Changes of Serum Lipids during Estrous Cycle in the Beagle

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ABSTRACT. Serum cholesterol levels were examined during two consecutive estrous cycles in female beagles. Estrogen and progesterone were also measured simultaneously. In addition, phospholipid, triglyceride and free fatty acid were measured. The peak levels of total cholesterol were 226±25 mg/100 ml at 5.3±0.5 weeks during the first estrus and 234±20 mg/100 ml at 4.8±1.0 weeks during the second estrus. The peak levels of 17β-estradiol and progesterone were 33.0±7.8 pg/ml at just after estrus and 18.7±1.6 ng/ml 2.0±1.0 weeks after the estrus, respectively. The changes in progesterone after estrus resembled those of serum cholesterol after estrus. The increase of phospholipid levels occurred slightly later and was more moderate than that of cholesterol levels. Triglyceride levels tended to be slightly higher at estrus and after several weeks of estrus, but free fatty acid did not change during estrous cycle in the female beagle.—KEY WRODS: beagle, cholesterol, estrous cycle, phospholipid, progesterone.


It is generally known that serum cholesterol levels change during pregnancy and the estrous cycle. The serum cholesterol levels in humans [13], guinea pigs [7], and rats [3, 5, 16] increase during pregnancy, while the levels decrease in monkeys [8–11, 21] and rabbits [14, 22]. The lowest serum cholesterol levels are observed at the time of ovolutions during the estrous cycle in humans [3,12, 13], but not in the ewe [15].

In dogs, the serum cholesterol level increases during pregnancy [2, 17, 19]. However, regarding the cholesterol level after estrus in beagles, Tietz et al. [19] reported an increase while Hayashi [2] reported a decrease. In some cases, high levels of cholesterol are observed with no abnormal finding on other parameters in female beagles after estrus [18]. Tietz et al. also experimented on mature male dogs under the same condition as the female [19]. Serum cholesterol of these males tended to reach minimum levels in summer and maximum levels in winter. For this reason seasonal differences were included in their results. In addition Ross and Zilversmit [14], in their study of plasma cholesterol in the pregnant rabbit, said that it was necessary for a study of this kind to keep food intake constant. Therefore, we attempt to reconfirm the changes of serum cholesterol levels in the female beagle under the controlled experimental condition in which serum cholesterol levels of male beagles remain constant [18]. Serum cholesterol levels during two consecutive estrous cycles were also measured in detail. The phospholipid, triglyceride and free fatty acid (FFA) levels were also measured, because these levels during estrous cycle have not been reported. In addition, the relationship between cholesterol, estrogen (17β-estradiol) and progesterone levels are discussed.

MATERIALS AND METHODS

Four 4-year-old female beagles, 7.0–11.2 kg, were used. These dogs were born and bred in our laboratory’s colony (Sankyo Co., Ltd., Japan), and individually caged in a controlled experimental animal room (temperature, 22±2°C, light cycle, 12 hours,
SERUM LIPIDS DURING ESTROUS CYCLE IN THE BEAGLE 875
determined by methods of GPO-p-chlorophenol, and of ACS-ACOD (Tri
glyceride G Test and NEFA C-Test; Wako
Pure Chemical Industries, Ltd.). 17β-
Estradiol and progesterone were deter-
bined by RIA methods (Estradiol H-3 kit
and Progesterone H-3 kit; Commissariat A
L’énergie Atomique, France).

RESULTS

Serum total cholesterol levels are indi-
vidually shown for two consecutive estrous
cycles in Fig. 1. The period between first
and second estrus ranged from 22 to 28
weeks. Serum total cholesterol levels in-
creased after estrus, showing the same
pattern in both estrous cycles. Its average
peak level during the first estrus was 226±25
(mean±SD) mg/100 ml, 145±19% of the
level just after estrus, at 5.3±0.5 week. That
during the second estrus was 234±20 mg/100
ml, 167±31% of the level just after estrus,
at 4.8±1.0 week. The levels of serum total
cholesterol declined to nearly the level just
after estrus between 10 and 15 weeks after
estrus in both cycles. In addition a tendency
to increase was recognized before the
second estrus.

The level of serum free cholesterol was
46±3.4 mg/100 ml and it increased after
estrus (Table 1). However, its peak was not
as marked as that of total cholesterol.

The level of serum phospholipid was
341±39 mg/100 ml and it increased after
estrus. Its peak level was 475±39 mg/100
ml, 132±13% of the level just after estrus,
at 5.8±1.0 week (Table 1). The C/P ratio
tended to remain at a higher level from the
4th to the 8th week.

The level of serum triglyceride was
47±13.7 mg/100 ml. It rose to over 50
mg/100 ml from the 2nd to 5th week and
dropped to under 40 mg/100 ml after 9th
week.

The level of serum FFA was 0.59±0.14

air ventilation, 12 times/hour). They were
fed with 200 g commercial dry dog food
once a day (DS; Oriental Yeast Co., Ltd.,
Japan), and water was given ad libitum. This
study was performed from the optimum day
of mating at estrus (0 week) to the 20th
week after estrus. The optimum day was
detected by vaginal smear method. Blood
samples were collected every seventh day
from the jugular vein between 8:30–9:00
a.m., prior to feeding. The serum was
obtained from blood centrifuged at 2,500
rpm for 10 minutes after standing 45
minutes at room temperature.

Total and free cholesterols and phospholip-
dids were determined by enzyme methods
(Cholesterol C-Test, Free cholesterol C-
Test; Wako Pure Chemical Industries, Ltd.,
Japan, Iatorset PL-E OM; Iatron Labora-
atories Medical and Chemical Products,
Japan). Cholesterol ester ratio and total
cholesterol-phospholipid ratio (C/P ratio)
were calculated. Triglyceride and FFA were

![Graph showing total cholesterol levels over time for two estrous cycles.](image-url)
Table 1. Cholesterol, phospholipid, triglyceride and FFA levels in serum after estrus in 4 female beagles

<table>
<thead>
<tr>
<th>Week</th>
<th>Total Cholesterol (mg/100 ml)</th>
<th>Free Cholesterol (mg/100 ml)</th>
<th>Ester Cholesterol ratio</th>
<th>Phospholipid (mg/100 ml)</th>
<th>C/P ratio (mg/100 ml)</th>
<th>Triglyceride (mg/100 ml)</th>
<th>FFA (mEq/liter)</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>153±15</td>
<td>46±3.4</td>
<td>70±1.0</td>
<td>341±39</td>
<td>43±2.8</td>
<td>47±13.7</td>
<td>0.59±0.14</td>
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<td>1</td>
<td>154±23</td>
<td>45±6.7</td>
<td>71±0.8</td>
<td>350±28</td>
<td>44±3.8</td>
<td>45±7.6</td>
<td>0.58±0.19</td>
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<tr>
<td>2</td>
<td>164±28</td>
<td>49±9.7</td>
<td>71±1.0</td>
<td>373±50</td>
<td>44±2.3</td>
<td>56±5.5</td>
<td>0.50±0.14</td>
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<tr>
<td>3</td>
<td>185±28</td>
<td>51±6.5</td>
<td>73±0.6</td>
<td>408±52</td>
<td>44±2.6</td>
<td>57±10.2</td>
<td>0.66±0.10</td>
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<td>4</td>
<td>208±38</td>
<td>57±11.4</td>
<td>73±1.6</td>
<td>449±60</td>
<td>46±2.6</td>
<td>52±2.5</td>
<td>0.67±0.17</td>
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<tr>
<td>5</td>
<td>218±38</td>
<td>59±10.1</td>
<td>73±1.4</td>
<td>455±60</td>
<td>48±3.9</td>
<td>54±9.1</td>
<td>0.71±0.29</td>
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<td>217±18</td>
<td>61±3.4</td>
<td>72±1.7</td>
<td>452±36</td>
<td>47±3.5</td>
<td>48±5.1</td>
<td>0.60±0.15</td>
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<td>203±19</td>
<td>58±1.9</td>
<td>71±2.3</td>
<td>443±31</td>
<td>46±5.3</td>
<td>40±14.6</td>
<td>0.64±0.25</td>
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<tr>
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<td>54±5.5</td>
<td>72±0.5</td>
<td>423±31</td>
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<td>46±7.7</td>
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<td>69±2.1</td>
<td>381±21</td>
<td>43±4.9</td>
<td>33±12.2</td>
<td>0.55±0.25</td>
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<td>70±1.3</td>
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<td>35±5.6</td>
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<td>69±1.9</td>
<td>301±16</td>
<td>40±3.0</td>
<td>32±10.8</td>
<td>0.58±0.14</td>
</tr>
<tr>
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<td>36±3.6</td>
<td>70±2.3</td>
<td>295±14</td>
<td>40±3.6</td>
<td>38±12.6</td>
<td>0.57±0.19</td>
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<td>69±2.2</td>
<td>303±25</td>
<td>40±3.2</td>
<td>33±10.7</td>
<td>0.62±0.23</td>
</tr>
<tr>
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<td>71±2.8</td>
<td>304±25</td>
<td>41±2.9</td>
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<td>0.73±0.31</td>
</tr>
<tr>
<td>18</td>
<td>124±20</td>
<td>37±5.9</td>
<td>70±1.9</td>
<td>306±22</td>
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<td>38±4.1</td>
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<tr>
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<td>302±12</td>
<td>39±3.1</td>
<td>30±11.6</td>
<td>0.61±0.15</td>
</tr>
</tbody>
</table>

a) Mean±SD.

Fig. 2. Changes of total cholesterol (mean±SD, n=4), 17β-estradiol (n=4), and progesterone (n=3) levels in serum after estrus in female beagles.
mEq/liter, and it varied from 0.50 mEq/liter to 0.73 mEq/liter throughout the experimental period (Table 1).

The level of 17β-estradiol just after estrus was 33.0±7.8 pg/ml, the highest throughout the experimental period, and decreased remarkably. The level of progesterone increased after estrus, and its average peak level 18.7±1.6 ng/ml (n=3) at 2.0±1.0 week, returning to its level just after estrus at 9 weeks (Fig. 2).

**DISCUSSION**

Serum cholesterol levels were examined during two consecutive estrous cycles in 4 female beagles. In this study, the peak levels of total cholesterol during the first estrus and the second estrus were 226±25 mg/100 ml, 45±19% rising, at 5.3 weeks, and 234±20 mg/100 ml, 67±31% rising, at 4.8 weeks, respectively. Free cholesterol levels also increased after estrus. These peak levels clearly show that the serum cholesterol increases after estrus in female beagles. It was also found that each changing pattern in the serum cholesterol level is similar during each estrus. This study showed first this fact.

Tietz et al. [19] reported that serum cholesterol levels in female multiparous beagles increased from 166±35 mg/100 ml at pre-estrus (1–5 weeks before estrus) to 307±65 mg/100 ml, 85% rising, at 7.8 weeks after estrus. There are some differences between their results and ours. These differences, in rising rate and peak time of the total cholesterol level, may be based on duration of estrus.

It is reported that serum cholesterol levels decreased following estrogen administration in rats [20] and cows [6]. In this study, however, the changes of serum cholesterol level after estrus resemble those of progesterone, rather than estrogen level. The progesterone is considered to be one factor which affects serum cholesterol increase after estrus in the female beagle.

Serum phospholipid also increased after estrus in the female beagle. The increase of phospholipid levels occurred slightly later and was more moderate than that of serum cholesterol, so that the C/P ratio remained at a high level. The changes of serum phospholipid were closely related to changes of serum cholesterol in beagles. It has been shown that serum phospholipid increase concomitantly with serum cholesterol in women during pregnancy and during the menstrual cycle [1, 12]. Serum phospholipid decrease at a constant C/P ratio are also reported in monkeys [8, 9] and rabbits [22].

It was observed that triglyceride levels in female beagles tended to be slightly high at estrus and after several weeks of estrus, while FFA levels do not change during the estrous cycle. Serum triglyceride levels in humans [4], guinea pigs [5], monkeys [9, 10] and rabbits [21] increase during pregnancy. The changing patterns of serum triglyceride levels in these mammals differ from those of their cholesterol and phospholipid levels. This suggests that increased serum triglyceride levels in the female beagle during estrous cycle are not directly related to the changes of cholesterol and/or phospholipid level.

Therefore, these results indicate that serum lipids in the beagles must be studied under a controlled condition, and that the estrous cycle must be closely observed in order to determine the cholesterol and/or phospholipid levels in female beagles.

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**REFERENCES**


