

## Regional Mechanisms Within Anterior Pituitary of Lactating Rats May Regulate Prolactin Secretion

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**Prolactin (PRL) release was compared after incubating the central and peripheral regions of the anterior pituitary of lactating rats, either nonsuckled or suckled in conditioned medium obtained by incubating medium with the same anterior pituitary regions from nonsuckled or suckled rats. To collect conditioned media, anterior pituitary regions were incubated in Earle's medium for 4 h, and conditioned medium was filtered and employed double concentrated. Each anterior pituitary was incubated in conditioned medium for 30 min. PRL released in vitro was determined by polyacrylamide gel electrophoresis. As a control, anterior pituitary regions from lactators were incubated in medium conditioned by male rat anterior pituitary regions, and they showed no changes of PRL release compared with those cultured in Earle's medium. In general, conditioned media from both anterior pituitary regions of nonsuckled and suckled rats inhibited PRL release in peripheral anterior pituitary regions, whereas PRL release was stimulated in central regions of both nonsuckled and suckled rats. A higher number of stimulatory effects was provoked by conditioned media from suckled than from nonsuckled rats, and most of these effects were from conditioned media of the peripheral region of suckled rats. Together, these results suggest the existence within anterior pituitary regions of factors that regulate PRL secretion and that their action depends on the physiologic condition of the animal.**

**Key Words:** Anterior pituitary; prolactin; lactation; regionalization.

### Introduction

The secretion of prolactin (PRL) by the anterior pituitary is a complex neuroendocrine phenomenon whose regulation is systemic, hypothalamic, and locally produced; that

is, autocrine and paracrine factors participate (see refs. 1 and 2 for reviews). Regional and lactotroph heterogeneity has been documented (3–5) regarding cell size, secretory capacity, responsiveness to secretagogues, as well as the influences of other PRL secretagogues from the posterior and/or neurointermediate lobes of the pituitary (PNIL), which, reaching the anterior pituitary via the short portal vessels, may physiologically stimulate PRL release and may influence PRL regionalization ([6–8]; see ref. 5 for review).

In a previous study, we analyzed the effect of removing the PNIL of the pituitary gland of lactating rats on the suckling-induced transformation and release of PRL and on regionalization of its secretion (9). The results of this and other studies (10–13) suggested that the PNIL may play a complementary role on in vivo and in vitro PRL release, and it may influence PRL regionalization, even though factors from the PNIL showed no effect on suckling-induced PRL transformation (9).

In the present study, we used a different approach to determine whether within the central (i.e., the region of the anterior pituitary surrounding the neurointermediate and posterior lobes of the pituitary) and the rest of the gland of lactating rats there exist mechanisms capable of influencing each others' secretion of PRL and whether the type of effect exerted varied according to the physiologic condition of the animal (i.e., suckled or nonsuckled). Conditioned media obtained by incubation of each region of anterior pituitaries from rats previously nonsuckled 6 h or from rats nonsuckled 6 h and then suckled for 15 min were employed to incubate anterior pituitary regions from animals in the same (i.e., nonsuckled and suckled) physiologic condition, and their effects were determined on PRL release.

### Results

#### *Effect of Conditioned Media from Incubation of Anterior Pituitary Regions of Male Rats on PRL Release from Anterior Pituitary Regions of Lactating (nonsuckled and suckled) Rats*

As shown in Table 1, conditioned media from anterior pituitary regions of male rats had no effect on PRL release from anterior pituitary regions of either nonsuckled or suckled rats.

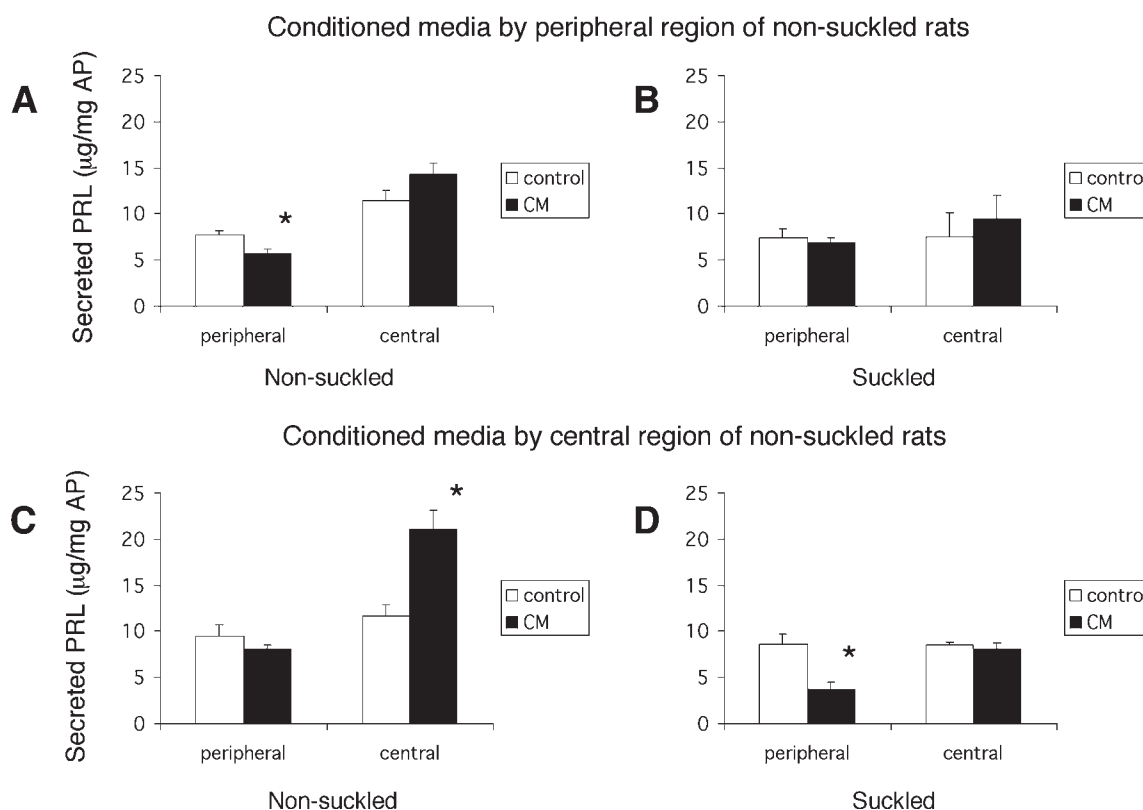
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**Table 1**

Effect on PRL Release ( $\mu\text{g}/\text{mg}$  of Anterior Pituitary) of Incubating Anterior Pituitary Regions from Male and Lactating (nonsuckled and suckled) Rats in Media Conditioned from Incubation of Anterior Pituitary Regions of Male Rats<sup>a</sup>

Incubated anterior pituitary region	Male rats		Nonsuckled rats		Male rats		Suckled rats	
	Control	Conditioned medium	Control	Conditioned medium	Control	Conditioned medium	Control	Conditioned medium
Peripheral	$0.72 \pm 0.32$	$1.06 \pm 0.18$	$8.47 \pm 0.51$	$7.84 \pm 0.56$	$1.02 \pm 0.22$	$1.26 \pm 0.06$	$9.61 \pm 1.93$	$8.25 \pm 1.05$
Central	$0.24 \pm 0.15$	$0.57 \pm 0.17$	$6.87 \pm 0.86$	$9.09 \pm 0.69$	$0.94 \pm 0.15$	$1.07 \pm 0.37$	$10.42 \pm 0.71$	$9.25 \pm 0.85$

<sup>a</sup>Control incubations were made in Earle's medium.



**Fig. 1.** Effect on PRL release of incubating nonsuckled and suckled rat anterior pituitary regions in conditioned media (CM) obtained from incubations of (A,B) peripheral and (C,D) central anterior pituitary regions of nonsuckled rats ( $n = 5$ ). \* $p < 0.05$  vs control.

### **Effect of Conditioned Media from Anterior Pituitary Regions of Nonsuckled and Suckled Rats on PRL Release**

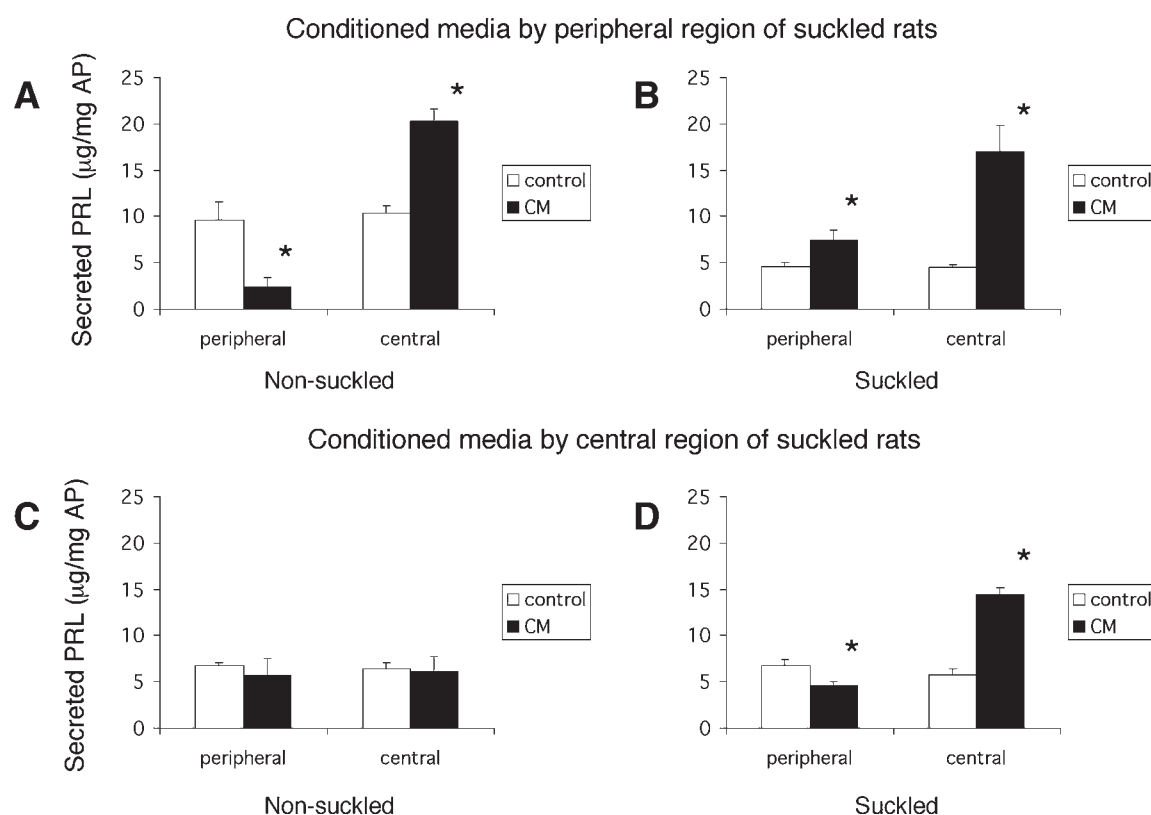
#### *Effect of Conditioned Media from Peripheral and Central Anterior Pituitary Regions of Nonsuckled Rats on Corresponding Anterior Pituitary Regions of Nonsuckled and Suckled Rats*

As shown in Fig. 1A,B, when incubated in conditioned media from the peripheral region of nonsuckled rats, PRL secretion decreased in the peripheral anterior pituitary region of nonsuckled rats, but no effect was observed in the central anterior pituitary region of nonsuckled rats, nor in either region of anterior pituitaries from suckled rats. As shown in Fig. 1C,D, PRL secretion was unchanged when either the peripheral region of nonsuckled rats or the central

region of suckled rats was incubated in media from the central region of nonsuckled rats. However, when incubated in media conditioned by the same tissue region, significant changes in PRL secretion occurred, namely increased secretion in the central region of nonsuckled rats and decreased PRL secretion in the peripheral region of suckled rats.

#### *Effect of Conditioned Media from Peripheral and Central Anterior Pituitary Regions of Suckled Rats on Respective Regions of Nonsuckled and Suckled Rats*

As shown in Fig. 2A,B, a clear stimulation of PRL secretion occurred when the central region of nonsuckled rats and both regions of suckled rats were incubated in conditioned media from the peripheral region of suckled rats.



**Fig. 2.** Effect on PRL release of incubating nonsuckled and suckled rat anterior pituitary regions in conditioned media (CM) obtained from incubations of (A,B) peripheral and (C,D) central anterior pituitary regions of suckled rats ( $n = 5$ ). \* $p < 0.05$  vs control.

However, inhibition of PRL secretion resulted from incubation of the peripheral region of nonsuckled rats in the same medium. On the other hand, as shown in Fig. 2C,D, no effect of conditioned media from the central region of suckled rats was observed on either anterior pituitary region of non-suckled rats, but incubation in this same medium resulted in a significant decrease or increase in PRL secretion in the peripheral and central anterior pituitary regions of suckled rats, respectively.

## Discussion

The results obtained in the present study using media conditioned by different anterior pituitary regions suggest that regional influences within the lactating rat anterior pituitary may regulate PRL secretion, and that the type of effect varies according to the physiologic condition of the animal. The fact that incubation of anterior pituitary regions from lactating rats in media from male rat anterior pituitary regions had no effect on PRL secretion indicates that the effects observed were specific for lactating rats. Incubations with conditioned media from lactating rats caused either stimulation, inhibition, or no effect on hormone release from each region, which suggests either that the conditioned media from male rats lack the factors existing in lactating female

anterior pituitaries, or that lactating female anterior pituitaries lack receptors for factors from male anterior pituitaries.

When considering the stimulatory and inhibitory effects observed, it is clear that the conditioned media from both anterior pituitary regions of suckled rats showed a higher number of effects than the conditioned media of nonsuckled rats; that is, four effects of conditioned media from suckled rats were stimulatory and two were inhibitory, whereas one effect of conditioned media from nonsuckled rats was stimulatory and two were inhibitory. In addition, no effects occurred in five conditions from nonsuckled rats and in two from suckled rats. Regarding the regions from which these conditioned media were obtained and those where they provoked the effects, for the suckled rats, four of the effects, three stimulatory and one inhibitory, were exerted by conditioned media from the peripheral anterior pituitary region of suckled rats, and its stimulatory effects were manifested both in anterior pituitary regions of suckled rats and in the peripheral region of nonsuckled rats. With respect to the effects shown by the conditioned media from the central region of nonsuckled rats, it inhibited PRL secretion from the peripheral region of suckled rats but stimulated it in the central region of nonsuckled rats. Finally, in contrast to conditioned media from the peripheral region of suckled rats, the conditioned media from the same region of nonsuckled

rats showed only an inhibitory effect on the same region of nonsuckled rats. A scheme showing the effects of conditioned media on anterior pituitary regions and those regions from which conditioned media were obtained is shown in Fig. 3.

Thus, within an integrative frame of PRL secretion by the lactating rat anterior pituitary, these results suggest that suckling may preferentially activate secretion and release of factors that stimulate rather than inhibit PRL release, whereas, in the absence of suckling stimulation, either no effects are manifested or only one of inhibitory and another of stimulatory type are expressed. Additionally, with respect to the action of suckling, it was clear that the origin of these factors was the peripheral, and not the central anterior pituitary region.

Previous studies have focused on the role that factors from the PNIL may exert, via the short portal vessels, on PRL secretion by the lactating rat anterior pituitary ([3,4]; see ref. 5 for review), and in particular on the influence of  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$ -MSH) on the central anterior pituitary region (5,10,11,14,15). In addition, the well-established hypothalamic mechanisms regulating PRL transformation and release (i.e., dopamine and secretagogues such as thyrotropin-releasing hormone [TRH]) (16,17), were found to occur in response to suckling only in the central and not in the peripheral region of the anterior pituitary, and the occurrence of these mechanisms was not affected by the absence of the PNIL (9). Taken together, the results of the present and previous studies suggest that suckling activates a complex series of interacting and perhaps sequential events involving hypothalamically activated PRL transformation and release, as well as activation and release of  $\alpha$ -MSH and of secretagogues, presumably from the posterior lobe, all of them impinging on the central region of the pituitary gland for PRL secretion. In this context, the results of the present study suggest that in addition to or interacting with other sources of regulation, the pituitary of the lactating rat produces factors capable of either stimulating or inhibiting PRL release, according to whether the rat has been suckled or not. Moreover, the findings that the number of stimulatory influences was found to increase in both regions of suckled rats and that most of those influences increased secretion from the central anterior pituitary region are consistent with the importance of this region for PRL secretion in the lactating rat.

At present not much can be said regarding the nature of the agents responsible for these effects, except that their size should be  $>10$  kDa, which was the limit employed for ultrafiltration and desalination of the conditioned media. Thus, based on size, the vast majority of known pituitary factors with autocrine-paracrine activity that have been reported (e.g., TRH, vasointestinal polypeptide [VIP], angiotensin II [AII], neurotensin, substance P,  $\alpha$ -MSH) (see refs. 1 and 2 for review) could be excluded. However, this would not imply that, under particular physiologic conditions, interactions of these and other PRL regulators may not occur.

Much work will be required to characterize the nature and mechanisms involved in the effects that were observed, and to define the role that these influences may have in the overall regulation of PRL secretion by the lactating rat anterior pituitary.

## Materials and Methods

### Animals

Primiparous lactating rats of the Wistar strain were used. They were kept in individual cages in a room with controlled light (light on from 7:00 AM to 9:00 PM) and temperature (23–25°C). Purina chow (Ralston Purina, Chicago, IL) and tap water were given ad libitum. Litter size was adjusted to 8–10 pups, and rats were used 7–10 d postpartum.

Some groups of mothers were killed by decapitation 6 h after their pups had been removed. These comprised a non-suckled group. Other groups were suckled by their litters for 15 min at the end of the nonsuckling period, then killed.

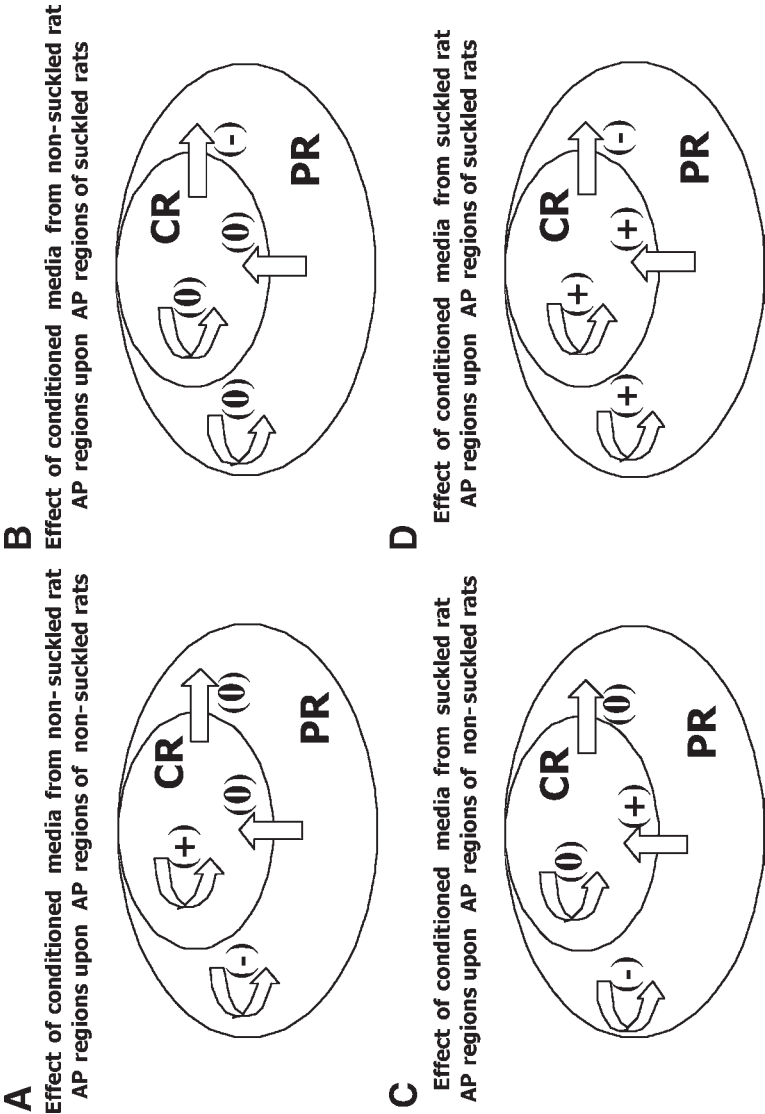
All the animals were sacrificed after light ether anesthesia, and the anterior pituitary of each rat was rapidly removed and dissected into a central region, surrounding the neuro-intermediate lobe of the pituitary gland (3,9), and the rest of the gland (i.e., the peripheral region) (see Fig. 4). All efforts were made to minimize the suffering and number of animals, and all our experiments conformed to local and international guidelines on the ethical use of animals.

### Conditioned Media

The conditioned media were obtained from 4-h incubations of anterior pituitary regions of nonsuckled and suckled rats. Incubations were made in a metabolic shaker in Earle's medium, pH 7.3, under 95% O<sub>2</sub>–5% CO<sub>2</sub>, at 37°C for 4 h. During incubation, media samples were collected at 30, 60, 90, 120, and 240 min. At each time, the media were collected and fresh Earle's medium was added. The collected media were pooled, ultrafiltered, and desalted in a Centricon microconcentrator with a 10-kDa cutoff at 4°C at 2055g in a Sorvall RC5C centrifuge (five cycles each for 30 min). Finally, the media were diluted with Earle's medium in a 2:1 concentration.

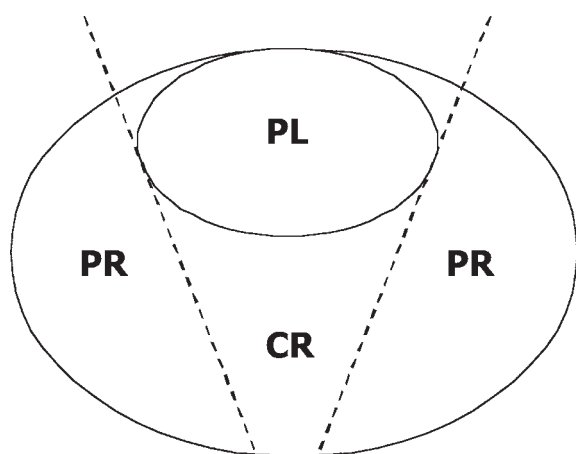
### PRL Determination and Data Analysis

The media samples were analyzed with the nondenaturing, nonreducing polyacrylamide disc gel electrophoresis densitometry procedure of Nicoll et al. (18) as previously described (9,17,19,20). Since monomeric (i.e., 23-kDa) PRL appears as a single band and the monomeric form is the main form released by the lactating rats, both in vivo and in vitro (21), this method can be used to quantitate changes in the PRL released. By contrast, radioimmunoassay measurements of PRL include not only the monomeric, but also other variants of PRL (see ref. 21 for review). The amount



**Fig. 3.** Schematic representation of data in Figs. 1 and 2 showing effects of conditioned media by central (CR) and peripheral (PR) anterior pituitary (AP) regions from 6-h nonsuckled or from 15-min suckled rats on PRL release from anterior pituitary regions of rats in same (A,D) or opposite (B,C) physiologic condition from which conditioned media was obtained. Arrows indicate the origin and site of action of conditioned media (+), (-), and (0) indicate stimulatory, inhibitory, or no effects, respectively. Note that the highest number of stimulatory effects was exerted by medium conditioned from incubations of suckled rat anterior pituitaries, particularly from the peripheral region.





**Fig. 4.** Scheme of pituitary of rats indicating posterior (PL), central (CR), and peripheral (PR) regions of anterior pituitary. Dashed lines indicate the site at which dissection of each region is made, after the posterior and neurointermediate lobes have been removed.

of PRL present in the conditioned media was determined, and its value was subtracted from the total amount of hormone secreted after incubation with anterior pituitary regions.

Data are expressed as the mean  $\pm$  SEM. Differences between experimental groups were analyzed using a one-way analysis of variance and Tukey HSD test. Differences with a  $p$  value of 0.05 or less were considered statistically significant.

### Experimental Design

A series of experiments was made to determine the effect on PRL secretion of incubating anterior pituitary regions from nonsuckled and suckled rats for 30 min in media conditioned from incubations of anterior pituitary regions of animals in the same conditions (i.e., nonsuckled and suckled).

Control incubations of anterior pituitary regions were made both in Earle's medium and in media conditioned from incubating anterior pituitary regions of male rats for 4 h, following the same procedure used to obtain conditioned media from lactating rats. Control incubations in Earle's medium were made in parallel with each experiment in which conditioned media from lactating rat anterior pituitaries were used.

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