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Author(s)	TSUTSUMI, Yoshio; TAKAHASHI, Masahiro; OGURI, Norihiko et al.
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TOTAL LEUCOCYTE COUNT OF THE FEMALE RABBIT AT THE VARIOUS REPRODUCTIVE PHASES

Yoshio TSUTSUMI, Masahiro TAKAHASHI, Norihiko OGURI, and Yoshio HACHINOHE

(Department of Animal Science, Faculty of Agriculture, Hokkaido University, Sapporo, Japan)

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There are numerous reports of the normal haematological ranges found in different species under various physiological conditions. Fluctuations in the blood cellular components have also been correlated with reproductive phases. Most of the latter, however, have been fragmentary with few continuous systematic studies. The present paper is the concluding report in a series of studies of body temperature (56) and blood cellular components (57) in the female rabbit during the stages of the reproductive cycle and deals with the variations in total leucocyte count.

MATERIALS AND METHODS

Fourteen female and two male, 3 to 4 kg, mature, Japanese native breed rabbits were isolated in individual cages in a room lighted by outside windows. They received water and feed *ad libitum*. Blood samples were taken twice daily (8 A.M. and 4 P.M.) by puncturing the marginal ear vein with a needle. The blood was drawn into a melangeur and diluted with Türk's solution. Total leucocytes were counted in duplicate on a hemocytometer. The stage of the estrous cycle was judged by variations in the level of free vaginal epithelial cells, in the crystal pattern of the dried vaginal smear, and the vaginal pH as described by TSUTSUMI (54).

RESULTS

1. Diurnal variation

From 9 A.M. July 8, 1966 to 12 A.M. July 9, blood samples were collected every 3 hrs. from 10 rabbits (8 females and 2 males). The leucocyte count was $104.18 \pm 0.85 \times 10^2/mm^3$ (Mean $\pm$ S.E.) for females and $55.12 \pm 1.34 \times 10^2/mm^3$ for males (Fig. 1). The difference between male and female may

have been due to strain since they were obtained from different sources. There was no significant diurnal variation although the average count was higher in the morning than in the afternoon.

A comparison of the morning and afternoon counts of the period January 24 to February 22 with the period May 24 to July 15 in 11 does showed significantly higher leucocyte counts in the morning than in the afternoon ($P<0.001$) 58.87 ± 1.11 and 52.79 ± 1.10 in the first period and 97.15 ± 2.05 and 89.25 ± 2.12 $\times10^2/mm^3$ in the second period for morning and afternoon respectively. It should be noted that the does used in these two periods were not the same rabbits and were obtained from different sources.

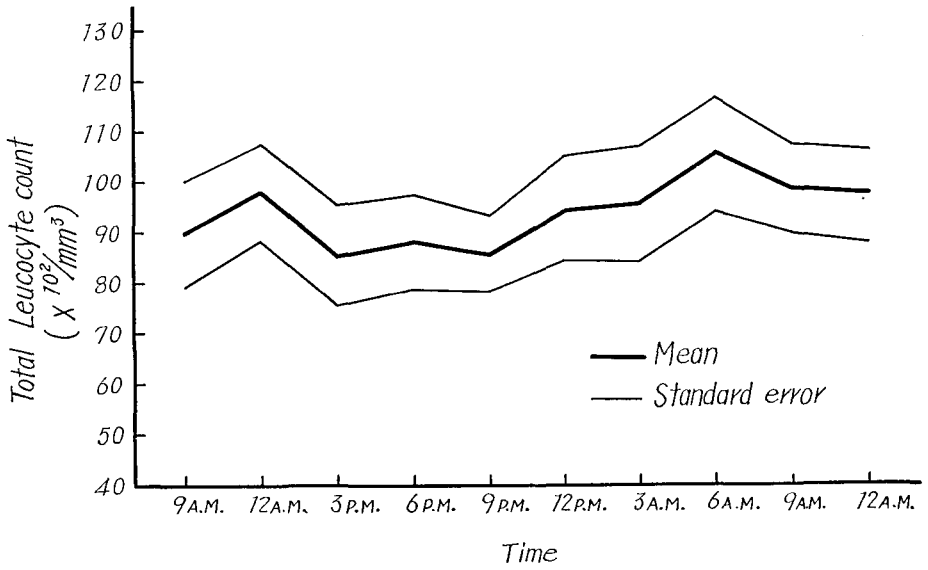

Fig. 1. Diurnal variation in the total leucocyte count for 10 rabbits.

2. Variations during the estrous cycle

During the 2 periods described in section 1, the stage of the estrous cycle was determined by observing vaginal mucus and smears each morning and afternoon daily. There was no clear fluctuation in the leucocyte count to correspond with the estrous cycle. Individual rabbits, however, did appear to have some cyclic variations especially in the morning counts. The afternoon count appeared to be markedly influenced by environmental conditions and therefore, this report will consider only the morning count. Figure 2 shows the results for one rabbit. The average time span between troughs was $5.78\pm$ 0.32 days for 11 does while the average estrous cycle length was 5.87 ± 0.50

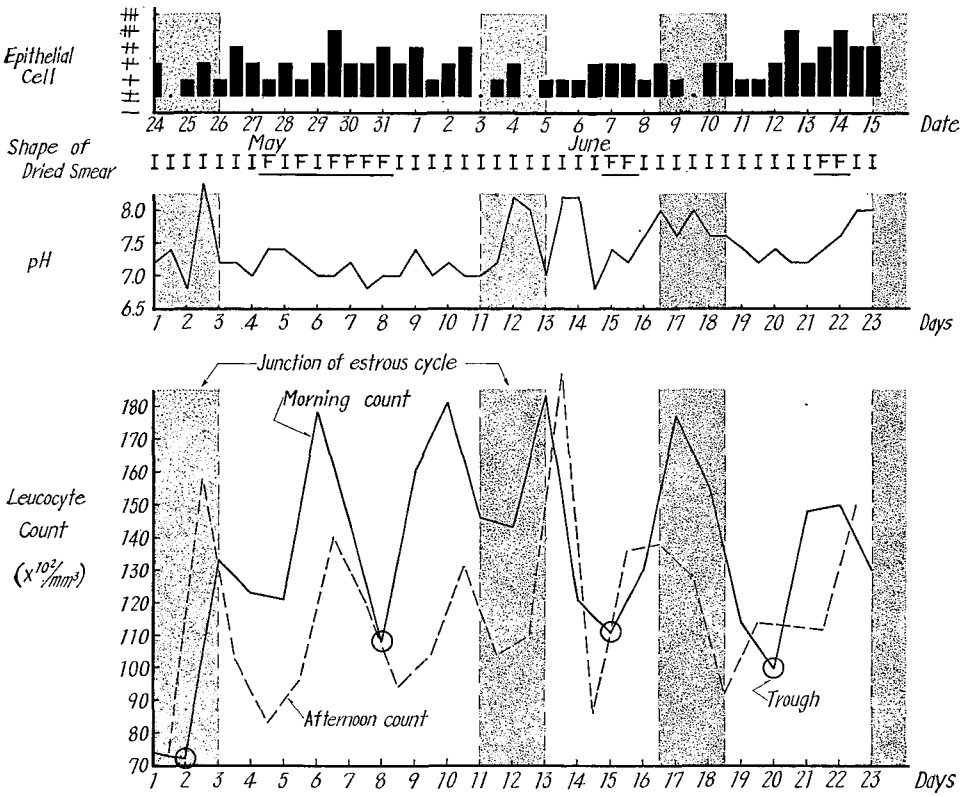

Fig. 2. Illustration of cyclic fluctuation in the vaginal mucus and vaginal smear, of pH, and of leucocyte count variation in one animal. The vestibule was flushed with physiological saline and the flushing placed on two slides. One slide was used for observation of the number of epithelial cells and the other for the shape of dried smear. The pH value of vaginal mucus was checked using pH test paper. In the dried smear, F type shows fern- and chrysanthemum-like figures, and I type shows polygonal and other irregular figures.

days for these does during the same period.

3. Variations after copulation

Leucocyte counts were made on 5 does at 2-hour intervals starting at copulation at 8 A.M. and ending 14 hrs. later at 10 P.M. July 26. Same procedure was repeated on the other 3 does the next day. The average count decreased markedly at 4 P.M. or 8 hrs. after copulation (Fig. 3). The average count varied from 94.75 ± 4.20 before copulation to 97.87 ± 6.06 from 4 hrs. after copulation to 83.25 ± 4.40 eight hrs. after copulation to 88.75 ± 3.33 twelve

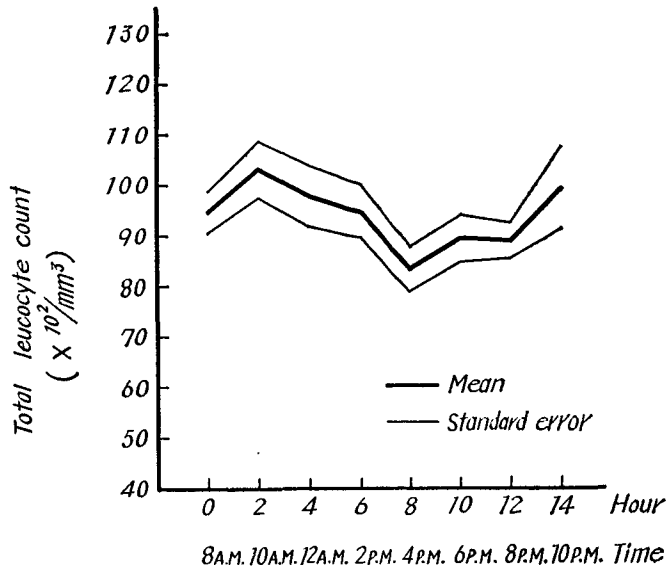

Fig. 3. Changes of total leucocyte count for 8 females from copulation to 14 hours post copulation.

hrs. after copulation. The value 8 hrs. after copulation was significantly lower than the other three values ($P<0.05$).

4. Variations during pseudopregnancy

Seven animals mated to bucks were pseudopregnant (Table 1). There was no significant fluctuation of the leucocyte count during pseudopregnancy (Fig. 4). A comparison of the leucocyte count during the 14 days preceding pseudopregnancy to the 14 days during pseudopregnancy (day 2 to 15 after copulation) showed morning counts of 85.26 ± 2.10 before and 87.15 ± 1.88 during pseudopregnancy (no significant difference). There was a tendency for the count to decrease on about the 18th day but this was not significant.

TABLE 1. The pseudopregnant does

Animal	Date of mating
811	February 14, 1966
812	February 14, 1966
C	July 26, 1966
D	July 26, 1966
E	July 26, 1966
A	July 27, 1966
F	July 27, 1966

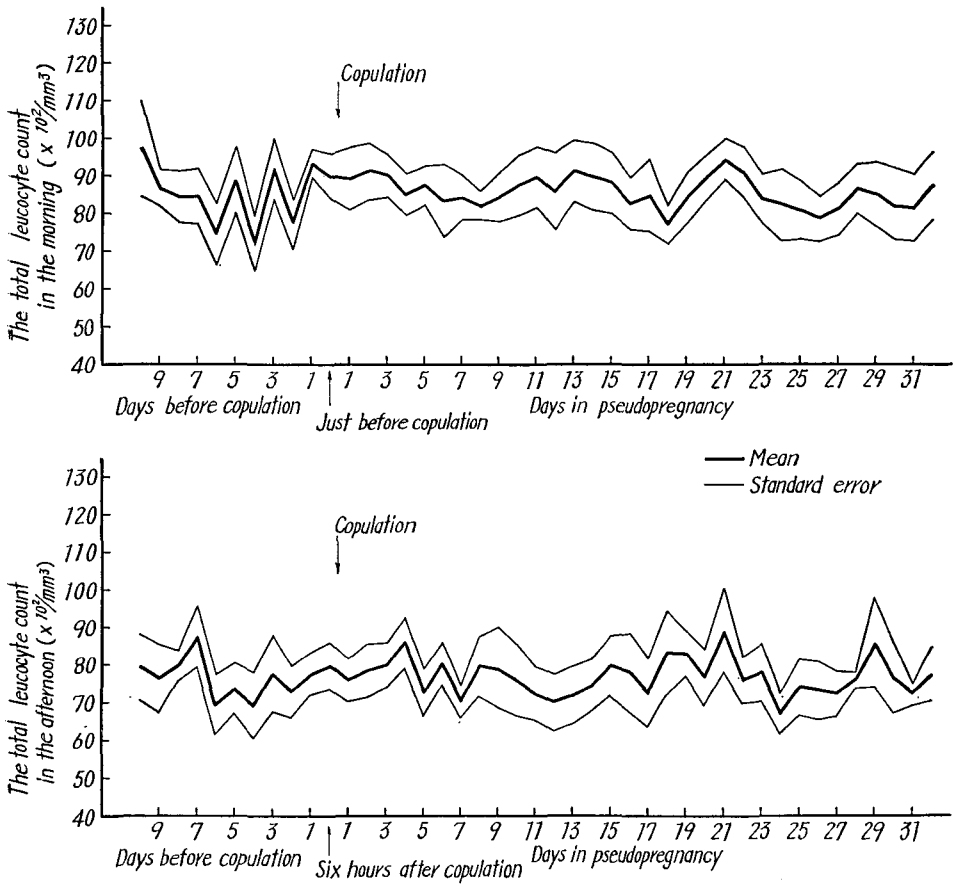

Fig. 4. Variation in total leucocyte count for 7 pseudopregnant rabbits.

5. Variations during pregnancy

Changes in the morning count of 13 does during pregnancy are shown in Figure 5. The counts were more variable during pregnancy than before, but an overall pattern of change was observed during pregnancy. The mean count increased gradually to reach a peak on the 16th day after which it decreased to a low on the 26th or 27th day of pregnancy. The average count was 84.68 ± 1.45 for the week before pregnancy, 94.17 ± 1.65 from the 5th to 18th day of pregnancy and 69.46 ± 2.03 from the 23rd day of pregnancy to the day before delivery. These differences were significant ($P<0.01$). A slight rise in the leucocyte count was observed in some does 1–4 days before parturition.

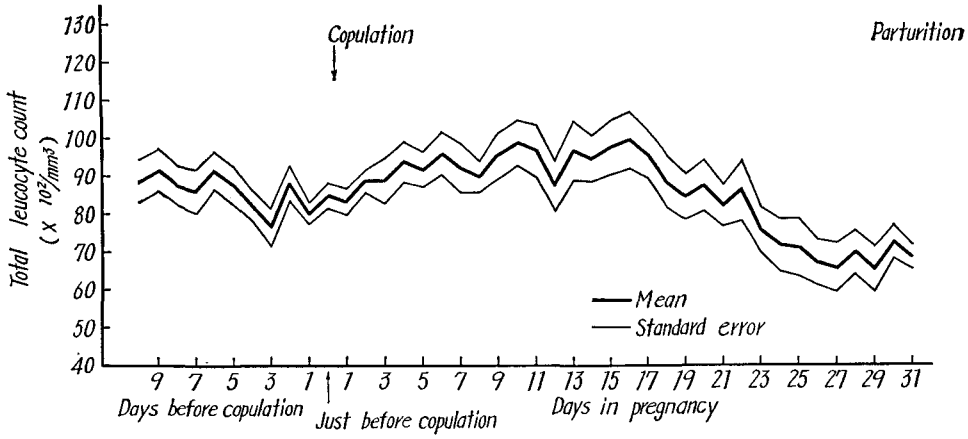

Fig. 5. Changes in total morning leucocyte count for 13 females during pregnancy.

On the basis of litter size and length of pregnancy the 13 does were divided into two groups for a closer inspection of the data. Group one was composed of 7 does with 5 or more young and gestation periods of 30 and 31 days. Group two was composed of 6 does, 4 with 2–3 young per litter and 2 with gestation periods of 27 and 29 days (Fig. 6, Table 2). During the last half of pregnancy the leucocyte counts for the does in group 1 showed

TABLE 2. Gestation length and litter size

Animal	Date of mating	Gestation length (day)	Litter size
F	September 26, 1966	27	9
I	August 19, 1966	29	9
B	July 27, 1966	30	7
717	September 9, 1963	30	10
A	September 26, 1966	30	7
811	October 5, 1966	30	6
710	June 9, 1963	31	5
712	June 9, 1963	31	9
D	September 24, 1966	31	3
E	September 24, 1966	31	8
309	June 9, 1966	31	2
C	October 3, 1966	32	2
611	September 9, 1963	33	2

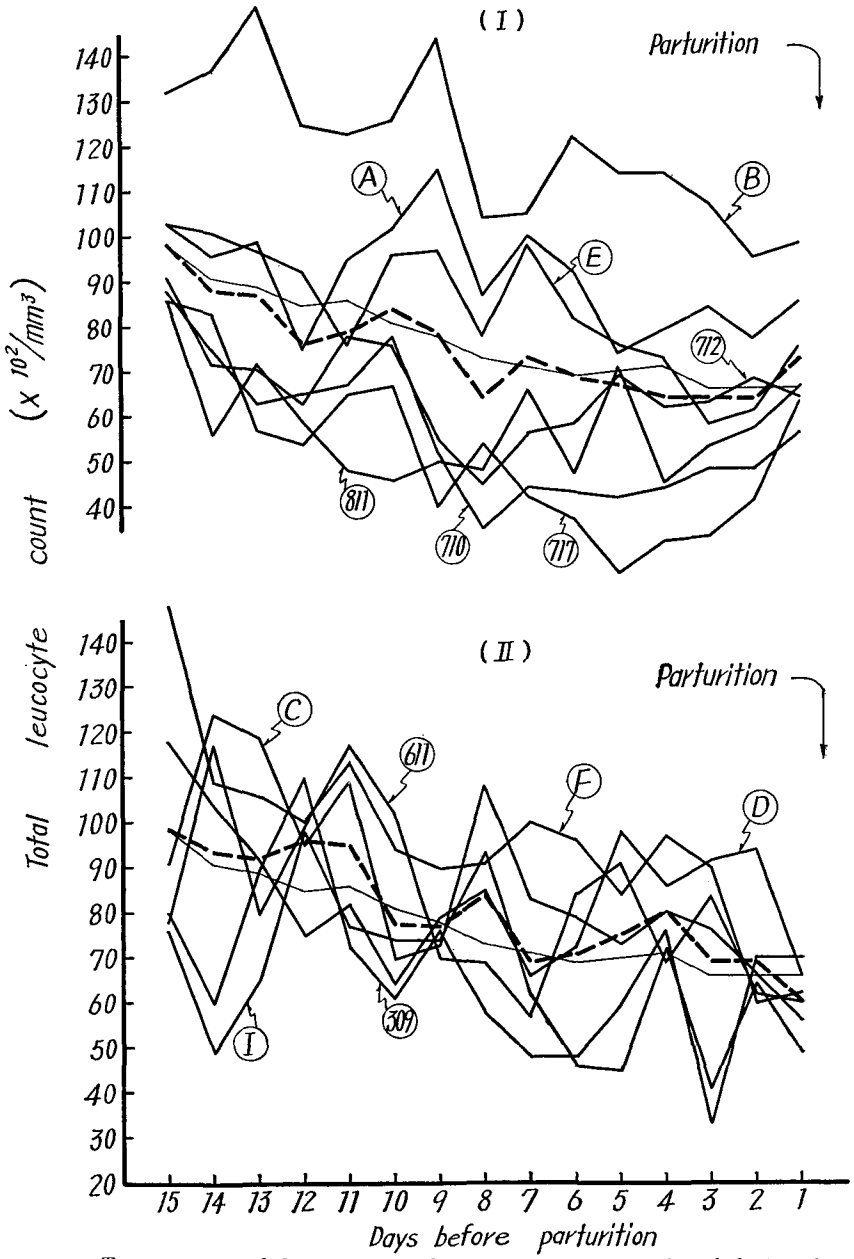

Fig. 6. Two patterns of fluctuations in leucocyte counts were found during the latter half of pregnancy. In group I fluctuations tended to be less extreme within each doe than in group II. The does in group II showed irregular fluctuations in the count as compared to group I.
—— Average count in all animals. ---- Average counts in each group.

some similarity with the count dropping slowly until 4 days before parturition and then rising slightly the last 4 days (Fig. 6). Group two does showed an irregular but steady decrease in leucocyte counts up to the day of parturition (Fig. 6).

6. Variations just prior to and following parturition

Leucocyte counts were made on 7 does (A, B, D, E, F, I, and 811 in the table 2) for 54 days following parturition. The counts increased significantly after parturition from 80.76 ± 2.63 for the 10 days preceding parturition to 98.70 ± 3.89 for the 10 days following parturition ($P < 0.001$) (Fig. 7). The highest level was reached from the 3rd to 5th day post delivery when the count averaged 170% of the level during the pre-parturition period.

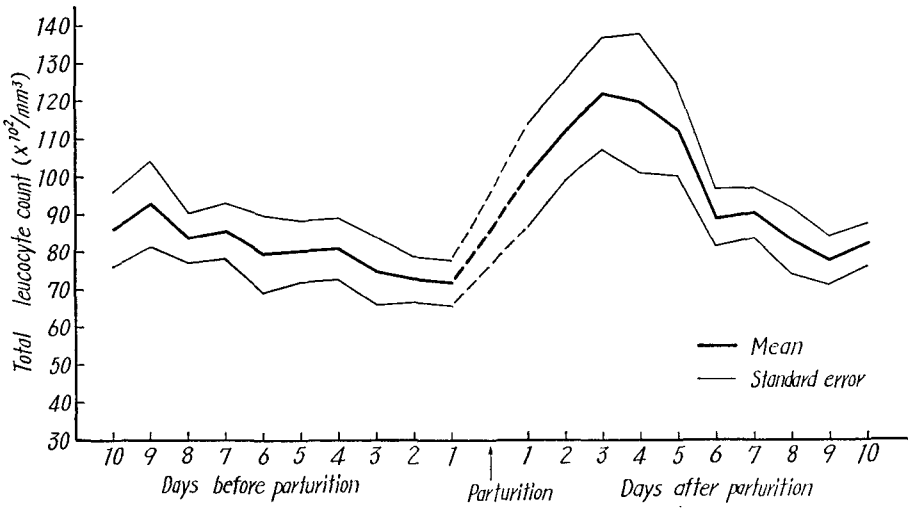

Fig. 7. Variation in total leucocyte count for 7 females before and after parturition.

TABLE 3. Average leucocyte counts of 7 does in different stages after parturition

Days after parturition From—To	Number of counts	Average leucocyte count ($\times 10^2/mm^3$) (Mean ± S.E.)
1— 6	42	109.05 ± 5.54
7—14	56	83.19 ± 2.28
15—22	56	100.78 ± 3.81
23—30	56	98.71 ± 2.58
31—42	84	106.36 ± 3.35
43—54	84	92.50 ± 1.89

All does nursed for 30 days. Figure 8 shows the counts during the 30 days of lactation and 24 days post lactation. The curve shows 3 periods of higher leucocyte activity at about the 4th, 18th and 37th days and lower activity around the 9th, 30th and 47th days. The average counts in each period are given in Table 3. Milk secretion continued until about the 41st day after which milk could not be squeezed from the nipples. The count fell markedly after the 42nd day until it reached normal levels on the 47th day after parturition or 17 days after weaning.

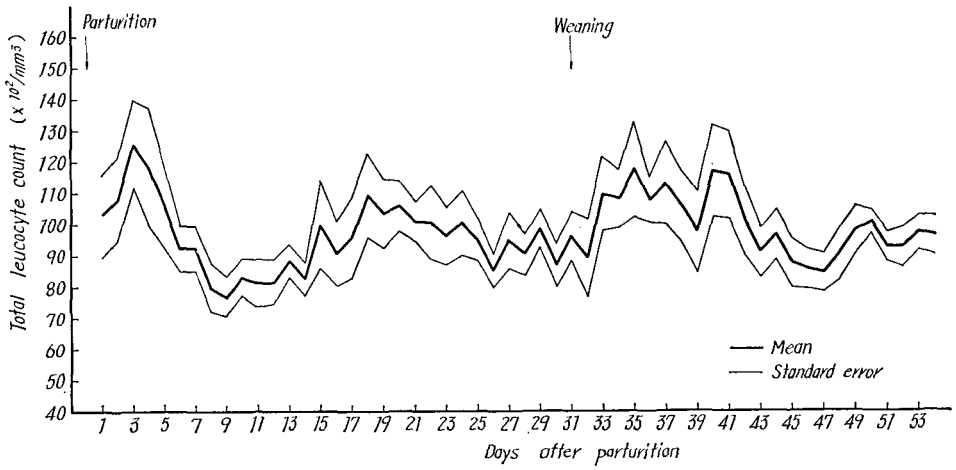

Fig. 8. Changes in total leucocyte count for 7 females for 54 days following parturition.

DISCUSSION

General haematological studies have been made in rabbits by many investigators showing total leucocyte fluctuations under various physiological conditions (6, 8, 27, 31, 38, 39, 41, 42, 44, 49, 51). Evidence of diurnal variation in leucocyte count has been described for man (45, 49), dog (1), and mouse (4). Diurnal variation has also been shown for rabbits but the reports have not been very consistent (5, 8, 21, 40). No clear pattern of diurnal fluctuation was seen in the present study with as 3 hr. interval between collections but morning counts over the entire study as a whole were higher than the afternoon counts as described by CHENG (8).

It is well known that a close relationship exists between adrenocortical activity and the estrous cycle. A correlation between adrenocortical function and eosinophil count is postulated by many investigators. In mice the total leucocyte count remains low in diestrum rising to its high in estrum (52).

In rats a leucopenia is shown at the peak of estrus (13). MOBERG (32, 33) states in cattle the highest leucocyte count which is associated with a definite neutrophilia is found at estrus, while KERR *et al.* (26) saw no marked reaction of the leucocytes during estrus. SOLIMAN and SELIM (46) report leucocytosis, neutrophilia and eosinopenia are associated with estrus in the water buffalo.

Some attempts have heen made to determine the physiological relationship between the reproductive cycle and the leucocyte count but results have been inconclusive. CRAFTS (9) reports a slight increase in leucocyte count, while VOLLMER *et al.* (61) saw no distinct trends in either total or differential counts after hypophysectomy in rats. Variations in leucocyte counts have been very inconsistent duing estrogen treatment in rate (47, 50). In dogs, however, large doses of estrogen produce a profound leucocytosis followed by a leucopenia (2, 7, 10, 11, 58). Monkeys did not show this variation in leucocyte count (10, 59). KALLELA and MOBERG (22) report a decrease in eosinophils and a tendency toward neutrophilia in sheep receiving fodder containing plant estrogens. Progesterone has been shown to produce a typical vaginal inflammatory response in germ-free mice (3).

The results in the present study show cyclic waves in the leucocyte count during the estrous cycle but no correlation with different parts of the cycle. SAKURAI and UEMATSU (43) reported a close correlation between the estrous cycle and eosinophil count in rabbit blood. The similarity of the estrous cycle length (5.87 days) and interval between leucocyte cyclic waves (5.78 days) suggests there may be some relationship between the estrous cycle and leucocyte count which was not seen in the present study.

In women many workers have shown the ovulatory response has a definite effect on the eosinophil count (reviewed by MATSUDA and FUJIMOTO, 28). CHENG (8) mentions a slight leucocytosis is observed in female rabbits 1–13 hrs. after copulation. The present study showed a slight decrease in leucocyte count at 4 P. M., about 8 hrs. after copulation, but it is not clear whether this is a diurnal variation or a true reflexion of the ovulatory response in the blood.

No significant fluctuations in leucocyte counts were seen during pseudopregnancy. There was a noticeable decrease in the morning count on about the 18th day which may have been associated with the end of pseudopregnancy.

Leucocytosis has been associated with pregnancy in women (14–16, 53, 63), rats, guinea pigs, and dogs (1, 24). In goats the percentage of lymphocytes is reported to increase with a concurrent decrease in neutrophils and eosinophils during pregnancy (34). In cattle MOBERG (33) mentions an increased leucocyte count reaching a maximum during the 3rd month while KHAJURIA and RAZDAN

(26) saw no change during pregnancy. Leucocytosis, neutrophilia, lymphopenia and eosinophilia are reported for the last few months of pregnancy in buffaloes (46).

In rabbits TATARA (51) briefly mentions a leucocytosis of pregnancy. The detailed observations on leucocyte counts by CHENG (8) could be interpreted as follows: (a) a slight leucocytosis during the first 2 weeks of pregnancy; (b) a steady decrease in counts during the 3rd week; (c) a marked leucopenia during the 4th week; (d) a slight rise 2–4 days prior to parturition but levels are still below normal non-pregnant counts. In general this agrees with the present study showing leucocytosis during the first half and leucopenia during the last half of pregnancy. The transitional period was between day 19 and 22 of pregnancy. TSUTSUMI and MATSUMOTO (55) report abundant reddish opaque vaginal mucus after about the 19th day of pregnancy while scanty mucus was obtained from the 5th to 18th days of pregnancy. This suggests some major physiological changes may be occurring around the 19th day of pregnancy.

The pregnant does in this study could be divided into two groups based on litter size and gestation period. Group one which might be designated normal had a litter size of 5 or more and a gestation of 30–31 days. Leucocyte counts were consistent among these does while the counts in group 2 were quite erratic suggesting possible differences in the physiological conditions of the systems in the two groups. The vaginal mucus reported by TSUTSUMI and MATSUMOTO (55) occurring around the 29th day of pregnancy may be associated with the slight rise in the leucocyte count seen in this study.

The bulk of reports on cellular constituents at parturition are for women (14, 36, 37, 53, 62, 63) and cows (19, 25, 29, 30, 35, 48). In general the leucocyte count increases markedly with the highest count achieved within a few hours following parturition. This response is thought to be related to the secretory activity of the adrenal cortex in response to the stress of delivery. Anemia is reported 3 hrs. after parturition in the buffalo with a rise in total leucocyte count. A significant eosinopenia, neutrophilia and lymphopenia results in leucocytosis (46). The tendency for leucocytosis is also reported in rats, guinea pigs and dogs (24). The present reports shows a marked leucocytosis in the rabbit by the 3rd to 5th day after parturition.

During active nursing a leucopenia is reported in the albino rat (12) suggesting that leucocytes participate in the activities of lactation. ANDERSEN and GEE (1) report partial recovery in the cellular constituents of the blood in dogs but complete recovery did not occur until after weaning. In cattle there are reports of leucocytosis and eosinophilia by some investigators (20,

26) while others report no marked fluctuation (19). HANSEN *et al.* (17) report lowered leucocyte levels in lactating mares. CHENG (8) reports (a) a leucocytosis in lactating as well as non-lactating rabbits during the 1st week after parturition; (b) a return to normal during the 2nd week of lactation with no further leucopenia. The present study showed 3 waves of increased leucocyte count with no leucopenia during the first 54 days following parturition. The 1st rise may be due to the stress of parturition, the 2nd to high lactation activity and the 3rd to weaning.

SUMMARY

The total leucocyte count was determined for 14 female rabbits during different reproductive states. During estrus there was no consistent correlation between changes in count and the stage of the cycle although the average time between peak counts (5.78 ± 0.32 days) was similar to the estrous cycle length (5.87 ± 0.50 days). The leucocyte count showed a slight decrease about 8 hrs. after copulation when samples were collected every 2 hrs. starting with copulation at 8 A.M. There was no consistent pattern of fluctuation during pseudopregnancy other than a temporary slight decrease about the 18th day. Pregnancy showed 2 phases in the count, a gradual rise to the 16th day followed by a gradual decrease to the 26th or 27th day. During the last half of pregnancy does which delivered 5 or more young with a gestation length of 30 to 31 days showed similar patterns of leucocyte fluctuation while does with less than 5 young and gestations of 27, 29, 32 or 33 days showed counts with much higher daily fluctuation. The leucocyte counts increased markedly after parturition reaching a peak on the 3rd or 4th day after which they decreased. There were 3 peaks in the leucocyte count during lactation and following weaning. The peaks occurred on about the 4th, 18th and 37th days after parturition. By the 47th day after parturition the total leucocyte counts had returned to the normal pre-pregnancy levels.

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