PITUITARY LH CONTENT DURING DELAYED AND POST-IMPLANTATION PERIODS IN THE ARMADILLO (DASYPUS NOVEMCINCTUS)

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Summary. The pituitary glands from female armadillos in various reproductive states were individually assayed for LH content by the OAD method of Parlow (1961). The glands from animals in the delayed implantation period contained significantly more LH than glands from the non-ovulated animals. Bilateral ovariectomy during the delayed implantation period resulted in an almost total depletion of pituitary LH stores when assayed 8, and 16 to 17 days later. Following implantation of the blastocyst, pituitary LH content declined progressively during gestation. No significant changes in the follicular population of the ovaries were observed in any of the experimental groups, although the weight of the ovary without the corpus luteum increased significantly during the post-implantation period.

INTRODUCTION

The female nine-banded armadillo (Dasypus novemcinctus) ovulates a single egg annually, usually between July and September. The blastocyst then undergoes a period of delayed implantation lasting about 3-5 months. Implantation of the blastocyst normally occurs in November or December, but it can be experimentally induced during the delay period by bilateral ovariectomy (Buchanan, Enders & Talmage, 1956; Enders & Buchanan, 1959; Enders, 1966). The corpus luteum of delayed implantation is smaller in size and contains less progesterone than the corpus luteum of the early post-implantation period. During the latter part of gestation, the corpus luteum shows morphological and functional signs of regression and the placenta probably forms an additional source of progesterone (Labhsetwar & Enders, 1968).

This report on the pituitary LH content in the armadillo during various reproductive states is a continuation of our studies on the endocrine relationships during the delayed and post-implantation periods.
MATERIALS AND METHODS

Animals
For the present study, female armadillos were captured in east Texas between October 1965 and September 1967, and received in the laboratory within a week of captivity. Further details are given elsewhere (Enders, 1966).

Operations
Animals were used for experiments within a week of their arrival in the laboratory. All surgical operations were performed under aseptic conditions using ether anaesthesia. All animals received antibiotics after surgery.

Experimental groups
Animals were classified into various groups as described in Table 1. A total of seven experimental groups with a minimum of four animals/group was studied. At autopsy pituitary glands were removed, freed of posterior lobe, Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Experimental conditions</th>
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<tbody>
<tr>
<td>Non-ovulated</td>
<td>Animals with no visible corpora lutea in the ovaries at autopsy.</td>
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<tr>
<td>Delayed implantation</td>
<td>Animals with corpora in the ovaries and free blastocysts in the uterus. The presence of blastocysts was established by flushing the uterus at autopsy.</td>
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<tr>
<td>Delayed implantation + ovarietomy</td>
<td>Animals with corpora in the ovaries were bilaterally ovarietomized and killed either 8, or 16 to 17 days later. At autopsy, uteri were examined for the presence of blastocysts or implantation sites.</td>
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<tr>
<td>Post-implantation</td>
<td>Animals carrying embryos of varying sizes. At autopsy, the animals were classified into three groups according to crown–rump length of foetuses: 1 to 3 cm, &gt; 3 to 6 cm, and &gt; 6 cm.</td>
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blotted, weighed on a torsion balance, and stored individually at $-20^\circ$ C for subsequent bio-assays. The ovary which did not contain the corpus luteum (hereafter called the non-luteal ovary) was also weighed and fixed in Bouin's solution. In non-ovulated animals, both ovaries were so treated. It may be noted that there is no evidence for asymmetry in weight of left and right ovary of the armadillo (Enders, 1966).

Ovarian histology
The non-luteal ovaries from twenty-one animals representing various reproductive states (Text-fig. 1) were serially sectioned (7 μ) and stained with a tetrachrome method. Every twelfth section was examined under a microscope equipped with an ocular micrometer and all follicles in which at least a portion of the ovum could be identified were counted and tabulated according to their largest diameter. The incidence of atresia was also tabulated.
LH bio-assay

The pituitary glands were thawed, homogenized in saline and bio-assayed individually for LH by the ovarian ascorbic acid depletion method of Parlow (1961) employing one ovary and a 3-hr interval. The details of this assay method as used in this laboratory have been described elsewhere (Labhsetwar, 1967). A total of thirty-two pituitary glands was assayed. Only one ovary of the previously intact assay animals was removed for ascorbic acid determination. A minimum of four assay rats/dose was used. Each assay included two doses of reference standard (NIH-LH-s-5 or -11) and one dose of pituitary tissue (1.5 mg). The assay data were analysed by the method recommended by Gaddum (1953) and the results were converted to NIH-LH-s-1. Further details are given under results.

RESULTS

Bio-assays

A considerable degree of variation in LH potency between individual animals of the same group was expected. Therefore, assay of individual rather than pooled pituitary glands was deemed essential. This necessitated a 2+1 assay design, since an armadillo pituitary gland is usually not large enough for a 2+2 assay design. However, validity of our assays was tested by assaying pooled pituitary glands with a 2+2 assay design and a fivefold interval between doses. Analysis of data by the method recommended by Gaddum (1953) for parallel
line assays showed no significant deviation from parallelism between dose-response lines of the pituitary (highest dose 3·5 mg/rat) and reference standard (highest dose 16 µg of LH-s-5 or 4 µg of LH-s-11/rat). Repetition of these assays on three different occasions has confirmed this parallelism. The dose of pituitary tissue (1·5 mg/rat) in the definitive assays was based on these trials.

As an additional check on the similarities between reference LH and ovarian ascorbic acid-depleting factor in the armadillo pituitary, both were bio-assayed simultaneously before and after heating in water bath for 1 hr at 100°C employing two doses (0·30 and 1·5 mg) of pituitary tissue and two doses (0·40 and 2 µg) of LH (five assay rats/dose). Before heating, both reference standard and pituitary tissue caused a significant ovarian ascorbic acid depletion, as evidenced by a significant slope of dose-response lines. In contrast, after heating, both materials failed to induce a significant ascorbic acid depletion with the dosages employed. This procedure is known to inactivate over 95% of LH (McCann, 1962; deGroot, 1967). This suggests that ovarian ascorbic acid depleting factor in the armadillo pituitary was inactivated under conditions which effectively destroy LH. On the basis of this evidence, i.e. parallelism between dose-response lines and inactivation following heating, it is assumed that OAAD method was measuring in the armadillo pituitary a substance which behaves like LH under the conditions employed.

**Ovarian weight**

During the delay period, animals without blastocysts tended to have a smaller non-luteal ovarian weight than animals with blastocysts, although the difference could not be established as statistically significant \(P>0·07\), Text-fig. 1). In non-ovulated animals the average weight per ovary (71 mg) was comparable to the mean weight of the non-luteal ovaries of the animals with or without blastocysts (88 mg). However, during the post-implantation period there was a progressive increase in the weight of the non-luteal ovary (Text-fig. 1). For each 1-cm increase in foetal crown-rump length, the weight of the non-luteal ovary increased by 10·7 mg.

**Number of vesicular follicles**

With the method of counting employed, no significant variation in the follicular count or extent of atresia in non-luteal ovaries was found in any of the groups. A majority of the follicles (on average, 71%) was found to be atretic. Classification of follicles in different size categories also failed to reveal any significant differences among any of the groups.

**Pituitary weight**

The pituitary weight showed no statistically significant changes in any of the experimental groups.

**Pituitary LH**

LH content (µg/gland) of the delay group was significantly higher than that of the non-ovulated group \(P<0·05\), Text-fig. 2), although the LH concentration (µg/mg) was not \(P>0·05\). Bilateral ovariectomy during the delay period
resulted in an almost total depletion of pituitary LH both 8, and 16 to 17 days after removal of the ovaries. During the post-implantation period there was a highly significant progressive decrease in the pituitary LH level (Text-fig. 2) with increasing gestation age.

Text-fig. 2. Changes in pituitary LH concentration (µg/mg wet pituitary) and LH content (µg/gland) during different reproductive states in the armadillo. Number at the base of the column in (a) indicates mean index of precision and in (b) the number of pituitary glands assayed. Bar at the top of the column indicates S.E. Noteworthy comparisons are shown.

**Blastocyst and implantation**

Uteri of all animals which were included in the delay group for LH determination contained free blastocysts. Eight days after ovariectomy, the uteri of three out of four animals contained a normal-sized blastocyst, while the remaining uterus showed evidence of incipient implantation. Sixteen or 17 days after ovariectomy all animals included in this group showed implantation sites, confirming earlier reports (Enders & Buchanan, 1959; Enders, 1966).
DISCUSSION

Although delayed implantation is known to occur regularly in several diverse forms (Enders, 1967), the role of the pituitary gland in this phenomenon has not been clearly elucidated. Effective suckling during the *post partum* period (which induces delayed implantation in the rat) has been found to be associated with lower pituitary LH content (Minaguchi & Meites, 1967). In contrast, pituitary LH content of the armadillo during the delay period was significantly higher as compared with animals without corpora lutea. It has been reported earlier that the corpora are functional in the armadillo during delayed implantation but probably at a lower level than corpora of the early post-implantation period (Talmage, Buchanan, Kraintz, Lazo-Wasem & Zarrow, 1954; Labhsetwar & Enders, 1968). It is possible that the increased pituitary LH content during the delay period represents increased retention resulting from the activity of the corpora lutea.

Removal of the ovaries during the delay period resulted in an almost total depletion of pituitary LH content. The pituitary LH stores, 8 days after bilateral ovariectomy, were significantly lower than in either the intact delay animals or the non-ovulated animals (2.9 µg/gland in ovariectomized group versus 35 µg/gland in non-ovulated group, *P*<0.05). In all other species studied, bilateral ovariectomy has been found to result in an increased pituitary gonadotrophin content (Chester-Jones & Ball, 1962). This is most dramatically illustrated in the rat where bilateral ovariectomy results in a several-fold increase in pituitary LH and FSH content within 10 to 13 days (Labhsetwar, 1967). The present study appears to be the first to record a decrease rather than an increase in LH content after ovariectomy. Whether this results from an increased release, a decreased synthesis or both remains to be determined. It is noteworthy that 16 to 17 days following ovariectomy, a decreased pituitary LH content was associated with implantation of the blastocyst. However, a similar decrease was present even 8 days after ovariectomy when blastocysts were not implanted.

The pituitary LH stores decreased markedly during the post-implantation period when, on the basis of previous evidence (Labhsetwar & Enders, 1968), the placenta appears to take over, at least partly, the luteal function of progesterone secretion. A recent study suggests that armadillo placental homogenates are capable of synthesizing oestrogens under *in vitro* conditions (Brinck-Johnsen, Benirschke & Brinck-Johnsen, 1967). It is conceivable, therefore, that the combined activity of progesterone and oestrogens results in depletion of pituitary LH stores. In the human female, where placental secretions of progesterone and oestrogens are well established, a similar decrease in pituitary gonadotrophin content during pregnancy has been observed (Bruner, 1951). The same combination in the armadillo does not appear to inhibit FSH output from the pituitary gland since the number and growth of vesicular follicles did not decrease during the latter part of gestation. In fact, the ovary without the corpus luteum increased in weight during this period. This weight increase might be due to an increase in the number of follicles which have lost their ova during the course of atresia since such follicles were not included in our count by the method employed.
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REFERENCES


