		OVARIAN WEIGHT		UTERINE WEIGHT	
	INITIAL	REMAINING	INITIAL (WET)/(mg/100 g b.w.)	REMAINING (WET)/(mg/100 g b.w.)	WET	(mg/100 g b.w.)
3185	171	162	26.2 / 15.3	17.0 / 10.5	58.4	36.0
3187	166	134	14.8 / 8.9	13.4 / 10.0	73.8	55.1
3188	177	156	26.2 / 14.8	22.8 / 14.6	59.6	38.2
3189	171	162	17.0 / 9.9	20.8 / 12.8	61.8	38.1
3191	161	152	19.4 / 12.0	19.0 / 12.5	65.0	42.8
3192	174	174	14.0 / 8.0	14.6 / 8.4	65.0	37.4
3193	176	166	20.3 / 11.5	22.1 / 13.3	68.2	41.1
3194	164	156	16.2 / 9.9	19.6 / 12.6	55.0	35.3
3195	190	180	21.4 / 11.3	15.8 / 8.8	91.4	50.8
3227	167	154	17.0 / 10.2	14.5 / 9.4	80.0	51.9
3228	167	162	19.5 / 11.7	22.0 / 13.6	83.8	51.7

Appendix E. Experimental data of Group 5 (Hypophysectomized ULO plus VAGOT).

ANIMAL NUMBER	BODY WEIGHT		OVARIAN WEIGHT		UTERINE WEIGHT	
	INITIAL	REMAINING	INITIAL (WET)/(mg/100 g b.w.)	REMAINING (WET)/(mg/100 g b.w.)	WET	(mg/100 g b.w.)
3155	162	142	20.8 / 12.8	13.3 / 9.4	56.0	39.4
3156	165	153	17.2 / 10.4	14.4 / 9.4	53.6	35.0
3157	154	121	15.6 / 10.1	14.2 / 11.7	64.8	53.6
3160	163	155	19.0 / 11.7	15.8 / 10.2	65.6	42.3
3163	162	126	22.8 / 14.1	12.0 / 9.5	71.4	56.7
3264	171	163	18.2 / 10.6	17.9 / 11.0	72.3	44.4
3265	174	139	19.7 / 11.3	6.5 / 4.7	83.0	59.7
3269	167	157	17.3 / 10.4	21.1 / 13.4	76.1	48.5
3270	160	132	10.3 / 6.4	13.2 / 10.0	61.0	46.2

Appendix F. Experimental data of Group 6 (Hypophysectomized ULO plus 6-HD plus VAGOT).

ANIMAL NUMBER	BODY WEIGHT		OVARIAN WEIGHT		UTERINE WEIGHT	
	INITIAL	REMAINING	INITIAL (WET)/(mg/100 g b.w.)	REMAINING (WET)/(mg/100 g b.w.)	WET	(mg/100 g b.w.)
3172	202	176	23.8 / 11.7	12.8 / 7.3	36.0	20.5
3215	172	153	20.5 / 11.9	18.0 / 11.8	85.9	56.1
3221	169	148	18.8 / 11.1	16.7 / 11.3	68.2	46.1
3271	175	136	10.0 / 5.7	13.9 / 10.2	64.0	47.1
3273	167	130	13.3 / 8.0	7.9 / 6.1	58.1	44.7
3279	165	145	16.5 / 10.0	18.0 / 12.4	68.1	47.0