COMPARISON OF INDUCED CORPORA LUTEA FROM PREPUBEERAL GILTS AND SPONTANEOUS CORPORA LUTEA FROM MATURE GILTS: HYDROXYSTEROID DEHYDROGENASE ACTIVITY

R. D. Kineman¹, R. R. Kraeling², G. B. Rampacek¹³ and G. J. Hausman²⁴

University of Georgia
Athens 30602

and

U.S. Department of Agriculture⁵
Athens, GA 30613

ABSTRACT

The activity of hydroxysteroid dehydrogenases was histochemically quantified in corpora lutea (CL) from prepuberal gilts induced to ovulate and mature gilts. Prepuberal (P) gilts, 120 to 130 d of age were induced to ovulate with 1,500 IU pregnant mare serum gonadotropin (PMSG) followed 72 h later by 500 IU human chorionic gonadotropin (hCG). Three P gilts and three mature (M) gilts each were ovariectomized on d 10, 14, 18, 22 and 26 (d 0 = day of hCG for P gilts and onset of estrus for M gilts). Gilts ovariectomized on d 14, 18, 22 and 26 were hysterectomized on d 6 to ensure luteal maintenance. At the time of ovariectomy, CL were frozen in liquid nitrogen and then stored at -80 C until analysis. Cryostat sections (12 μm) were histochemically analyzed for Δ⁴-3β-hydroxysteroid dehydrogenase (Δ⁴ OHSD), 17α-hydroxysteroid dehydrogenase (17α OHSD) and 20α-hydroxysteroid dehydrogenase (20α OHSD). The intensity of staining (greater enzyme activity resulted in darker staining) was quantified using a Zeiss SF microscope integrated with a Zonax photometer, which measured the percentage of light transmitted through a given area (22,500 μm²) of the tissue section. Data were subjected to analysis of variance using the general linear models procedure of Statistical Analysis System (SAS). The Δ⁴ OHSD activity did not change over days, but the mean activity (throughout all days) in the P gilts (32.6 ± 1.8) tended (P<.08) to be elevated above that of M gilts (27.9 ± 1.7). The 17α OHSD activity was similar for P and M gilts but 17α OHSD activity was different (P<.05) between days (d 10, 5.3 ± .9; d 14, 9.2 ± .9; d 18, 7.1 ± .8; d 22, 5.7 ± .9 and d 26, 5.5 ± 1.0). The 20α OHSD activity was greater (P<.05) in P gilts (6.7 ± .5) than in M gilts (5.1 ± .5); there was also an effect (P<.01) of day (d 10, 3.9 ± .8; d 14, 10.7 ± .9; d 18, 5.6 ± .8; d 22, 6.5 ± .8 and d 26, 3.4 ± .9). The results indicate that the progesterone metabolic pathway may be different in the induced CL from P gilts and the spontaneous CL from M gilts. These differences may contribute to abnormal function of the induced CL and subsequent inability of the P gilt to maintain pregnancy.

(Key Words: Progesterone, Enzymes, Corpus Luteum, Histochemistry, Pigs.)

Introduction

Pregnancy is rarely maintained to 25 d of gestation in prepuberal gilts induced to ovulate (Shaw et al., 1971; Segal and Baker, 1973; Rampacek et al., 1976b). The inability of the prepuberal gilts to maintain pregnancy was attributed to inadequate steroid production of the induced corpora lutea (CL) because the percentage of prepuberal gilts that maintained pregnancy was increased by administration of steroids or gonadotropins (Shaw et al., 1971; Ellicott et al., 1973; Segal and Baker, 1973; Rampacek et al., 1976b). Hysterectomy following induced ovulation maintained CL to at least d 30 (Segal and Baker, 1973; Rampacek et al.,

¹ Dept. of Anim. and Dairy Sci.
² USDA-ARS.
³ To whom reprint requests should be addressed, Anim. and Dairy Sci. Dept., Livestock-Poultry Bldg., Univ. of Georgia, Athens, GA 30602.
⁴ The authors thank Ms. M. Hart, Dr. C. R. Barb, Mrs. G. Thomas, Mrs. E. Price-Taras, Mrs. C. Estienne, Mr. M. Estienne and Mr. B. Johnson for their technical assistance and Ruel L. Wilson Biometrician, Southern Region, ARS for his statistical advice. Mention of trade name, proprietary product or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply approval to the exclusion of other products that may be suitable.
Received December 27, 1985.
Accepted August 8, 1986.
1976a), indicating adequate luteotropic support if the uterine luteolysin was removed.

Previous work from this laboratory (Kineman et al., 1985) indicated that luteal cells from prepuberal gilts induced to ovulate have a decreased progesterone response to varying doses of gonadotropins in vitro as compared with luteal cells from mature gilts. These data might indicate an abnormal steroidogenic enzyme system in the induced CL. Therefore this study was conducted to compare the activity of 3β-hydroxysteroid dehydrogenase (3β OHSD), 17α-hydroxysteroid dehydrogenase (17α OHSD) and 20α-hydroxysteroid dehydrogenase (20α OHSD) in CL of prepuberal gilts induced to ovulate and spontaneous CL of mature gilts as an indication of the ability to produce and metabolize progesterone.

Materials and Methods

Animals. Prepuberal (P) crossbred gilts, 120 to 130 d of age, and mature (M) gilts, from the same population as the P gilts and that had displayed one or more estrous cycles of 18 to 22 d (onset of estrus = d 0), were used. The P gilts were induced to ovulate with a single im injection of 1,500 IU pregnant mare serum gonadotropin (PMSG) followed 72 h later by an im injection of 500 IU human chorionic gonadotropin (hCG; day of hCG = d 0). Three P gilts and three M gilts each were ovariectomized on d 10, 14, 18, 22 and 26. Gilts ovariectomized on d 14, 18, 22 and 26 were hysterectomized on d 6 to ensure luteal maintenance. A portion of an ovary containing CL was frozen in liquid nitrogen and stored at -80 C until analysis. On d 26, sufficient quantities of luteal tissue were recovered from only two P and two M gilts.

Histocbemistry. Corpora lutea were sectioned at 12 /m on a cryostat microtome at -20 C. The sections were placed on clean, dry glass slides and allowed to set at room temperature for a minimum of 1 h prior to incubation. The incubation medium used to demonstrate the steroid dehydrogenases was prepared according to the procedure of Balogh (1964). Five milligrams of nitro-blue tetrazolium (Nitro-BT)6 were dissolved in 2 ml of TRIS-HCl buffer. In addition, 5 mg of nicotinamide adenine dinucleotide (NAD)6 was added as a cofactor for 3β OHSD and 17α OHSD or 5 mg nicotinamide adenine dinucleotide phosphate (NADP)6 was added as a cofactor for 20α OHSD. In order to control diffusion of the enzyme, 2 ml of 50% polyvinyl alcohol6 solution in TRIS-HCl buffer, pH 7.5 was added to the medium. Also, 1 ml of N, N-dimethylformamide6 containing 5 mg of pregnenolone7, 17α-hydroxyprogesterone7 or 20α-hydroxyprogesterone7 was used as a substrate for 3β OHSD, 17α OHSD and 20α OHSD, respectively. Finally, 100 /l of 500 mM NaN38, an inhibitor of the cytochrome oxidase system, and 100 /l of 16.3 mM phenazine methosulfate6, which facilitates the transfer of the hydrogen to Nitro-BT, were added. Medium was adjusted to pH 7.5 for 3β OHSD and 17α OHSD and to pH 8.0 for 20α OHSD. Alternate sections were designated as controls. Control incubation solutions were prepared in an identical manner but individually excluded the respective steroid substrates. Sections were incubated for 1 h at 37 C.

Slides were rinsed in distilled H2O for 1 to 2 min and the incubation was terminated by fixing the sections in Baker's Formalin at 4 C for 15 min and rinsing for 5 min in distilled H2O. Coverslips were mounted with glycerogel6.

Analysis. The intensity of staining (the darker the stain, the greater the enzyme activity) was quantified using a Zeiss SF microscope integrated with a Zonax photometer9, which measured the percentage of light transmitted through a given area (22,500 μm2) of the tissue section. A section of each slide containing no tissue was considered 100% transmittance. Enzyme activity was indicated by the difference between percentage of transmittance in treated sections and control sections; the greater the difference, the greater the enzyme activity. Data were subjected to analysis of variance using the General Linear Models procedure of SAS (1982). To evaluate differences between ages within a given day and trends throughout days of the study, a regression analysis across time was applied using pigs within age as errors for testing age.

Results

Mean steroid dehydrogenase activities for P and M gilts and for d 10, 14, 18, 22 and 26 are presented in tables 1 and 2, respectively. The 3β OHSD activity did not change over days, but
TABLE 1. MEAN 3β-HYDROXYSTEROID DEHYDROGENASE (3β OHSD), 17α-HYDROXYSTEROID DEHYDROGENASE (17α OHSD) AND 20α- HYDROXYSTEROID DEHYDROGENASE (20α OHSD) ACTIVITY OF INDUCED CORPORA LUTEA (CL) FROM PREPUBERAL (P) GILTS AND SPONTANEOUS CL FROM MATURE (M) GILTS

<table>
<thead>
<tr>
<th>Age</th>
<th>3β OHSD</th>
<th>17α OHSD</th>
<th>20α OHSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>32.6 ± 1.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.1 ± .5</td>
<td>6.7 ± .5&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>M</td>
<td>27.9 ± 1.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.1 ± .5</td>
<td>5.1 ± .5&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean ± pooled SE.
<sup>b</sup>,<sup>c</sup>Means in a column that do not have a common superscript letter differ (P<.08).
<sup>d</sup>,<sup>e</sup>Means in a column that do not have a common superscript letter differ (P<.05).

The mean activity throughout all days for the P gilts was greater (P<.08) than that of M gilts. The 17α OHSD activity was similar for P and M gilts but was different (P<.05) between days; activity was greatest on d 14. The 20α OHSD activity was greater (P<.05) in P gilts than in M gilts, and was also greater (P<.01) on d 14 compared with all other days.

**Discussion**

The activity of 3β OHSD has been used as an indicator of the progesterone-secreting capacity of the porcine CL (Bjersing, 1967; Gregorasz-czuk and Wojtusiak, 1982). Interestingly, luteal tissue from P gilts exhibited greater activity for 3β OHSD than that from M gilts. One might expect the opposite because supplementation of P gilts with steroids increased the percentage maintaining pregnancy (Shaw et al., 1971; Elliott et al., 1973; Segal and Baker, 1973; Rampacek et al., 1976b), implying inadequate progesterone production of the induced CL from P gilts. The physiological significance of the difference in 3β OHSD activity between P and M gilts is not clear. Human chorionic gonadotropin increased progesterone production in rat luteal tissue, in vivo (Yoshinaga et al., 1967), yet acute administration of hCG to rats failed to alter ovarian 3β OHSD activity (Rubin et al., 1963). Therefore, histochemical evaluation of 3β OHSD might not provide a sensitive index of the capacity of the ovary to synthesize progesterone. Caffrey et al. (1979) reported that 3β OHSD could be inhibited by its product, progesterone, in the ovine CL. Therefore, prod-

TABLE 2. MEAN ACTIVITY OF 3β-HYDROXYSTEROID DEHYDROGENASE (3β OHSD), 17α-HYDROXYSTEROID DEHYDROGENASE (17α OHSD) AND 20α-HYDROXYSTEROID DEHYDROGENASE (20α OHSD) OF INDUCED CORPORA LUTEA (CL) FROM PREPUBERAL GILTS AND SPONTANEOUS CL FROM MATURE GILTS ON D 10, 14, 18, 22 AND 26 FOLLOWING OVULATION

<table>
<thead>
<tr>
<th>Day</th>
<th>3β OHSD</th>
<th>17α OHSD</th>
<th>20α OHSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>27.0 ± 2.7</td>
<td>5.3 ± .9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.9 ± .8&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>14</td>
<td>29.6 ± 2.7</td>
<td>9.2 ± .9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.7 ± .9&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>18</td>
<td>27.8 ± 2.6</td>
<td>7.1 ± .8&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>5.6 ± .8&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>22</td>
<td>34.0 ± 2.8</td>
<td>5.7 ± .9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.5 ± .8&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>26</td>
<td>34.3 ± 3.2</td>
<td>5.5 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.4 ± .9&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean ± pooled SE.
<sup>b</sup>,<sup>c</sup>Means in a column that do not have a common superscript letter differ (P<.05).
<sup>d</sup>,<sup>e</sup>Means in a column that do not have a common superscript letter differ (P<.01).
uct inhibition of 3β-OHSD could explain a decreased 3β-OHSD activity in spontaneous CL compared to induced CL.

Luteal tissue from P and M gilts had similar 17α-OHSD activities, indicating that there probably is not a difference between induced and spontaneous CL in the metabolism of progesterone to androgens and estrogens. However, luteal tissue from P gilts had a greater activity of 20α-OHSD than luteal tissue from M gilts. The 20α-OHSD activity has been associated with regression of the rat CL (Balogh, 1964). 20α-Hydroxyprogesterone had reduced progestational activity in bioassay systems as compared with progesterone (Hecht-Lucari et al., 1961), and exhibited less than 5% of competitive binding for the progesterone receptor (McLaughlin and Richardson, 1976).

Exogenous administration of prostaglandin F$_2$α (PGF$_2$α) to rats increased ovarian 20α-OHSD activity, suggesting that PGF$_2$α stimulated the 20α-OHSD enzyme (Fuchs and Mok, 1974). Therefore, PGF$_2$α-induced luteolysis could be due, in part, to the conversion of progesterone to an inactive metabolite. Because P gilts induced to ovulate are more sensitive to the uterine luteolysin (Puglisi et al., 1979, 1978), luteal PGF$_2$α production (Mattioli et al., 1984) might have caused the significant increase in P luteal tissue 20α-OHSD activity that was seen in this study.

Results of this study indicate that the progesterone catabolic pathways may be different between induced CL of P gilts and spontaneous CL of M gilts. These differences may contribute to abnormal function of the induced CL and subsequent inability of the P gilt to maintain pregnancy.

**Literature Cited**


