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The effect of large doses of mammalian (sheep) gonadotrophins on the reproductive cycle of immature female rana pipiens

Robert Arthur Washington
Atlanta University

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THE EFFECT OF LARGE DOSES OF MAMMALIAN (SHEEP) GONADOTROPHINS ON THE REPRODUCTIVE CYCLE OF IMMATURE FEMALE RANA PIPIENS

A THESIS
SUBMITTED TO THE FACULTY OF ATLANTA UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE

BY
ROBERT ARTHUR WASHINGTON

DEPARTMENT OF BIOLOGY

ATLANTA, GEORGIA
AUGUST 1968
ABSTRACT

BIOLOGY

WASHINGTON, ROBERT ARTHUR

B. S., Morehouse College, 1949

Effect of Large Doses of Mammalian (Sheep) Anterior Pituitary Gonadotrophins on the Reproductive Cycle of Immature Female Rana pipiens

Advisor: Dr. George E. Riley

Master of Science degree conferred August 8, 1968

Thesis dated August, 1968

This investigation was undertaken to determine the possible effects of large doses (5 times the minimal) of sheep gonadotrophins (FSH, LH, LTH) plus estrone and cortisone on the reproductive cycle of immature female Rana pipiens. Twenty-seven frogs were grouped and injected daily for 4 days with FSH, LH, LTH, cortisone, and estrone separately and in combination. On the fifth day all frogs were sacrificed and their ovaries weighed and examined for possible ovulation.

Although none of the injected frogs ovulated, the eggs of two groups showed significant effects as a result of the injections (Group IV--FSH, LH, estrone, cortisone and Group VII--FSH, LH, LTH, cortisone, estrone), the eggs of these two groups showed advanced oogonial development. The black pigment granules covered almost all of the animal pole. The eggs of all other injected frogs remained in the same condition as those of the control.

It was concluded that large doses of combinations of FSH, LH, estrone and cortisone, or FSH, LH, LTH, cortisone and estrone will
cause acceleration of oogonial development in immature female R. pipiens.
ACKNOWLEDGEMENTS

My acknowledgement goes to Dr. George E. Riley, my advisor, for vital assistance and intellectual stimulation, as well as his great sense of humor, all of which contributed so very much in the selection, design and completion of this investigation.

I extend, also, appreciation to the entire biology staff of Atlanta University for its aid in the completion of this study.
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CHAPTER I

INTRODUCTION

The anterior pituitary gland of mammals produces at least three hormones that have a direct physiological and morphological effect on the reproductive organs of mammals. These three hormones are FSH (Follicle-Stimulating Hormone), LH (Luteinizing Hormone), and ITH (Luteotrophic Hormone). Because of their actions, these fractions are called gonadotrophins.

It has been well established by many investigators that these extracts, injected separately or in combination, induce marked changes in the reproductive processes of the donor species and many related species. The direct and indirect effects of these gonadotrophins are many and varied, ranging from simple increases in the weight of the reproductive organs to ovulation in the female and spermatogenesis in the male.

The mechanism of these effects within a species and related species has been carefully worked out by many investigators; but there is some disagreement as to the effect of these gonadotrophins on the reproductive process of distantly related species. Therefore, it is of continuous interest to investigators as to what possible effects that the gonadotrophins from one class of vertebrates may have on the reproductive cycle of another class of vertebrates.

This investigation was concerned with the effects of large doses of the anterior pituitary fractions (FSH, LH, LTH) from a mammal (sheep) on the reproductive cycle of immature female *Rana pipiens*.
The focus of attention in this study was the possibility of early ovulation, which is normally three years after metamorphosis, as a result of injections of sheep FSH, LH, and LTH.

In order to augment the possible effects of FSH, LH, and LTH, estrone and cortisone were used.

From studies of gross morphological changes of the intact frogs and microscopic examination of the ovaries of sacrificed frogs, this investigator sought to find: (1) whether or not large injections of these gonadotrophic hormones, used separately can shorten the initial reproductive cycle of R. pipiens, and (2) whether or not any combination of any of these hormones can shorten the reproductive cycle.

A study of the following morphological and physiological changes was made: (1) changes in body weight due to an increase in ovary weight; (2) extent of ovulation within the ovary as determined by microscopic examination of the gross structure; and (3) comparison of the eggs ovulated by induction with normally ovulated eggs.
CHAPTER II

REVIEW OF LITERATURE

The first experiments on the induction of ovulation in amphibia by implanting pituitary tissue were done by Wolf (1929) on *R. pipiens* and Houssay, Giusti, and Lascano-Gonzales (1929) on *Bufo marinae*. Their observations have been confirmed and extended by many investigators since that time to include various species of both Anura and Urodela.

Before 1939, some investigators believed that the anterior pituitary did not secrete two separate and distinct gonadotrophic substances, FSH and LH. Fevold (1939) described methods of extraction, separation and assay of pituitary follicle stimulating and luteinizing hormones. Other investigators (Riddle, Bates, and Dykshorn, 1933) have not only confirmed that FSH and LH are separate hormones, but they have established the existence of a third anterior pituitary hormone called prolactin (LTH).

Whereas many workers, such as Rugh (1948), have reported failure to induce ovulation in *R. pipiens* with mammalian gonadotrophins, Wright and Hisaw (1946) succeeded. Chang and Witschi (1957) suggested that cortisone played a supporting part in the process of ovulation in *R. pipiens* and that the ovary could be brought to a threshold by implanting one or two pituitary glands to enable the supporting agent to work.

The investigations of Ramaswami and Lackshman (1958) confirmed the observations of Rugh (1948), since their frogs were refractory to mammalian gonadotrophins. Their results agreed with those of Chang and Witschi (1957) in that hormones not directly involved with ovulation, such as cortisone, may also act as augmenting agents.
In *R. pipiens* the growth of the eggs takes place over a period of 3 years. The young oocytes begin to grow after the tadpoles metamorphose into young frogs. One-year old and two-year old frogs do not have mature eggs, but by the third year the eggs have reached maturation and the frog may spawn for the first time (Balinsky, 1960).

Grant (1953) observed that the growth of oocytes in *R. pipiens* is fairly slow during the first two seasons, but becomes more rapid in the summer of the third year of the frog's life. By the following autumn, the eggs reach their maximum dimensions which is about 1500 microns.

Rugh (1935) stated that the males of *R. pipiens* and the sexually immature females have no coelomic cilia. Stebbins (1954) observed that male *R. pipiens* are smaller than the female, and juveniles may have reduced spotting or sometimes may be unspotted. The body length of sexually mature female *R. pipiens* ranges between 65 mm and 72 mm (MacDonald, 1967). This was also found to be true by Burgos and Ladman (1955), and Wright and Wright (1933).

According to Rugh (1948) the female *R. pipiens* is not considered mature unless it is at least 72 mm in body length.
CHAPTER III
MATERIALS AND METHODS

Materials

Seventy immature female *Rana pipiens* from J. W. Prince Company, Silver Springs, Florida, were used in this investigation. They ranged in body length from 55 mm to 65 mm and in body weight from 14.8 to 25.1 g. Thirty-five of the frogs were used in the first experiment and thirty-five were used in a replication experiment. The materials and methods used in the first experiment were used in the replication experiment.

The following is an account of the materials and method for both original and replication experiments.

Twenty-six of the frogs were randomly divided into thirteen groups of twos. Three remaining frogs were randomly divided into three groups with one frog in each group. Each frog of a particular group was placed in a separate battery jar and the battery jars were labeled Group I through Group XVI. In cases where there were two frogs in a group, each frog was labeled A and the other B. The remaining ungrouped frogs were placed in a large battery jar and labeled controls.

The mammalian (sheep) hormones, FSH, LH, LTH, cortisone and estrone, used in this investigation came from Nutritional Biochemical Corporation Cleveland, Ohio. A stock solution of FSH, containing 1.25 mg/cc, was prepared by dissolving 48.0 mg of FSH in 38.4 cc of sterilized distilled water. A stock solution of LH, containing 0.1 mg/cc, was prepared by dissolving 2.5 mg of LH in 25 cc of sterilized distilled water. A stock solution of LTH, containing 10 IU/cc, was prepared by dissolving 1000 IU of LTH, containing 100 cc of sterilized distilled water. The IU (International Unit) is equivalent to 0.1 mg of the standard
preparation. A stock solution of cortisone containing 5 mg/cc, was prepared by suspending 200 mg of cortisone acetate in 40 cc of olive oil. A stock solution of estrone, containing 50 IU/cc, was prepared by dissolving 10,000 IU of estrone in 200 cc of sterilized distilled water.

In order to be sure the daily dose of hormones was sufficiently large 5 times the minimal dosage for each hormone was injected daily. For LH, a minimal dose of 5 micrograms per cc, standardized by the Weaver Finch Reaction (WFR), was used. For FSH, a minimal dose of 250 micrograms per cc, standardized by the National Institutes of Health (NIH-FSH-SI) based on an assay of ovarian weight in the rat was used. For LH, which was prepared in a rather pure form, the International Unit (IU) of 0.1 mg of the standard preparation, was used. The same was true of estrone. For cortisone, used in combination with estrone as an ovulation supporting agent, the minimal dosage of 5 mg per cc was used.

All injections were made subcutaneously with a Glaspac Disposable syringe and needle No. 2-101 (A-1).

All photographs of R. pipiens ovaries were made with a 35 mm Kodak Pony Camera.

Methods of Procedure

Upon arrival, all frogs were weighed and measured, randomly grouped, and placed in labeled battery jars containing approximately one inch of water. During the experimental period, all groups were kept at a controlled temperature of approximately 21 C. They were not fed during this period.

The first daily injection began at 8 o'clock the morning after the frogs arrived and lasted approximately 2 hours. Evening injections
began at 8 o'clock the same evening. Table 1 shows the groups, number of frogs in each group, average body weight and length upon arrival, hormones injected, daily dosage, and number of days injected. Group XVII of Table 1 represents the controls.

Before making injections each frog was weighed and the weight recorded for future tabulation.

On the fifth day of the first injections, the frogs in the ten groups were weighed and then sacrificed. The body cavities and ovaries were examined for eggs.
Table 1. The average weight, length of all groups of *R. piniens* and amounts of hormones injected.

+= injected  
-= not injected

<table>
<thead>
<tr>
<th>Group No. in Group</th>
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<th>XIII</th>
<th>XIV</th>
<th>XV</th>
<th>XVI</th>
<th>XVII</th>
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<tbody>
<tr>
<td>Avg. wt. (g)</td>
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<td>16</td>
<td>18.5</td>
<td>21.35</td>
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<td>Avg. lgt. (mm)</td>
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<td>60</td>
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<td>FSH 1.25 mg daily</td>
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<td>LH 0.1 mg daily</td>
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<td>LTH 5 IU daily</td>
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<td>Cortisone 5 mg</td>
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<td>Estrione 12.5 IU</td>
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<td>Days inj.</td>
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CHAPTER IV

OBSERVATIONS AND DISCUSSION

An examination of the body cavities of all experimental frogs did not reveal any ovulated eggs; but a comparison of the ovarian weights, and a study of the eggs under the dissecting and phase contrast microscope did reveal the extent to which the large doses of mammalian hormones affected the reproductive cycle of immature female *R. pipiens*.

The effect of large doses of gonadotrophins on the ovarian weight is presented in Table 2 and shows that the two frogs in Group IV injected with FSH, LH, cortisone and estrone had an increase in ovarian weight of almost twice that of the other experimental frogs regardless of body weight or body length. These findings tend to confirm those of Ramaswami and Lackshman (1958). Table 2 also reveals that none of the experimental groups had significant ovarian weight increases over those of the control groups. Based on the investigations of Berger and Li (1960), this was expected.

A microscopic examination of the ovaries was much more revealing than a comparison of ovarian weights in determining the effects of large doses of the gonadotrophins FSH, LH, LTH, used in combination with cortisone and estrone. The eggs of Group XVI, taken from non-injected immature *R. pipiens* and used as a basic standard for comparison, revealed that there were no black pigment granules formed, but there were widely dispersed yolk granules (Fig. 1). This ovarian condition indicates that the frog is between 1½ and 2 years old (Balinsky, 1960). The eggs of Groups I-III remained in the same conditions as the non-injected frogs of the control Group XVII (Fig. 1). This indicates that large doses of
Table 2. Effect of mammalian gonadotrophins plus cortisone on ovarian weight of immature female *R. pipiens.*

* = cortisone  
** = estrone  

Note: Group IV has ovarian weight more than two times that of any other group.

<table>
<thead>
<tr>
<th>Group</th>
<th>I</th>
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<td>No. of frogs</td>
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<td>FSH</td>
<td>LH</td>
<td>LTH</td>
<td>FSH</td>
<td>FSH</td>
<td>LH</td>
<td>FSH *</td>
<td>**</td>
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<td>INJ.</td>
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<td>LH</td>
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<td>Avg. ovary inj. frogs (g)</td>
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<td>Avg. ovary wt.</td>
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<td>0.16</td>
<td>0.39</td>
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<td>0.19</td>
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</table>
Fig. 1. Photograph showing eggs of non-injected immature female *R. pipiens*. Note relatively small yolk granules and absence of black pigment granules (Group XVI).
the fraction FSH, LH, and LTH used separately and in combination with cortisone and estrone had no effect on the rate of development of the oocytes.

The eggs in group IV (Fig. 2) and Group VII (Fig. 3) revealed the greatest effect of the injected hormones.

The frogs of Group IV were injected with large doses of FSH and LH in combination with cortisone and estrone. A microscopic examination of their eggs revealed that the black pigment granules had accumulated in the peripheral ooplasm of the animal hemisphere lending a black appearance to this region (Fig. 2). The follicle sheath had flattened and become approximately one cell layer in thickness indicating advanced oogonial development. This is in agreement with the findings of Huettner (1965). These eggs had almost twice the diameter of those found in any other group. This is correlated with the ovarian weight of Group IV, being almost twice that of any other group. These findings, to a large degree, support the investigations of Chang and Witschi (1957) and Ramaswami and Lackshman (1958). Their investigations indicated that cortisone acted as an augmenting agent in ovulation. Although the eggs of Group IV did not ovulate, the advanced stages of oogonial development confirmed the supporting role of cortisone and estrone.

The frogs in Group VII received large doses of FSH, LH, and LTH in combination with cortisone and estrone. Their eggs revealed some effects of the injected hormones (Fig. 3). The yolk granules of these eggs were much larger than those of the control group (Fig. 1). The black pigment granules had not only developed, but had begun to disperse
Fig. 2. Photograph showing eggs of immature female *R. pipiens* injected with FSH, LH, cortisone, and estrone (Group IV).
Fig. 3. Photograph showing eggs of immature female *R. pipiens* injected with FSH, LH, LSH, cortisone, and estrone. Note the density of black pigment granules (Group VII).
throughout the animal pole. This suggested that their developmental stage was more advanced than those of any group except Group IV.

This investigator believes that the luteinizing effect of LH, coupled with that of LTH reduced the effectiveness of the FSH. The possibility has been reported by Huestner (1965).

The eggs from experimental frogs in Groups V and VI and the frogs in Groups VIII through XV (Fig. 1) showed no stages of oogonial development beyond that of the eggs of the non-injected frogs. This seems to indicate that without the augmenting effect of the cortisone and estrone the ovaries of immature females remain refractory to anterior pituitary fractions.

The observations of the replication experiment showed no significant change in the observation of the original experiment. This can be determined by an examination of Table 3 and Figures 4, 5, and 6.

It should be noted here that the condition of the ovaries and the degree of development of the eggs, rather than the increase in ovarian weight, furnished most of the basis for the conclusion drawn in the summary. But it should be remembered that the increase in ovarian weight of Group IV is supportive.
Table 3. Effect of mammalian gonadotrophins plus cortisone on ovarian weight of immature female *R. pipiens*. (Replication experiment)

* = cortisone  
** = estrone

Note: As in original experiment Group IV ovarian weight twice or more than that of any other group.

<table>
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<th>Group</th>
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<th>V</th>
<th>VI</th>
<th>VII</th>
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<td>FSH</td>
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<th>Avg. Ovary Wt.</th>
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<td>Avg. Ovary Inj. (g)</td>
<td>0.17 0.16 0.15 0.37 0.17 0.18 0.15 0.14 0.17 0.16 0.16 0.15 0.18 0.17</td>
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Fig. 4. Photograph showing eggs of non-injected immature female *R. piniens.* Note relatively small yolk granules as in Fig. 1b (Group XVI).
Fig. 5. Photograph showing eggs of immature R. pipiens injected with FSH, LH, cortisone, and estrone (Group IV). Note the presence of large amount of black pigment granules as in Fig. 2.
Fig. 6. Photograph showing eggs of female R. minima injected with FSH, LH, LH, estrone, and cortisone. Note relatively light deposit of black granules in two of eggs as in Fig. 3. (Group VII).
CHAPTER V

SUMMARY

When injected with 5 times the minimal dosage of mammalian anterior pituitary hormones (FSH, LH, LTH) in combination with cortisone and estrone, immature female *Rana pipiens* failed to ovulate; but the combination of FSH and LH did produce an advanced stage of oogonial development when cortisone and estrone were used as augmenting agents.

Immature female *R. pipiens* injected with the combination of FSH, LH, and LTH in combination with cortisone and estrone did not ovulate. The eggs, however, showed an advanced stage of oogonial development. However, this advanced oogonial development was not to the degree of those eggs from injected frogs of FSH and LH in combination with estrone and cortisone. This is believed to be due to reduced potency of FSH caused by the excess luteinizing effect of LH when coupled with the luteinizing effect of LTH.
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