INFLUENCE OF OVARIAN STATUS AND INTRAUTERINE DEVICES ON THE DIAMINE OXIDASE AND HISTAMINE CONTENT OF SHEEP ENDOMETRIUM

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THE presence of a silk thread in the lumen of one uterine horn of ovariectomized rats caused the diamine oxidase (DAO) content to increase by more than a hundredfold over that of the opposite horn (Scommegna and Chatnoraj, 1968). Treating ovariectomized rats with estradiol also caused a marked increase in the DAO content of the uterus, and treatment with estradiol after placing a thread in the uterus resulted in an additive increase.

In sheep, a plastic intrauterine device (IUD) changed the composition of endometrial ground substance, heightened the level of endometrial vascular function, and intensified the acute inflammatory response to induced infection in the uterine lumen (Cooper and Hawk, 1968; 1970a, b). The ovarian status of the ewe also influenced each of these aspects of uterine function. However, neither endogenous ovarian hormones nor an IUD caused such marked changes in the sheep uterus as estradiol or an IUD caused in the DAO content of the rat uterus. This study was done to determine whether endogenous ovarian hormones and intrauterine devices would affect the DAO content of the sheep endometrium. Since DAO degrades histamine, the concentration of histamine in the endometrium was also determined.

Materials and Methods

The ewes were 4 years old, parous and of Targhee breeding. They were checked for estrus twice daily by the use of vasectomized rams.

The intrauterine devices were cylindrical spirals made from polyethylene plastic rods as described previously (Cooper and Hawk, 1968). Each IUD measured about 12 cm in length and 8 mm in outside diameter, with 1 cm between turns of the spiral. An IUD was inserted by surgery, as described previously (Cooper and Hawk, 1968), into one uterine horn of each of 28 ewes. At the same time, both ovaries were removed from seven of the ewes. An IUD was inserted into one horn of each of 24 control ewes and removed immediately, the ovaries being removed from six of these ewes. The operations were performed between the 11th and 14th days of the estrous cycle, at which time each ewe with intact ovaries was assigned to a day at which she was to be autopsied during a later estrous cycle (table 1).

The ewes were killed about 10 weeks postsurgery, the cycling ewes being killed on the day to which they were previously assigned. The presence of an IUD in one uterine horn of ewes causes erratic estrous cycles, usually characterized by combinations of normal and short 6 to 9 day cycles, so each ewe assigned to the 10-day group was killed in a cycle in which she had not returned to estrus by 10 days. At autopsy, a mature corpus luteum was found in each 10-day ewe.

Immediately after the death of each ewe, the portion of uterine horn containing the IUD and a comparable portion of the other horn were opened longitudinally and cut into strips. The endometrium was excised, then the caruncles removed from the endometrium. The caruncular and non-caruncular tissue from each horn was divided in half and one portion used for determination of histamine and the other for diamine oxidase. Each of these samples generally weighed between 0.5 and 2g, the smallest samples being obtained from the ovariectomized ewes. The tissue was frozen on dry ice immediately after the samples were weighed.

Histamine determinations were made at Beltsville by the fluorometric method of Shore, Burkhalter and Cohn (1959), using a Farrand Model A Fluorometer.

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TABLE 1. EFFECT OF OVARIAN STATUS AND INTRAUTERINE DEVICES ON THE PROTEIN CONTENT OF THE ENDOMETRIUM

<table>
<thead>
<tr>
<th>Ewe group and tissue sample</th>
<th>Status of ewes</th>
<th>3 days post-estrus</th>
<th>10 days post-estrus</th>
<th>After ovariectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In estrus</td>
<td>10 days post-estrus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control ewes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroma</td>
<td>68</td>
<td>67</td>
<td>69</td>
<td>80</td>
</tr>
<tr>
<td>Caruncles</td>
<td>76</td>
<td>87</td>
<td>77</td>
<td>82</td>
</tr>
<tr>
<td>IUD ewes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IUD horn</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroma</td>
<td>56</td>
<td>87</td>
<td>68</td>
<td>121</td>
</tr>
<tr>
<td>Caruncles</td>
<td>65</td>
<td>98</td>
<td>74</td>
<td>98</td>
</tr>
<tr>
<td>Non-IUD horn</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroma</td>
<td>67</td>
<td>89</td>
<td>81</td>
<td>85</td>
</tr>
<tr>
<td>Caruncles</td>
<td>63</td>
<td>93</td>
<td>72</td>
<td>85</td>
</tr>
</tbody>
</table>

Protein expressed as milligrams per gram of wet tissue.

Tissue to be analyzed for diamine oxidase was sent by air, frozen on dry ice, to the Michael Reese Hospital and Medical Center. Diamine oxidase was measured according to the method of Okuyama and Kabayashi (1961), using putrescine --C<sup>14</sup> as substrate. Radioactivity of putrescine metabolism products was measured in a Packard Tri-Carb liquid scintillation counter. Tissue protein content was determined by the Folin phenol reagent method (Lowry et al., 1951).

The data were analyzed statistically by least squares analysis of variance. Data from the two uterine horns of each control ewe were pooled for statistical analysis.

In the following text, the ewes with a spiral in one uterine horn will be designated “IUD ewes.” The horn containing the IUD will be the “IUD horn,” and the other the “non-IUD horn.”

TABLE 2. EFFECT OF OVARIAN STATUS AND INTRAUTERINE DEVICES ON DIAMINE OXIDASE CONCENTRATION AND ACTIVITY IN THE ENDOMETRIUM

<table>
<thead>
<tr>
<th>Ewe group and tissue sample</th>
<th>Status of ewes</th>
<th>3 days post-estrus</th>
<th>10 days post-estrus</th>
<th>After ovariectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Activity&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Concentration&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Activity&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control ewes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroma</td>
<td>117</td>
<td>1.75</td>
<td>77</td>
<td>1.30</td>
</tr>
<tr>
<td>Caruncles</td>
<td>45</td>
<td>0.63</td>
<td>24</td>
<td>0.30</td>
</tr>
<tr>
<td>IUD ewes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IUD horn</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroma</td>
<td>53</td>
<td>0.95</td>
<td>98</td>
<td>1.12</td>
</tr>
<tr>
<td>Caruncles</td>
<td>36</td>
<td>0.56</td>
<td>55</td>
<td>0.60</td>
</tr>
<tr>
<td>Non-IUD horn</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroma</td>
<td>108</td>
<td>1.47</td>
<td>131</td>
<td>1.47</td>
</tr>
<tr>
<td>Caruncles</td>
<td>38</td>
<td>0.58</td>
<td>57</td>
<td>0.64</td>
</tr>
</tbody>
</table>

<sup>a</sup> Diamine oxidase units per gram of wet tissue.
<sup>b</sup> Diamine oxidase units per milligram of protein.
<sup>c</sup> Six control and seven IUD ewes in each ovarian state.
Concerning the DAO concentration in caruncular tissue, there was a significant interaction between treatment and ovarian status (control vs. IUD ewes x status of ewes, table 4). This interaction seemed to be due more to a consistently low DAO concentration in 3-day control ewes than to an effect of the IUD on the caruncles.

**Histamine.** Including all of the ewes represented in table 3, the concentration of histamine was greater in stromal tissue than in caruncular tissue (P<.01).

Both in stromal and caruncular tissue, the histamine concentrations varied significantly among ewes of the four endocrine states (tables 3 and 4). The effect of endocrine state was due largely to the relatively high concentrations of histamine in ovariectomized ewes, suggesting that endogenous ovarian hormones tended to suppress the histamine content of the endometrium.

In stromal tissue of IUD ewes, the histamine concentration was significantly lower in the IUD than in the non-IUD horns (tables 3 and 4). However, the effect of the IUD was influenced significantly by the ovarian state of the ewe, this interaction being due largely to the lack of effect of the IUD in 3-day ewes. The IUD did not decrease the histamine content of caruncular tissue significantly, possibly because the coils of the IUD position themselves between caruncles and thus have less contact with the caruncles than with the stromal tissue.

Except for higher concentrations of both histamine and DAO in stromal than in caruncular tissue (tables 2 and 3), there was no consistent relationship between histamine and DAO concentrations.

**Discussion**

In contrast to the marked increase caused by an IUD in the DAO content of the ovariectomized rat uterus (Chattoraj and Scommegna, 1968), an IUD tended to decrease the DAO content of sheep endometrium. Suggesting another species difference was the fact that exogenous estradiol injected into ovariectomized rats greatly increased the DAO content of the uterus while endogenous ovarian hormones of the estrous ewe decreased the DAO content of the endometrium.

The histamine content of the sheep endometrium was quite stable through the estrous cycle, but increased after ovariectomy, suggesting that endogenous ovarian hormones maintained the histamine content of the endometrium at a sub-maximum level. An IUD

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**TABLE 3. EFFECT OF OVARIAN STATUS AND INTRAUTERINE DEVICES ON HISTAMINE CONCENTRATIONS IN THE ENDO-METRIUM OF THE EWE**

<table>
<thead>
<tr>
<th>Ewe group and tissue sample</th>
<th>Status of ewes</th>
<th>3 days post-estrus</th>
<th>10 days post-estrus</th>
<th>After ovariectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control ewes b</td>
<td>Stroma</td>
<td>1.39</td>
<td>1.44</td>
<td>1.31</td>
</tr>
<tr>
<td>Caruncles</td>
<td>1.30</td>
<td>1.06</td>
<td>1.19</td>
<td>2.61</td>
</tr>
<tr>
<td>IUD ewes b</td>
<td>Stroma</td>
<td>1.33</td>
<td>1.31</td>
<td>0.86</td>
</tr>
<tr>
<td>Caruncles</td>
<td>1.16</td>
<td>1.07</td>
<td>1.24</td>
<td>2.80</td>
</tr>
<tr>
<td>Non-IUD horn</td>
<td>Stroma</td>
<td>1.66</td>
<td>1.27</td>
<td>1.35</td>
</tr>
<tr>
<td>Caruncles</td>
<td>1.08</td>
<td>1.54</td>
<td>1.16</td>
<td>2.66</td>
</tr>
</tbody>
</table>

a Micrograms of histamine per gram of wet tissue.

b Six control and seven IUD ewes in each ovarian state.

**TABLE 4. DEGREES OF STATISTICAL SIGNIFICANCE OBTAINED BY ANALYSIS OF VARIANCE OF THE DATA SUMMARIZED IN TABLES 1, 2 AND 3**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Protein</th>
<th>Diamine oxidase concentration</th>
<th>Diamine oxidase activity</th>
<th>Histamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Status of ewes</td>
<td>Stroma</td>
<td>Caruncles</td>
<td>Stroma</td>
<td>Caruncles</td>
</tr>
<tr>
<td>Control vs. IUD ewes</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>NS NS</td>
<td>NS NS</td>
<td>NS NS</td>
<td>NS NS</td>
<td>NS NS</td>
</tr>
<tr>
<td>Control vs. IUD ewes x status of ewes</td>
<td>NS</td>
<td>&lt;.01</td>
<td>NS</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>IUD vs. Non-IUD horn within IUD ewes</td>
<td>NS</td>
<td>NS</td>
<td>&lt;.05</td>
<td>NS</td>
</tr>
<tr>
<td>IUD vs. Non-IUD horn x status of ewes</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

<.01, significant at the .01 level; NS, not significant.
tended to depress the histamine content of stromal tissue in 10-day luteal phase and in ovariec-tomized ewes. With rats, Parr (1967) reported that an IUD increased the concentra-
tion of histamine in the uterus during diestrus
or pregnancy, but Wrenn, Wood and Bitman
(1969) found a decrease in histamine concen-
tration of rat uteri around the time of implanta-
tion.

An IUD evokes a chronic inflammation of
the endometrium, which is characterized in
sheep by a mild leukocytic infiltration and
elevated vascular function (Hawk, 1967;
Cooper and Hawk, 1970a). It is possible that
the depressing effect of an IUD on DAO and
histamine content of the endometrium are con-
sequences of the chronic inflammatory re-
response of the uterus to the presence of the
IUD.

Summary

A plastic spiral (IUD) was inserted by sur-
gery into one uterine horn of 28 ewes; 24 other
ewes were sham-operated. The ovaries were
removed from one-fourth of the ewes of each
group. All ewes were autopsied about 10 weeks
later, when the cycling ewes were in estrus or
3 or 10 days post-estrus. Diamine oxidase
(DAO) and histamine concentrations were
determined in stromal and caruncular tissue
of the endometrium.

Diamine oxidase concentrations were con-
siderably higher in stromal than in caruncular
tissue. The concentrations of both DAO and
histamine varied significantly among ewes of
the four endocrine states; DAO levels were
highest in 10-day luteal phase and in ovariec-
tomized ewes, and histamine levels were higher
in ovariec-tomized than in cycling ewes. The
plastic IUD tended to decrease the DAO and
histamine concentrations in the IUD-bearing
uterine horn.

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