Ketoconazole Impairs Early Pregnancy and the Decidual Cell Response via Alterations in Ovarian Function

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Ketoconazole (KCZ) is an imidazole antifungal agent that also affects P450 enzymes of the mammalian steroidogenic system. Several steps in the ovarian steroidogenesis pathway are known to be inhibited by KCZ, but previous work has failed to address the ramifications of such inhibition with respect to early pregnancy. In initial studies, Holtzman rats (8-10/group) were administered 10-100 mg/kg KCZ during days 1-8 of pregnancy. On day 9, evaluations revealed a reduction at both 75 and 100 mg KCZ/kg in the number of implantation sites and serum progesterone levels as well as an increase in ovarian weight. The decidual cell response (DCR) was blocked by KCZ in parallel with decreased serum progesterone and increased ovarian weight, indicating direct interference with uterine function. KCZ had no effect when given to long-term-ovariectomized rats that were hormone supplemented to permit the DCR, indicating that the ovary was at least one site of KCZ action on early pregnancy. Measurement of ovarian progesterone production in vitro from ovaries removed from rats treated in vivo with KCZ indicated a decline in progesterone production, suggesting a direct effect of KCZ on ovarian steroidogenesis. These data demonstrate that KCZ can compromise early pregnancy and appears to do so by inhibiting progesterone synthesis in the ovary.

Ketoconazole (KCZ; cis-1-acetyl-4-(4-(2,4-dichlorophenyl)-2-(1-H-imidazole-1-ylmethyl)-1,3-dioxolane-4-yl))) is a member of the family of antifungal agents known as azoles which includes imidazoles (such as ketoconazole) and triazoles, characterized by the nitrogen component of their structure (Como and Dismukes, 1994). The antifungal activity of azoles is mediated via the inhibition of the synthesis of ergosterol, a component of the fungal cell membrane. This inhibition occurs through an interaction with a P450-dependent enzyme, 14α-demethylase (Como and Dismukes, 1994). Additional effects of antifungal azoles include the inhibition of respiration and the inhibition of the transformation of yeasts to the mycelial form (Como and Dismukes, 1994).

Oral ketoconazole is an effective treatment for fungal infections. In addition to its antifungal activity, the drug also acts on the P450 enzymes of the mammalian steroidogenesis system. Ketoconazole was shown to lower circulating testosterone levels in men following the oral doses of 200-600 mg/day standard for antifungal therapy (Pont et al., 1982a). Additional studies in vitro demonstrated a direct effect of ketoconazole on rat testicular cells (Feldman, 1986). Experiments in men by Santen et al. (1983) revealed an effect of ketoconazole on C17,20 lyase, the enzyme in testicular steroidogenesis that catalyzes the synthesis of androstenedione from 17α-OH progesterone. Work in vitro using rat testes demonstrated an additional blockade of steroidogenesis at 17α-hydroxylase (Sikka et al., 1985). Following the finding of an inhibition of adrenal steroidogenesis by KCZ by Pont et al. (1982b), Loose et al. (1983) reported the mechanism of this inhibition and demonstrated blockade by KCZ of two adrenal P450 steroidogenic enzymes, at 11-hydroxylase (catalyzing deoxycorticosterone to corticosterone) and at the side chain cleavage enzyme (catalyzing cholesterol to pregnenolone).

Research on the ovarian effects of KCZ originally focused on aromatase and 17β-estradiol synthesis (Watanabe and Menzies, 1985, 1986a; Malozowski et al., 1986; Latrille et al., 1987). KCZ has since been shown to affect several steps in human ovarian steroidogenesis including 3β-hydroxysteroid dehydrogenase/soromerase (3β-HSD), 17-hydroxylase (17-OH), aromatase (DiMattina et al., 1988), and the cholesterol side-chain cleavage enzyme (P450scC) (Gal et al., 1991). Thus, the fungicide appears to impair mammalian steroidogenesis by inhibiting multiple P450 enzymes.

There is also evidence of adverse effects of KCZ on pregnancy. The administration of the drug to rats (10, 25, or 50 mg/kg; gestation days 6-21) or mice (10, 20, or 40 mg/kg; GD 6-18) resulted in a high incidence of resorptions, increased number of stillbirths, and delayed parturition in the mice at 20 and 40 mg/kg KCZ (Buttar et al., 1989). The
chemical also produced a 100% incidence of failed parturition in rats at 50 mg/kg KCZ associated with a high incidence of resorptions found at necropsy (Buttar et al., 1989). Reductions in birth weights and late descent of testes and vaginal opening were found in both rats and mice (Buttar et al., 1989). The administration, between GD 14 and 19, of 100 mg/kg KCZ to rats resulted in a 100% pregnancy loss characterized by resorption (L. Earl Gray, Jr., personal communication).

The previously observed adverse effects of KCZ on ovarian steroidogenesis suggested that there may be ramifications of KCZ exposure on ovarian steroid hormone-regulated events such as implantation and early pregnancy. Since circulating progesterone is critical for implantation and pregnancy maintenance, then an inhibitory effect of KCZ on ovarian steroidogenesis is likely to impair the success of the events of early pregnancy. Our approach to test this hypothesis was to use a panel of techniques capable of both detecting chemical effects on very early pregnancy and identifying specific physiological mechanisms of such effects (Cummins, 1990). These methods included the early pregnancy protocol, which examines implantation rate and related parameters, and the decidual cell response (DCR) technique with and without ovariectomy and hormone replacement. The DCR technique is a method which tests the ability of a chemical to interfere with the development of uterine sensitivity for implantation and/or the growth of decidual (temporal) tissue following surgical induction or implantation. Sufficient E2 and P4, in the proper balance, are required for a normal response. Thus, a chemical may directly affect the uterus or may interfere with ovarian function, resulting in an ovarian steroid hormone milieu that will not support the decidual response. While the simpler DCR technique can rule out or confirm an effect on some part of the reproductive system, the ovariectomized hormone-replaced model can determine that at least part of the effect of the chemical is on the ovary. Following these studies, we evaluated ovarian steroid hormone secretion \textit{ex vivo} in order to directly examine the impact of KCZ on ovarian progesterone secretion.

\section*{METHODS}

\textbf{Animals.} Female Holtzman rats were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN) at 70 days of age and acclimated for 1 week before use. Animals were housed in pairs in clear plastic cages containing laboratory grade heat-treated pine shavings for bedding (Northeastern Products Corp., Warrensburg, NY). Tap water and feed (Prolab rat, mouse, hamster 3000, Agway, Syracuse, NY) were provided \textit{ad libitum}. Photoperiod was 14 h light:10 h dark, with lights on at 0500 h EST. Temperature and humidity were maintained at 20–24°C and 40–60%, respectively.

Only rats that displayed normal vaginal cytology for two cycles were used in this study. In pregnancy studies, females were caged on the night of proestrus with untreated, proven-fertile males; the finding of a sperm-positive vaginal smear on estrus was considered gestation day 0.

\textbf{Experiment I.} Groups of 8–10 rats were dosed, by gavage, with 0, 10, 30, or 100 mg KCZ/kg/day on days 1–8 of pregnancy. This regimen of dosing begins after fertilization, includes the day of implantation (GD 4), and encompasses both the preimplantation interval and a short postimplantation period. This study also included 300 mg KCZ/kg/day, but the 300 mg/kg/day dose was discontinued when it became obvious that it was overly toxic. KCZ was suspended in sesame oil and diluted for each dose such that animals received a volume of 3 ml/kg body wt. Blood was collected from the tail vein on day 4 of pregnancy (the day of implantation), and serum was stored at −70°C until assayed for 17β-estradiol (E2), progesterone (P4), and luteinizing hormone (LH). Animals were killed on gestation day 9 at which time trunk blood was collected following decapitation under CO2 anesthesia, and serum was stored at −70°C until assayed for E2, P4, and LH. Endpoints evaluated included body weight, number of implantation sites and their weight, number of resorptions (defined as those sites having obvious necrosis or extravasation of blood), uterine weight (including pups and fluids), ovarian weight, and total number of corpora lutea (CL). Preimplantation loss was calculated by the formula: (No. of CL) − (No. of implantation sites) = preimplantation loss. E2 and P4 were assayed using radioimmunoassay kits for the appropriate hormone (Diagnostic Products, Los Angeles, CA). LH was assayed by a previously validated RIA using materials supplied by the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases: iodination preparation, 1-9; reference preparation, RP-3; antisera, S-11.

\textbf{Experiment II.} In light of the decline in body weight seen at 100 mg KCZ/kg in the first study, a second dose–response evaluation was performed. Groups of eight rats were dosed during days 1–8 of pregnancy with 0, 25, 50, 75, or 100 mg KCZ/kg/day on days 1–8 of pregnancy. Decidual induction was performed surgically by the knife scratch method of De Feo (1963) on day 4, at which time tail vein blood was also collected for hormone measures. All rats were killed on day 9 of pseudopregnancy, and evaluations included the measurement of E2, P4, and LH in serum collected on days 4 and 9, uterine weight (decidual response), and ovarian weight.

\textbf{Experiment IV.} In the next set of experiments, two groups of female rats (treated with 0, 10, 30, or 100 mg KCZ/kg/day during days 1–8 of pregnancy) were killed on day 9, ovaries were removed from all animals and placed individually in 1.5-ml vials on ice for subsequent incubation. Ovary culture was performed on day 4 of pregnancy (the day of implantation), and serum was stored at −70°C until assayed for 17β-estradiol (E2), progesterone (P4), and luteinizing hormone (LH). Animals were administered 0, 25, 50, 75, or 100 mg KCZ/kg/day on days 1-8 of pregnancy. Decidual induction was performed surgically by the knife scratch method of De Feo (1963) on day 4, at which time tail vein blood was also collected for hormone measures. All rats were killed on day 9 of pseudopregnancy, and evaluations included the measurement of E2, P4, and LH in serum collected on days 4 and 9, uterine weight (decidual response), and ovarian weight.

\textbf{Experiment V.} As soon as animals from Experiment I (treated with 0, 10, 30, or 100 mg KCZ/kg/day during days 1–8 of pregnancy) were killed on day 9, ovaries were removed from all animals and placed individually in 1.5-ml vials on ice for subsequent incubation. Ovary culture was performed immediately, as previously described (Cummins and Laskey, 1993). Each minced ovary was incubated in 1 ml of supplemented Medium 199 for 1 h at 34°C under 5% CO2. Medium 199 (Gibco) was supplemented with NaHCO3 (0.71 g/L; J. T. Baker), Hepes (2.1 g/L; Sigma), bovine serum albumin (1.0 g/L; Schwarz-Mann), soybean trypsin inhibitor (25 mg/mL; Sigma), and human chorionic gonadotropin (100 IU/mL; Calbiochem). Following centrifugation (200g, 3 min), the decanted supernatant was stored
at −40°C until assayed for P_4 by an RIA kit (Diagnostic Products). Data were calculated for expression as nanograms P_4 production/ovary/hour.

**Statistics.** Data for all parameters except percentages were analyzed by the General Linear Models (GLM) procedure (SAS, 1985); percentages were transformed to the arcsine of the square root prior to analysis. When significant effects on the overall analysis of variance were detected (p < 0.05), post hoc comparisons among treatments were made using t tests (Least Squares Means; SAS, 1985) according to *a priori* hypotheses.

**RESULTS**

Pregnant rats were administered KCZ using a dose range of 0, 10, 30, and 100 mg KCZ/kg/day on days 1–

![Graphs showing the effect of ketoconazole on parameters of early pregnancy: log-dose range. Pregnant rats were treated days 1–8 with KCZ at the indicated dosages and evaluated on day 9 of pregnancy. Doses of 100 mg KCZ/kg/day significantly reduced the number of implantation sites, uterine weight, implantation site weight, and serum progesterone. Ovarian weight and serum LH were significantly increased at this dosage level. Data are expressed as the means ± SE. **Significantly different from vehicle-treated controls at p < 0.005.](image)
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TABLE 1

Effect of Ketoconazole on Early Pregnancy

<table>
<thead>
<tr>
<th>Dose of KCZ (mg/kg)</th>
<th>0</th>
<th>10</th>
<th>30</th>
<th>100*</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Resorptions*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preimplant. loss'</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100*</td>
</tr>
<tr>
<td>No. Cl'</td>
<td>14.10 ± 0.59</td>
<td>16.88 ± 0.61*</td>
<td>14.40 ± 0.64</td>
<td>17.63 ± 0.75*</td>
</tr>
<tr>
<td>Body wt gain (g)'</td>
<td>26.10 ± 2.24</td>
<td>25.00 ± 2.77</td>
<td>16.20 ± 1.88*</td>
<td>-1.00 ± 4.26*</td>
</tr>
<tr>
<td>Serum P₄ (ng/ml), day 4</td>
<td>55.48 ± 1.47</td>
<td>63.14 ± 4.61</td>
<td>41.79 ± 4.54*</td>
<td>11.65 ± 1.42*</td>
</tr>
<tr>
<td>Serum LH (ng/ml), day 4</td>
<td>0.24 ± 0.05</td>
<td>0.23 ± 0.03</td>
<td>0.31 ± 0.06</td>
<td>1.07 ± 0.21*</td>
</tr>
<tr>
<td>Serum E₂ (pg/ml), day 9</td>
<td>8.46 ± 1.51</td>
<td>9.68 ± 0.91</td>
<td>6.66 ± 0.70</td>
<td>11.84 ± 2.54</td>
</tr>
<tr>
<td>N</td>
<td>10</td>
<td>9</td>
<td>10</td>
<td>8</td>
</tr>
</tbody>
</table>

B. Dose–response evaluation II*

| % Resorptions*     |   |    |    |      |
| Preimplant. loss'  | 1.75 ± 1.16 | 4.00 ± 2.32 | 0.25 ± 0.16 | 6.75 ± 2.52 |
| No. Cl'            | 14.50 ± 0.80 | 13.75 ± 0.75 | 13.38 ± 0.32 | 13.88 ± 0.95 |
| Body wt gain (g)'  | 13.88 ± 5.99 | 13.88 ± 3.47 | 3.38 ± 6.10 | 9.00 ± 2.07 |
| Serum P₄ (ng/ml), day 4 | 47.46 ± 3.00 | 42.65 ± 6.86 | 34.59 ± 9.19 | 16.05 ± 3.84* |
| Serum LH (ng/ml), day 9 | 0.28 ± 0.04 | 0.26 ± 0.02 | 0.30 ± 0.06 | 0.64 ± 0.11* |
| Serum E₂ (pg/ml), day 9 | 10.78 ± 0.96 | 11.05 ± 0.43 | 10.71 ± 0.40 | 11.92 ± 0.81 |
| N                  | 8  | 8  | 8   | 8    |

Note. Data except % resorptions are expressed as means ± SE; % resorptions are expressed as the mean of individual percentages.

* KCZ was administered to groups of rats on days 1–8 of pregnancy using two dose ranges, as shown, and the listed evaluations were performed on day 9.

* % Resorptions = (No. resorptions/No. implantations sites) × 100.

* Preimplantation loss = (No. CL) – (No. implantation sites).

* Corpora lutea were larger and had a white rather than pink appearance in KCZ-treated rats.

* Body weight gain = (body weight on day 9) – (body weight on day 1 of pregnancy).

* Statistically significant difference from vehicle treated control group at p < 0.05.

Upon evaluation of pregnancy-related parameters on day 9, the number of implantation sites, uterine weight, and implantation site weight were all found to be significantly decreased in the 100 mg/kg group (Fig. 1). The number of resorptions, which was 100% of implantation sites remaining (after preimplantation loss), and preimplantation loss were significantly increased at 100 mg KCZ/kg/day (Table 1A). Ovarian weight in animals treated with 100 mg KCZ/kg/day was approximately double that of controls (Fig. 1); the small but significant increase in the number of CL at 10 and 100 mg KCZ/kg/day (Table 1A) is more likely a reflection of interanimal variation in CL number than an effect related to the change in ovarian weight. Gross observation of the ovaries revealed that the organs from animals treated with 100 mg KCZ/kg/day were white in color with large CL as opposed to the normal pink color of the ovaries from control animals. Serum progesterone levels showed a severe decline on both days 4 and 9 of pregnancy following treatment with 100 mg KCZ/kg/day (Fig. 1, Table 1A). Concomitantly, serum LH levels rose dramatically following treatment with 100 mg KCZ/kg/day as measured on days 4 and 9 (Fig. 1, Table 1A). Serum estradiol showed no change, on day 9, at any dose of KCZ. A potential problem with the initial dose–response study was that 100 mg KCZ/kg/day produced a reduction in body weight gain between days 1 and 9 (Table 1A), suggesting the possible presence of maternal toxicity. Thus, a dose–response evaluation (Experiment II) was performed in which the highest dosage was 75 mg KCZ/kg/day.

In Experiment II, dosages of 0, 25, 50, and 75 mg KCZ/kg/day were used. No significant alteration in body weight gain was seen on day 9 following any of these treatments (Table 1B). At 75 mg KCZ/kg/day, the number of implantation sites and uterine weight were significantly decreased (Fig. 2). There was no significant increase in resorptions or preimplantation loss at this dose, although there was a trend toward increased preimplantation loss; the litters had either a full complement
FIG. 2. Effect of ketoconazole on early pregnancy: dose response. Rats were treated with KCZ days 1-8 of pregnancy and assessment of parameters was done on day 9. KCZ, at 75 mg/kg/day, reduced the number of implantation sites, uterine weight, and serum progesterone while increasing ovarian weight and serum LH. Data are expressed at means ± SE. * **Significantly different from vehicle-treated controls at p < 0.05 and p < 0.005, respectively.

of implantation sites or no sites at all (all or nothing effect; Table 1B). There was no effect of KCZ on implantation site weight at these doses (Fig.2). Ovarian weight was significantly increased at 25 and 75 mg KCZ/kg/day (Fig.2) with no significant change in the number of CL found at any dose (Table 1B). Ovaries from rats treated with 75 mg KCZ/kg/day were, again, white instead of pink. Serum progesterone levels were significantly decreased on days 4 and 9 after treatment with KCZ at 75 mg/kg/day, and serum LH was increased to more than double control levels on days 4 and 9 following the same treatment (Fig.2, Table 1B). No change in serum estradiol level was observed at any dose of KCZ (Table 1B).

When the DCR technique was applied to the evaluation of KCZ, decidual growth (uterine weight) was significantly
FIG. 3. Ketoconazole blocks the decidual cell response. Surgical induction of the decidual cell response was performed on day 4 of pseudopregnancy, and rats were treated days 1–8 of pseudopregnancy with KCZ at the indicated dosages. Uterine weight, the measure of decidual growth, was significantly reduced at 75 and 100 mg KCZ/kg/day. Serum progesterone was significantly decreased on days 4 and 9 whereas serum LH was increased on both days. Data are expressed as means ± SE. * **Significantly different from vehicle-treated controls at $p < 0.05$ and $p < 0.005$, respectively.

impaired at 75 and 100 mg KCZ/kg/day, administered during days 1–8 of pseudopregnancy (Fig.3). Ovarian weight was again increased, at 50, 75, and 100 mg KCZ/kg/day (Fig.3). Serum progesterone showed a sharp, dose-dependent decline on both days 4 and 9 of pseudopregnancy (Fig.3). Serum LH patterns showed a significant increase on both days 4 and 9 at 100 mg KCZ/kg (Fig.3).

To assess the role of the ovary in KCZ action, long-term (3-week) ovariectomized rats were treated with progesterone and estrone to mimic pseudopregnancy. Decidual induction on day 4 produced a maximal level of decidual growth in controls treated by gavage with vehicle (Fig.4). When ovariectomized, hormone-treated animals were also treated with 75 mg/kg/day KCZ on days 1–8 of the artificial pseudopreg-
FIG. 4. Ketoconazole and the decidual cell response: dependence of effect on ovarian function. Rats were ovariectomized and treated with estrone and progesterone to mimic pseudopregnancy, and surgical induction of the decidual cell response was performed. In the absence of ovaries, with exogenous hormonal support, KCZ had no effect on decidual growth as measured by uterine weight. Serum progesterone (administered) and serum estradiol were unchanged while serum LH was significantly decreased. Data are expressed as the mean ± SE. *Significantly different from vehicle-treated controls at \( p < 0.05 \).

Investigation of the potential ovarian effect of KCZ was performed by incubating ovaries, in vitro, from animals treated with KCZ in vivo, the same animals described in Experiment I. Progesterone production and secretion into the media, in ng/ovary/hour, are shown in Fig. 5. Ovaries from rats treated with 100 mg KCZ/kg/day produced significantly less progesterone in vitro than vehicle-treated controls.

DISCUSSION

In the dose–response evaluations of the effect of KCZ on early pregnancy, both 100 and 75 mg KCZ/kg significantly reduced the number of implantation sites found on day 9, indicating an adverse effect on some aspect of early pregnancy. A reduction in body weight gain was noted at the 100 mg/kg dose but not at 75 KCZ/kg, suggesting a potential systemic toxic effect of the higher dose. On gestation day 9, the pregnant uterus contributes a significant proportion of body weight; thus this decline in body weight at 100 mg/kg is not attributable to embryonic loss. The administration of 75 mg KCZ/kg on gestation days 1–8 resulted not only in pregnancy loss but also in significantly lower serum \( P_4 \) concentrations on both day 4 (day of implantation) and day 9 of pregnancy. Since sufficient circulating \( P_4 \) is essential for implantation and pregnancy maintenance, these data suggest that the decline in the concentration of \( P_4 \) produced by chemical exposure may have contributed to the observed early pregnancy failure. The increase in serum LH seen concurrently with the progesterone decline is likely due to the disruption of the steroid hormone feedback loop between
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Previous data indicating an effect of KCZ on ovarian steroidogenesis both in vivo (Watanabe and Menzies, 1986b; Gal et al., 1994) and in vitro (Malazowski et al., 1986; Weber et al., 1991) led us to examine the effect of KCZ on progesterone production in vitro using an ovarian organ culture system. KCZ, administered in vivo, decreased the in vitro production of progesterone by ovarian tissue. In studies using human granulosa-luteal cells in culture, Gal et al. (1991) demonstrated an apparent effect of KCZ on the cholesterol side-chain cleavage enzyme (P450ccc), the enzyme that catalyzes the synthesis of pregnenolone from cholesterol prior to progesterone formation. Such an effect in our rats would have led to an accumulation of cholesterol in the ovaries, perhaps resulting in the observed increase in ovarian weight and change in color from pink to white. An alternative mechanism potentially mediating the increase in ovarian weight is that as progesterone production is decreased and pituitary LH secretion is concomitantly increased, the ovary may respond to the increase in LH by hypertrophy.

While KCZ has been shown to inhibit ovarian aromatase (Watanabe and Menzies, 1985, 1986a; Lattrille et al., 1987, 1989; Weber et al., 1991), no effect of KCZ on serum estradiol was found in any of our studies. This is likely due to the fact that estradiol is already very low during early pregnancy, and some inhibition of aromatase activity may have little effect on the measured levels.

The significance of these results lies in several arenas. First, it is apparent that exposure to these levels of ketoconazole, and perhaps to similar substances which significantly impair progesterone synthesis in the rat and human, is a hazard to the initiation and maintenance of pregnancy. Insufficient progesterone can interfere with the preparation of the uterus for implantation as well as prevent the maintenance of pregnancy after implantation by impairing decidual growth (Yochim and De Feo, 1962, 1963). In addition, these studies demonstrate that the early pregnancy protocol and accompanying techniques can identify an indirect effect of a chemical on uterine function. KCZ was shown to impair implantation and early pregnancy via its effect on the ovary. However, these studies do not eliminate the possibility that the decrease in progesterone in vivo may result from decreased pituitary prolactin secretion or effects on P450 enzymes in tissues other than the ovary. Further work, in vitro, provided evidence that corroborated previous data on the ability of KCZ to inhibit ovarian steroidogenesis.

In summary, KCZ reduced both the number of implantation sites and the serum levels of progesterone following exposure during early pregnancy in the rat. Physiologically, the mechanism of this effect appears to be ovarian. KCZ inhibited the production of ovarian progesterone ex vivo in a manner consistent with a blockade of the cholesterol side-chain cleavage enzyme. These data point to KCZ-induced

the ovary and the hypothalamic–pituitary axis. Serum estradiol was unaffected. In previous unrelated studies, ovaries from KCZ-treated rats (100 mg KCZ/kg) have indicated an accumulation of cholesterol, suggested by staining that showed excess lipid in the ovaries (John W. Laskey, personal communication).

The decidual cell response is a model in which the induction of artificially induced decidua can be used to quantitatively assess whether a chemical impairs implantation and/or early pregnancy via an effect, either direct or indirect, on the uterus. Our finding of an impaired decidual response at 75 and 100 mg KCZ/kg, concurrent with significantly decreased serum progesterone, suggests that the blockade of implantation and induction of embryonic resorption by KCZ is mediated through a decrease in ovarian progesterone production. When long-term ovariectomized rats received a hormone regimen to permit the DCR, KCZ had no effect. Data showing (1) that the ovaries are required to see an effect of KCZ on the DCR and (2) that exogenous progesterone replacement can restore the KCZ-induced inhibition of the DCR seen in intact rats indicate that the ovaries are involved in mediating the effect of KCZ. The chemical had an indirect effect on the uterus under these conditions. The finding of a decrease in serum LH under these conditions, while contrary to the effect of KCZ in intact rats, is consistent with a report by Irsy and Koranyi (1990) indicating that the suppressive action of exogenous estrogen on LH in ovariectomized rats was enhanced by KCZ. This suggests the potential for a CNS, or at least pituitary, effect of KCZ.

**Significantly different from vehicle-treated controls at p < 0.005.**

FIG. 5. *In vitro* ovarian progesterone production following *in vivo* exposure to ketoconazole. Animals received KCZ during days 1–8 of pregnancy, and ovaries were removed and incubated *in vitro* on day 9. The *in vivo* administered dosage level of 100 mg KCZ/kg/day dramatically reduced the ovarian production of progesterone. Data are expressed as the mean ± SE of progesterone output from individual ovaries. **Significantly different from vehicle-treated controls at p < 0.005.**
changes in ovarian function that ultimately lead to adverse effects on embryonic well-being during very early pregnancy.

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REFERENCES


