Deciduomata: Their Antilutbolytic Effect and Influence on Serum Levels of Ovarian Steroids, Luteotropins, and Prostaglandin F(2alpha) During the Onset of Luteolysis in Pseudopregnant Hamsters.

Paul Frank Terranova
Louisiana State University and Agricultural & Mechanical College

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A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The Department of Zoology and Physiology

by

Paul Frank Terranova
B.S., McNeese State University, 1969
M.S., McNeese State University, 1971
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. TITLE PAGE</td>
<td>i</td>
</tr>
<tr>
<td>II. ACKNOWLEDGMENTS</td>
<td>ii</td>
</tr>
<tr>
<td>III. TABLE OF CONTENTS</td>
<td>iii</td>
</tr>
<tr>
<td>IV. LIST OF TABLES</td>
<td>iv</td>
</tr>
<tr>
<td>V. LIST OF FIGURES</td>
<td>v</td>
</tr>
<tr>
<td>VI. ABSTRACT</td>
<td>vii</td>
</tr>
<tr>
<td>VII. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>VIII. MATERIALS AND METHODS</td>
<td>9</td>
</tr>
<tr>
<td>IX. RESULTS</td>
<td>22</td>
</tr>
<tr>
<td>X. DISCUSSION</td>
<td>25</td>
</tr>
<tr>
<td>XI. SUMMARY</td>
<td>69</td>
</tr>
<tr>
<td>XII. LITERATURE CITED</td>
<td>71</td>
</tr>
<tr>
<td>XIII. VITA</td>
<td>76</td>
</tr>
</tbody>
</table>
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Analysis of variance of progesterone levels of pseudopregnant hamsters with and without deciduomata</td>
<td>31</td>
</tr>
<tr>
<td>II</td>
<td>Analysis of variance of follicle stimulating hormone levels of pseudopregnant hamsters with and without deciduomata</td>
<td>32</td>
</tr>
<tr>
<td>III</td>
<td>Analysis of variance of follicle stimulating hormone levels of pseudopregnant hamsters with deciduomata</td>
<td>33</td>
</tr>
<tr>
<td>IV</td>
<td>Analysis of variance of luteinizing hormone levels of pseudopregnant hamsters with and without deciduomata</td>
<td>34</td>
</tr>
<tr>
<td>V</td>
<td>Analysis of variance of luteinizing hormone levels of pseudopregnant hamster with deciduomata</td>
<td>35</td>
</tr>
<tr>
<td>VI</td>
<td>Analysis of variance of luteinizing hormone levels of pseudopregnant hamsters without deciduomata</td>
<td>36</td>
</tr>
<tr>
<td>VII</td>
<td>Analysis of variance of prolactin levels of pseudopregnant hamsters with and without deciduomata</td>
<td>37</td>
</tr>
<tr>
<td>VIII</td>
<td>Analysis of variance of estradiol levels of pseudopregnant hamsters with and without deciduomata</td>
<td>39</td>
</tr>
<tr>
<td>IX</td>
<td>Analysis of variance of estradiol levels of pseudopregnant hamsters without deciduomata</td>
<td>40</td>
</tr>
<tr>
<td>X</td>
<td>Analysis of variance of prostaglandin $F_{2\alpha}$ levels of pseudopregnant hamsters with and without deciduomata</td>
<td>41</td>
</tr>
<tr>
<td>XI</td>
<td>Analysis of variance of prostaglandin $F_{2\alpha}$ levels of pseudopregnant hamsters with deciduomata</td>
<td>42</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gel filtration pattern of products from the separate iodination of NIH-Rat FSH-1-1, LH-1-3, and PRL-1-1</td>
<td>44</td>
</tr>
<tr>
<td>2</td>
<td>Gel filtration pattern of $^{125}$I-FSH, $^{125}$I-LH, and $^{125}$I-PRL</td>
<td>46</td>
</tr>
<tr>
<td>3</td>
<td>Standard curves for reference hormones, and dose response curves for pooled sera in FSH, LH, and prolactin radioimmunoassays</td>
<td>48</td>
</tr>
<tr>
<td>4</td>
<td>Standard curve for progesterone radioimmunoassay</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>Standard curve for estradiol radioimmunoassay</td>
<td>52</td>
</tr>
<tr>
<td>6</td>
<td>Standard curve for prostaglandin $F_{2α}$ radioimmunoassay</td>
<td>54</td>
</tr>
<tr>
<td>7</td>
<td>Progesterone levels at the indicated hours after sterile mating in pseudopregnant hamsters with and without deciduomata</td>
<td>56</td>
</tr>
<tr>
<td>8</td>
<td>Percent pseudopregnant hamsters with and without deciduomata exhibiting hyperemic corpora lutea at the indicated hour after sterile mating</td>
<td>58</td>
</tr>
<tr>
<td>9</td>
<td>Arterial serum concentrations of FSH in pseudopregnant hamsters with and without deciduomata</td>
<td>60</td>
</tr>
<tr>
<td>10</td>
<td>Arterial serum concentrations of LH in pseudopregnant hamsters with and without deciduomata</td>
<td>62</td>
</tr>
<tr>
<td>11</td>
<td>Arterial serum concentrations of prolactin in pseudopregnant hamsters with and without deciduomata</td>
<td>64</td>
</tr>
<tr>
<td>12</td>
<td>Estradiol levels of ovarian venous serum in pseudopregnant hamsters with and without deciduomata</td>
<td>66</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>13</td>
<td>Arterial serum concentrations of prostaglandin $F_{2\alpha}$ in pseudopregnant hamsters with and without deciduomata</td>
<td>68</td>
</tr>
</tbody>
</table>
ABSTRACT

The effects of deciduomata (DCRs) on ovarian progesterone production near the end of pseudopregnancy (PS) and the relationship between progesterone levels and the termination of luteal hyperemia in hamsters with and without DCRs were investigated. In addition, serum levels of FSH, LH, prolactin (PRL), estradiol, and prostaglandin F$_{2\alpha}$ (PGF) were measured by radioimmunoassay on the days associated with the terminal decline of progesterone in pseudopregnant hamsters to determine the effect of DCRs, if any, on levels of these hormones.

Cyclic female hamsters were sterile mated to induce PS on the evening of proestrus at 2100 hrs (day 4 of cycle). Eighty-three to 89 hrs later all pseudopregnant females were anesthetized by a single intraperitoneal injection of Nembutal, fifty were subjected to bilateral uterine trauma to induce DCRs by sewing thread loops into each uterine horn, and fifty were used as nontraumatized controls. Blood from pseudopregnant controls under Nembutal anesthesia was collected from the right ovarian vein near its confluence with the postcava at either 132, 138, 144, 150 or 156 hrs after sterile mating, and ovarian venous blood from traumatized hamsters was collected at either 156, 162, 168, 174 or 180 hrs after sterile mating. Immediately after blood collection the left ovary in situ was inspected for the presence of hyperemic corpora lutea and each site of uterine trauma was examined grossly for DCRs. Thereupon, 3-5 ml of blood was collected by cardiac puncture from the left ventricle. Serum obtained from arterial blood was assayed for LH, FSH, PRL, and PGF. Serum collected from ovarian venous blood was assayed for progesterone and estradiol.

The presence of DCRs in pseudopregnant hamsters delayed the onset of
The terminal decline in circulating progesterone occurred abruptly overnight in both groups and was synchronous with termination of luteal hyperemia.

The onset of the terminal decline in progesterone secretion was not immediately preceded or accompanied by observed decreases in serum levels of hamster luteotropic hormones, FSH and PRL. However, PRL levels may have been altered by operational stress and Nembutal anesthesia. Providing that the serum PRL levels are valid, luteal regression in pseudopregnant hamsters with or without DCRs cannot be explained by withdrawal of luteotropic support from the pituitary.

During the photoperiod immediately preceding luteal regression the mean progesterone level was higher in hamsters with DCRs than in hamsters lacking DCRs.

High serum PGF levels 6-12 hrs prior to the onset of the terminal decline of progesterone in pseudopregnant hamsters lacking DCRs suggest that PGF is related to luteolysis. In hamsters with DCRs, serum PGF levels did not fluctuate significantly during the 24 hr observation period; however, they might well account for luteolysis since they were not at baseline levels for PS at the onset of luteolysis.

Serum LH levels in pseudopregnant hamsters with DCRs were low and did not fluctuate significantly during the 24 hr observation period. Serum LH levels of hamsters lacking DCRs peaked during the onset of luteal regression and returned to baseline the following morning.

Serum PRL levels of pseudopregnant hamsters with and without DCRs fluctuated significantly during the 24 hr observation period. It is
likely that a daily rhythm of serum PRL levels exists in pseudopregnant hamsters.

DCRs had no effect on ovarian venous estradiol levels until the morning after luteal regression and at that time estradiol levels were higher in hamsters with DCRs. This postregressional rise in ovarian venous estradiol may be associated with ovulation.
INTRODUCTION

The fact has been known for many years that traumatizing the uterine endometrium of rats and hamsters under appropriate conditions results in a differentiation of endometrial cells that become a deciduoma (DCR). Ershoff and Deuel (1943) showed that deciduomata (DCRs) in rats prolong the duration of pseudopregnancy (PS) to that of pregnancy (21 days) as evidenced by postponement of psychic estrus. Subsequently, Olsen et al. (1951) found that the degree of prolongation of PS in rats is dependent on the amount of decidual tissue. In hamsters, on the other hand, the duration of PS (9 days) is not significantly affected by massive DCRs (Kent and Atkins, 1959).

The functional life span of corpora lutea (CL) of PS is prolonged in rats with DCRs as evidenced by persistence of elevated levels of progesterone in peripheral serum (Pepe and Rothchild, 1974) and in ovarian venous plasma (Hashimoto et al., 1968). These researchers reported that progesterone levels began to decline on day 9 of PS in rats without DCRs and on day 16 of PS in rats with DCRs.

In pseudopregnant hamsters with DCRs luteal hyperemia, usually considered an indicator of functional CL, persists 17 hours (hrs) longer than in pseudopregnant hamsters lacking DCRs (Tate, 1964). Luteal hyperemia terminates on day 6 of PS in the absence of DCRs (Tate, 1964; Choudary and Greenwald, 1967), and progesterone levels in ovarian venous blood decline at that time (Shaikh et al., 1973).

A presumed role of prolactin (PRL) in pseudopregnant rats is stimulation of luteal progesterone production (Rabii and Kragt, 1972; Bischof et al., 1973). There is evidence that in rats PRL antagonizes the action of 20α-hydroxysteroid dehydrogenase (an enzyme that mediates the cata-
bolism of progesterone to 20α-dihydroprogesterone) and mobilizes pre­
cursors for progesterone synthesis (Weist et al., 1968), thereby main­
taining progesterone synthesis (Bischof et al., 1973). On the other
hand, McClean and Nikotovitch-Winer (1973) reported that injection of
anti-PRL into pseudopregnant rats at any time after CL formation was in­
effective in preventing DCR formation or in causing any significant
fluctuation of serum progestin levels. In intact pseudopregnant rats,
implantation of PRL in ovarian bursas before day 9 had no effect on
duration of PS (Laio et al., 1974). PRL does not support maximal DCRs
in hypophysectomized rats unless luteinizing hormone (LH) or estrogen
is also supplied (Armstrong, 1968; Greenwald and Rothchild, 1968).

PRL levels have been determined in serum of pseudopregnant rats by
several investigators (Bishop et al., 1971; Bast and Melampy, 1972;
Rabii and Kragt, 1972; Freeman and Neill, 1972; Freeman et al., 1974).
Freeman et al. reported a nocturnal and diurnal surge of serum PRL on
each day of PS in rats lacking DCRs. The diurnal surge started at 1500-
1700 hrs, peaked at 1700-1900 hrs, and returned to baseline by 2300 hrs.
The nocturnal surge of PRL began at 0100 hrs, peaked at 0300-0500 hrs
and terminated by 0900-1100 hrs (lights on from 0500-1700 hrs). On days
9 and 10 of PS the diurnal and nocturnal surges of PRL were of decreased
magnitude when compared with those on days 1 through 8. Bast and
Melampy (1972), collecting blood once daily from pseudopregnant rats
with and without DCRs, reported a decline in serum levels of PRL during
the latter half of PS, and serum PRL levels in neither pseudopregnant
group were significantly different during the days of luteal regression.

Several investigators have reported that LH maintains luteal pro­
gesterone secretion in pseudopregnant rats. McClean and Nikotovitch-
Winer (1973) reported that single intraperitoneal (ip) injections of anti-LH considerably diminished the magnitude of DCRs and lowered serum levels of progestin when given as late as day 4 of PS. Rothchild et al. (1974) reported that injection of anti-LH on day 7 of PS in DCR-bearing rats had no effect on the peripheral progesterone level, duration of PS, or DCR weight, whereas injection of anti-LH on days 8 or 9 of PS caused a fall in the progesterone level 72 hrs later, a shortening of PS, and regression of DCRs. These reports suggest that progesterone production in pseudopregnant rats with DCRs is dependent on LH at various times of PS. In pseudopregnant rats lacking DCRs, Rothchild et al. (1974) reported that injections of anti-LH on days 7 or 9 had no effect on the peripheral progesterone level. However, Shaikh and Yoshinaga (1970) reported that intravenous (iv) or ip injection of 25 µg LH on day 4 or on day 6 in rats prolonged PS to approximately 16 days, the duration of life span of CL in rats with DCRs (Pepe and Rothchild, 1974). Somewhat similar results were reported by Laio et al. (1974), who showed that implantation of LH in ovarian bursas before day 9 of PS in rats prolonged PS to 16 days.

LH levels in pseudopregnant rats have been reported by several investigators (Bishop et al., 1971; Rabii and Kragt, 1972; Bast and Melampy, 1972). Bast and Melampy reported that daily plasma LH levels increased during the last several days of PS in rats with and without DCRs. In contrast, Rabii and Kragt reported that plasma LH levels were relatively uniform throughout PS in rats lacking DCRs. In pseudopregnant rats with DCRs, Bishop et al. (1971) reported plasma LH levels lower on days 4 and 9 of PS than during cyclic diestrus.

A role of follicle stimulating hormone (FSH) in maintaining func-
tional rat CL during PS has recently been implicated. Laio et al. (1974) reported that FSH implanted in ovarian bursas of pseudopregnant rats lacking DCRs prolonged PS to 16 days, which indicates the possibility that FSH has the ability to maintain functional CL under these conditions.

FSH levels in pseudopregnant rats have been reported by Rabii and Kragt (1972) who demonstrated that plasma FSH levels peaked on day 3 of PS, declined to day 6, and thereafter rose steadily through day 11 of PS. Bishop et al. (1971) noted that FSH levels on days 4 and 9 of PS in DCR-bearing rats were elevated compared with those during cyclic diestrus.

The role of estrogen in maintaining functional CL in rats has been described by several investigators (Bogdanove, 1966; Ahmad, 1971; Keys, 1971; Labhsetwar, 1971). Estrogen and no other hormone thus far studied, will increase the size of pseudopregnant rat CL to that of late pregnancy (Deansley, 1966). Large functional CL are produced in rats by daily injections of estrogen beginning at estrus or by estrogen implanted at that time (Everett, 1964). On day 4 of PS in rats, plasma estrogen rises (Shaikh and Abraham, 1969) and luteal weights exceed those of the estrous cycle. Whether estrogen is acting directly on CL or via stimulation of luteotropic release from the pituitary has not been clearly demonstrated. However, estrogen has been shown to increase serum PRL in rats (Chen and Meites, 1970; Lu and Meites, 1971).

That DCRs of pseudopregnant rats produce a luteotropic hormone that sustains progesterone secretion in the relative absence of PRL has been proposed by Gibori et al. (1974), but no such hormone has yet been identified. An antibody to rat decidual extracts has been prepared (Yoshinaga, 1972), and a homogenate of decidual tissue showed no cross reactivity with anti-rat PRL (Gibori et al., 1974).
Rat uterine endometrium produces a luteolysin, prostaglandin F$_{2\alpha}$ (PGF) (Pharriss et al., 1972; Labhsetwar and Watson, 1974). That induction of DCRs prevents a luteolytic effect of the uterus has been reported by several investigators (Melampy et al., 1964; Pakurar and Rothchild, 1972; Gibori et al., 1974). Hysterectomy in pseudopregnant rats prolongs PS proportionately to the amount of uterine tissue removed (Melampy et al., 1964). Uterine autotransplants or injection of rat endometrial suspensions shortens PS (Anderson et al., 1969). Injection of PGF into pseudopregnant rats induces rapid CL regression (Pharris and Wyngarden, 1969). Recently, Weems et al. (1974) demonstrated that PGF content of uteri and uterine venous plasma were elevated on day 9 of PS (day of onset of luteal regression) in rats lacking DCRs.

Bast and Melampy (1972) suggested that LH stimulates 20α-hydroxy-steroid dehydrogenase activity on days 9-13 of PS in rats lacking DCRs. Thus far, no reports have demonstrated that LH is luteolytic in pseudopregnant rats. However, in hypophysectomized rats exogenous LH causes luteal regression (Rothchild, 1965).

PRL is luteolytic in hypophysectomized rats if administered more than 80 hrs after an ovulating injection of LH (Malven, 1969). Rat pituitaries placed under the kidney capsule 10-12 days after hypophysectomy had the same effect (Piacsek and Meites, 1967). PRL has been reported to be luteolytic in cyclic rats (Wuttke and Meites, 1971). However, there is no indication that PRL is luteolytic in intact pseudopregnant rats.

In 1959, Novoselsky reported vaginal mucification and lengthening of the diestral period in PRL treated hamsters. In the same year Hursey (1959) noted an increase in CL size in PRL treated hamsters.
Kent and Lytle (1960) found enhanced DCRs in PRL treated hamsters, and Terranova and Kent (1974) reported similar findings from progesterone treated ovariectomized hamsters administered PRL. In 1968, Grady and Greenwald induced PS in hamsters injected with PRL and FSH, and proposed that the maintenance of functional CL in hamsters requires a luteotropic complex of PRL, FSH and (since their FSH was contaminated with trace amounts of LH) probably LH. Since then, several reports have confirmed that PRL and FSH constitute a minimal hormone complex required to maintain functional CL in hamsters (Greenwald, 1973). In addition to the minimal luteotropic complex hormones, PRL and FSH, Jaggannadha Roa et al. (1970) found in hamsters that LH was essential to maintain pregnancy between days 6 and 11.

Thus far, the only reports of pituitary hormone levels in pseudo-pregnant hamsters are those of Qazi (1962) and Orsini and Schwartz (1970). Qazi, using a pigeon crop sac bioassay, demonstrated that pituitary content of PRL rose until day 2 of PS and then leveled off to day 6. Orsini and Schwartz, using an ovarian ascorbic acid depletion assay, determined that pituitary LH content rose until it reached a peak on day 7 of PS in hamsters without DCRs.

The uterus exerts a luteolytic effect on CL of PS in hamsters. The duration of PS in hysterectomized hamsters is 18 days, twice that of normal PS (Gatipon, 1964; Lukaszewska and Greenwald, 1969). Hyperemic CL of hysterectomized hamsters persist 268 hrs after sterile mating, approximately 2 times the duration of hyperemic CL of normal PS (Gatipon, 1964). Caldwell et al. (1967) demonstrated that transplantation of a uterine horn or endometrial sections to the cheek pouch reduced the duration of PS in hysterectomized hamsters to 13.5 days. Mazer and Wright (1968)
reported that the aqueous submitochondrial fraction of pseudopregnant uteri of days 6-7 contains a luteolytic factor which shortens the duration of PS in hysterectomized hamsters. In contrast, Lukazewska et al. (1972) reported peak PGF content of PS hamster uteri on days 7-8 and that a uterine lipid fraction, rather than the aqueous submitochondrial fraction, contained the luteolytic substance at that time.

Gutknecht et al. (1971) demonstrated a significant drop in progesterone levels in hamsters within 24 hrs after an injection of PGF, and Labhsetwar (1972) induced CL regression in pseudopregnant and pregnant hamsters by injections of PGF. In pseudopregnant hamsters lacking DCRs, Shaikh et al. (1973) reported that progesterone levels began to decline on day 6, and this decline was not correlated with any rise of peripheral plasma levels of PGF. They also found that PGF levels peak on day 4. Correlated with this result are data from our lab on hysterectomy and rejunction of uteri in pseudopregnant hamsters. Kent (1967) reported that the duration of PS as measured by the postponement of psychic estrus was prolonged to 15.6 ± 0.4 days when pseudopregnant hamsters were hysterectomized (thus interrupting the uterine vascular and nerve supply), and the uteri immediately rejoined on days 1, 2 or 3, but that it was shorter (11.6 ± 0.5 days) when the operation was postponed until days 5, 6 or 7. Whether or not the PGF peak on day 4 is important in CL regression on days 6-7 of PS in hamsters has not yet been determined.

The present study was undertaken to determine whether the presence of decidual tissue in hamsters has any effect on ovarian progesterone secretion near the end of PS and how declining levels of progesterone at that time in decidua bearing animals correlate with the termination of luteal hyperemia. In addition, the serum levels of FSH, LH, PRL,
estradiol, and PGF were measured on the days associated with the onset of luteal regression in pseudopregnant hamsters to determine the effect of DCRs, if any, on levels of these hormones.
MATERIALS AND METHODS

Female golden hamsters (Mesocricetus auratus Waterhouse) aged 2-3 months were maintained in a room at 22°C with a 12L:12D lighting schedule, 2400 hrs being the midpoint of the dark period. They were provided with Purina laboratory chow and water ad libitum and maintained singly in cages. Vasectomized male hamsters were caged in the same room with females.

After exhibiting two successive four-day estrous cycles as evidenced by postovulatory discharges, female hamsters were placed with vasectomized hamsters on the evening of proestrus at 2100 hrs (day 4 of cycle) and mating was observed. Day 1 of PS is herein defined as the first 24-hour (hr) period commencing at 2400 hrs on the night of sterile mating. Eighty-three to 89 hrs after sterile mating all females were anesthetized by a single intraperitoneal injection of Nembutal\(^1\) (9.0 mg/100 g body weight). Fifty pseudopregnant anesthetized animals were subjected at that time to bilateral uterine trauma to induce DCRs by sewing 2 thread loops (size 00 surgical silk\(^2\)) 1.5 cm apart into each uterine horn. Fifty additional pseudopregnant hamsters served as nontraumatized controls. If sterile mated female hamsters did not exhibit psychic estrus at 2100 hrs on day 5 of PS and a postovulatory discharge the following morning, they were adjudged pseudopregnant.

Blood from pseudopregnant controls under Nembutal anesthesia was collected from the right ovarian vein near its confluence with the postcava at either 132, 138, 144, 150 or 156 hrs after sterile mating.

\(^1\)Abbott Laboratories

\(^2\)Ethicon, Inc.
Ovarian venous blood from traumatized pseudopregnant hamsters given Nembutal was collected at either 156, 162, 168, 174 or 180 hrs after sterile mating. Ovarian venous blood collection lasted from 10-15 minutes, 11 minutes being the most common, and 0.4-0.8 ml ovarian venous blood was collected within this time. Immediately after ovarian venous blood collection the left ovary was inspected in situ for the presence of hyperemic CL. Pink CL were adjudged not hyperemic. In addition, each site of uterine trauma was examined for gross evidence of DCRs. Following uterine inspection and within 20 minutes after anesthetization, 3.0-5.0 ml blood was collected by cardiac puncture. All collected blood was allowed to clot for not more than 12 hrs at 4°C and then centrifuged (1000 X g) for 30 minutes, after which serum was withdrawn and stored at -20°C until its use in a radioimmunoassay (RIA). Arterial serum obtained from cardiac blood of the left ventricle was assayed for LH, FSH, PRL, and PGF. Serum collected from ovarian venous blood was assayed for progesterone and estradiol.

Procedures for RIA of FSH, LH, and PRL

RIA procedures for FSH, LH, and PRL were in accordance with methods supplied by the Rat Pituitary Hormone Distribution Program, National Institute of Arthritis and Metabolic Diseases (NIAMD), National Institutes of Health (NIH). Since FSH, LH, and PRL immunoassay procedures are similar, only one description of radiolabeling with $^{125}$I and one description of immunoassay of standards and samples is given. Modifications of each peptide hormone assay are indicated where pertinent.

$^{1}$New England Nuclear Co., NEZ 033
Radioiodination of FSH, LH, and PRL. NIAMD-Rat PRL-1-1 (30 IU/mg), FSH-1-1 (100 X NIH-FSH-S1), and LH-1-3 (1 X NIH-LH-S1) were radiolabeled with \(^{125}\text{I}\). One-half or 1 millicurie \(^{125}\text{I}\) in 25 \(\mu\)l 0.1N NaOH was neutralized with an equal volume of 0.1N HCl in a 13X100 mm disposable glass culture tube containing 25 \(\mu\)l 0.1M NaH\(_2\)PO\(_4\). Twenty \(\mu\)l (2.0 ug) of hormone for iodination previously stored at \(-20^\circ\text{C}\) in 0.01M NaKHPO\(_4\) containing 0.15M NaCl with 0.1% NaN\(_3\), phosphate buffered saline (PBS), pH 7.6, was added to the reaction vessel and this was followed immediately by the addition of 10 \(\mu\)l of chloramine-T\(^1\) (25.0 mg/ml PBS without 0.1% NaN\(_3\)) . The reaction vessel was gently agitated for 60 seconds, after which 25 \(\mu\)l sodium metabisulfite\(^2\) (25.0 mg/ml PBS without 0.1% NaN\(_3\)) was added and the entire reaction mixture was applied to a Bio Gel P-60\(^2\) gel filtration column. The gel column was prepared for the reaction mixture at room temperature by equilibrating with PBS. Then 2.0 ml 2.0% bovine serum albumin (BSA) in PBS was added to the equilibrated column and this was followed by 5.0 ml PBS. The reaction mixture was eluted with PBS. Twenty 0.5 ml fractions were collected singly in 13X100 mm culture tubes containing 50 \(\mu\)l 2.0% BSA in PBS. After a single use the Bio Gel column was discarded. Ten \(\mu\)l of each diluted fraction was then placed in a liquid scintillation counting vial containing 10 ml scintillation fluid, SF, (2 parts toluene, 1 part Triton X-100\(^3\) and containing 0.27% 2,5-phenyloxazole). Each vial was shaken and then counted (counting error is 2.0%) in the full tritium window of a Beckman LS-100C scintillation

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\(^1\)solution prepared 5-10 minutes prior to use

\(^2\)Bio Rad Laboratories

\(^3\)Sigma Chemical Co.
Radioactivities of each vial were recorded as counts per minute (cpm).

**Results of radioiodinations.** Two peaks of radioactivity were detected in the 10 µl aliquots of the twenty 0.5 ml fractions. The first peak began in tube 2 or 3 and had trailed off in tube 5 or 7 (Fig. 1). A second peak containing Na$^{125}$I began in tube 6 or 8 and had trailed off in tube 18 (Fig. 1). The radiolabeled hormone was contained in the first peak and this fraction was stored at 4°C until its use in an assay. If more than 24 hrs elapsed between iodination of hormone and its use in an assay, the iodinated hormone was repurified on a Bio Gel column according to the method described for the original separation of iodination products. Results of repurification of iodinated hormones are shown in Figure 2.

**Assay of standards and samples for FSH, LH, and PRL.** In preparing standard curves NIAMD-Rat PRL-RP-1 (11 IU/mg), FSH-RP-1 (2.1 X NIH-FSH-S1) and LH-RP-1 (0.03 X NIH-LH-S1) were employed as reference preparations, i.e., standards. Antisera utilized in the assays were NIAMD-Anti-Rat PRL S-2, Anti-Rat FSH S-6, and Anti-Rat LH S-1. The minimum and maximum volumes of serum used in the assays were 50 µl and 100 µl in the FSH and LH assays, and 25 µl and 75 µl in the PRL assay. All serum samples from individual animals and all standards were assayed in triplicate.

Reagents and serum samples were added to 13x100 mm culture tubes for the assay of FSH, LH, and PRL. Twenty-five to 100 µl of arterial serum was added to an appropriate amount of 1.0% BSA in PBS to produce a final sample volume of 500 µl. Standards were dissolved in 500 µl 1.0% BSA in PBS in concentrations ranging from 0 to 1.0 µg per tube. Radiolabeled hormone (approximately 20,000 cpm/100 µl 1.0% BSA in PBS)
was added to each tube containing standard or serum. Then, 200μl of antisera previously diluted (1:2,500 for anti-FSH, 1:37,500 for anti-LH, and 1:25,000 for anti-PRL) with 3.0% normal rabbit serum in PBS containing 0.5M ethylenediamine-tetraacetic acid (EDTA) was added to each tube. The tubes were agitated on a vortex stirrer for approximately 5 seconds and then incubated for 5 days ± 6 hrs at 4°C. At the end of the incubation period 200μl of sheep or goat anti-rabbit gamma globulin serum in PBS was added in a concentration sufficient to precipitate maximally the antibody-bound labeled rat hormone when incubated for 3 days at 4°C. After this incubation, all tubes were centrifuged (1000 x g) for 30 minutes at room temperature (22°C-25°C). The supernatants were discarded and the precipitates dissolved in 50μl 0.05N NaOH, after which 1.0 ml of distilled water was added to each tube. The entire mixture was placed in a scintillation vial containing 10 ml SF. Each vial was agitated and counted (counting error is 2.0%) as previously described for the iodinated products. CPM were recorded and standard curves were constructed. Only the linear portions of standard curves were used in determining sample hormone concentrations. For assay validation, pooled sera from pseudopregnant hamsters was assayed in aliquots ranging from 25-200μl. The curve resulting from assays of pooled sera was not significantly different from the standard curve (Fig. 3).

Procedure for progesterone RIA

Ovarian venous serum (OVS) was assayed for progesterone by modification of a previously reported RIA method (Furuyama et al., 1971). Since this method was modified in the present investigation, a description of procedures employed for determination of progesterone in OVS is given. Antibody to progesterone-11-succinate-BSA conjugate (P-11-192) was
supplied by Endocrine Sciences and exhibited a 7% cross reaction with 17α-hydroxyprogesterone and a 0.5% cross reaction with cortisol, estrone, and estradiol-β. Progesterone standard was obtained from Sigma Chemical Company and used without further purification. 1,2 $^3$H-progesterone was purchased from New England Nuclear Company.

Extraction procedure and percent recovery of 1,2 $^3$H-progesterone. OVS samples (20-50 μl) were added to clean extraction tubes. The volume of all tubes containing OVS was adjusted to 0.25 ml with solvent (hexane: ethyl acetate, 100:1) extracted distilled water and then gently agitated on a vortex stirrer for approximately 5 seconds. Two ml of extraction solvent (hexane:ethyl acetate) was added to the diluted OVS samples, the sample was vortexed at high speed for 1.5 minutes, the aqueous and organic layers were allowed to separate for 5 minutes, and then a 0.1-0.5 ml aliquot of each organic phase was transferred to a 13×100 mm culture tube. The volume of aliquots was adjusted to 0.5 ml with solvent and then evaporated to dryness in a water bath (45°C) with an air stream. These dried extracts were to be assayed for progesterone. Percent recovery of progesterone from OVS samples was determined by extracting 10 samples containing an internal standard (5,000 cpm 1,2 $^3$H-progesterone). An aliquot (0.5 ml) of extraction solvent from each sample was transferred to a counting vial containing 10 ml SF. Each vial was counted (counting error is 2.0%) in the full tritium window of a Beckman LS-100C scintillation counter. This solvent extracted approximately 82% of the 1,2 $^3$H-progesterone. The percent recovery did not vary with volume of serum used in the extraction procedure.

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1 Tarzana, California
Assay of standards and samples for progesterone. A standard curve was prepared by adding 0-1.0 ng of progesterone in 0.5 ml aliquots of solvent to clean 13X100 mm culture tubes. The 0.5 ml aliquots were evaporated to dryness as previously described for OVS extracts. One hundred fifty μl of a 1:12,000 dilution of progesterone antibody in 0.05M borate buffer (pH 8.0) containing 0.2% BSA, 0.05% bovine gamma globulin, 0.1% NaN₃, and 2,260 cpm 1,2³H-progesterone was added to each tube containing dried extracts of standards or samples. Each tube was vortexed for 30 seconds to dissolve the dried standards or samples. Foaming of the borate-protein buffer was avoided while vortexing. The standards and samples were incubated for 2 hrs at room temperature with the progesterone antiserum solution.

Separation of free and bound progesterone. Two hundred fifty μl 72.5% (NH₄)₂SO₄ in distilled water was added to each tube after the 2 hr incubation. The contents were vortexed at medium speed for 5 seconds. The tubes were then centrifuged (1000 X g) for 30 minutes at room temperature. Then 0.3 ml of supernatant, containing unbound progesterone, was transferred to a counting vial containing 10 ml of Aquasol and 1.5 ml of distilled water. The resulting mixture was shaken until clear, allowed to settle for 30 minutes, and counted (counting error is 1.5%) in the full tritium window of a Beckman LS-100C scintillation counter. CPM of standards were recorded and a standard curve was constructed (Fig. 4). Only the linear portion of the standard curve was used in

1 added immediately before use in the assay

2 New England Nuclear Company
determining sample hormone concentrations. All progesterone values were corrected for procedural losses as determined by percent recovery of the internal standards.

**Procedure for estradiol-β RIA**

OVS was assayed for estradiol-β by modification of a previously reported RIA procedure (Baranczuk and Greenwald, 1973). Since the method (Baranczuk and Greenwald, 1973) was modified in the present investigation, a description of extraction procedure, column chromatography, and assay of standards and samples is given. Antibody to estrone-17-oxime conjugate (E17-94) was purchased from Endocrine Sciences. Estradiol-β was obtained from Sigma Chemical Company and used without further purification. 2,4,6,7 \(^3\)H-estradiol was supplied by New England Nuclear Company.

Extraction procedure and percent recovery of 2,4,6,7 \(^3\)H-estradiol.

OVS, 100-150 μl, was added to 13X100 mm culture tubes and volumes were adjusted to 500 μl with solvent (hexane:ethyl acetate, 75:25) extracted water. Each tube was then gently agitated on a vortex stirrer for approximately 5 seconds. Thereafter, each diluted sample was extracted with 2.5 ml of solvent for 1 minute by vortexing at high speed, the aqueous and organic layers were allowed to separate for 5 minutes, and then 2.0 ml aliquots of each organic phase were evaporated to dryness under air in a 45°C water bath. The dried residue was dissolved in 0.8 ml of benzene:methanol (85:15) and stored at room temperature until chromatographed for collection of the estradiol fraction. Percent recovery of estradiol from OVS samples was determined by extracting 10 samples containing an internal standard (5,000 cpm 2,4,6,7 \(^3\)H-estradiol). An aliquot (0.5 ml) of extraction solvent from each sample was transferred to counting vials containing 10 ml SF. Each vial was counted
(counting error is 1.5%) in the full tritium window of a Beckman LS-100C scintillation counter. The solvent extracted approximately 56% of the 2,4,6,7 \(^3\)H-estradiol from OVS samples when extracted for 1 minute. The percent recovery was not dependent on the volume of serum extracted.

**Column chromatography of estradiol extracts.** Sephadex LH-20\(^1\) was allowed to swell in a flask with column eluting solvent (benzene:methanol, 85:15) for at least 24 hours. The packing was slurried 4-5 times and then poured into a large (25X40 cm) clean-up column. The packing was washed 5 times with benzene:methanol (1:1) and 4 times with benzene:methanol (85:15). The packing was dispersed with each wash by gently shaking the column and then stored in a glass stoppered flask.

Microcolumns (2.0 ml all glass syringes) were used for the collection of estradiol. The filter support was Whatman GF/B glass fiber filter paper. Each column was thoroughly cleaned with extraction solvent before slow addition of 2.0 ml of prepared Sephadex LH-20 (0.4 g dry weight).

Before re-use, the packed columns were cleaned by removing the contents, soaking in 25% potassium dichromate in sulfuric acid for 1 hr, and rinsing with eluting solvent. Then each column was repacked with unused Sephadex LH-20.

Two hundred \(\mu\)l of the 0.8 ml estradiol extract was added to the microcolumn and eluted by the following additions of solvent to packed microcolumns:

- a) 0.3 ml solvent, discard eluate
- b) 1.0 ml solvent, " "
- c) 0.5 ml solvent, " "
- d) 0.3 ml solvent, " "

\(^1\)Sigma Chemical Co.
e) 0.8 ml solvent, discard eluate
f) 0.8 ml solvent, collect estradiol
g) 1.2 ml solvent, discard eluate

Sixty-six percent of the estradiol was collected in the second 0.8 ml fraction as revealed by chromatography of $^3$H-estradiol. Seven hundred μl of the estradiol fraction was transferred to assay tubes and evaporated to dryness under air in a 45°C water bath. The dried residue was subsequently assayed for estradiol.

Assay of standards and samples for estradiol. A standard curve was prepared by adding 0-650 pg estradiol-β in 0.5 ml benzene:methanol (85:15) to assay tubes. The contents of each tube were evaporated to dryness under air in a 45°C water bath. The dried residues were dissolved in 0.25 ml of a 1:8,000 dilution of estradiol antibody in 0.05M borate buffer (pH 8.0) containing 0.01% BSA, 0.05% bovine gamma globulin, 3330 cpm 2,4,6,7 $^3$H-estradiol$^1$ and 0.1% NaN₃, by vortexing for 30 seconds (foaming avoided). The standards and samples were incubated for 3 hrs at room temperature with estradiol antiserum solution.

Separation of free and bound estradiol. Two hundred fifty μl of saturated (NH₄)$_2$SO₄ was added to each tube after 3 hrs incubation with the antiserum solution. The contents were then gently vortexed for approximately 5 seconds and centrifuged (1000 X g) for 30 minutes at room temperature. Four hundred μl of the supernatant, containing unbound estradiol, was transferred to a counting vial containing 10 ml of Aquasol and 1.5 ml of water. Each vial was counted (counting error is 1.5%) on a Beckman LS-100C scintillation counter. CPM were recorded and sample hormone concentration was determined by using only the linear portion of

$^1$added immediately prior to its use
the standard curve (Fig. 5). All estradiol values were corrected for procedural losses as determined by percent recovery of the internal standard.

**Procedure for RIA of prostaglandin F₂α**

The PGF RIA was in accordance with a method supplied by Clinical Assays, Inc.¹ and is a modification of procedures previously described (Caldwell et al., 1971; Jaffee et al., 1971). The procedure has not yet been published, but is provided to those who purchase their PGF RIA kit. Therefore, a description of this assay is given herein.

**Extraction procedure and percent recovery of PGF.** Two hundred to 700 µl of arterial serum was extracted with 2 times the volume of methylal:ethanol (3:1) by the slow addition of solvent to serum. The extract was then vortexed for 1 minute at high speed and allowed to stand at room temperature for approximately 1 hr. The contents of the extraction tube were filtered through Whatman No. 1 filter paper using an aspirator for vacuum, and the filtrate was collected in a test tube. The extraction tube and residue were thoroughly rinsed with 10 ml of extraction medium, and then the contents were filtered. When the residue was dry it was discarded. Ninety-six percent (± 5%; n = 10) of ³H-PGF was recovered from filtrates resulting from extraction and vacuum filtering. The filtrate was evaporated to dryness with a stream of air in a water bath (40°C). After evaporation was complete, 1.1 ml 0.01 M Tris-HCl buffer (pH 7.4) containing 0.1% gelatin, 0.9% NaCl, 0.8% MgSO₄, and 0.8% CaCl₂ was added to each dried residue. Each tube was then agitated for 15 minutes at room temperature on a mechanical shaker (1 cycle

¹Cambridge, Massachusetts
per second; 1 cycle = 80 mm) to dissolve the residue. Then the contents of each tube were transferred to a dialysis chamber (1.5 ml Karush type) and dialyzed against 1.1 ml of Tris buffer for 18 hrs at room temperature while being agitated on a mechanical shaker (1 cycle per second). Dialysis tubing was pretreated before use by boiling in 1 liter of distilled water containing 1.0 g EDTA and 0.5 g NaHCO₃ until the tubing was soft and odorless. The dialysis tubing was then stored at 4°C in distilled water containing 0.1% NaN₃ until its use. The solution located in the dialysis cell that formerly contained only Tris buffer was transferred to a tube for assay. Dialysis equilibrium was attained after 12 hrs as revealed by dialysis of ³H-PGF. Forty-eight percent (+ 2%; n = 12) of the ³H-PGF was recovered from the dialysis chamber that formerly contained only buffer.

Assay of standards and samples for PGF. Standards and samples in 1.0 ml Tris buffer were added to 10X75 mm culture tubes. Zero to 43.0 ng PGF standard were contained in each 1.0 ml of Tris buffer. Fifty µl ³H-PGF (8,000 cpm) was added to each standard and sample tube. Then 10 µl of a 1:10,000 dilution of PGF antibody in Tris buffer was added to each tube. The contents were mixed thoroughly on a vortexer and incubated for 3 hrs in a 37°C water bath. After the 3 hr incubation 0.1 ml 10% normal rabbit serum in Tris buffer and 0.1 ml of goat anti-rabbit gamma globulin serum were added to each tube. The contents of each tube were mixed thoroughly by vortexing and incubated at 4°C for 48 hrs. After incubation all tubes were centrifuged (1000 X g) at room temperature for 30 minutes, the supernatants were decanted, and the tubes were

¹Fisher Scientific Company, No. 8-667C
placed upside down in a test tube rack for 5-10 minutes. The inside of each tube was wiped with a Kimwipe in such a way as not to agitate the precipitate. Fifty μl 0.01N NaOH was added to each tube in order to dissolve the precipitate. One ml of distilled water was then added and the entire contents of each tube were then transferred to counting vials containing 10 ml SF. Each vial was shaken and then counted (counting error is 2.0%) in the full tritium window of a Beckman LS-100C scintillation counter. Radioactivities of each vial were recorded as cpm and a standard curve constructed (Fig. 6). Only the linear portion of the standard curve was used in determining sample hormone concentrations. All PGF values were corrected for procedural losses as determined by percent recovery of the internal standard.

**Statistical analyses**

An analysis of variance was conducted on data from each hormone assay to determine the effect of DCRs on hormone levels, the effect of time on hormone levels, and the interaction of the DCR effect and time on the pattern of hormone secretion. In addition, orthogonal comparisons were employed to identify significant differences.
RESULTS

**Progesterone and hyperemia.** The presence of DCRs delayed the onset of decline in ovarian venous levels of progesterone in pseudopregnant hamsters (Fig. 7). Ovarian venous progesterone levels in pseudopregnant hamsters with DCRs at 156 hrs after sterile mating were significantly higher \((p<0.001)\) than those in pseudopregnant hamsters without DCRs at the same hr (Fig. 7 and Table I, Source A). The presence of DCRs also delayed by 24 hrs the onset of luteal regression as indicated by hyperemia CL (Fig. 8). An analysis of variance revealed that DCRs had an overall significant effect \((p<0.03)\) on progesterone levels (Table I, DCR), and this was evidenced by significantly higher \((p<0.01)\) progesterone levels in hamsters with DCRs during the photoperiod immediately preceding luteal regression than in hamsters lacking DCRs during the corresponding photoperiod (Fig. 7 and Table I, Source B). Luteal regression occurred overnight as evidenced by a significant decline \((p<0.001)\) of ovarian venous progesterone levels by early morning in hamsters with and without DCRs (Fig. 7 and Table I, Time).

**FSH** (Fig. 9). Serum FSH levels of pseudopregnant hamsters with DCRs during the 24 hr observation period were not significantly different from those in pseudopregnant hamsters lacking DCRs during the corresponding time interval (Table II, DCR). Serum FSH levels of pseudopregnant hamsters with DCRs did not fluctuate significantly during the 24 hr observation period as revealed by ANOVA (Table III, Time). However, serum FSH levels of hamsters lacking DCRs were significantly higher \((p<0.05)\) at 2100 hrs than at 0900 hrs before luteal regression as revealed by orthogonal comparison A (Table II).
LH (Fig. 10). The presence of DCRs in pseudopregnant hamsters was correlated with lower serum LH levels at 1500 hrs \( (p<0.05) \) than those of pseudopregnant hamsters lacking DCRs at the corresponding hr of luteal regression (Table IV, Source A). This was not so at 2100 hrs (Table IV, Source B). Serum LH levels in pseudopregnant hamsters with DCRs did not fluctuate significantly during the 24 hr observation period as revealed by ANOVA (Table V, Time). However, serum LH levels of hamsters lacking DCRs fluctuated significantly during the 24 hr observation period (Table VI, Time). This was evident by significantly higher serum LH levels at 2100 hrs than at 0900 hrs \( (p<0.002) \) before and 0900 hrs \( (p<0.01) \) after luteal regression (Table IV, Sources C and D, respectively).

PRL (Fig. 11). The mean serum PRL level in hamsters with DCRs during the photoperiod immediately before luteal regression was significantly lower than that in hamsters without DCRs during the corresponding photoperiod (Table VII, part A). However, serum PRL levels were not significantly affected by DCRs after the onset of the dark period, and both groups exhibited significant fluctuations of serum PRL levels during the 24 hr observation period. The serum PRL level of hamsters with DCRs at 2100 hrs was significantly higher than that at any other time in this group (Table VII, Sources B-E). In hamsters without DCRs serum PRL levels were significantly higher at 2100 hrs \( (p<0.01) \) and at 0300 hrs \( (p<0.05) \) than at 0900 hrs of the following morning (Table VII, Sources F and G, respectively).

Estradiol (Fig. 12). Ovarian venous serum levels of estradiol in pseudopregnant hamsters were not significantly different from those in pseudopregnant hamsters lacking DCRs until 0900 hrs \( (p<0.001) \) of the
morning after the onset of luteal regression (Table VIII, Source A). Estradiol levels in hamsters lacking DCRs did not fluctuate significantly during the 24 hr observation period as revealed by ANOVA (Table IX). However, hamsters with DCRs exhibited significantly higher (p<0.001) estradiol levels at 0900 hrs after luteal regression than at any other hr of the observation period (Table VIII, Sources B-E).

PGF (Fig. 13). Serum PGF levels in pseudopregnant hamsters without DCRs at 0900 hrs of the morning immediately preceding luteal regression were significantly higher (p<0.001) than those in pseudopregnant hamsters with DCRs at the corresponding hr (Table X, Source A). The serum level of PGF in hamsters lacking DCRs declined significantly (p<0.001) by 1500 hrs preceding luteal regression and was significantly (p<0.05) lower at that time than that in decidua bearing hamsters at the corresponding time (Table X, Sources C and B, respectively). Serum PGF levels of hamsters with DCRs did not fluctuate significantly during the 24 hr observation period (Table XI, Time).
DISCUSSION

Certain of the preceding data indicate that the presence of DCRs in pseudopregnant hamsters prolongs ovarian progesterone secretion and luteal hyperemia, that the presence of DCRs is correlated with higher progesterone levels during the photoperiod immediately preceding luteal regression, that luteolysis occurs abruptly overnight in pseudopregnant hamsters with and without DCRs as evidenced by the terminal decline in ovarian venous progesterone secretion and synchronous termination of luteal hyperemia, that the onset of the terminal decline in progesterone secretion is not associated with observed decreases in serum levels of luteotropic hormones, and that serum PGF levels in pseudopregnant hamsters lacking DCRs decline significantly 6-12 hrs before the terminal decline of progesterone.

That the presence of DCRs delayed the onset of decline in ovarian progesterone secretion and postponed the termination of luteal hyperemia by 1 full day indicates either an antiluteolytic or a luteotropic effect of decidual tissue in pseudopregnant hamsters. Since PGF is luteolytic in hamsters (Gutknecht et al., 1971), has vasoconstrictive properties (Pharriss, 1970), and has been identified in pseudopregnant hamster uterine extracts (Lukaszewska et al., 1972), sustained secretion of progesterone and persistence of hyperemic CL in hamsters with DCRs may be due to inhibition of synthesis, release or action of PGF. Alternatively, that a luteotropic effect of decidual tissue delayed the onset of decline in ovarian progesterone secretion and postponed the termination of luteal hyperemia is possible. A luteotropic hormone from decidual tissue in pseudopregnant rats with DCRs has been proposed and it may be a factor in maintaining progesterone secretion by the ovary on
days 8 through 11 (Gibori et al., 1974), but no such hormone has yet been isolated from decidual tissue.

An explanation for the antiluteolytic effect of decidual tissue in pseudopregnant rats proposed by Melampy et al. (1964) was that the capability of the endometrium to produce a luteolysin is lost when endometrium is converted to decidual tissue. However, Weems et al. (1974) reported higher levels of PGF in uterine venous plasma and uteri of pseudopregnant rats with DCRs than in rats lacking DCRs on day 9 of PS (day of onset of luteal regression in rats lacking DCRs). Therefore, in pseudopregnant rats with DCRs any luteolytic action of PGF on day 9 of PS is apparently prevented.

The presence of DCRs is correlated with higher progesterone levels during the photoperiod immediately before luteal regression which suggests the possibilities that DCRs may have promoted progesterone secretion, and that CL of pseudopregnant hamsters with DCRs secrete more progesterone than those of hamsters lacking DCRs.

The higher progesterone levels, before luteal regression in hamsters with DCRs, may be responsible for the maintenance of the DCR. It has been shown that progesterone is necessary for induction and maintenance of DCRs in ovariectomized hamsters (Kehl et al., 1964).

The higher levels of progesterone before luteal regression in hamsters with DCRs were not accompanied by any significant elevation of PRL, FSH or LH. In fact, the mean serum PRL level and the LH level at 1500 hrs before the terminal decline in progesterone were lower in pseudopregnant hamsters with DCRs than in hamsters lacking DCRs (Tables IV and VII, Source A). Hashimoto et al. (1968) reported a similar difference in ovarian venous progesterone levels in pseudo-
pregnant rats. Pepe and Rothchild (1974), however, reported no significant difference in peripheral levels of progesterone in serum of pseudopregnant rats with and without DCRs on days prior to luteal regression and attributed the high levels of progesterone in Hashimoto's report to methodological problems during blood collection from the ovarian vein. In the present investigation, however, the difference in progesterone levels between DCR and non-DCR bearing animals is not the result of methodological problems during ovarian venous blood collection, since the changes of arterial levels of progesterone in both groups (unpublished) were similar to those of ovarian venous blood.

Luteolysis, as evidenced by the terminal decline in ovarian venous progesterone secretion, occurred abruptly overnight in pseudopregnant hamsters with and without DCRs and was synchronous with termination of luteal hyperemia. This is in agreement with the results reported by Greenwald (1973), who demonstrated that functional and structural luteolysis occur simultaneously in hamsters.

The terminal decline in progesterone secretion was not accompanied by an observed decrease in serum levels of the basic hamster luteotropic hormone complex, FSH and PRL. In fact, serum levels of these two hormones were higher during CL regression in pseudopregnant hamsters with and without DCRs than those observed previously in cyclic hamsters during luteal regression (Bast and Greenwald, 1974a; Terranova, unpublished). Serum FSH levels during luteal regression in both pseudopregnant groups were higher than those observed previously in pregnant hamsters on days associated with the terminal decline of progesterone (Bast and Greenwald, 1974b). Serum PRL levels during luteal regression in pseudopregnant hamsters with and without DCRs were equal to or higher than those
PRL levels reported for pregnant hamsters during a corresponding time (Bast and Greenwald, 1974b). However, the stress associated with manipulation during blood collection, and the effect of Nembutal anesthesia could have altered the serum PRL concentrations in the present investigation. Providing that the serum PRL levels are valid, luteal regression in pseudopregnant hamsters with and without DCRs cannot be explained by withdrawal of luteotropic support from the pituitary.

High serum PGF levels in pseudopregnant hamsters lacking DCRs at 0900 hrs prior to the onset of the terminal decline of progesterone suggest that PGF is related to the terminal decline of progesterone (Fig. 13). In hamsters with DCRs, serum PGF levels did not fluctuate significantly \( (p > 0.05) \) during the 24 hr observation period (Fig. 13); however, they might well account for luteolysis, since they were not at baseline levels for PS at the onset of luteolysis (Fig. 13). In hamsters with and without DCRs, PGF may have abruptly increased hrs before the initial 0900 hrs samples were collected. Earlier and more frequent sampling, or sampling from the uterine vein may be required to detect increases in PGF during the hrs prior to luteal regression.

In pseudopregnant hamsters lacking DCRs (Shaikh et al., 1973), and in pregnant rats (Labhsetwar and Watson, 1974), plasma PGF levels did not fluctuate on the day prior to or on the day of, the onset of the terminal decline of progesterone. That PGF of ovarian origin may be involved in the terminal decline of progesterone in pregnant rats was proposed by Labhsetwar and Watson (1974). If the ovarian PGF is metabolized within the rat ovary itself, then no significant increase in plasma PGF would be observed during CL regression. However, fluctuations in PGF secretion during a single day could not be detected by the single
daily samples collected by Shaikh or Labhsetwar and Watson.

The determination of PGF from serum (at 4°C) rather than from plasma may have altered the assayable PGF, but the levels of PGF reported for hamsters with and without DCRs in this investigation are within the range of levels reported from peripheral plasma of pseudopregnant hamsters lacking DCRs by Shaikh et al. (1973). Also, PGF levels of human plasma and serum collected from blood which stood at 4°C for 24 hrs are not significantly different (Challis and Tulchinsky, 1973).

FSH levels in hamsters lacking DCRs were not significantly different from those in hamsters with DCRs at any time during the 24 hr observation period (Table II, DCR). If the DCRs had any effect on arterial FSH levels, it was not at the time of luteal regression. Whether DCRs affect arterial levels of FSH at any time has not been reported for rats or hamsters.

FSH levels in hamsters with and without DCRs were higher than those reported previously for cyclic hamsters (Bast and Greenwald, 1974a) and for pregnant hamsters (Bast and Greenwald, 1974b) during the terminal decline in progesterone associated with luteolysis in both groups. The high FSH levels in the present investigation are probably indicative of ovarian follicular growth.

The presence of DCRs in pseudopregnant hamsters was correlated with a significantly lower LH level at 1500 hrs than in hamsters lacking DCRs at the corresponding hr (Fig. 10, and Table IV, Source A). LH levels of hamsters with DCRs did not fluctuate significantly during the 24 hr observation period, whereas LH levels of hamsters lacking DCRs did fluctuate (Tables V and VI). LH levels in both pseudopregnant groups were lower than, or equal to, those observed previously in cyclic hamsters (Bast
and Greenwald, 1974a; Terranova, unpublished) or in pregnant hamsters (Bast and Greenwald, 1974b) during the terminal decline of progesterone associated with luteolysis. The significance of these differences in LH levels is not clear at this time.

In the present investigation, endogenous levels of PRL in both groups were found to fluctuate during the 24 hr observation period (Fig. 11). It is noteworthy that diurnal and nocturnal surges of PRL have been reported in serum of pseudopregnant rats lacking DCRs (Freeman et al., 1974), and in pregnant rats (Butcher et al., 1972). Recently, Bast (unpublished observation; see Bast and Greenwald, 1974b) found a diurnal rhythm of serum PRL during the early days of pregnancy in hamsters. That a daily rhythm of PRL exists in pseudopregnant hamsters is likely.

DCRs had no effect on ovarian venous estradiol levels until sometime between 0300 hrs and 0900 hrs after luteal regression. This postregressional rise in ovarian venous estradiol may be associated with ovulation (Shaikh et al., 1973), since the onset of psychic estrus would have occurred during the next dark period in hamsters with DCRs (Tate, 1964).
TABLE I

ANALYSIS OF VARIANCE OF PROGESTERONE LEVELS OF PSEUDOPREGNANT HAMSTERS WITH AND WITHOUT DECIDUOMATA

<table>
<thead>
<tr>
<th>Source</th>
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<th>p</th>
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<tbody>
<tr>
<td>DCR</td>
<td>1</td>
<td>65132.49</td>
<td>65132.50</td>
<td>5.07</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>A</td>
<td>1</td>
<td>320045.00</td>
<td>320045.00</td>
<td>24.91</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>103022.50</td>
<td>103022.50</td>
<td>8.02</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Time</td>
<td>4</td>
<td>931875.02</td>
<td>232968.75</td>
<td>18.13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DCR X Time</td>
<td>4</td>
<td>48026.38</td>
<td>12006.60</td>
<td>0.93</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Error</td>
<td>87</td>
<td>1117780.36</td>
<td>12848.05</td>
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</tr>
<tr>
<td>Total</td>
<td>96</td>
<td>2175294.68</td>
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</tr>
</tbody>
</table>

a - mean progesterone level of hamsters lacking DCRs at 156 hrs after sterile mating compared orthogonally with the mean progesterone level of hamsters with DCRs at the same hr

b - mean progesterone level of hamsters lacking DCRs at 132 and 138 hrs after sterile mating compared orthogonally with the mean progesterone level of hamsters with DCRs at 156 and 162 hrs after sterile mating
# TABLE II

ANALYSIS OF VARIANCE OF FOLLICLE STIMULATING HORMONE LEVELS
OF PSEUDOPREGNANT HAMSTERS WITH AND WITHOUT DECIDUOMATA

<table>
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<td>DCR</td>
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<td>33087.61</td>
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<td>1.138</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Time</td>
<td>4</td>
<td>106379.24</td>
<td>26594.81</td>
<td>0.915</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1</td>
<td>172496.73</td>
<td>172496.73</td>
<td>5.935</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>DCR X Time</td>
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<td>191341.24</td>
<td>47835.31</td>
<td>1.646</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Error</td>
<td>90</td>
<td>2615640.50</td>
<td>29062.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>99</td>
<td>2946448.59</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> - mean FSH level of hamsters lacking DCRs at 0900 hrs before luteal regression compared orthogonally with the mean FSH level of hamsters lacking DCRs at 2100 hrs.
<table>
<thead>
<tr>
<th>Source</th>
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</thead>
<tbody>
<tr>
<td>Time</td>
<td>4</td>
<td>74961.90</td>
<td>18740.48</td>
<td>0.75</td>
<td>&gt;0.05</td>
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<tr>
<td>Error</td>
<td>45</td>
<td>1122389.89</td>
<td>24942.00</td>
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<td>Total</td>
<td>49</td>
<td>1197351.79</td>
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TABLE IV
ANALYSIS OF VARIANCE OF LUTEINIZING HORMONE LEVELS OF PSEUDOPREGNANT HAMSTERS WITH AND WITHOUT DECIDUOMATA

<table>
<thead>
<tr>
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<td>DCR</td>
<td>1</td>
<td>7761.60</td>
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<td>3.48</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>A</td>
<td>1</td>
<td>15680.00</td>
<td>15680.00</td>
<td>7.03</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>10125.00</td>
<td>10125.00</td>
<td>4.54</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Time</td>
<td>4</td>
<td>18082.54</td>
<td>4520.64</td>
<td>2.03</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>27691.68</td>
<td>27691.68</td>
<td>12.42</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>D</td>
<td>1</td>
<td>19263.42</td>
<td>19263.42</td>
<td>8.64</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DCR X Time</td>
<td>4</td>
<td>26151.34</td>
<td>6537.84</td>
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<td>&lt;0.05</td>
</tr>
<tr>
<td>Error</td>
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<td>200719.10</td>
<td>2230.21</td>
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<tr>
<td>Total</td>
<td>99</td>
<td>252714.58</td>
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</tr>
</tbody>
</table>

a - mean LH level of hamsters lacking DCRs at 1500 hrs compared orthogonally with the mean LH level of hamsters with DCRs at the corresponding hr

b - mean LH level of hamsters lacking DCRs at 2100 hrs compared orthogonally with the mean LH level of hamsters with DCRs at the corresponding hr

c - mean LH level of hamsters lacking DCRs at 0900 hrs before luteal regression compared orthogonally with the mean LH level of hamsters lacking DCRs at 2100 hrs

d - mean LH level of hamsters lacking DCRs at 0900 hrs after luteal regression compared orthogonally with the mean LH level of hamsters lacking DCRs at 2100 hrs
TABLE V

ANALYSIS OF VARIANCE OF LUTEINIZING HORMONE LEVELS
OF PSEUDOPREGNANT HAMSTERS WITH DECIDUOMATA

<table>
<thead>
<tr>
<th>Source</th>
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<th>Mean squares</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>4</td>
<td>3487.81</td>
<td>871.95</td>
<td>0.45</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Error</td>
<td>45</td>
<td>87825.63</td>
<td>1951.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>91313.44</td>
<td></td>
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</table>
### TABLE VI

**ANALYSIS OF VARIANCE OF LUTEINIZING HORMONE LEVELS**

**OF PSEUDOPREGNANT HAMSTERS WITHOUT DECIDUOMATA**

<table>
<thead>
<tr>
<th>Source</th>
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<th>Sum of squares</th>
<th>Mean squares</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>4</td>
<td>40851.06</td>
<td>10212.77</td>
<td>4.08</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Error</td>
<td>45</td>
<td>112643.91</td>
<td>2503.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>153494.97</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
### TABLE VII

**ANALYSIS OF VARIANCE OF PROLACTIN LEVELS OF PSEUDOPREGNANT HAMSTERS WITH AND WITHOUT DECIDUOMATA**

<table>
<thead>
<tr>
<th>Source</th>
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<th>Mean squares</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCR</td>
<td>1</td>
<td>36.69</td>
<td>36.69</td>
<td>0.202</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1</td>
<td>1214.40</td>
<td>1214.40</td>
<td>6.69</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Time</td>
<td>4</td>
<td>4868.96</td>
<td>1217.24</td>
<td>6.70</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1</td>
<td>3269.12</td>
<td>3269.12</td>
<td>18.00</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1</td>
<td>3928.40</td>
<td>3928.40</td>
<td>21.63</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>D&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1</td>
<td>959.11</td>
<td>959.11</td>
<td>5.28</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>E&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1</td>
<td>1829.78</td>
<td>1829.78</td>
<td>10.07</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>F&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1</td>
<td>1620.00</td>
<td>1620.00</td>
<td>8.92</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>G&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1</td>
<td>945.31</td>
<td>945.31</td>
<td>5.20</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>DCR X Time</td>
<td>4</td>
<td>1878.96</td>
<td>469.74</td>
<td>2.59</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Error</td>
<td>90</td>
<td>16346.90</td>
<td>181.63</td>
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</tr>
<tr>
<td>Total</td>
<td>99</td>
<td>23131.51</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **a** - mean prolactin level of pseudopregnant hamsters lacking DCRs at 0900 hrs before luteal regression and at 1500 hrs compared orthogonally with the mean prolactin level of hamsters with DCRs at corresponding hrs

- **b** - the mean prolactin level of hamsters with DCRs at 0900 hrs before luteal regression compared orthogonally with the mean prolactin level of hamsters with DCRs at 2100 hrs

- **c** - the mean prolactin level of hamsters with DCRs at 1500 hr compared orthogonally with the mean prolactin level of hamsters with DCRs at 2100 hrs.

- **d** - the mean prolactin level of hamsters with DCRs at 0300 hr compared orthogonally with the mean prolactin level of hamsters with DCRs at 2100 hrs

- **e** - the mean prolactin level of hamsters with DCRs at 0900 hrs of the morning following luteal regression compared orthogonally with the mean prolactin level of hamsters with DCRs at 2100 hrs
TABLE VII continued

f - the mean prolactin level of hamsters without DCRs at 2100 hrs compared orthogonally with the mean prolactin level of hamsters without DCRs at 0900 hrs after luteal regression

g - the mean prolactin level of hamsters without DCRs at 0300 hrs compared orthogonally with the mean prolactin level of hamsters without DCRs at 0900 hrs after luteal regression
TABLE VIII
ANALYSIS OF VARIANCE OF ESTRADIOL LEVELS
OF PSEUDOPREGNANT HAMSTERS WITH AND WITHOUT DECIDUOMATA

<table>
<thead>
<tr>
<th>Source</th>
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<th>Mean squares</th>
<th>F</th>
<th>p</th>
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<tr>
<td>DCR</td>
<td>1</td>
<td>12.45</td>
<td>12.45</td>
<td>8.30</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>A(^a)</td>
<td>1</td>
<td>43.48</td>
<td>43.48</td>
<td>28.99</td>
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</tr>
<tr>
<td>Time</td>
<td>4</td>
<td>29.60</td>
<td>7.40</td>
<td>4.93</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>B(^b)</td>
<td>1</td>
<td>31.07</td>
<td>31.07</td>
<td>20.71</td>
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</tr>
<tr>
<td>C(^c)</td>
<td>1</td>
<td>44.76</td>
<td>44.76</td>
<td>29.84</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>D(^d)</td>
<td>1</td>
<td>25.74</td>
<td>25.74</td>
<td>17.16</td>
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</tr>
<tr>
<td>E(^e)</td>
<td>1</td>
<td>41.64</td>
<td>41.64</td>
<td>27.76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DCR X Time</td>
<td>4</td>
<td>38.41</td>
<td>9.60</td>
<td>6.40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>90</td>
<td>135.24</td>
<td>1.50</td>
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</tr>
<tr>
<td>Total</td>
<td>99</td>
<td>215.70</td>
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</table>

\(^a\) - the mean level of estradiol in hamsters with DCRs at 0900 hrs after luteal regression compared orthogonally with the mean estradiol level at the corresponding hr in hamsters lacking DCRs

\(^b\) - the mean estradiol level of hamsters with DCRs at 0900 hrs after luteal regression compared orthogonally with the mean estradiol level of hamsters with DCRs at 0300 hrs

\(^c\) - the mean estradiol level of hamsters with DCRs at 0900 hrs after luteal regression compared orthogonally with the mean estradiol level of hamsters with DCRs at 2100 hrs

\(^d\) - the mean estradiol level of hamsters with DCRs at 0900 hrs after luteal regression compared orthogonally with the mean estradiol level of hamsters with DCRs at 1500 hrs

\(^e\) - the mean estradiol level of hamsters with DCRs at 0900 hrs after luteal regression compared orthogonally with the mean estradiol level of hamsters with DCRs at 0900 hrs before luteal regression
TABLE IX
ANALYSIS OF VARIANCE OF ESTRADIOL LEVELS
OF PSEUDOPREGNANT HAMSTERS WITHOUT DECIDUOMATA

<table>
<thead>
<tr>
<th>Source</th>
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<th>Mean squares</th>
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<th>p</th>
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</thead>
<tbody>
<tr>
<td>Time</td>
<td>4</td>
<td>7.95</td>
<td>1.99</td>
<td>2.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Error</td>
<td>45</td>
<td>43.53</td>
<td>0.97</td>
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<td></td>
</tr>
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<td>Total</td>
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<td>Mean squares</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>----------------------</td>
<td>--------------------</td>
<td>----------------</td>
<td>--------------</td>
<td>-------</td>
<td>------</td>
</tr>
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<td>DCR</td>
<td>1</td>
<td>0.000576</td>
<td>0.000576</td>
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<td>&gt;0.05</td>
</tr>
<tr>
<td>A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1</td>
<td>8.27</td>
<td>8.27</td>
<td>22.97</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1</td>
<td>2.04</td>
<td>2.04</td>
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<td>&lt;0.05</td>
</tr>
<tr>
<td>Time</td>
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<td>20.19</td>
<td>5.05</td>
<td>14.03</td>
<td>&lt;0.001</td>
</tr>
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<td>C&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1</td>
<td>19.38</td>
<td>19.38</td>
<td>53.83</td>
<td>&lt;0.001</td>
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<td>DCR × Time</td>
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<td>11.21</td>
<td>2.80</td>
<td>7.78</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>90</td>
<td>32.52</td>
<td>0.36</td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>99</td>
<td>63.92</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>- mean PGF level of hamsters lacking DCRs at 0900 hrs before luteal regression compared orthogonally with the mean PGF level in hamsters with DCRs at the corresponding hr

<sup>b</sup>- mean PGF level of hamsters lacking DCRs at 1500 hrs compared orthogonally with the mean PGF level in hamsters with DCRs at the corresponding hr

<sup>c</sup>- mean PGF level of hamsters lacking DCRs at 0900 hrs before luteal regression compared orthogonally with the mean PGF level of hamsters lacking DCRs at 1500 hrs
TABLE XI

ANALYSIS OF VARIANCE OF PROSTAGLANDIN F$_{2\alpha}$ LEVELS
OF PSEUDOPREGNANT HAMSTERS WITH DECIDUOMATA

<table>
<thead>
<tr>
<th>Source</th>
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<th>Sum of squares</th>
<th>Mean squares</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
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<td>1.79</td>
<td>0.45</td>
<td>1.96</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Error</td>
<td>45</td>
<td>10.33</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>12.12</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 1. Gel filtration pattern of products from the separate iodination of NIAMD-Rat FSH-1-1, LH-1-3 and PRL-1-1. Each hormone was individually iodinated with $^{125}$I and the products of each iodination applied to a Bio Gel P-60 gel filtration column (0.7X20.0 cm) previously equilibrated at room temperature with phosphate buffered saline and 2% bovine serum albumin in buffered saline. Fractions of 0.5 ml were collected. Each point represents counts per minute (counting error is 2%) in a 10 μl aliquot. The first and second peak of each curve represents the maximum concentrations of radiolabeled hormone and Na$^{125}$I, respectively.
Counts per minute/10 ul aliquot x 2 x 10^-4

Fraction number

Iodinated hormones

NIAMD-Rat FSH-1-1 and Na\textsuperscript{125}I

LH-1-3

PRL-1-1
Figure 2. Gel filtration pattern of $^{125}\text{I}-\text{FSH}$, $^{125}\text{I}-\text{LH}$ and $^{125}\text{I}-\text{PRL}$ on Bio Gel P-60 (0.7x20.0 cm) previously equilibrated with phosphate buffered saline and 2% bovine serum albumin in buffered saline. Two hundred $\mu$l of fraction 3 or 4 from the original purification was repurified by gel filtration. Fractions of 0.5 ml were collected. Each point represents counts per minute (counting error is 2%) in one 10 $\mu$l aliquot. Each peak represents the maximum concentration of radiolabeled hormone.
Figure 3. Standard curves for reference hormones NIAMD Rat-FSH-RP-1, Rat-LH-RP-1, and Rat-PRL-RP-1, and dose response curves for pooled sera from pseudopregnant hamsters in each assay. Antisera employed in the assays were NIAMD-Anti-Rat FSH S-6, Anti-Rat LH S-1, and Anti-Rat PRL S-2. Each point represents counts per minute (counting error is 2%) and is the mean of triplicate determinations. Fifty percent binding represents 10,000 counts per minute.
Percent l-hormone bound

Reference preparation
Pooled sera

Reference preparation (ng)

Percent l-hormone bound

0.1 0.2 0.5 1.0 2.0 5.0 10.0 20.0 50.0 100.0 200.0 500.0 10000
Figure 4. Standard RIA curve for progesterone derived by using progesterone-11-succinate-bovine serum albumin antibody at a 1:12,000 dilution. Each point represents counts per minute (counting error is 1.5%) and is the mean of triplicate determinations. Fifty percent binding represents 1,130 counts per minute.
Figure 5. Standard RIA curve for estradiol-β derived by using estrone-17-oxime conjugate antibody at a 1:8,000 dilution. Each point represents counts per minute (counting error is 1.5%) and is the mean of triplicate determinations. Fifty percent binding represents 1,665 counts per minute.
Figure 6. Standard RIA curve for prostaglandin $F_{2\alpha}$. Each point represents counts per minute (counting error is 2.0%) and is the mean of triplicate determinations. Fifty percent binding represents 4,000 counts per minute.
Figure 7. Progesterone levels (ng/ml ovarian venous serum) at the indicated hrs after sterile mating in pseudopregnant hamsters with and without deciduomata (DCRs) and maintained on a 12L:12D lighting schedule in which the dark period ended at 0600 hrs. Each point represents a mean of 10 animals except those at 174 and 180 hrs after sterile mating, which represent mean of 8 and 9 animals, respectively.
Nanograms progesterone/ml serum

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<th>Hours after sterile mating</th>
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Figure 8. Percent pseudopregnant hamsters with and without deciduomata (DCRs) exhibiting hyperemic corpora lutea in the left ovary at the indicated hr after sterile mating. All hamsters were maintained on a 12L:12D lighting schedule. The dark period that began at 141 hrs after sterile mating in hamsters without DCRs and at 165 hrs after sterile mating in hamsters with DCRs is represented by a black bar at the bottom of the figure.
No DCRs

With DCRs

Percent hamsters exhibiting hyperemis corpora lutea

Hours after sterile mating
Figure 9. Arterial serum concentrations (mean ± SE, n = 10) of follicle stimulating hormone (FSH) in pseudopregnant hamsters with and without deciduomata (DCRs). Fifteen hundred hrs designates 138 hrs after sterile mating in pseudopregnant hamsters without DCRs and 162 hrs after sterile mating in pseudopregnant hamsters with DCRs. Day 1 of pseudopregnancy is the first 24 hr period commencing at 2400 hrs on the night of sterile mating. FSH levels are expressed in terms of NIAMD-Rat FSH-RP-1 (potency 2.1 X NIH-FSH-S1). Black and white bars at the bottom of the figure represent dark and light periods, respectively.
- Days 6-7, no DCRs
- Days 7-8, with DCRs

Nanograms FSH/ml serum

Hours
Figure 10. Arterial serum concentrations (mean ± SE, n = 10) of luteinizing hormone (LH) in pseudopregnant hamsters with and without deciduomata (DCRs). Fifteen hundred hrs designates 138 hrs after sterile mating in pseudopregnant hamsters without DCRs and 162 hrs after sterile mating in hamsters with DCRs. Day 1 of pseudopregnancy is the first 24 hr period commencing at 2400 hrs on the night of sterile mating. LH levels are expressed in terms of NIAMD-Rat LH-RP-1 (0.03 X NIH-LH-S1). Black and white bars at the bottom of the figure represent dark and light periods, respectively.
Nanograms LH/ml serum

- - Days 6-7, no DCRs
○○ Days 7-8, with DCRs

Nanograms LH/ml serum

Days 6-7, no DCRs
Days 7-8, with DCRs

Hours

0900 1500 2100 0300 0900
Figure 11. Arterial serum concentrations (mean ± SE, n = 10) of prolac­
tin (PRL) in pseudopregnant hamsters with and without deciduomata (DCRs). Fifteen hundred hrs indicates 138 hrs after sterile mating in hamsters without DCRs and 162 hrs after sterile mating in pseudopregnant hamsters with DCRs. Day 1 of pseudopregnancy is the first 24 hr period commencing at 2400 hrs on the night of sterile mating. PRL levels are expressed in terms of NIAMD-Rat PRL-RP-1 (11.0 IU/mg). Black and white bars at the bottom of the figure represent dark and light periods, respectively.
Nanograms Prolactin/ml serum

Days 6-7, no DCRs
Days 7-8, with DCRs

Nanograms Prolactin/ml serum

Hours

0900 1500 2100 0300 0900
Figure 12. Estradiol levels (mean ± SE, n = 10) of ovarian venous serum in pseudopregnant hamsters with and without deciduomata (DCRs). Fifteen hundred hrs indicates 138 hrs after sterile mating in hamsters lacking DCRs and 162 hrs after sterile mating in hamsters with DCRs. Day 1 of pseudopregnancy is the first 24 hr period commencing at 2400 hrs on the night of sterile mating. Black and white bars at the bottom of the figure represent dark and light periods, respectively.
Nanograms estradiol/ml serum

Days 6-7, no DCRs

Days 7-8, with DCRs
Figure 13. Arterial serum concentrations (mean ± SE, n = 10) of prostaglandin F$_{2\alpha}$ in pseudopregnant hamsters with and without deciduomata (DCRs). Fifteen hundred hrs indicates 138 hrs after sterile mating in hamsters lacking DCRs and 162 hrs after sterile mating in hamsters with DCRs. Day 1 of pseudopregnancy is the first 24 hr period commencing at 2400 hrs on the night of sterile mating. Black and white bars at the bottom of the figure represent dark and light periods, respectively.
Nanograms prostaglandin/ml serum

Days 6-7, no DCRs
Days 7-8, with DCRs

Nanograms prostaglandin/ml serum

Hours

0900 1500 2100 0300 0900
SUMMARY

1. The presence of DCRs in pseudopregnant hamsters delayed the onset of decline in ovarian progesterone secretion by one full day, which indicates an antiluteolytic effect of decidual tissue.

2. During the photoperiod immediately preceding luteal regression the mean progesterone level was higher in hamsters with DCRs than in hamsters lacking DCRs.

3. The terminal decline in circulating progesterone occurred abruptly overnight in both groups and was synchronous with termination of luteal hyperemia.

4. The onset of the terminal decline in progesterone secretion was not immediately preceded or accompanied by observed decreases in serum levels of hamster luteotropic hormones, FSH and PRL. However, PRL levels may have been altered by operational stress and Nembutal anesthesia. Providing that the serum PRL levels are valid, luteal regression in pseudopregnant hamsters with or without DCRs cannot be explained by withdrawal of luteotropic support from the pituitary.

5. High serum PGF levels 6-12 hrs prior to the onset of the terminal decline of progesterone in pseudopregnant hamsters lacking DCRs suggest that PGF is related to luteolysis. In hamsters with DCRs, serum PGF levels did not fluctuate significantly during the 24 hr observation period; however, they might well account for luteolysis since they were not at baseline levels for PS at the onset of luteolysis.

6. Serum FSH levels of pseudopregnant hamsters with and without DCRs during the day of luteal regression were not significantly different.

7. Serum LH levels in pseudopregnant hamsters with DCRs were low and
did not fluctuate significantly during luteal regression. Serum LH levels of hamsters lacking DCRs peaked during the onset of luteal regression and returned to baseline the following morning.

8. Serum PRL levels of pseudopregnant hamsters with and without DCRs fluctuated significantly during the 24 hr observation period. It is likely that a daily rhythm of serum PRL levels exists in pseudopregnant hamsters.

9. DCRs had no effect on ovarian venous estradiol levels until the morning after luteal regression and at that time estradiol levels were higher in hamsters with DCRs. This postregressional rise in ovarian venous estradiol may be associated with ovulation.
did not fluctuate significantly during luteal regression. Serum LH levels of hamsters lacking DCRs peaked during the onset of luteal regression and returned to baseline the following morning.

8. Serum PRL levels of pseudopregnant hamsters with and without DCRs fluctuated significantly during the 24 hr observation period. It is likely that a daily rhythm of serum PRL levels exists in pseudopregnant hamsters.

9. DCRs had no effect on ovarian venous estradiol levels until the morning after luteal regression and at that time estradiol levels were higher in hamsters with DCRs. This postregressional rise in ovarian venous estradiol may be associated with ovulation.
LITERATURE CITED


Labhsetwar, A.P. (1972). Effects of prostaglandin \text{F}_{2\alpha} \text{ on some reproductive processes of hamsters and rats. J. Endocrinol. 53, 201-213.}


VITA

Paul F. Terranova was born July 10, 1946, in Lake Charles, Louisiana. He was graduated from Lake Charles High School in May, 1964, and attended Tulane University from September, 1964, to May, 1965. He attended Tyler Junior College from September, 1965, to May, 1966. In the fall of 1966, he entered McNeese State College from which he was graduated with a Bachelor of Science degree on May 17, 1969. In June, 1969, he entered McNeese State University Graduate School to pursue the Master of Science degree with a major in Biology. In August, 1971, he received the Master of Science degree from McNeese State University. In the fall of 1971, he entered Louisiana State University to pursue the degree of Doctor of Philosophy in the Department of Zoology and Physiology. At present he is a candidate for the Ph.D. degree. He has been awarded a National Institute of Child Health and Human Development Postdoctoral Traineeship in the Departments of Obstetrics and Gynecology, and Anatomy, at the University of Kansas Medical Center, subject to successful completion of all remaining requirements for the Doctor of Philosophy degree.
EXAMINATION AND THESIS REPORT

Candidate: Paul Frank Terranova

Major Field: Zoology

Title of Thesis: Deciduomata: Their Antiluteolytic Effect and Influence on Serum Levels of Ovarian Steroids, Luteotropins, and Prostaglandin F$_{2\alpha}$ During the Onset of Luteolysis in Pseudopregnant Hamsters

Approved:

[Signatures]

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

[Signatures]

Date of Examination:

April 10, 1975