The effects of Nolvadex (tamoxifen citrate; ICI 46,474) on pregnancy in rabbits

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In the rabbit there is evidence that oestrogen, derived from the ovarian follicles, forms part of the luteotrophic complex responsible for maintenance of the corpora lutea (CL) of pregnancy and pseudopregnancy (Hilliard, 1973). Labhsetwar (1971) showed that administration of the anti-oestrogen, Nolvadex, to rabbits either early in pregnancy or between Days 10 and 20 of pseudopregnancy results in a reduction in the size and number of CL and concluded that the drug directly counters the luteotrophic action of oestradiol. However, there is no supporting information available on the hormonal changes following treatment with Nolvadex. We therefore treated rabbits with Nolvadex and measured plasma progesterone and oestradiol concentrations.

Twelve adult female Dutch-belted rabbits were mated to a fertile buck (= Day 1 of pregnancy) and divided randomly into 3 groups. Nolvadex (tamoxifen citrate, ICI 46,474; trans-1-(p-β-dimethylaminoethoxyphenyl)-1,2-diphenylbut-1-ene citrate) was administered in 0.5% aqueous Tween 80. Animals in Group 1 (control) received only Tween 80; those in Group II were treated orally with Nolvadex (2 mg/kg/day) beginning on Day 10 of pregnancy; and those in Group III received the same dose of Nolvadex from Day 20 of pregnancy. The animals were killed either on the day following parturition or abortion or on Day 35 after mating. The numbers of CL and implantation sites were counted and the mammary tissue was examined for the presence of milk.

During pregnancy, blood (~2 ml) was drawn from an ear vein into a heparinized tube at intervals of 1–2 days. After centrifugation at 1500 g for 15 min at 4°C, the plasma was separated and stored at −20°C. The concentrations of plasma progesterone and oestradiol were measured by radioimmunoassay (Challis, Davies & Ryan, 1973; Furr, 1973). The solvent blanks were equivalent to 0.03 ± 0.03 ng/ml (mean ± S.D.) for progesterone and 2.4 ± 3.8 pg/ml for oestradiol. The inter-assay coefficients of variation for progesterone at 0.5 ng/ml and for oestradiol at 250 pg/ml were 15.2% and 12.7% respectively. Nolvadex had negligible cross-reaction (< 0.001%) in both radioimmunoassays.

The effect of Nolvadex treatment on the outcome of pregnancy is presented in Table 1. In Group II, 2 rabbits had not given birth by Day 35 and at autopsy fetal resorptions were found. Although the other 2 animals littered, one did so prematurely and the other showed uterine bleeding between Days 23 and 26. Since the number of implantation sites was similar in all groups and the mean number of young born was significantly (P < 0.05) less in Group II than in Group I, the major effect of the drug was to induce fetal resorption. Treatment later in pregnancy (Group III) caused a significant reduction in the length of gestation (P < 0.01) and number of live young born. The amount of milk present in the mammary glands of the Nolvadex treated animals at parturition was variable but apparently less than in the control group.

The concentrations of progesterone and oestradiol in the plasma of these rabbits are shown in Text-fig. 1. Administration of Nolvadex from Day 10 provoked a significant decrease in plasma progesterone concentration within 4–6 days (P < 0.002) and values of < 4 ng/ml were found throughout the remainder of gestation. After Nolvadex treatment from Day 20, there was a rapid fall in plasma progesterone and the values on Days 21 and 22 were significantly lower than those on Day 20 (P < 0.01). The differences in plasma progesterone concentrations were not due to differences in the numbers of CL in the different groups (Table 1). Treatment with Nolvadex from Day 10 had no significant effect on
Table 1. Effects (mean ± S.E.M.) of Nolvadex on the outcome of pregnancy in the rabbit

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of animals</th>
<th>Length of pregnancy (days)</th>
<th>No. of young born</th>
<th>% Young born alive</th>
<th>No. of implantation sites</th>
<th>No. of CL</th>
<th>Presence of milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Controls</td>
<td>4</td>
<td>32-5 ± 0-3</td>
<td>5-8 ± 1-6</td>
<td>95-7</td>
<td>6-0 ± 1-4</td>
<td>7-3 ± 0-3</td>
<td>4+</td>
</tr>
<tr>
<td>II</td>
<td>Nolvadex (2 mg/kg/day orally from Day 10)</td>
<td>4</td>
<td>26,31†</td>
<td>1-75 ± 1-2*</td>
<td>28-6</td>
<td>6-25 ± 1-1</td>
<td>7-25 ± 1-5</td>
<td>2†; 2—</td>
</tr>
<tr>
<td>III</td>
<td>Nolvadex (2 mg/kg/day orally from Day 20)</td>
<td>4</td>
<td>26-8 ± 1-3**</td>
<td>5-0 ± 2-0</td>
<td>65-0</td>
<td>8-0 ± 1-5</td>
<td>8-0 ± 1-5</td>
<td>1†; 3†</td>
</tr>
</tbody>
</table>

Significantly different from controls, *P < 0-05; **P < 0-01.
† Only 2 rabbits gave birth.

Text-fig. 1. Effects of Nolvadex on plasma concentrations (mean ± S.E.M.) of (a) progesterone and (b) oestradiol in pregnant rabbits. ○, Group I, controls; ▲, Group II, Nolvadex from Day 10; ■, Group III, Nolvadex from Day 20.

plasma oestradiol concentrations (P > 0-05), but these were consistently lower in the animals in Group III (although after Day 26 the values given represent only the two animals which had not aborted).

These results have confirmed and extended the observations of Labhsetwar (1972), who demonstrated the antifertility effects of Nolvadex in rabbits. We have shown that administration of the drug
from Day 10 of gestation results in considerable embryonic loss whilst administration from Day 20 causes premature parturition/abortion. Both of these effects were associated with a significant reduction in plasma progesterone concentration, although abortion did not occur immediately following the decline in progesterone, perhaps because of the presence of a myometrial inhibitory factor (Porter, 1974).

The unaltered plasma oestradiol values in the treated animals suggest that oestradiol secretion was unimpaired and that the lutetotropic stimulus (Hilliard, 1973) was maintained. Watson, Anderson, Alam, O’Grady & Heald (1975) have shown that Nolvadex does inhibit the preimplantation surge of oestradiol in the rat and have suggested that the drug inhibits oestradiol synthesis in the ovary. This apparent discrepancy may be due either to a difference in response by the two species or to a difference in drug dose. An effect of Nolvadex on gonadotrophin secretion (Aiyer & Fink, 1974) also seems to be precluded by the unchanged plasma oestradiol concentrations.

The study of Labhsetwar (1971) showed that the reduction in weight of the CL occurred in pseudo-pregnant and pregnant rabbits, indicating that the effect of Nolvadex was unlikely to result from a reduction in the lutetotropic support from the placenta (Porter, Becker, & Csapo, 1968). Skidmore, Walpole & Woodburn (1972) have shown that Nolvadex competes with oestradiol for cytosol binding sites in the rabbit uterus and the rat uterus and pituitary gland, and the most probable explanation for the present results, and for those of Labhsetwar (1971), therefore, would be that Nolvadex competes with oestradiol for binding sites in the CL (Lee, Keyes & Jacobson, 1971), thereby directly antagonizing the lutetotropic effect of the steroid.

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References


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