THE EFFECTS OF PROLACTIN ON THE

POPULATIONS OF GONADOTROPES AND BLOOD SERUM LEVELS

OF GONADOTROPINS IN OVARIECTOMIZED RATS

Marshall Craig Simpson

This study represents an effort to correlate the known
hypogonadal effects of hyperprolactinemia with serum gonadotropin
levels and pituitary gonadotrope cell volume and numerical density.
To induce hyperprolactinemia, pituitary allograft transplantation to
the renal capsule was performed on 49 rats. This was followed by
serum luteinizing hormone and follicle-stimulating hormone
determinations.

Eleven rats were sham-operated and served as controls. Animals
were sacrificed one, three, and five days after pituitary
transplantation. Pituitaries were then examined ultrastructurally
from both groups three days after pituitary transplantation.
Gonadotrope cell volumes and numerical density were determined
morphometrically.

The results showed a suppression (p<.05) of serum LH one, three,
and five days after induction of hyperprolactinemia (PRL>100 ng/ml).
Serum FSH levels were not suppressed until five days after induction
of hyperprolactinemia (p<.05).

When autogenous pituitary tissue was taken three days after induction of hyperprolactinemia and examined by electron microscopy, the numerical density (number of cells / mm^3 of tissue) of gonadotropes was increased when compared with the sham-operated animals ($p<.05$). Gonadotrope cell volume (um^3 / cell) was decreased ($p<.05$) three days after hyperprolactinemia.

These results indicate that hyperprolactinema does cause a decrease within 24 hours of serum LH levels, and a decrease of FSH levels later, demonstrating a differential effect upon the two gonadotropins. Pituitary gonadotrope cell volume may have been decreased due to depletion of hormone stores. The numerical density may have been increased in a compensatory mechanism, but further ultrastructural and biochemical correlative studies will have to be done to understand fully these results.

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INTRODUCTION

Hyperprolactinemia is the presence of elevated prolactin (PRL) levels in the blood. Prolactin, a large peptide hormone, is normally secreted at low levels in the blood from the anterior pituitary. During lactation, PRL reaches its greatest physiological levels due to the suckling stimulus.

The gonadotropic hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH), are large glycoprotein hormones that are also released into the blood from the anterior pituitary. In the mammalian female, FSH causes follicular growth and ova development while LH stimulates ovulation and corpus luteum formation. Thus the gonadotropins maintain the integrity of the estrous and menstrual cycles.

Post-partum breast feeding frequently inhibits regular menstruation (amenorrhea) in the human. This has been utilized as a means of birth control in many developing nations. Furthermore, amenorrhea in lactating women has been shown to be due to the antigonadotropic effect of PRL during lactation, when PRL levels reach their physiological maximum (Evans et al., 1982).

Occasionally amenorrhea occurs in women who are not breast feeding. This is an abnormal condition, and is usually caused by an hormonal imbalance at either the hypophyseal (pituitary) or ovarian

level. However, many cases of amenorrhea are characterized only by high serum PRL levels. In these cases PRL is being secreted at a much greater rate than is normal for nonlactating women. Many times this is due to PRL secretory pituitary tumors, and PRL is presumably exerting its antigonadotropic effect just as it does during lactation.

The gonadotropins are thought to be regulated by the same releasing factor. Previous studies show that high PRL levels suppress both hormones and that PRL may be inhibiting the releasing factor. However, recent studies show that one, but not both, gonadotropins may be suppressed.

The classical theory of hormone production and release states that each particular hormone is produced by a separate cell type (the one hormone/one cell hypothesis). However, in recent years the two gonadotropic hormones have been immunocytochemically located in the same cell type within the anterior pituitary. Thus it appears as if there may be a differential suppression of one and a release of the other gonadotropin within the same cell.

The present study is designed to determine (1) the precise transplantation procedure necessary to promote hyperprolactinemia, (2) whether there is a selective suppression of the gonadotropins during hyperprolactinemia and (3) whether there are any detectable differences in the gonadotrope populations during hyperprolactinemia.

LITERATURE REVIEW

In 20-30 percent of all women with functional amenorrhea, elevated serum PRL levels have been observed (Jacobs et al., 1971, Badano et al., 1979). This is due to the suppression of LH and/or FSH which is vital to normal cyclicity. Galactorrhea is often associated with amenorrhea in these patients, indicating that a probable PRL imbalance is present. Hyperprolactinemia constitutes the second most common cause of amenorrhea. It also causes hypogonadism in laboratory animals (Schechter et al., 1981; Evans et al., 1982), and in both men (Thorner et al., 1977) and women (Thorner, 1977).

Radiologic and tomographic evidence of a pituitary tumor is often observed in hyperprolactinemic women, but the incidence of these tumors is difficult to establish accurately without surgical exploration or at least pituitary biopsy. Therefore, many individuals with hyperprolactinemia may possess microadenomas indistinguishable by normal radiological methods (Lloyd et al., 1975). These tumors some-times develop spontaneously at an early age and prevent the develop-ment of secondary sex characteristics, but usually the pituitary tumors arise in older individuals (Kovacs et al., 1977; Prysor-Jones and Jenkins, 1981; Schechter et al., 1981).

Prolactin secretion is under an inhibitory influence from the hypothalamus, and separation of the pituitary from the hypothalamus

leads to reduced secretion of all the adenohypophyseal (anterior pituitary) hormones except PRL, which increases (Everett, 1954; Thorner, 1977).

A physiological releasing factor for PRL has not been identified (Evans et al., 1982). However, the catecholamine, dopamine (DA), is probably the most physiologically important inhibitory factor (MacLeod, 1976). This neurotransmitter is synthesized in neurons whose cell bodies lie in the basal hypothalamus, and whose axons lie within the capillary loops which feed the hypophyseal portal vessels. As these neurons are stimulated, DA is released into the hypophyseal portal vessels which supply the anterior pituitary gland, and mammotropes (PRL-secretory cells) react to DA by suppressing PRL release. Any abnormality in this tubero-infundibular dopamine (TIDA) system can cause excessive PRL release (hyperprolactinemia). Prolactin in turn can increase DA turnover in the hypothalamus thus acting as short-loop negative feedback on its own secretion (Evans et al., 1982).

Bromocriptine (CB-154), a dopamine agonist, was found to reduce serum PRL levels in humans with amenorrhea (Besser et al., 1972; del Pozo et al., 1972) and to restore cyclic hormone release, ovulation, and fertility. Furthermore, the same drug inhibited galactorrhea in hyperprolactinemic women who cycled normally. Bromocriptine is very effective in restoring cyclicity to women with hyperprolactinemia, and is used extensively. Similar findings were shown with metoclopramide (MCP), another DA agonist (Quigley et al.,

1979). While the previous drugs act primarily at the hypothalamic level, ergocornine has been shown to inhibit the release of PRL in transplanted pituitaries removed from the hypothalamic influence (Melven and Hoge, 1971). Therefore it has been suggested that some drugs inhibit PRL secretion directly at the pituitary (Graf et al., 1977), although results are contradictory (Kamberi et al., 1970).

Induced hyperprolactinemia resulting from lactation (Fuxe et al., 1969), exogenous administration of PRL (Hökfelt and Fuxe, 1972), and pituitary transplants beneath the kidney capsule (Olson et al., 1972) cause increased DA turnover in the hypothalamus. This effect of PRL on DA turnover becomes important when considering the possible antigonadotropic actions of PRL. If DA is inhibitory to gonadotropic releasing hormone (GnRH) at the hypothalamic level, then increased PRL secretion increases DA turnover in the hypothalamus, which lowers GnRH and subsequently gonadotropin secretion. However, the effects of DA on gonadotropin release are still unclear and this mechanism is not proven (Evans et al., 1982).

Many other factors have been implicated in the control of gonadotropin release. These include norepinephrine, opioids (both endogenous and exogenous), prostaglandins, and estrogens (Evans et al., 1982). Estrogens are known to override the inhibitory effects of DA on PRL secretion by one of two possible pathways. These steroids may act indirectly by inhibiting the secretion of DA into hypophyseal portal vessels (Cramer et al., 1978), or by depleting the DA stores (Jacobs et al., 1971; Meites and Clemens, 1972). In addition

estrogens may bind to estrogen receptors in the pituitary (Muldoon, 1977) and thus directly affect PRL secretion (Blake et al., 1972; Raymond et al., 1978). This direct mechanism may also involve prostaglandins PGE_1 and PGE_2 (Ojeda et al., 1977). Estrogens can induce PRL-secretory tumors in rats (Furth and Clifton, 1975), and can also elevate blood PRL levels in the rat and human in the absence of any tumorigenesis (Chen and Meites, 1970). See Figure 1 for a diagrammatic view of the possible feedback loops involved in the reproductive axis (Hypothalamus-pituitary-ovary).

The antigonadotrophic effect of PRL is not easy to study in the intact adult female rat because high PRL levels in the rat invariably stimulate corpus luteal function resulting in increased progesterone secretion and pseudopregnancy (Dohler and Wuttke, 1974). These conditions make it impossible to differentiate between PRL and progesterone effects (Beck et al., 1977). Ovariectomized adult rats were used in the present study to reduce the possibility of progesterone effects.

High PRL levels may be induced by exogenous injection, transplantation of PRL-secreting tumors, or the transplantation of pituitary glands beneath the kidney capsule of experimental recipients. The transplanted pituitary grafts from donor animals secrete high concentrations of PRL and very little LH and FSH, since the glands are removed from hypothalamic control (Meites et al., 1963). Desclin (1950) pioneered pituitary grafting to the renal capsule. Since then,

Figure 1. Basic pathways involved in the hypothalamic-pituitary-
ovarian axis. Feedback loops are shown.
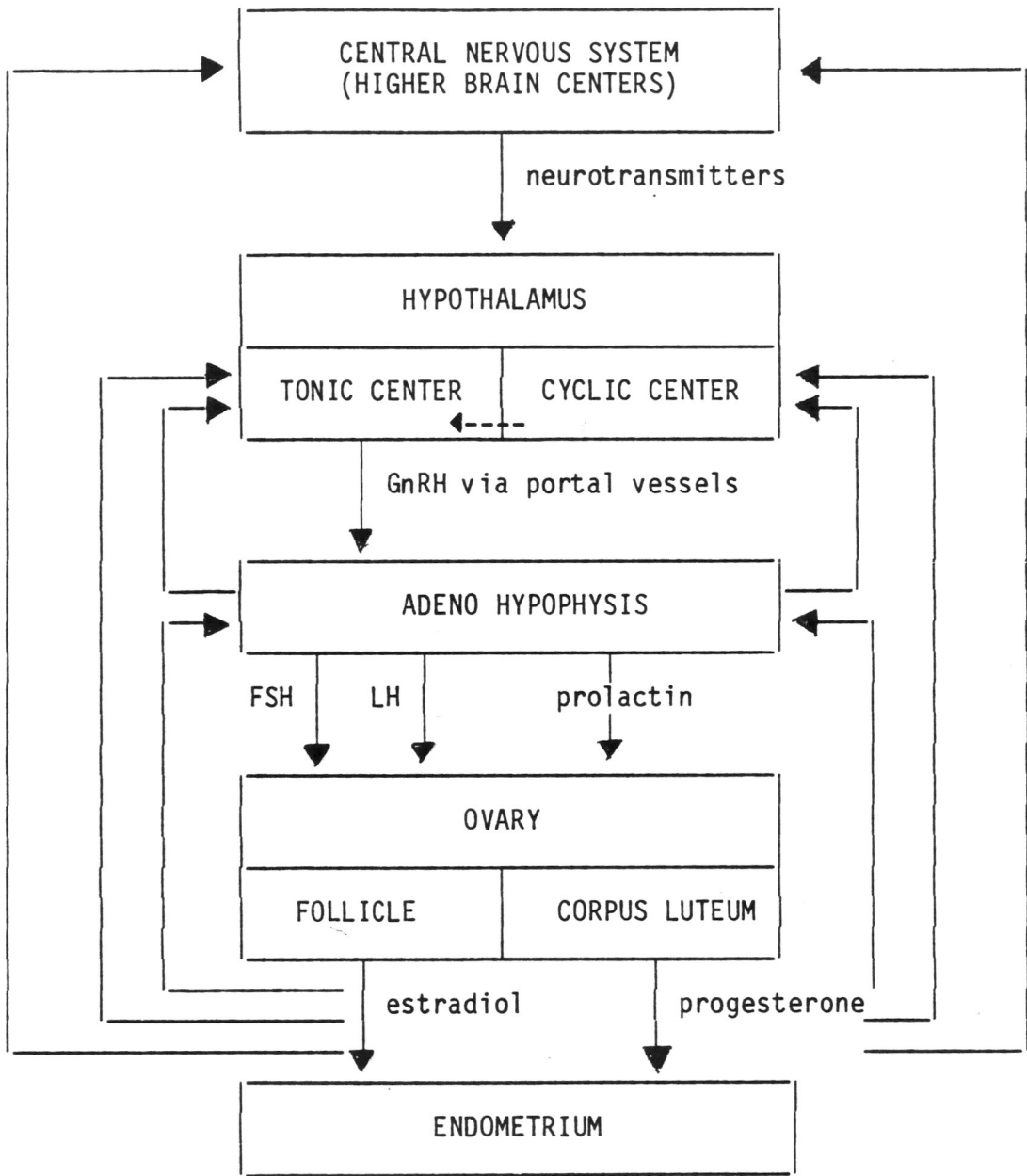
numerous other investigations have been reported using different methodological approaches with this technique. LH and FSH are either not secreted (Negro-Vilar et al., 1977) or secreted at levels much lower than in intact pituitaries (Lu et al., 1979). A recent immunocytochemical study confirms that LH and FSH cells are present but do not readily secrete their hormones (Aguado et al., 1981).

There has been considerable controversy concerning whether the LH secreting cell is functionally different from the FSH secreting cell. The classical view of pituitary hormone storage is that each cell produces only one specific hormone, and stores it in a granule unique in morphology (size, shape, and distribution) to that cell type. This concept has been confirmed by immunocytological findings in the case of PRL, growth hormone, and thyroid stimulating hormone (Childs and Ellison, 1980). There is increasing evidence, however, that LH and FSH may be secreted by the same cell type (Childs et al., 1982).

The control of gonadotropin release by GnRH is unclear, however, there is usually a marked LH release accompanied by a much smaller FSH response. In hyperprolactinemic models, a differential suppression of LH, but not FSH, has been demonstrated (Beck et al., 1977; Tresguerres and Esquifino, 1981). The gonadotropic releasing hormone (GnRH) was originally thought to regulate both LH and FSH, but this may not be the only controlling

hormone. On the other hand, LH and FSH may react differently to the GnRH dose, as well as to the GnRH pulse size, shape, and frequency (Knobil, 1980).

The gonadotropes were originally distinguished using Periodic Acid Schiff's stain. Follicle stimulating hormone (FSH) cells contained granules that stained purple and LH cells stained red. Criteria for identification of gonadotopes were later put forth by Barnes (1962) and later adopted by Kurosumi and Oota (1968) for electron microscopical identification. The classical Kurosumi-Oota FSH cell usually possessed a large rounded cell body and was found along the capillary wall. Two types of secretory granules were observed, smaller granules 200-250 nm in diameter and electron dense, and larger granules (700nm) that had less electron density. LH cells are somewhat smaller that FSH cells. They are pleomorphic and contain less variable secretory granules of about 250 nm in size.

The first electron microscopic immunocytochemistry studies (Kawarai and Nakane, 1970; Nakane, 1971) showed that LH was in the "classical FSH cells" of Kurosumi and Oota (1968). Tougard et al., (1973) demonstrated that LH and its subunits were found in both the classical FSH and LH cells in males. Thus, it appeared that LH and FSH cells could not be distinguished morphologically and the question arose how functional variants of the same cell type could exist. Immunocytochemical evidence indicates that one pituitary cell may secrete more than one hormone. Childs and Ellison (1980) offer a good review of this evidence, and show with serial sections that two

hormones may even be stored within the same secretory granule.

Most studies to date have considered immunoassayable concentrations of serum LH and FSH, and a few have studied pituitary concentrations of LH and FSH. However, none have considered serum gonadotropin levels correlated with gonadotrope intracellular changes during hyperprolactinemia. Pituitary gonadotrope populations may be characterized, localized, and quantified using quantitative electron microscopy (morphometry) and standard stereological formulas formulated by Weibel and Bolender (1973).

The distribution of gonadotropes as well as other anterior pituitary cell types in the cycling female rat has been discussed (Poole and Kornegay, 1982). They described the gonadotrope population as consisting of two cell types. The Type II cell was the same as the "LH" cell type described by Kurosumi and Oota (1968) and has been confirmed immunocytochemically as a cell type containing only LH (Moriarity, 1975). The Type I cell was the same cell as the "FSH" cell described by Kurosumi and Oota (1968). However, Moriarity (1976) showed that this cell type reacts immunocytochemically with both LH and FSH antibodies. Later Childs et al., (1982) demonstrated the presence of both hormones in 91% of male castrate gonadotropes, often within the same cisternae of rough endoplasmic reticulum. No study to date has described the gonadotrope populations in ovariectomized rats, nor has morphometry been utilized to study the dynamics of the gonadotrope population during hyperprolactinemia.

MATERIALS AND METHODS

Animals

The animals used in this study were adult female Harlan Sprague-Dawley rats weighing between 200 and 300 grams. They were maintained in a temperature controlled environment ($25 \pm 1^{0}C$) and a 14/10 light-dark photoperiod. The animals were given food and water ad libitum and were caged in pairs.

Ovariectomy

Vaginal smears were taken daily to determine the time for ovariectomy. Bilateral ovariectomy was performed on all control and experimental animals on the second day of diestrus (Diestrus II) following at least two normal estrous cycles. A three week recovery period was allowed before proceeding with the pituitary transplants.

Induction of Hyperprolactinemia

The transplantation of pituitary glands beneath the kidney capsule of the rat will selectivly release PRL from the transplant and not LH and FSH (Thorner, 1977). In order to attain a broad range of serum PRL concentrations, until the level of serum PRL exceeded 90 ng/ml, the approximate physiological level in the lactating rat (Terkel et al., 1972), the following protocol was used.

Initially two pituitaries were transplanted underneath the kidney capsules of 17 ovariectomized rats. In order for the transplanted pituitaries to be relatively constant in their degree of activity, the donor rats were all sacrificed during the diestrus II period of their cycle. The day of transplantation will henceforth be designated as Day 0. On this day the donor and experimental rats were transported by cage to the surgical area. A towel was draped over their cages to avoid undue stress.

At the surgical area, three stations were set up: one for the decapitation of the donors and removal of their pituitaries, one for ether anesthesia of the experimental animals, and the third station for the transplant surgery. As one experimental animal was being anesthetized, both donor animals were decapitated and their pituitaries removed. Each gland was placed in a phosphate buffered saline solution and passed to the table where the experimental animals were waiting.

The experimental animals' kidneys were exposed by a one inch incision slighly anterior and medial to the earlier ovariectomy site. Retractors were used to open the incision and the kidney was exposed. The kidney capsule was raised with small forceps and one pituitary was inserted. The capsule was allowed to reseal itself and the kidney was placed back into its original position. A similar procedure was performed on the contralateral kidney. The peritoneum was sutured and the entire incision was clamped with autoclips. When the animals regained consciousness they were taken back to the animal facilities.

Three animals were sham-operated in a procedure similar to above, however, no pituitaries were transplanted to this group. A second group of 14 experimental animals received transplants identical to the first, but with two pituitary glands transplanted under each kidney capsule (four transplanted pituitaries). Eight rats were sham-operated along with this second experimental group.

The third group received pituitary transplants from animals which had been primed with estrogen. A dosage of 1.0 microgram of estradiol benzoate in corn oil was injected subcutaneously into 72 donor rats. They received this dosage daily for 5 days, including the day of the pituitary transplant. On Day 0 the estrogen primed pituitaries from the donor rats were removed as in the earlier procedures and two were transplanted underneath each kidney capsule of the 18 experimental rats.

Experimental Design

In order to determine what effects PRL had on LH and FSH over a period of time, the rats with transplanted pituitaries were sacrificed after three different time intervals. On Day 1, eighteen of the experimental and three of the sham-treated group were sacrificed (Table 1). On Day 3, fifteen of the experimental animals and five of the sham treated animals were sacrificed. On Day 5, sixteen animals from the three experimental groups and three sham treated animals were sacrificed.

TABLE 1

NUMBER OF ANIMALS PER TRANSPLANT GROUP

	One day		Three Day		Five Day	
	LH	FSH	LH	FSH	LH	FSH
Two pituitaries	7	5	4	4	6	6
Four pituitaries	5	5	5	5	4	4
Four pituitaries plus estrogen	6	6	5	6	6	6
Sham Operated	3	3	5	5	3	3

Collection of Pituitaries and Blood Serum

On the day of sacrifice, the experimental animals were
decapitated and their trunk blood collected into test tubes. The
blood from each animal was allowed to clot overnight at room
temperature and was then centrifuged at 2000 rpm on a Beckman Model
I-6 centrifuge. The blood serum was separated and stored at -20'C.
It was later used for radioimmunoassay (RIA) of PRL, LH, and FSH.

The pituitary gland was removed from each experimental animal,
the pars nervosa removed and the adenohypophyseal portion was weighed.
Each adenohyopophyseal portion was divided into two hemipituitaries by
a cross-sectional cut through the isthmus. One hemipituitary was
placed in phosphate-buffered (pH 7.4) saline and frozen at -20'C for
RIA at a later date. The other portion was prepared for electron
microscopy.

Electron Microscopy

One hemipituitary was divided by two perpendicular cuts to yield two central regions and two peripheral regions. The tissue was initially fixed in small cork-stoppered vials containing approximately 2.0 ml of a 2% glutaraldehyde/paraformaldehyde solution (Karnovsky, 1965) and buffered with 0.1 M phosphate buffer at pH 7.3 and maintained at 4^0C. This temperature and pH were kept constant throughout fixation. The specimens were washed several times in phosphate buffer, then dehydrated by increasing concentrations of ethanol and infiltrated with increasing concentrations of Araldite 502 resin/propylene oxide. The pituitary tissue was embedded in BEEM capsules with Araldite 502 and hardened in a vacuum oven for 48 hours.

Sectioning was performed with an ultramicrotome using a diamond knife. The sections were placed on 200 mesh copper grids and subsequently stained with 2% uranyl acetate for 10 minutes and counterstained with lead citrate (Reynolds, 1963) for 5 minutes. They were thoroughly washed with distilled water and examined with a Philips 201 electron microscope. Micrographs for morphometry were taken at 648X on Kodak 35 mm Fine Grain Release Positive film. A calibration grid was shot at the beginnning of each roll of film. The film was developed and prints were enlarged to 5249X.

Identification of Gonadotropes

Ovariectomy in rats leads to hyperactivity of the gonadotropes characterized by cells greatly increased in size, with granules

dispersed throughout the cytoplasm and greatly dilated cisternae of endoplasmic reticulum. This is due to the lack of a negative feedback of ovarian steroids on the pars distalis. Immunological studies indicate that LH and FSH are contained within the same cell (Moriarity, 1976). Therefore, this study did not utilize the basic morphological criteria of Kurosumi and Oota (1968) in identifying the classical FSH- and LH-containing cells. Instead, gonadotropes were classified into two morphological types. Type 1 gonadotropes contained secretory granules that ranged from 200 to 250 nm in diameter, and possessed dilated cisternae of endoplasmic reticulum comprising less than 15% of the cytoplasmic area. Type 2 gonadotropes were much larger and contained cisternae that covered more than 15% of the cytoplasmic area of these cells, however, their granules were of the same size (200 to 250 nm) as those of Type 1 (see Figure 2).

Morphometric Methods

1. Sampling Protocol

To determine whether elevated PRL levels had any effect on the number and distribution of gonadotropes in the pituitary gland, three groups of four animals each were chosen for morphometric analysis based upon their serum PRL concentrations. The first group consisted of animals that had greatly elevated serum PRL levels (>90 ng/ml) the second group slightly elevated PRL levels (<90 ng/ml) , and the last group consisted of four sham-operated animals (10-15 ng/ml).

One hemipituitary from each animal yielded two blocks from both the central and peripheral regions. One block from each region was

Figure 2. Electron micrograph demonstrating the two types of

gonadotropes recognized by this study. The Type I cell

containing less than 15% endoplasmic reticulum is seen in

plate A, flanked by two Type 2 cells. Plate B illustrates

the typical, larger Type 2 cell, with much dilated

endoplasmic reticulum. 5702X.

A

TYPE 1

5 μm

B

TYPE 2

5 μm
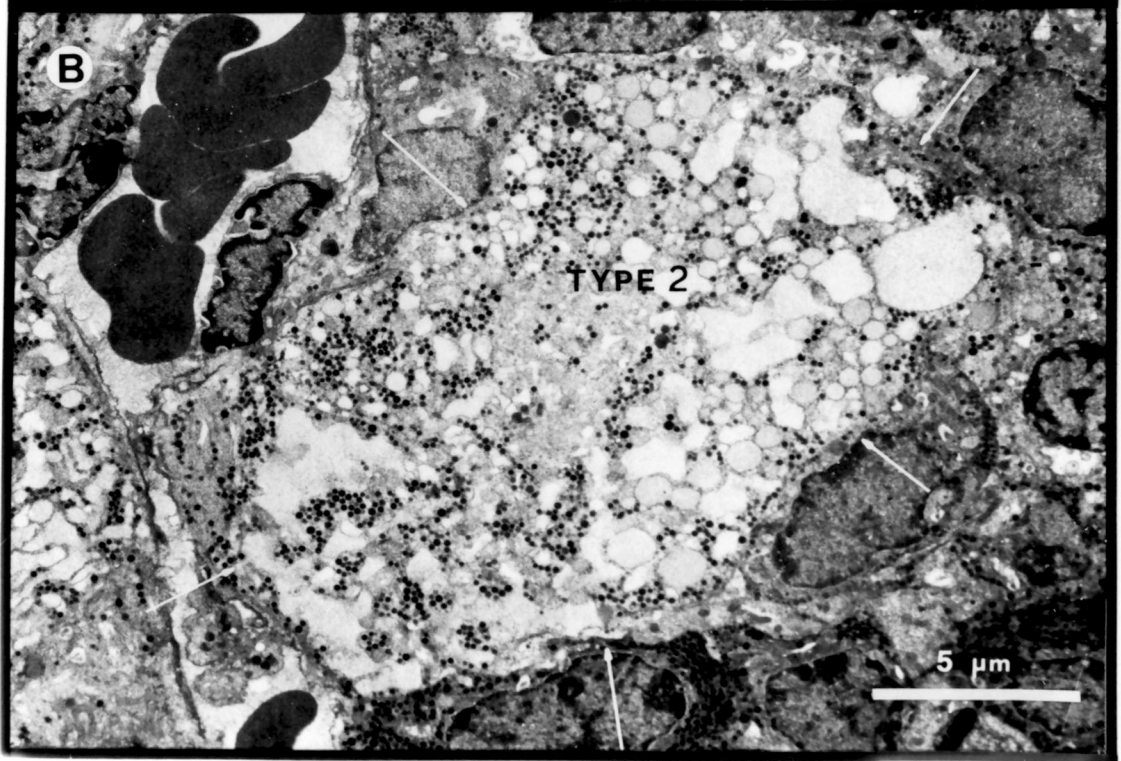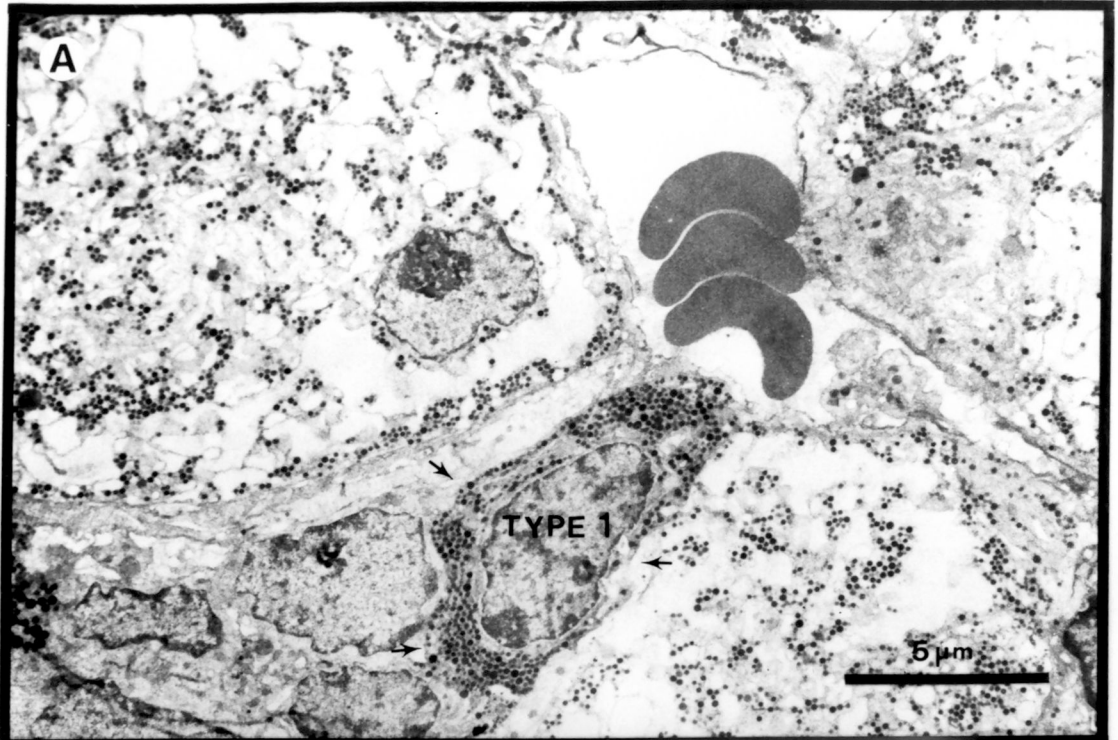
chosen randomly for sectioning and three grids of tissue were collected from each block. Each grid was examined with the electron microscope and every fifth grid square containing tissue was photographed. At least six micrographs were taken from each grid, yielding at least 36 micrographs per animal (Figure 3). Sampling variance was ascertained at three different levels. Sampling variance at the animal level should point to differences in the three groups used. Sampling variance at the regional (block) level should determine whether differences occur in the number or size of gonadotropes in the different regions of the pars distalis. The sampling variance at the grid level was also monitored (the number of grids per block and the number of micrographs per grid).

2. Grid Design

The grid used in this study to determine the volumes and numerical densities of gonadotropes consisted of a square lattice (about 10" x 12") with spacings of 1.0 cm and with 720 intersections. Each intersection constituted one test point and the lattice was drawn on an acetate transparency. The grid transparency was laid over each micrograph and the number of points falling on each type of gonadotrope, the nuclei of each type of gonadotrope, and the total number of points constituting tissue on each micrograph was recorded.

For these data, the volume of an average gonadotrope of each type as well as the numerical density of the gonadotropes (the number of

Figure 3.   Flowchart demonstrating the sampling protocol utilized in

this experiment for morphometric analysis of gonadotropes.

Micrographs

Grid 1
---- 1
---- 2
---- 3
---- 4
---- 5
---- 6

Block 1

Grid 2
---- 1
---- 2
---- 3
---- 4
---- 5
---- 6

Peripheral

Grid 3
---- 1
---- 2
---- 3
---- 4
---- 5
---- 6

Block 2
(Unused)

Hemipituitary -

Block 2
(Unused)

Central

Grid 1
---- 1
---- 2
---- 3
---- 4
---- 5
---- 6

Grid 2
---- 1
---- 2
---- 3
---- 4
---- 5
---- 6

Block 1

Grid 3
---- 1
---- 2
---- 3
---- 4
---- 5
---- 6
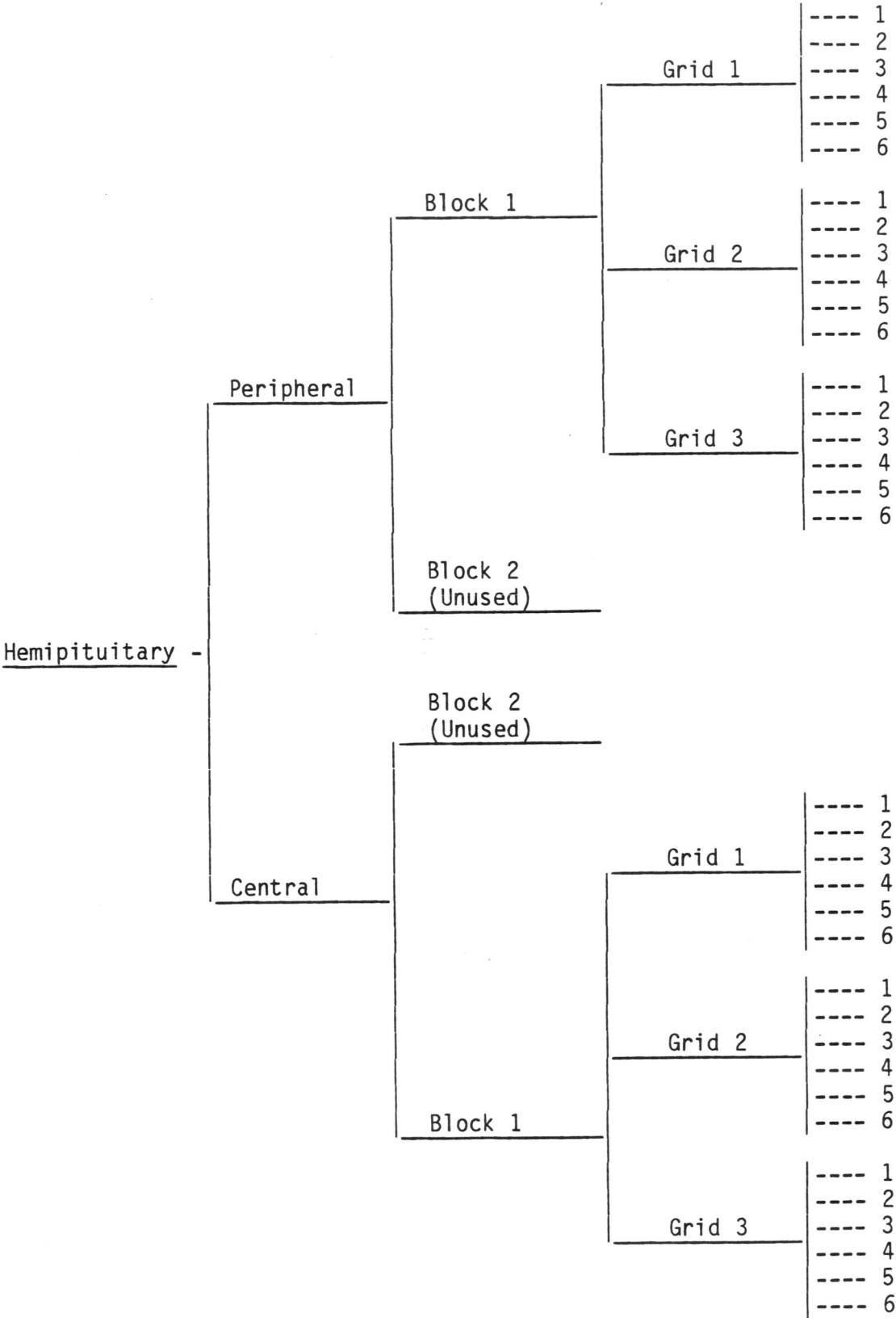
gonadotropes per unit volume of tissue) could be determined using standard stereologic formulas (Weibel and Bolender, 1973; Weibel 1979). The data were then incorporated into the computer program of Poole and Costoff (1979).

Radioimmunoassay

Serum LH, FSH, and PRL were quantified by the double antibody RIA technique using material provided by the National Institute of Arthritis, Metabolism and Digestive Diseases. PRL determinations were made according to Niswender et al. (1969). Iodinations were performed using the chloramine-T method as modified from Greenwood et al. (1963). Anti-rabbit gamma globulin was prepared in our laboratory.

Statistical Analysis

All data were analyzed using a Hewlett-Packard 9845 T mini-computer. RIA data were analyzed using a program developed by Poole (personal communication) and the results were expressed as a mean $\pm$ standard error of the mean. Differences between subgroups were determined by the Kruskal-Wallace multiple sample test at the p=.05 level of significance. The data generated from morphometric studies were analyzed using a series of computer programs developed by Poole and Costoff (1979). An additional program (Kornegay and Poole, 1983) was utilized to test the sampling protocol using a nested analysis of variance. All morphometric results were expressed as the mean $\pm$ standard error of the mean. The Mann-Whitney test was used for

testing significance between two groups at the p=.05 level (Sokal and Rohlf, 1969).

RESULTS

The PRL levels in rats receiving two pituitaries were
significantly higher (p<.05) than the average level of 10.7 ng/ml
found in the sham treated groups (Table 2). The length of time the
transplant remained in the hosts had no significant effect on the
level of PRL produced. Although the animals receiving two pituitaries
did show about fourfold increases in serum levels of PRL, these levels
were less than those reported in lactating rats (90 ng/ml). In the
second group each host received four pituitaries. These results are
presented in Table 2.

TABLE 2

MEAN PROLACTIN LEVELS PER TRANSPLANT GROUP

Transplant Group	One Day	Three Day	Five Day	Average
Two Pituitaries	45.5+17.9*	35.4+ 6.1	37.8+10.0	40.5+13.4
Four Pituitaries	72.8+10.7	51.4+19.8	53.8+12.4	59.7+17.1
Four Pituitaries (Estrogen-primed)	136.8+ 8.8	132.8+19.7	148.2+19.5	139.1+17.2
Sham Operated	5.8 + 0.6	13.9+ 9.4	10.2+ 3.0	10.7+ 7.1

*Expressed in ng/ml as mean + S.E.M.

The length of time that the hosts were exposed to the transplants had no significant effect on serum PRL levels; however, all three groups differed significantly from the sham treated group (p<.01). Although PRL was seen to increase in these animals, the PRL concentrations were still less than 90 ng/ml. A third group received four pituitaries from donors that had been estrogen-primed. The levels of seum PRL for the one, three, and five day treatment periods were 136.8, 132.3 and 148.2 ng/ml, respectively (Table 2). Although there was no significant difference between the treated groups of this experiment, they were all significantly higher (p<.001) than was the PRL levels of the sham treated groups. More importantly, they had higher PRL levels than is typical for lactating rats.

Changes in the PRL levels were correlated with changes in FSH and LH. A total of 49 experimental animals were used in comparing PRL levels with those of FSH and LH. Of this total, 18 animals were sacrificed at day one; 15 at day three, and 16 at day five (Table 1). The animals were divided into three subgroups based upon their level of PRL; 0.50, 51-100, and 101-150 ng/ml for each of days one, three, and five (see Table 3). From these data it could be determined whether LH or FSH was affected by increasing levels of PRL on each day of sacrifice.

In the group of animals sacrificed one day after receiving pituitary transplants, LH concentrations were unchanged from the sham treated group until PRL levels reached 100 ng/ml. In this subgroup of animals there was a significant (p<.05) decrease in serum LH levels.

TABLE 3

LH CONCENTRATIONS

Groups	One Day	Three Day	Five Day
Sham Values	564.7 + 7.0*	524.8 + 40.4	527.6 + 25.9
PRL 0- 50 ng/ml	508.7 + 38.6	529.2 + 33.3	636.1 + 40.2
PRL 51-100 ng/ml	508.2 + 23.5	505.2 + 0.3	571.8 + 40.6
PRL 101-150 ng/ml	437.9 + 12.9	416.2 + 42.5	487.6 + 23.8

*Mean + S.E.M.

FSH CONCENTRATIONS

Groups	One Day	Three Day	Five Day
Sham Values	5169.0 + 77.0*	5748.4 + 854.2	7317.3 + 1036.3
PRL 0- 50 ng/ml	5915.4 + 809.1	5909.8 + 260.9	6552.2 + 493.2
PRL 51-100 ng/ml	5654.9 + 375.8	4843.0 + 144.0	6379.4 + 1148.0
PRL 101-150 ng/ml	6336.9 + 359.9	7039.9 + 322.8	4672.3 + 311.7

*Mean + S.E.M.

Likewise, the group of animals sacrificed three days after pituitary transplantation showed no significant difference in LH values from the sham treated group until PRL levels were 101-150 ng/ml. Again, in that subgroup there was a significant decrease ($p < .05$) in LH values. On Day 5 post-transplantation, LH levels were significantly higher in animals containing up to 50 ng/ml PRL than in the sham treated animals. Luteinizing hormone (LH) levels were significantly lower in animals with more than 100 ng/ml than in the sham treated animals (see

Figure 4 for a graphical representation of these data).

Serum LH levels were compared within each range of PRL to determine if a time difference existed in LH levels after one, three, and five days of pituitary transplantation. When PRL was less than 50 ng/ml, LH values were not different after one and three days of transplantation, but the LH values on the fifth day were significantly higher. The same result was observed when PRL was 51-100 ng/ml and when PRL was greater than 100 ng/ml. Five days after transplantation, LH values increased significantly in each subgroup of PRL.

Serum FSH levels were compared in the same manner as LH levels (Table 3). After one day of transplantation, FSH values from each PRL subgroup were not significantly different from the sham treated values. The third day after transplantation, FSH values were lower in the 50-100 ng/ml PRL subgroup and the sham treated subgroup than in the other two subgroups (Figure 5). Five days after transplantation, FSH was decreased from the sham treated values only when PRL was greater than 100 ng/ml. As a function of time, FSH levels were significantly different ($p<.05$) only when PRL exceeded 100 ng/ml. At this level of PRL, the Day 5 FSH levels were less than the levels on Days one and three.

The numerical density (number of gonadotropes/mm^3 tissue) calculations were made using standard morphometric techniques for the two types of cells. The numerical densities were compared between their central or peripheral location, and between each experimental group of animals (sham treated animals and experimental animals with

less than and greater than 90 ng/ml PRL), all sacrificed three days post-transplantation. At the cellular level, the number of Type 2 cells (RER>15%) was significantly greater than the number of Type 1 cells (RER<15%) in every group of animals tested (Table 4).

TABLE 4

NUMERICAL DENSITY OF GONADOTROPES

Groups	Type 1 Cell	Type 2 Cell
Sham Operated Animals		
Central*	36416 $\pm$ 7466**	163482 $\pm$ 20890
Peripheral	36392 $\pm$ 3559	154930 $\pm$ 31862
Total	38120 $\pm$ 4255	159817 $\pm$ 16513
PRL < 90 ng/ml		
Central	168564 $\pm$ 48637	454607 $\pm$ 34809
Peripheral	137097 $\pm$ 17614	351986 $\pm$ 44919
Total	155078 $\pm$ 27579	410627 $\pm$ 32623
PRL > 90 ng/ml		
Central	149835 $\pm$ 41575	332775 $\pm$ 96405
Peripheral	78560 $\pm$ 5438	363893 $\pm$ 77122
Total	107070 $\pm$ 22057	351446 $\pm$ 52648

 *Denotes pituitary region

**Num. density (#cell/mm^3 tissue) $\pm$ S.E.M.

The only regional differences shown were between the central and peripheral region of Type 2 cells (RER>15%) when PRL was less than 90 ng/ml, and between the central and peripheral regions of Type 1 cells (RER<15%) when PRL was greater the 90 ng/ml. All the other animals showed remarkable uniformity in the number of cells from each region. A graphical representation of these data is given in Figure 6.

When the number of each cell type in the sham treated animals was compared with the numerical density of its respective cell type in animals with PRL both less than and greater than 90 ng/ml, a significant increase (p<.05) was observed in the experimental animals. There was no difference between the two experimental groups.

The gonadotrope cell volumes in each region (central and peripheral) and in each group (sham, PRL less than 90 mg/ml, PRL greater than 90 ng/ml) were determined using standard morphometric techniques and were expressed as um^3/cell. The Type 2 cell (RER>15%) was, as expected, larger than the Type 1 Cell (RER<15%) in each group of animals tested (Table 5). At the regional level, there was no overall difference in cell volumes within each group of animals tested, although significantly larger (p<.05) Type 2 cells were seen in the peripheral region of the animals with less than 90 ng/ml (Figure 7). Cell volumes of gonadotropes in experimental animals having a PRL serum level of less than 90 ng/ml were smaller (p<.05) than in the sham treated group. Similarly, when PRL levels were greater than 90 ng/ml the volumes were reduced (p<.05) from that of the sham animals. When the two experimental groups were compared to each other, there was no

significant difference between their cell volumes (Figure 7).

TABLE 5

CELL VOLUMES OF GONADOTROPES

Groups	Type 1 Cell	Type 2 Cell
Sham Operated Animals		
Central*	447.5 $\pm$ 93.9**	1122.9 $\pm$ 55.8
Peripheral	352.8 $\pm$ 112.6	1229.5 $\pm$ 170.6
Total	406.7 $\pm$ 68.5	1168.6 $\pm$ 74.2
PRL < 90 ng/ml		
Central	206.4 $\pm$ 13.5	613.1 $\pm$ 78.2
Peripheral	214.1 $\pm$ 23.7	952.2 $\pm$ 117.4
Total	209.7 $\pm$ 11.6	758.4 $\pm$ 91.7
PRL > 90 ng/ml		
Central	181.8 $\pm$ 0.9	834.8 $\pm$ 41.6
Peripheral	195.6 $\pm$ 16.1	792.2 $\pm$ 37.1
Total	190.0 $\pm$ 9.5	809.2 $\pm$ 26.3

 *Denotes pituitary region

**Cell volume (um^3/cell) $\pm$ S.E.M.

Figure 4. Serum LH concentrations are plotted against rising PRL

levels at each day of sacrifice. Asterisk denotes

significant (p<.05) difference from sham levels on each

particular day. All figures expressed as mean $\pm$ S.E.M.
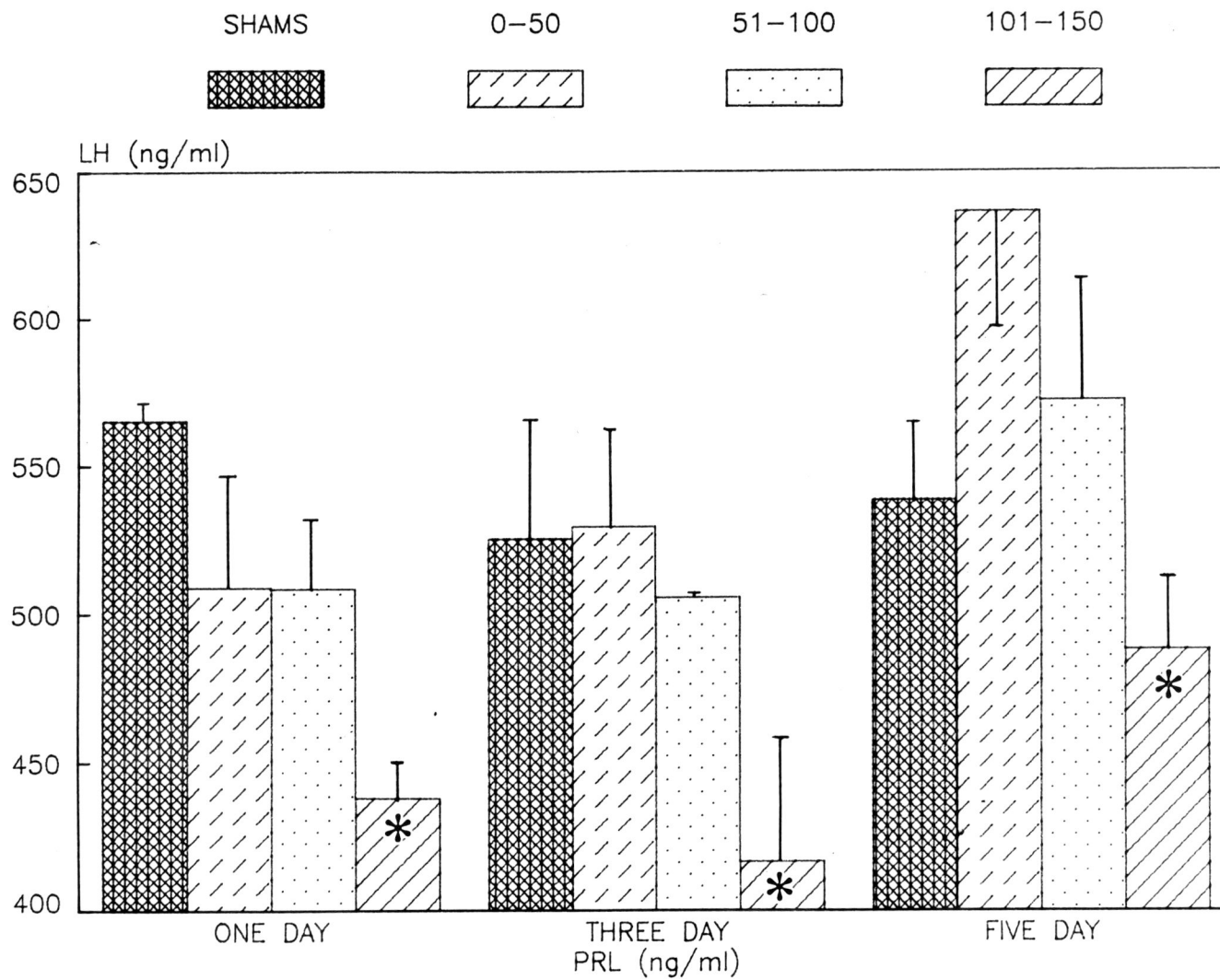
LH CONCENTRATION VS. PRL CONCENTRATION

Figure 5.   Serum FSH concentrations are plotted against rising PRL

levels at each day of sacrifice.   Asterisk denotes

significant difference (p<.05) from sham levels on each

particular day.   Figures expressed as mean ± S.E.M.
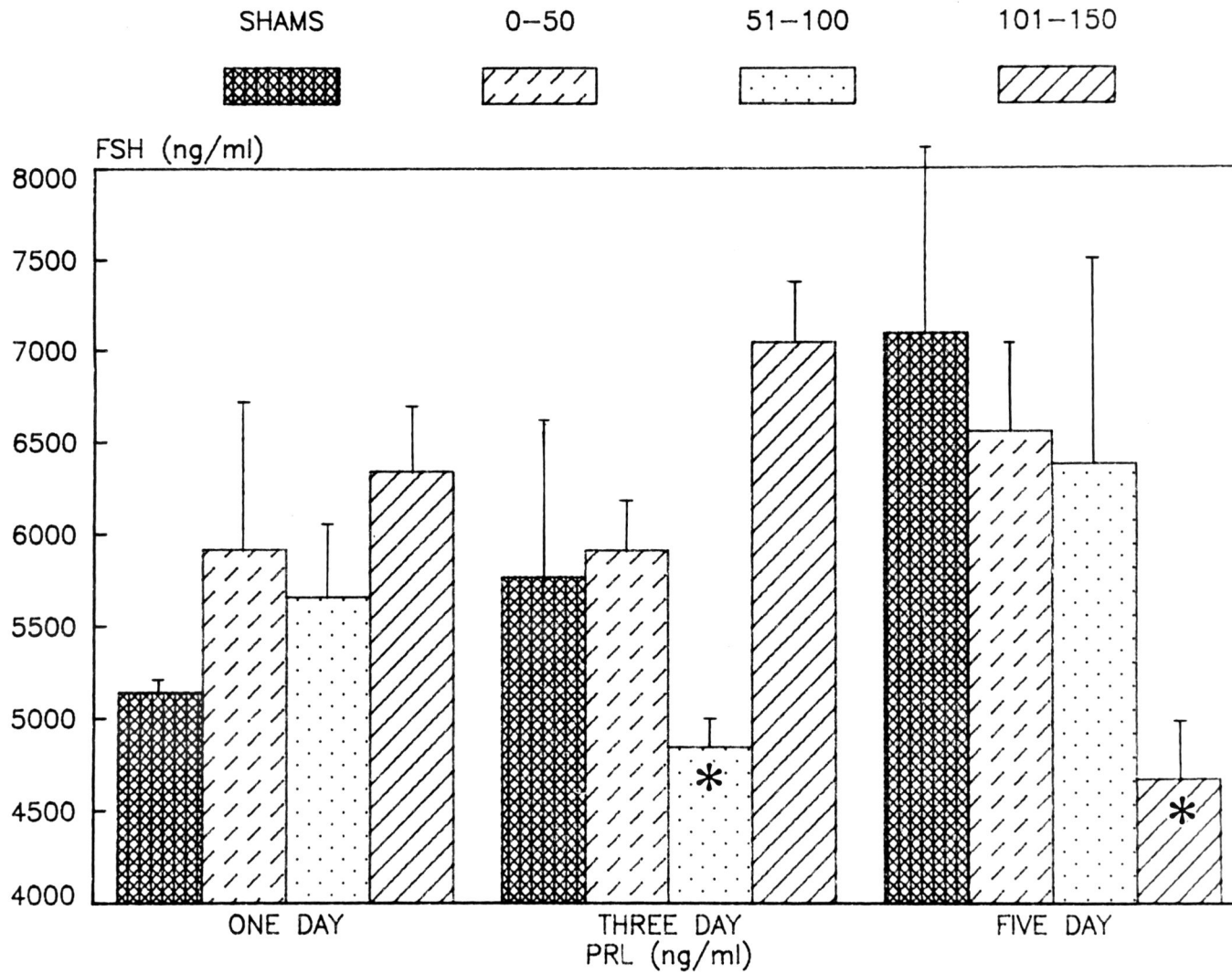
FSH CONCENTRATION VS. PRL CONCENTRATION

Figure 6. The effects of prolactin on the numerical density of

pituitary gonadotropes in ovariectomized rats. Regional

figures are also illustrated. All values shown are

mean $\pm$ S.E.M.

EFFECTS OF PRL ON GONADOTROPES:
NUMERICAL DENSITY

PRL<90 ng/ml   PRL>90 ng/ml   Ovx Shams

NUMERICAL DENSITY (# CELLS x1000/mm3 GLAND)

CENTRAL   PERPHRL   WHOLE
TYPE 1 CELL (RER<15%)

CENTRAL   PERPHRL   WHOLE
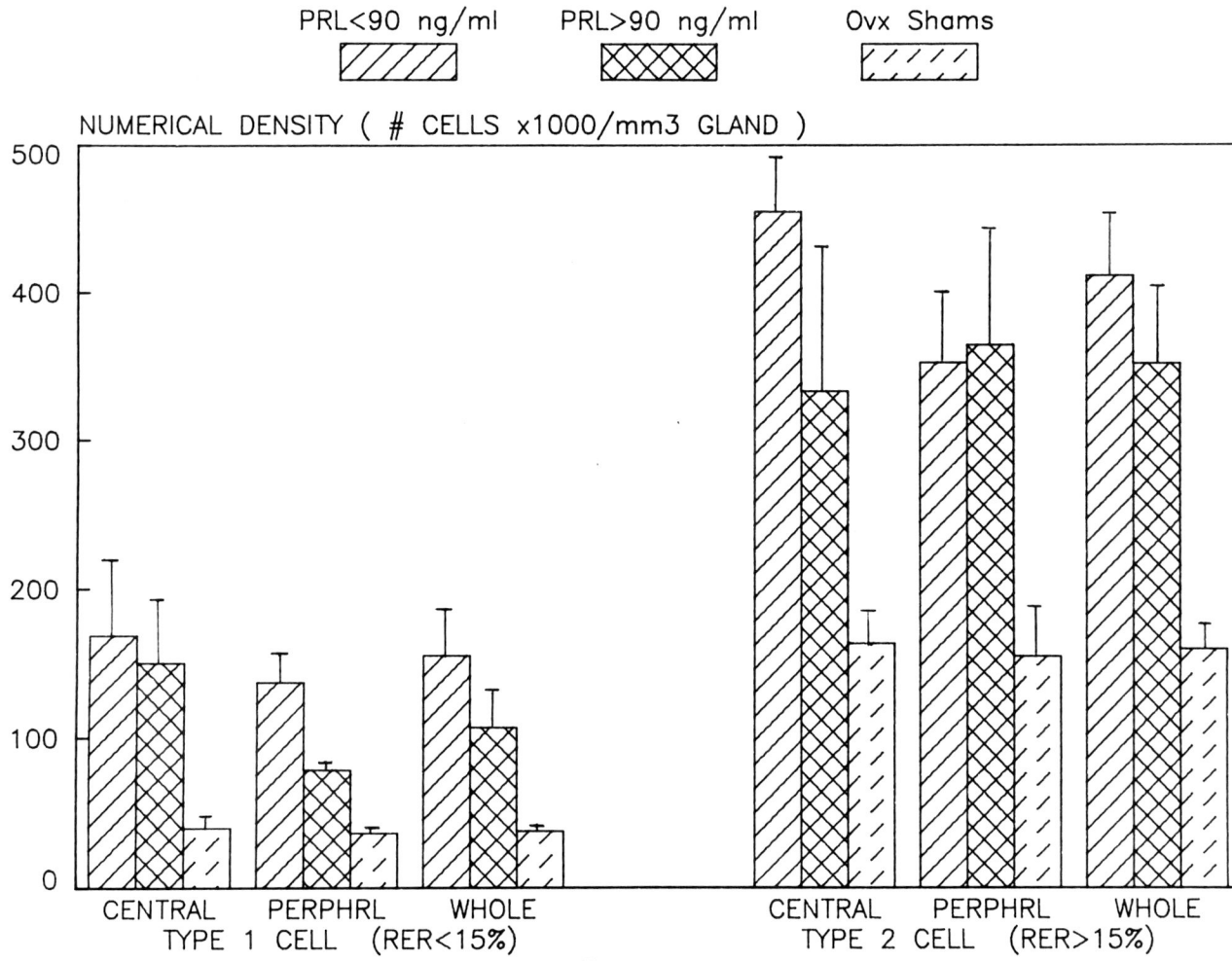TYPE 2 CELL (RER>15%)

FIGURE 6

Figure 7. The effects of prolactin on the cell volume of pituitary
gonadotropes in ovariectomized rats. Regional figures are
also illustrated. All values shown are mean $\pm$ S.E.M.

EFFECTS OF PRL ON GONADOTROPES:
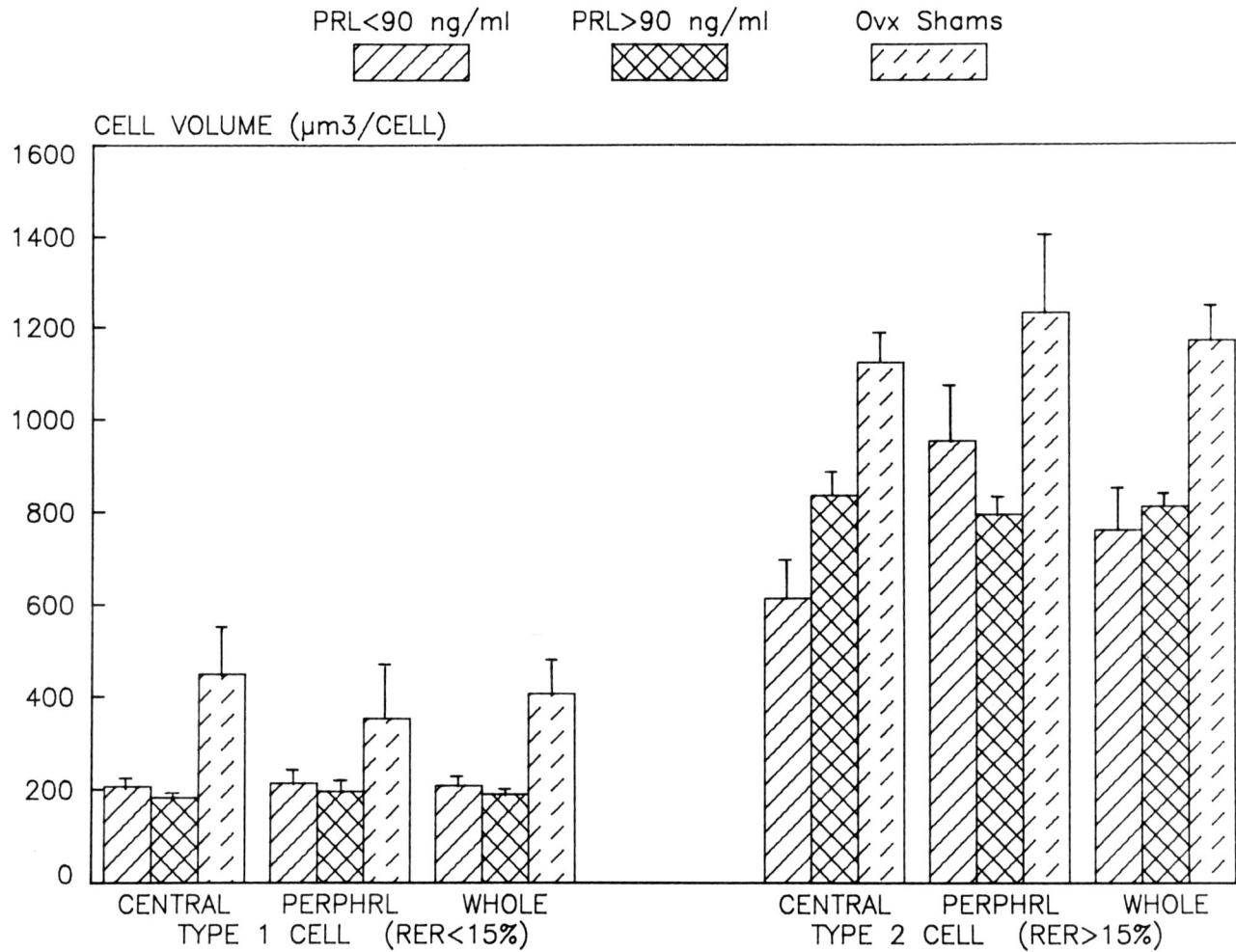CELL VOLUME

FIGURE 7

DISCUSSION

The transplantation of pituitaries beneath the kidney capsule of
donor rats has been shown to elevate PRL levels (Beck et al., 1977;
Tresguerres and Esquifino, 1981), since the pituitaries were removed
from the inhibitory influence of the hypothalamus. The results from
Beck et al. (1977) showed a considerably higher increase (mean of
120 ng/ml). However male rats were used in their study and their
animals were cannulated under light ether anesthesia, which can raise
serum PRL significantly due to ether stress. Tresguerres and
Esquifino (1981) used littermate donor pituitaries in their study of
both male and female prepubertal rats. The PRL level in their study
was 75 $\pm$ 12 ng/ml. The PRL levels of the present study compare
favorably with their results.

Estrogen was first shown by Reece and Turner (1937) to stimulate
lactogenic hormone (PRL) production and release in the pituitary.
Thus after estradiol benzoate was given for five days prior to trans-
plantation in this study there was a twofold increase in serum PRL.
This is consistent with the finding of Chen and Meites (1970) that a
twofold increase of pituitary PRL was present in a group of
ovariectomized rats receiving approximately the same dosage of
estrogen as those in this study.

The precise mechanism of action of estrogen on PRL secretion is
not known and in the present study it should be pointed out that only

the donor rats received estradiol benzoate. The experimental rats received the donors' pituitaries only, and it is assumed that these transplanted pituitaries did not release any estrogens. The experimental rats were ovariectomized so that there could be no effect of estrogens or progesterone on PRL, LH, and FSH secretions.

The effects of PRL on serum gonadotropin concentration shows that as PRL concentrations exceeded 90 ng/ml, they cause a suppression of LH in ovariectomized rats. This points to a direct action of PRL on the hypothalamo-hypophyseal pathways controlling gonadotropin secretion. These results agree with those obtained using different hyperprolactinemia models, including the immature female rat (Beck et al., 1977), the castrated male rat (Hodson et al., 1980; McNeilly et al., 1980) bearing prolactin-secreting tumors, and the ovariectomized rat bearing pituitary transplants (Beck et al., 1977).

From Figure 4 it is apparent that on each of Days 1, 3, and 5 there is a suppression of LH by increasing PRL concentrations. However, the basal levels of LH are increasing with time and on Day 5 there is an increase of LH over the sham level; this is in agreement with Beck et al., (1977) and Beck and Wuttke (1977). It should be pointed out that there is approximately the same amount of suppression on Day 5 as on Days 1 and 3, even though the basal levels of LH have increased.

Serum FSH, on the other hand, is increased above sham levels on Days 1 and 3 with levels increasing concurrently with PRL levels. On

Day 5 serum FSH is below sham levels, and FSH decreases here as PRL concentrations are increasing.

It is shown that on Days 1 and 3 serum FSH levels are rising above sham levels as serum PRL levels are increased. In addition, LH levels are declining as PRL concentrations increased. The two gonadotropins are responding to hyperprolactemia in an inverse manner. On Day 5, however, both gonadotropins are decreased by rising PRL levels, so the inverse relationship has disappeared by that time.

On Days 1 and 3 there was a differential suppression of LH, which agrees with a number of previous studies (Beck and Wuttke, 1977; Winters and Loriaux, 1978; Greeley and Kizer, 1979; McNeilly et al., 1980). However, on Day 5 here was a suppression of both LH and FSH at higher (above 90 ng/ml) concentrations. It appears that serum FSH suppression occurs only after refractory period of 4-5 days following the induction of hyperprolactinemia.

The mechanism of this gonadotropin suppression by PRL is unclear at the present. It could involve any component of the reproductive axis including suprahypothalamic neurons, the hypothalamus, the pituitary, or the gonad. Since the present experiment used ovariectomized animals, only one or more of the former three components must be involved, and a combination of these components may actually cause the suppression of LH by PRL.

The prevailing thought is that high circulating PRL increases the turnover of tuberoinfundibular DA (TIDA), and that the resulting release of DA mediates the suppression of gonadotropins, probably by

inhibiting GnRH release (Evans et al., 1982). It should be emphasized that current studies are confusing in this area and although ample data supports the inhibitory role of DA on gonadotropin release, there is sufficient evidence to suggest other possible mechanisms of action of hyperprolactinemia and its antigonadal effects. These include the actions of endogenous opiates and norepinephrine, and possible roles of the adrenal gland and prostaglandins on prolactin and gonadotropin release.

There is little evidence to show that there is a direct effect of PRL on the pituitary gland although a recent study (Wuchenich and Cheung, 1981) shows that there is a decreased basal and GnRH-stimulated LH release from rat pituitaries that were incubated (in vitro) with ovine PRL. The increased use of organ culture with the addition of specific hormones and neurohormones will likely lead to a clearer understanding of hormonal interactions.

Gonadotrope population dynamics, shown using morphometric techniques, indicates that there is no regional difference in both numerical densities and cell volumes of the respective gonadotropes. This was true in both the sham animals and those that were hyper-prolactinemic. It had been previously shown that there was no regional variation in cell volumes of gonadotropes (Garner and Blake, 1979; Poole and Kornegay, 1982). However, this study utilized ovariectomized rats with high circulating PRL levels unlike the others. Thus, ovariectomy and high PRL levels must affect gonadotrope cell size and numerical density equally throughout the pituitary gland.

The data concerning regional distribution of gonadotropes with respect to numbers of cells (numerical density) has pointed to a regional variation in the numbers of the classical Type I FSH and Type II LH cell (Herlant, 1964; Poole and Kornegay, 1982). Again, the present study utilized ovariectomized rats under high PRL influences and differently defined gonadotropes, whereas the others did not. Poole and Kornegay (1982) found a regional variation only in the Type I gonadotrope on the left side on the pituitary in their study of cellular distribution in the anterior pituitary. They found that all the other cell types in the pituitary were not significantly localized in either the central or peripheral region.

The morphometric data on gonadotrope numerical densities and cell volumes all came from the Day 3 sham treated and experimental animals. It should be pointed out that on this day, as PRL levels rose, LH levels declined. FSH levels increased above sham levels on Day 3. The numerical densities of both the small cell type (Type 1) and the large cell type (Type 2) increased while under the influence of PRL levels above the shams. This indicated that either cell division occurred or that there was a transformation of one of the other cell types into gonadotropes. Since mitotic figures were not prevalent in the pituitary tissue, the latter explanation seems to provide the probable answer to this finding. Indeed, Yoshimura et al. (1969) have presented evidence that chromophobes, of otherwise unknown functional significance, may be stem cells capable of differentiating into other cell types. At any rate, this transformation occurs when PRL levels

are less than 90 ng/ml and does not increase at levels above 90 ng/ml.

While the numbers of both types of gonadotropes increased when circulating PRL levels were elevated, the volumes of the individual cells decreased. This was seen in both the small Type 1 and the larger Type 2 gonadotrope. Since mitotic figures were not prevalent, this could not be due to dividing cells, although this explanation would account for both the increase in numbers and the decrease in size of the cells. Instead, a decreased synthesis of LH may result in the extrusion of the endoplasmic matrix, or increased lysosomal activity may have occurred.

It now seems clear that PRL suppresses LH differentially, and also both LH and FSH simultaneously - depending upon the time involved. There is a correlation reported here for the first time between high PRL levels and the numbers and size of gonadotropes. However, the precise mechanisms involved are not clear, and will require further elucidation. Quantitative morphometric techniques will be necessary to ascertain the intracellular dynamics involved in changing functional states.

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