CHANGES IN THE HORMONE CONTENT OF SWINE PITUITARIES DURING THE ESTRUAL CYCLE

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IN an extensive study conducted at the University of Illinois during the past few years qualitative and, where possible, quantitative data on the anatomic, endocrine and genetic causes of sterility and impaired fertility of female swine have been obtained (Wilson, Nalbandov and Krider, 1949). In addition to a large number of partially or completely sterile females a group of presumably normal females was studied. The past reproductive performance of these normal control animals was accurately known, permitting us to obtain some interesting correlations between the hormone content of their pituitaries and the ovarian changes of each animal during the normal estrual cycle.

That a cyclic variation of the gonadotrophic and thyrothrophic hormone content of the pituitary exists among animals has been amply shown in endocrine literature. Wolfe (1931) found swine pituitaries to be most potent during proestrus and least potent during heat. Similar results for swine were obtained by Faermark and Singerman (1938). Studies with females from other species support the conclusion that the gonadotrophic potency of pituitaries from animals in estrus is lower than during the remainder of the cycle. But no systematic attempt has been made in earlier studies to correlate assayable potency of the pituitary throughout the estrual cycle with the ovarian activity of the donor. Such a study of normal females, it seemed, might further the understanding of the role of this gland in sexual development and function, and could be used as a standard for comparison with females with impaired fertility.

The object of this study was to measure the day-by-day changes in the gonadotrophic and thyrotrophic activity of normal swine pituitaries during the 21-day estrual cycle. This study was also designed to show the simple and multiple correlations between (1) day of cycle and number of follicles, (2) day of cycle and gonadotrophic potency, (3) number of follicles and gonadotrophic potency, (4) day of cycle and pituitary weight, (5) day of cycle and number of corpora lutea and (6) day of cycle and thyrotrophic potency.
Materials and Methods

The pituitaries used in this study were obtained from nulliparous and multiparous females which had shown normal estrual cycles prior to being killed. Statistical analysis revealed that there were no significant differences between nulliparous (gilts) and multiparous (sows) females as far as follicle size and number (follicle index) and the number of corpora lutea were concerned. There was no significant difference between the gonadotrophic potency of identical amounts of pituitary powder from the two ages. In view of the significantly heavier pituitaries found in sows the total amounts of gonadotrophic and thyrotrophic hormone present per gland were much greater in sows than in gilts. Due to the larger body weight of the sows the amount of gonadotrophic hormone per unit body weight was found to be about the same in the two age groups. For these reasons it appears proper not to differentiate between these two types of females in the subsequent discussion, especially since they are distributed at random throughout the different days of the estrual cycle. The differences in pituitary weights between the animals of different ages were taken into account in the statistical analysis.

The animals were permitted to go through at least two complete cycles after being placed on experiment and prior to being killed. Heat periods were established by the use of teaser males. The pituitaries were removed from the heads 15 to 30 minutes after the animals had been killed. Individual glands were placed into small vials, properly numbered, and kept frozen until they were ready for use. In all cases a careful description was made of the ovaries of each female, including the number and diameters of all visible follicles and the number of corpora lutea or corpora albicantia.

Day-old male chicks were chosen as assay animals because, in addition to being suitable test animals for both hormones in which we were interested, it was possible to run the two assays simultaneously on the same animal. The response of the two end-points to the hormone is probably independent of each other. The chicks used were of the Single Comb White Leghorn breed and were received at the laboratory the day following hatching. During injections they were kept in standard chick brooders and were fed an adequate growing ration.

The glands were allowed to thaw just before they were to be used for assay and the pituitary stalk and the adhering connective tissue were removed. Each pituitary was weighed after trimming, placed between two glass plates and flattened by an appropriate amount of
pressure. The glass plates with the adhering mangled gland were placed in a calcium chloride desiccator at room temperature for dehydration which was completed in about 24 hours.

The dry glands were ground into a fine powder which was stored in a calcium chloride desiccator until ready for use a few days later. The exact amount of powder to be injected was suspended in distilled water and this aqueous suspension was kept in the refrigerator during the period of injections. Each pituitary was assayed individually.

Preliminary tests showed that 10 mgs. of swine pituitary powder produced a good response for both hormones to be measured. Since most of the glands yielded a little more than 30 mgs. of powder, it was decided to let the amount of powder obtained determine the number of chicks to be used for each gland. The birds were two to three days old at the time of the first injection. A total of 10 mgs. of pituitary powder per chick was injected subcutaneously in eight equal doses during four days. At each injection, the chick received the powder suspended in 0.25 ml. of water. The animals were sacrificed on the fifth day, their testes and thyroids were removed, cleaned, fixed in Bouin’s solution and later weighed. The increments in the weights of the testes and thyroids of the injected chicks compared with those of the uninjected controls were used as the criteria of hormone effects.

In order to reduce errors due to differences in season, assay animals, and other environmental causes, the pituitaries were assayed in series. Each series included at least one pituitary for each day of the cycle for which glands were available; thus glands representing the early and late portions of both the follicular and luteal phases of the cycle were assayed simultaneously. Two complete series consisting of glands distributed throughout the cycle were run while the third series consisted of glands representing only days -1, -2, -6, and -13 of the cycle. No significant differences were found to exist between these series and they were therefore combined. For each series an adequate number of uninjected control chicks was retained.

It was found most advantageous in this laboratory to date the estrual cycle of the sow beginning with the first day of heat which was called day-1 of the cycle. Ovulation is known to occur on the second or third day of heat depending on the age of the female. On the basis of uterine histology the luteal phase extends to the thirteenth day of the cycle (eleventh day after ovulation) while the follicular phase begins on the fourteenth day and includes the first and second days of the new cycle (Creen, 1950).
In order to correlate the ovarian activity of the donor with the gonadotrophic hormone content of her pituitary a quantitative description of the ovary had to be devised. A simple follicle count was not thought adequate because late in the follicular phase and early in the luteal phase about the same number of follicles was found in the ovaries but those in the follicular phase were much larger than the ones in the luteal phase (table 1). Tabulation of the follicular com-

<table>
<thead>
<tr>
<th>Day of cycle</th>
<th>Percent follicles in class</th>
<th>Aver. no. follicles in both ovaries</th>
<th>Gonadotrophic hormone in pituitary</th>
<th>Stage in cycle</th>
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<td>&gt;5</td>
<td>5-7</td>
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1 Testes weight, mgs.

ponents of the ovary showed the shifts occurring during the estrual cycle both in number and in size of follicles (table 1).

In an attempt to avoid the difficulty mentioned above the approximate area of the surface of the ovary that was occupied by macroscopically visible follicles was determined by multiplying the number of follicles by their diameter. The figure thus obtained was designated as the "follicle index." The sum of the follicle indices of both ovaries of an animal was used in subsequent statistical analyses. The same calculations were made using the uncorrected follicle count and the follicle index and the two showed sufficiently close agreement to justify the use of the latter.
Results and Discussion

Thirty-three normally cycling sows were killed at certain intervals during the estrual cycle and their pituitaries assayed for gonadotrophic and thyrotrophic hormones.

During the first third of the estrual (days-1 through -7) the gonadotrophic potency was low and the number of follicles and the follicle index was also low (tables 1 and 2, figure 1). It should be noted that this period includes days-1 and -2 of the cycle during which all of the eight females killed on these two days were in heat. One of the females killed on day-2 had ovulated and all the females past day-2 had ovulated at autopsy. The low gonadotrophic potency of the pituitaries from females in heat may be due to the inhibition of the pituitary by follicular estrogen during heat, and is primarily due to a shift in the proportion of FSH and LH.

On day-8 the gonadotrophic hormone titer suddenly increased and remained high during the remainder of the cycle. This abrupt increase
was accompanied by an abrupt increase in the follicle numbers found in the ovaries (tables 1 and 2, figure 1). The follicle index and follicle number reached peaks on days-13 and -20. One might be justified in explaining these two peaks and the trough between them on the basis of insufficient numbers of animals involved in this study. However, in an independent companion study in this laboratory by Green (1950) in which 58 females were used, a remarkably similar curve was obtained also showing peaks of maximum follicle numbers on days-13 and -20 separated by a trough.

Figure 1. Simultaneous changes in the gonadotrophic hormone of the pituitaries and the follicle number in the ovaries of female swine during the estrual cycle. Animals were in heat on days-1 and -2. Ovulation occurred on days-2 or -3.

Of interest are the shifts in the proportion of follicles of various sizes occurring during the estrual cycle (table 1).

Coinciding with the increasing secretion of gonadotrophic hormone on day-8, there is an increase in the number, but not in the size of the follicles. The beginning of the follicular phase as judged by uterine histology (Green 1950) is the result of the increase in follicle size which does not begin until day-14 of the cycle (table 1). Major changes in follicular size continue until the second day of heat. From the 20th day to the 1st day of heat there is also a very pronounced drop in the number of follicles from about 60 to the ovulatory number which
coincides with the pronounced decrease in the gonadotrophic hormone content of the pituitary. The reduction of the follicle number is due to degeneration (Green 1950) and is undoubtedly caused by the decrease in the amount of FSH secreted by the hypophysis.

While the presence of follicles classified as "cysts" (table 1) and appearing during the luteal phase will be discussed in more detail in another paper (Green and Nalbandov, unpublished), a brief explanation appears necessary. One or two such cysts, measuring up to 30 mm. in diameter, are frequently found during the luteal phase in swine. They do not affect the reproductive performance and are "retained" follicles which have failed to ovulate after the last previous heat. They degenerate before the next heat.

According to the Moore-Price law one would expect a close correlation between the gonadotrophic hormone content of the pituitary and the events in the ovaries. But in spite of the fact that many pituitary assays have been and are being reported in the literature, the question has never been answered what is actually being measured in these assays. Three alternatives are possible: (1) that the actual rate at which gonadotrophic hormones are being secreted is determined in the assay, (2) that the secretory rate in the gland does not change and that the amount of hormone found in the gland reflects only the quantity stored, and (3) that the hormone enters into circulation as fast as it is being produced and that therefore the assay reveals only the residual amounts of gonadotrophin in the gland. If either of the last two alternatives are correct then the information that one obtains from bio-assays loses most of its value.

It is evident that it is theoretically and practically important to know with which of these three possibilities one is dealing when bio-assays of the pituitary gland are undertaken. As far as the authors are aware, no one has attempted to correlate the gonadotrophic potency of the pituitary as revealed by test animals with the ovarian picture of donors with long estrual cycles.

From the data presented it seems justifiable to propose that we are not only measuring the gonadotrophic potency of the pituitary at the time of the animal's death but that we are also measuring its rate of secretion (tables 1 and 2, figure 1). This assertion is based on the fact that changes in the number of follicles in the ovaries closely follow changes in the gonadotrophic potency of the pituitary.

In order to determine the degree of association between the various factors in which we were interested a number of correlation coefficients
were calculated (figure 2). The correlations between the gonadotrophic hormone, the follicle index, and the day in the cycle were high and statistically significant (p=0.01) and indicated that the former two were mutually interdependent and that both of them increased with the progressing estrual cycle. These figures further support the con-

Figure 2. Correlation coefficients between the various factors studied.
clusion that as far as the gonadotrophic hormone is concerned the potency of the pituitary accurately reflects the rate of secretion of that hormone by the hypophysis.

There was no change in the weight of the pituitary during the estrual cycle and no correlation between its weight and its gonadotrophic or thyrotrophic hormone content.

It is commonly assumed that the prolificacy of polytocous animals is genetically controlled. It is of interest to note in this connection, that there was no significant correlation between the gonadotrophic potency and the number of corpora lutea. This may be due to the fact that the number of corpora lutea found is the result of the amount of hormone secreted in the previous cycle and thus the result of the number of follicles matured and ovulated then. In this study we were not measuring differences in the gonadotrophic hormone output between genetically different strains, but were only concerned with changes occurring during the estrual cycle. That genetically different strains do produce significantly different amounts of gonadotrophic hormone has been demonstrated in another phase of this study (Robinson and Nalbandov, unpublished).

The amount of thyrotrophic hormone secreted also increases during the cycle ($r=0.45$, $p=0.05$) but it does not appear to be associated with ovarian activity ($r=0.21$, ns.) or the gonadotrophic potency of the pituitary ($r=0.33$, $p=0.06$).

**Summary**

Assay of individual pituitaries from 33 female swine killed on almost every day of the 21-day estrual cycle showed that the gonadotrophic hormone was lowest during the two days of heat, remained low after ovulation until the eighth day of the cycle when it increased suddenly and remained high until the 20th day.

There is complete correspondence between the rise or fall in gonadotrophic hormone in the pituitary and the rise or fall in the number but not the size of follicles. The follicular phase, as judged by uterine histology, begins on the 14th day of the cycle which coincides with the increase in follicle size and is independent of the increase in follicle number.

A very high positive association was found between the day of the cycle and the gonadotrophic potency ($r=0.62$, $p=0.01$) and between the gonadotrophic potency and the number and size of follicles in the ovaries ($r=0.69$, $p=0.01$).
These correlations and other considerations lead to the conclusion that so far as the gonadotrophic hormone is concerned the bio-assay of the pituitary actually reflects the rate at which this hormone is being secreted.

There is an increase in the amount of thyrotrophic hormone secreted during the cycle (r=0.42, p=0.02), a questionable association between this hormone and the rate of secretion of gonadotrophic hormone (r=0.33, p=0.06) and no association between the thyrotrophic hormone and the events in the ovaries (r=0.21, ns.).

There is no change in the weight of the pituitary during the cycle and no association between its weights and the rate of hormone secretion.

**Literature Cited**


