PROGESTERONE-DEPENDENT BLASTOCYST SURVIVAL DURING ALTERED THYROID ACTIVITY IN THE RAT

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(Received 16th March 1970)

Summary. On the 8th day of pregnancy, ova were flushed from the uteri of ovariectomized albino rats maintained on progesterone. Hyperthyroidism and even a dose of L-thyroxine as low as 8 µg/day counteracted the detrimental effects of progesterone deficiency upon blastocyst survival during this delay period, while thyroidectomy contributed to the detrimental effect. This study clarifies the earlier reported effect of the thyroid upon delayed implantation of blastocysts by showing that (1) the effect occurs during the progesterone-dependent maintenance period rather than during the implantation process, and (2) the effect can be obtained with a dose of L-thyroxine which is within the physiological hypersecretory potential of the rat thyroid.

There are conflicting reports in the literature concerning the effects of thyroid hormone upon pregnancy. Investigators have reported beneficial effects, disadvantageous effects and some have reported that it exerts no effect. Examples of these investigations and possible reasons for the conflicting results were presented in an earlier publication (Holland, Dorsey, Harris & Johnson, 1967). The previous studies in our laboratory have employed the technique of delayed implantation of blastocysts (which involves ovariectomy of rats on Day 3 of pregnancy, administration of progesterone from Days 3 to 8, laparotomy on Day 8 to confirm delay, supplementation of the daily progesterone with oestrone beginning on Day 8 in order to induce implantation and, finally, observation of the number of implantation sites at autopsy on Day 13 of pregnancy). With this technique, it was demonstrated (Holland et al., 1967) that, in rats receiving daily injections of 48 µg L-thyroxine, this hyperthyroid condition significantly counteracts the detrimental effects of progesterone deficiency upon the number of implantation sites observed on Day 13 of pregnancy, while surgical thyroidectomy has the opposite effect. More recently (Holland, Calhoun, Harris & Walton, 1968), the subnormal levels of uterine alkaline phosphatase which occur during progesterone deficiency were shown to be restored to normal by thyroxine and further depleted by hypothyroidism in uteri examined at a time
corresponding to Day 8 of pregnancy, i.e. the final day of the delay period. The present study was conducted to determine whether the alterations in the thyroid state were influencing the intra-uterine maintenance of the blastocysts during the progesterone-dependent delay period (Meyer & Nutting, 1964) of Days 3 to 8 rather than influencing the effectiveness of the oestrogen-dependent implantation process (Prasad, Mohla & Rajalakshmi, 1969).

Seventy-nine Holtzman, albino, virgin rats of between 90 and 120 days of age were housed, maintained and ovariectomized as described earlier (Holland et al., 1967). These rats were ovariectomized on Day 3 of pregnancy and treated

### Table 1

**The Effect of Progesterone and Alterations in Thyroid Condition upon the Intra-Uterine Survival of Delayed Blastocysts in Ovariectomized Rats**

<table>
<thead>
<tr>
<th>Progesterone treatment</th>
<th>Thyroid condition</th>
<th>No. of rats</th>
<th>No. of rats with no surviving blastocysts</th>
<th>Blastocysts recovered from uterine flushing (average no./rat ± S.E. of mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-0 mg/day</td>
<td>Normal</td>
<td>15</td>
<td>0</td>
<td>5.63 ± 0.44 *</td>
</tr>
<tr>
<td>0-4 mg/day</td>
<td>Normal</td>
<td>15</td>
<td>0</td>
<td>3.06 ± 0.50</td>
</tr>
<tr>
<td>0-4 mg/day</td>
<td>48 µg L-T₄/day</td>
<td>14</td>
<td>0</td>
<td>7.00 ± 0.74 **</td>
</tr>
<tr>
<td>0-4 mg/day</td>
<td>15 µg L-T₄/day</td>
<td>8</td>
<td>0</td>
<td>7.25 ± 0.53 **</td>
</tr>
<tr>
<td>0-4 mg/day</td>
<td>8 µg L-T₄/day</td>
<td>9</td>
<td>1</td>
<td>5.50 ± 0.92 **</td>
</tr>
<tr>
<td>0-4 mg/day</td>
<td>Thyroidectomized</td>
<td>18</td>
<td>6</td>
<td>1.50 ± 0.38 ****</td>
</tr>
</tbody>
</table>

All animals were ovariectomized on Day 3 of pregnancy and maintained on progesterone until autopsy on Day 8.

* Significantly different from 2-0-mg controls at 5% level.
** Significantly different from 0-4-mg controls at 1% level.
*** Significantly different from 0-4-mg controls at 5% level.
**** Significantly different from 0-4-mg controls at 2% level.

on Days 3 to 8 with either of two doses of progesterone (the standard dosage of 2-0 mg/day normally used to maintain delayed blastocysts or a deficiency dosage of 0-4 mg/day). Hyperthyroidism was induced by daily injection of 48, 15 or 8 µg L-thyroxine beginning at least 10 days before insemination, while surgical thyroidectomies were performed at least 1 month before insemination. All rats were killed on Day 8 (the time comparable to the final day of the delay period; oestrogen stimulation of implantation was omitted). Uteri were excised, each horn was separated and with the use of a 1-ml syringe containing physiological saline, the contents of each horn were flushed on to a depression slide. The flushings were examined under ×40 and ×100 magnification. The findings are presented in Table 1.

As was the case when blastocysts were induced to implant, a reduction of the daily maintenance dose of progesterone from 2-0 to 0-4 mg caused a significant decrease in the number of blastocysts recovered by flushing the uterus on Day 8. Again, 48 µg L-thyroxine effectively counteracted the effects of progesterone deficiency while thyroidectomy further decreased the survival of pre-implantation blastocysts.
Since 48 µg L-thyroxine exceeds the hyperthyroid secretory levels in the rat, lower thyroxine dosages were investigated. A daily dosage of 15 µg was highly effective and 8 µg daily has also proved to be quite effective in counteracting progesterone deficiency. In earlier studies of delayed implantation, it was reported (Holland et al., 1967) that a high percentage of hypothyroid rats were devoid of implantation sites. In the present study, a high percentage of the thyroidectomized rats were devoid of blastocysts, as determined by uterine flushings. The earlier report presented evidence to show that the state of the thyroid did not influence ovulation.

Seemingly, this effect upon blastocyst survival is not a pharmacological occurrence but may be physiologically important. For example, Feldman (1956) reported that thyroid activity increases by 100% during normal oestrus as compared with di-oestrus in the rat, while Grosvenor & Turner (1958) reported that lactation may also produce a two-fold increase in thyroid secretion in the rat. Thyroxine secretion rate in the rat has been reported (Grosvenor & Turner, 1958) to be 1.24 to 1.75 µg/100 g body wt/day or about 2.8 to 4.0 µg/day in rats of the size used in our experiments. Although the earlier studies reported by our laboratory utilized 48 µg L-thyroxine/day to induce the hyper-thyroid condition, we have now shown that 15 µg/day yield identical results concerning numbers of blastocysts which can be flushed from the uterus following the period of progesterone maintenance. Preliminary studies indicate that a dose of 8 µg L-thyroxine/day is equally effective. This latter dosage is only about twice the normal daily secretion level. This two-fold increase has been approached during normal oestrus and during lactation, as cited above. Our current investigations indicate that dosages of thyroxine which approach the physiological hypersecretory potential are effective in compensating for progesterone deficiency. The differences in numbers of implanted blastocysts are due to an influence upon the progesterone maintenance of the blastocysts rather than upon an alteration of the oestrogen stimulus for nidation.

No studies were conducted to determine the effect of administration of a dose of L-thyroxine comparable to the secretory level (e.g., 2 to 4 µg/day) since this would possibly be a duplication of the control or normal thyroid groups if given to thyroidectomized rats as a replacement therapy or if given to intact rats (since endogenous thyroid secretion would be suppressed). Studies of the plasma half-life of thyroxine in these experiments would be useful in clarifying this possibility and in determining more critically the peripheral levels of thyroxine necessary for the reproductive effects reported in this paper.

The level of thyroid hormone has been reported by Gallagher (1964) to be an important determinant of the direction of metabolism of steroid hormones. Since intra-uterine maintenance of the blastocysts during the delay period is progesterone-dependent and since thyroxine enhances the action of low doses of progesterone upon the survival of delayed blastocysts and enhances the stimulatory action of progesterone upon uterine alkaline phosphatase, there is a possibility that these findings reflect a more rapid and/or effective utilization or metabolism of progesterone during hyperthyroidism. Preliminary studies in our laboratory with [14C]progesterone indicate this possibility. Further studies with gas chromatography are in progress.
This investigation was supported by the National Science Foundation research grant GB-8241.

REFERENCES


