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Author(s)	TSUTSUMI, Yoshio; TAKAHASHI, Masahiro; OGURI, Norihiko; HACHINOHE, Yoshio
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RED BLOOD CELL COUNT IN RELATION TO REPRODUCTIVE PHASES IN THE FEMALE RABBIT

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

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

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It is an accepted practice to count the erythrocytes in the blood stream on the correlation of the reproductive activity in women, with anemia during pregnancy (31, 37, 62, 75, 76, 97-99, 107, 110, 128, 130). In farm animals the red blood cell counts have, also, been reported in relation to reproductive phenomena. Most of them are treated fragmentarily, with few systematic and continuous studies. There are many works on the normal range of the red blood cell counts in various animals: dairy cows (54), goats (7, 42, 43), sheep (50, 81, 122), dogs (87, 129), and rabbits (13-15, 48, 51, 55, 58, 79, 80, 84, 129).

HIRAGA *et al.* (40), STRAUB *et al.* (96), MIYAKE *et al.* (68) and MURAKAMI (70) observed the fluctuations of the number of erythrocytes in pre- and post-parturition of cows, and ISHII (46) described the effect of lactation on the red blood cell count. The blood picture in connection with persistency of follicles in cows was reported by MOBERG (69). SOLIMAN and SELIM (90) observed the blood picture of buffaloes at the various reproductive phases and stated that pregnancy was accompanied by anemia, characterized by a drop in the erythrocyte number and hemoglobin content, and the blood picture was manifested by a drop in the erythrocyte number soon after labour. In mares, HANSEN *et al.* (39) observed the blood pictures of lactating and non-lactating ones. ROY *et al.* (85) described briefly changes in the red blood cell count in goats during pregnancy, parturition and post-parturition periods. Similar observations were made by ANDERSEN and GEE (4), KAWACHI (53) and TIETZ, JR. *et al.* (108) on dogs. In rabbits, HORGER and ZARROW (44), TATARA (106) and ZARROW and ZARROW (131) described the anemia of pregnancy.

It seems that red blood cell concentration is constant in healthy animals because of regulatory control of the red cell production and destruction. It has long been known that in the adult animals the red blood cells are formed

under ordinary circumstances in the red bone marrow, and anoxia is regarded as the fundamental stimulus for erythropoiesis. Furthermore, it became clear recently that erythropoietin (erythropoiesis stimulating factor) act on the erythropoiesis directly or primarily (11, 25-27, 33, 34, 41, 47, 60, 66, 72, 73, 82, 93, 94).

Many works on erythropoiesis have been made on a basis of endocrinology. The anemia following hypophysectomy in rats is well known evidence (17, 21, 120, 125-127). And the anemia was induced by a resultant hypothyroidism, hypoadrenocorticalism, and lack of growth hormone that occur after hypophysectomy. These losses do not exert themselves directly upon the bone marrow but secondarily, through a decrease in general metabolism; this decrease in metabolism leads to decreasement of the animal's need for oxygen, and this decreased oxygen-need results in a decrease erythropoiesis in the bone marrow, possibly through a decreased amount of erythropoietin in the blood plasma (CRAFTS and MEINEKE, 21).

Thyroidectomy or hypothyroidism cause anemia in animals such as rabbits (57), and rats (17, 32, 121). It seems that thyrioid hormones increase the body metabolic rate and these induce the production of erythropoietin (73). The functional alterations in the thyroid and in the serum or pituitary thyrotropin level were reported as correlating with the estrous cycle (8, 9, 12, 28, 88, 89), and with the pregnancy and lactation in rats (117). A number of investigators have reported definite changes in the basal metabolic rate during the menstrual cycle in women. LEE (59) recognized that heat production increased toward the end of the diestrum and the first of pro-estrus in rats.

A sex difference in red blood cell count has been observed. The count, in general, has been found to be higher in the male than that in the female animals and birds (1, 24, 49, 52, 77, 83, 84, 95, 100), although a few reports describe the number of erythrocytes as about equal in the blood of male and female animals (56, 78). These results lead to research on the effects of castration, and of sex hormones on red blood cell counts of various animals. It is generally agreed that androgen stimulates the erythropoiesis and estrogen produce anemia by an inhibiting effect on the erythropoiesis: men (61, 101), monkeys (18, 118), dogs (5, 16, 20, 119), hamsters (91), rats (19, 22, 32, 92, 102-105, 125, 127), mice (29) and birds (100). MIRAND *et al.* (65) investigated the mechanism of testosterone action in erythropoiesis and showed that the increased erythropoietic activity of the plasma of animals receiving androgen is due to the erythropoietin. MIRAND and GORDON (64) describe how estrogen inhibits erythropoiesis by suppressing the production of an extrarenal precursor of erythropoiesis-stimulating factors which requires activation by a kidney

mechanism for elaboration of the functional circulating erythropoiesis-stimulating factor.

Recently it was suggested that red blood cells might be an important vehicle for steroids. VERMEULEN (124) showed that corticoids were absorbed at the red cell surface. AGARWAL and GARBY (3) observed an inhibitory action by a number of various corticosteroids on the hemolysis of human red blood cells *in vitro*. AGARWAL and CARSTENSEN (2) postulated that the differences observed in the inhibitory activity of several steroids could be due to differences in uptake on or in the red blood cells from the medium. A binding affinity of progesterone and aldosterone to red blood cells was investigated by DEVENUTO (23) to elucidate the role played by erythrocytes in steroid transport. The uptake of progesterone by the erythrocytes was of the order of 70-85% of the total steroid in contact with the red blood cell, whereas the uptake of aldosterone is insignificant. And it was concluded that the steroid does not enter into the red blood cells, but could be absorbed at the cells' surface.

The recent progress reviewed above induced an interest in the role of the red blood cell in various reproductive states. The present study investigated the fluctuations of the number of erythrocytes in blood of estrous, pseudopregnant, pregnant and lactating rabbits.

MATERIALS AND METHODS

Eight adult female and two adult male rabbits, Japanese native white breed, ranging in weight from 3 to 4 kg were used. They were kept isolated in cages under identical conditions in a single room and under natural lighting condition through windows, and received food and water *ad libitum*.

Blood was secured by puncturing the marginal ear vein. The sample of blood was collected immediately in a melangeur and diluted with Hayem's solution. The red blood cell counts were made with Thoma-Zeiss's hemocytometer.

The stage of the female estrous cycle was judged by fluctuations in levels of free vaginal epithelial cells, in crystal patterns of dried vaginal smear, and vaginal pH described by TSUTSUMI (111). During two periods (May 24 to June 13, and July 13 to July 26, 1966) the estrous cycle was observed. From July 27 to November 15, 1966 pregnant, pseudopregnant and lactating rabbits were investigated.

The blood samples were obtained every morning (8 a.m.) and afternoon (4 p.m.) during these periods.

RESULTS

1. Diurnal variation

From 9 o'clock in the morning of July 8 to noon the next day, blood samples in 10 animals were obtained at three-hour intervals. Room temperatures remained nearly constant, averaging 19.8°C. The results are demonstrated in Figure 1. Mean red blood cell count in this case was $609.6 \pm 52.43 \text{ } 10^4/\text{mm}^3$ (Mean \pm S.D.). No clear diurnal variation was recognizable, because of the irregular and wide variance in the counts.

A comparison of the red blood cell count in the morning and in the afternoon during two periods showed no significant difference in either two period. Mean red blood cell counts were $601.85 \pm 64.73 \text{ } 10^4/\text{mm}^3$ in the morning and $597.84 \pm 98.64 \text{ } 10^4/\text{mm}^3$ in the afternoon for the first period, and $614.58 \pm 49.77 \text{ } 10^4/\text{mm}^3$ in the morning and $615.4 \pm 51.23 \text{ } 10^4/\text{mm}^3$ in the afternoon for the second period.

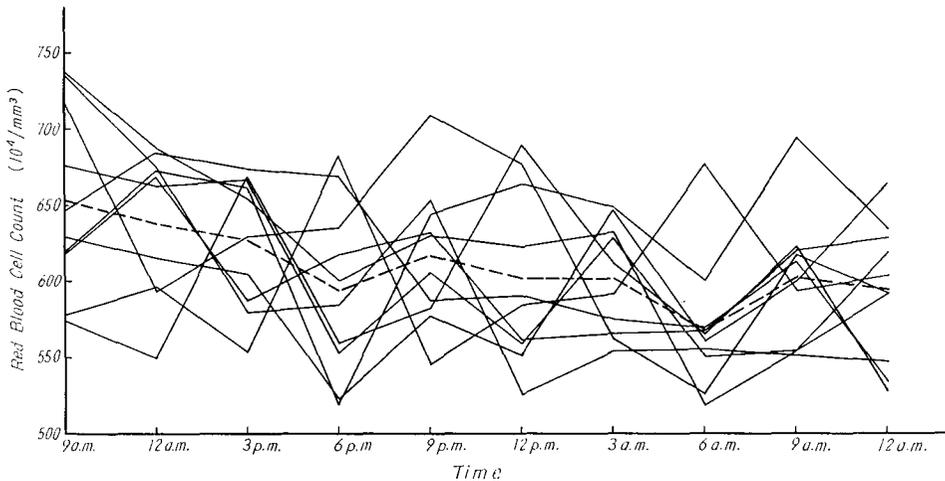


Fig. 1. Diurnal variation in the red blood cell count.
--- Average of 10 rabbits.

2. Estrous cycle variation

The estrous cycle was checked by observations of the vaginal mucus and by vaginal smears every morning and afternoon. There was no clear corresponding fluctuation between the red blood cell count and the estrous cycle, although the fluctuations of the red blood cell counts for each doe showed some cyclic waves in both morning and afternoon counts. In the present study the morning counts were noted, because it seemed that body conditions in the

morning were more stable than in the afternoon. The average length of time between troughs in the morning curves of the red blood cell counts of all females was 5.56 ± 1.56 days and average length of estrous cycles was 5.87 ± 2.65 days. There was no significant difference between these values. As an example, the results of observations on one animal are illustrated in Figure 2.

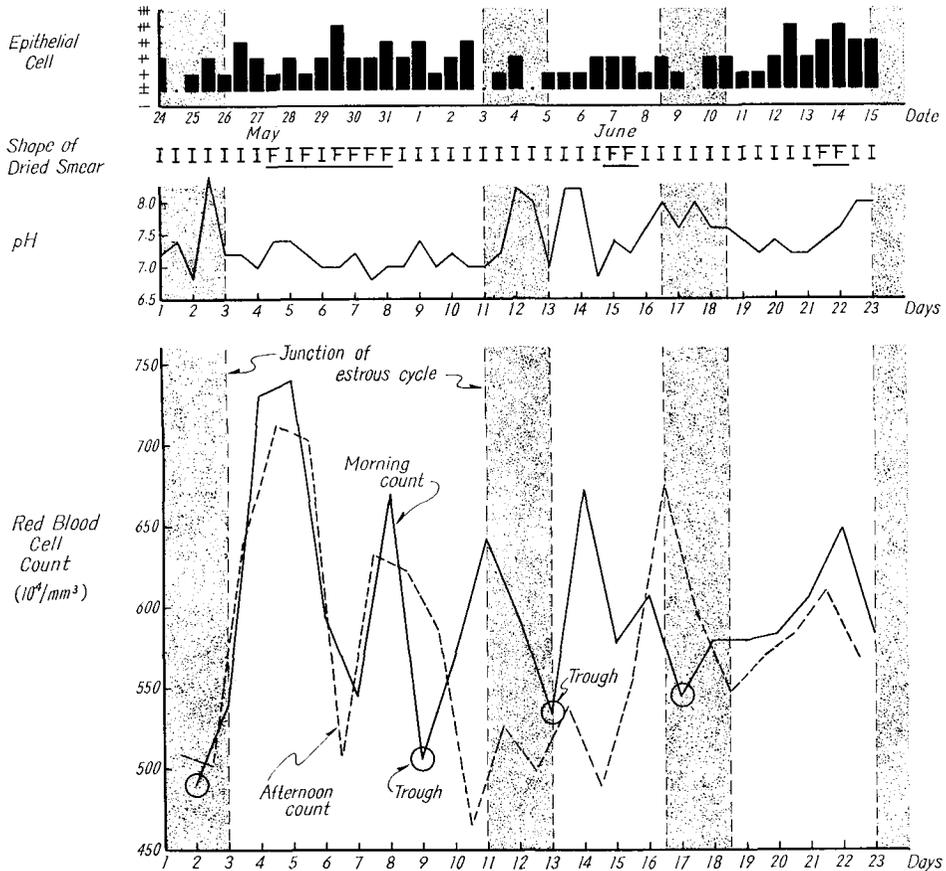


Fig. 2. Illustration of cyclic fluctuation in the vaginal mucus and vaginal smear, of pH, and of red blood cell count variations in one animal. The vestibule was flushed with physiological saline and the flushing placed on two slides. One slide was used for observation of the number of epithelial cells and the another 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.

3. Variation after copulation

The red blood cell counts of eight females were made at 2-hour intervals, beginning just before copulation at 8 a.m. and continuing until the 14th hour at 10 p.m. on July 26. The results are given in Figure 3. No variation associated with copulation or ovulation was noticeable.

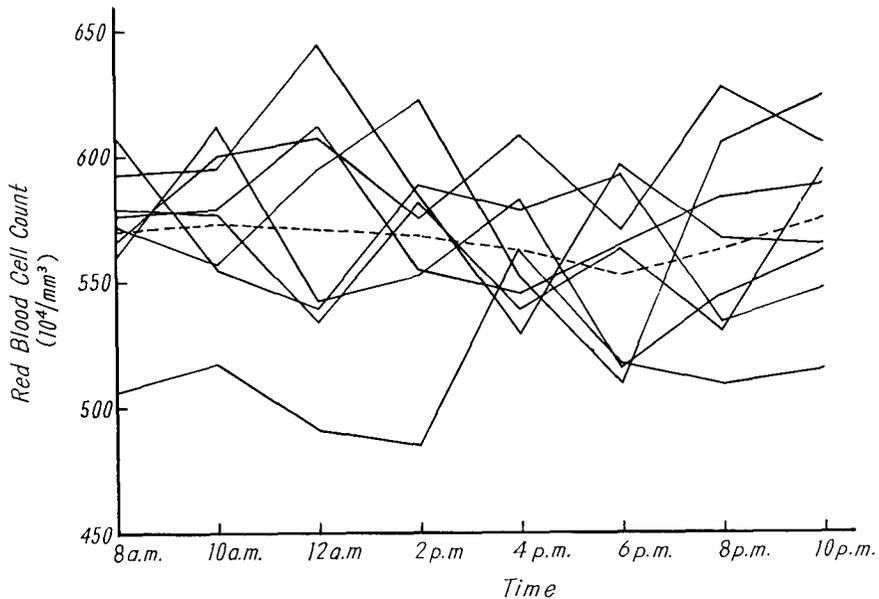


Fig. 3. Changes of red blood cell count after copulation to 14 hours later.
 --- Average of 8 females.

4. Variation during pseudopregnancy

Five females which had been mated with bucks were identified as being pseudopregnant. Variations of the red blood cell counts in these animals are presented in Figure 4.

In general, it was hard to observe some tendency of the fluctuations of the red cell counts during pseudopregnancies due to wide variance in each doe, except a sharp and temporary fall in the morning count on the 12th or 13th day of pseudopregnancy. Such fall was not recognizable in the afternoon count. The reduction in the morning counts of red blood cells on the 13th day was significant at 1 per cent level in comparison with two periods; the 10th to 12th day and the 14th to 16th day in pseudopregnancy.

Comparison was made of the red blood cell count during 10 days before copulation with that from the first to eleventh day in pseudopregnancy. Means

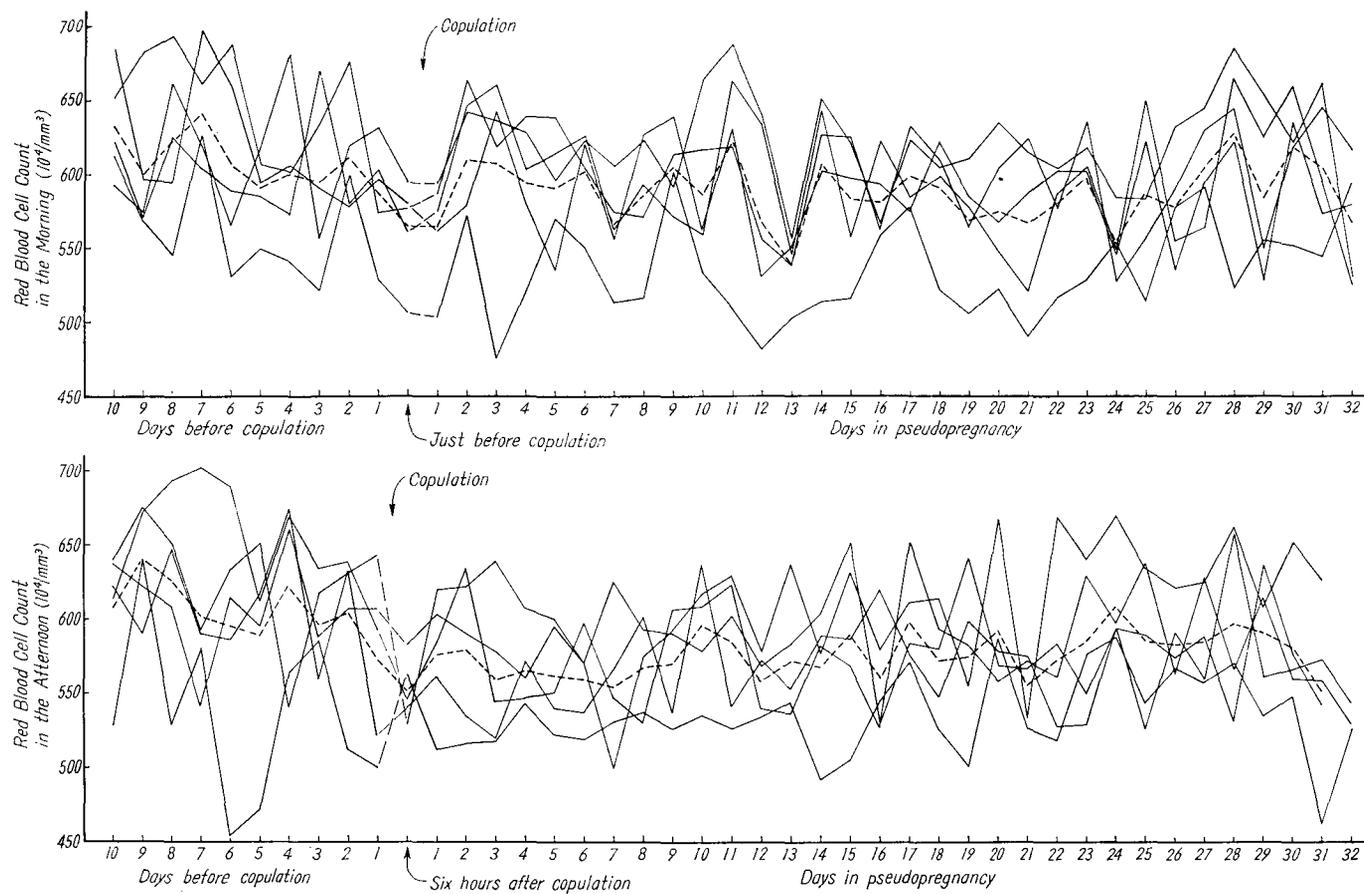


Fig. 4. Variations in red blood cell count in five pseudopregnant rabbits.
 --- Average count.

of the morning counts were $607.86 \pm 37.33 \text{ } 10^4/\text{mm}^3$ before copulation and $592.87 \pm 34.13 \text{ } 10^4/\text{mm}^3$ after copulation, and means of the afternoon counts were $606.52 \pm 40.77 \text{ } 10^4/\text{mm}^3$ before copulation and $569.94 \pm 28.67 \text{ } 10^4/\text{mm}^3$ after copulation. Both cases showed that the numbers of erythrocytes were slightly reduced after copulation, and the differences before and after copulation were significant at 5% level in the morning count and at 1% level in the afternoon count. The reduction of the red blood cell count was more marked in the afternoon (mean reduction, 6.0%) than in the morning (2.5%), in spite of identical counts before copulation. The difference between the morning and afternoon counts after copulation was significant at 1% level.

5. Variation during pregnancy

Since no clear diurnal variation and no difference between the morning and afternoon counts were recognizable in the number of erythrocytes in the normal state, and since no clear difference in the pattern of the changes in the afternoon count between normal state and pseudopregnancy, consideration was given thereafter only to the morning count. Changes in the morning red blood cell count during pregnancy in 8 females are shown in Figure 5.

Generally the red blood cell counts began to decrease slowly after copulation, and marked decrease was recognizable on the 18th or 19th day of pregnancy. During the 19th to the 26th day, the level of the red blood cell count remained the lowest of the whole pregnancy. In the succeeding period before parturition, the count increased slightly.

Comparison was made of the red blood cell count during four periods of pregnancy; 7 days before copulation (stage A), the 5th to 17th day of pregnancy (stage B), the 19th to 26th day (stage C) and the 27th day to before parturition (stage D). Mean counts in each stage in this case were $598.51 \pm 34.36 \text{ } 10^4/\text{mm}^3$ for stage A, $581.71 \pm 32.08 \text{ } 10^4/\text{mm}^3$ for stage B, $536.18 \pm 25.38 \text{ } 10^4/\text{mm}^3$ for stage C, and $561.37 \pm 33.71 \text{ } 10^4/\text{mm}^3$ for stage D. Ratios of decrease to stage A were 2.8% for stage B, 10.14% for stage C, and 6.20% for stage D. Differences of the counts among these four stages were highly significant at 0.1% level. Each stage in pregnancy (stages B, C, and D) showed significantly lower values of erythrocyte counts on the average than that before copulation (stage A). Differences of the count between stage B and C, between stage C and D, and between stage B and D were significant at 0.5% level, respectively.

6. Variation before and after parturition

The red blood cell count of six pregnant rabbits during the 10 days before parturition was compared with that of 10 days after parturition, because one rabbit showed cannibalism for her offspring and the other one represented

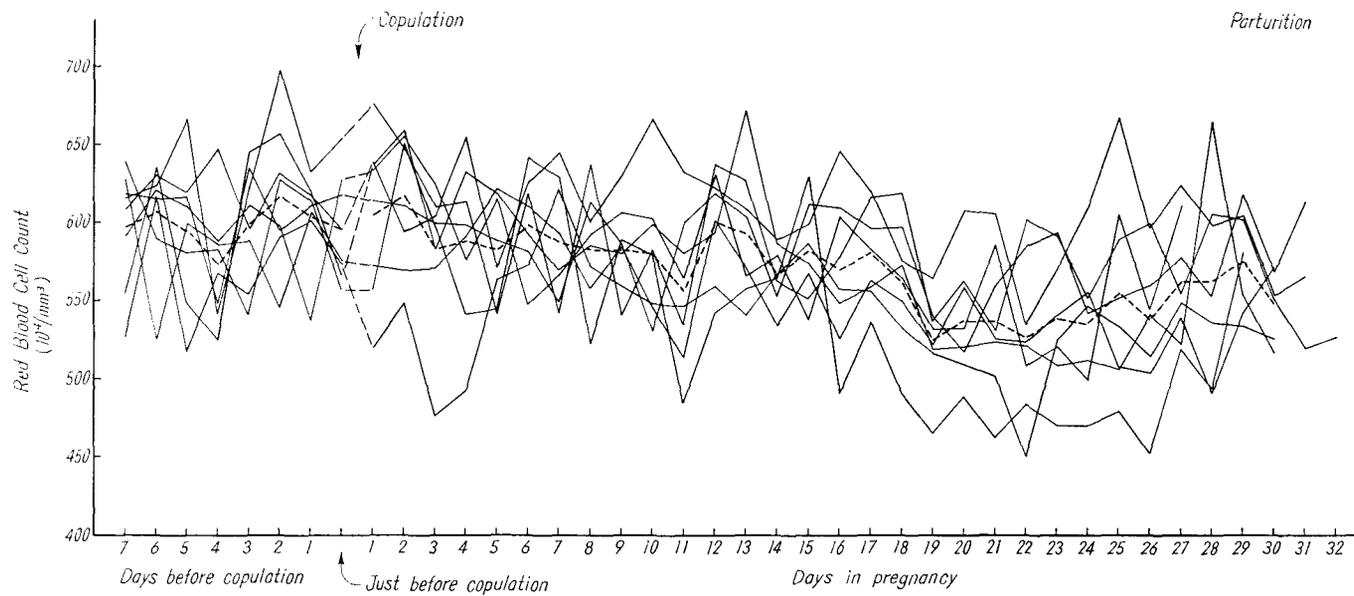


Fig. 5. Changes in morning red blood cell counts during pregnancy.
--- Average count in 8 females.

a marked decrease in the erythrocyte count. Mean count for the former group was $542.33 \pm 31.39 \text{ } 10^4/\text{mm}^3$ and the latter was $544.9 \pm 31.03 \text{ } 10^4/\text{mm}^3$. There was no difference in two groups.

However, one rabbit showed a marked decrease after parturition as noted above, in spite of normal appearance in nursing behavior. Mean counts of this animal were $578.0 \text{ } 10^4/\text{mm}^3$ during three days before parturition and $345.0 \text{ } 10^4/\text{mm}^3$ during three days after parturition. The decrease in the count of this

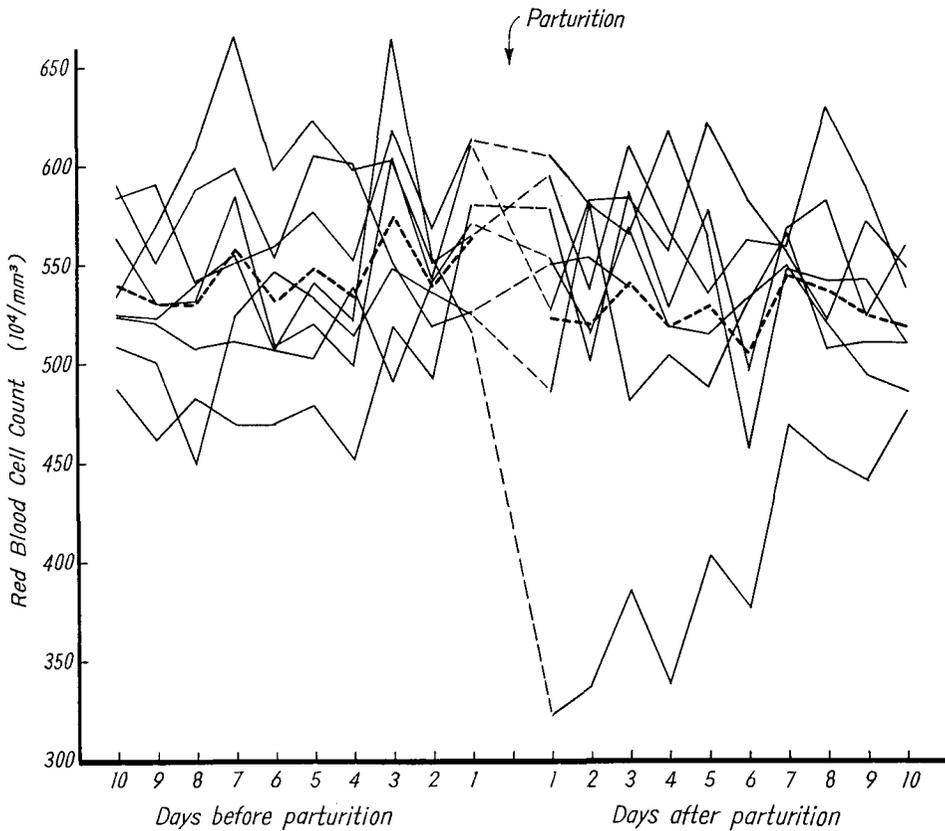


Fig. 6. Variation in red blood cell count before and after parturition. --- Average count.

animal after parturition reached 40.3%. The data of 7 animals are illustrated in Figure 6.

7. Variation after parturition to the 54th day

The red blood cell count was made in 7 female animals during 54 days following parturition. They nursed for 30 days after delivery. The results

are illustrated in Figure 7.

The count in the one animal which showed severe anemia after parturition increased day by day to reach the normal level on the 11th day in puerperium, and after this period the changes in the count followed the general tendency of the other six female animals.

In the six female animals, in general, the erythrocyte count decreased slowly after parturition, and it showed the lowest values during the 15th–22nd day. After this stage, the count increased gradually until about the 33rd day to reach the normal level.

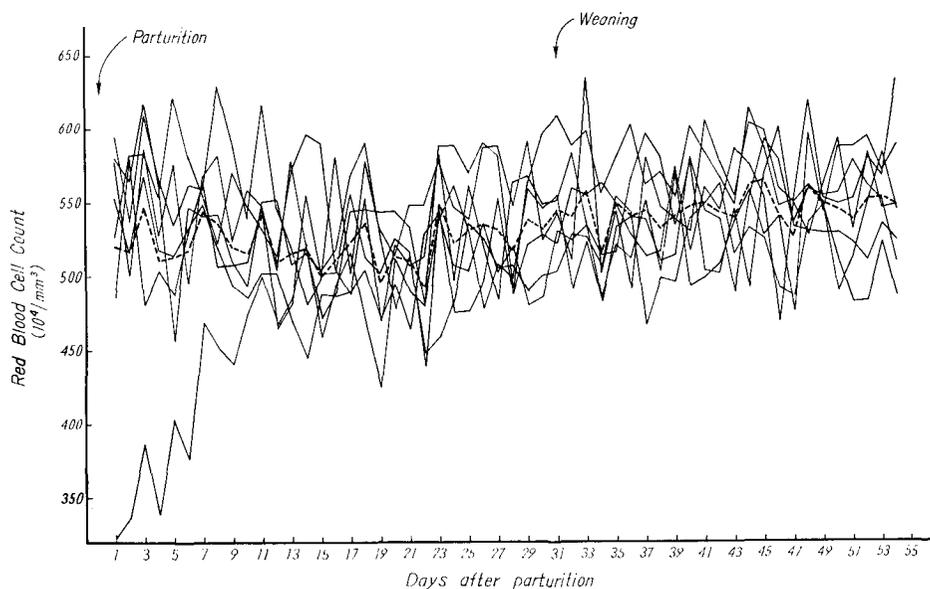


Fig. 7. Changes in morning red blood cell counts in 7 females for 54 days following parturition.

--- Average count.

Milk secretion of these animals continued until about the 41st day, and milk could not be squeezed from their nipples on the 42nd day as a rule.

At this point, comparison was made among following five stages; the 7th to 14th day (stage I), the 15th to 22nd day (stage II), the 23rd to 30th day (stage III), the 31st to 42nd day (stage IV), and the 43rd to 54th day (stage V). Mean red blood cell counts of the six female animals were 533.22 ± 33.02 $10^4/\text{mm}^3$ for the stage I, 508.85 ± 31.70 $10^4/\text{mm}^3$ for the stage II, 525.14 ± 26.97 $10^4/\text{mm}^3$ for the stage III, 535.40 ± 30.21 $10^4/\text{mm}^3$ for the stage IV, and 543.65 ± 29.09 $10^4/\text{mm}^3$ for the stage V. The counts of the stages II and

III were significantly low, though there was no significant difference in stage I, IV and V.

DISCUSSION

It is well known that there is considerable variation in the erythrocyte count in duplicate examinations (BUSHNELL and BANGS, 13). Therefore, the present study tried to compare by grouping the data. As a few signs of the diurnal variation in the red blood cell count were recognizable, the present study emphasized diurnal variation. But no clear diurnal variation was obtainable, though ANDERSEN and GEE (4) stated that the number of erythrocytes in dogs decreased $\frac{1}{2}$ to $\frac{3}{4}$ of a million during mid-day and early morning hours.

Estrous cycle variation in women has been reported by many investigators. However, only a few observations have been made on the estrous cycle variation in animals. TOMOEDA (109) mentioned that the number of erythrocytes in blood of mice was at the lowest level in diestrus and at the highest level in estrus. FRUHMANN (30) studied splenic erythropoiesis in relation to estrous cycle and concluded that splenic erythropoiesis was heightened during proestrus, estrus and metestrus-1 relative to metestrus-2 and diestrus. MATSUOKA (63) measured the size of erythrocytes and claimed that the erythrocytes of a smaller diameter appeared more abundantly in the blood during proestrus and estrus than in other stages. In buffaloes a slight decrease in the erythrocyte count at estrus was observed by SOLIMAN and SELIM (90). In the progress of the reproductive physiology in rabbits, a conception of estrous cycle was confirmed by several workers (HAMILTON, 36; MYERS and POOLE, 71; TSUTSUMI, 111; TSUTSUMI and MATSUMOTO, 112, 114). It has been demonstrated by many that there is a close relationship between erythropoiesis and sex hormones. Similarly there is some possibility that a relationship between fluctuation in the red blood cell count and stages in the estrous cycle may be recognizable in rabbits. However, in our study there was generally a poor correlation between fluctuation in the count and the estrous cycle, though the average time interval between troughs in curves made by the red blood cell counts in each doe was similar to the length of the estrous cycle.

The count did not show any ovulatory response during 14 hours after copulation. However, it was noted that the level of the red cell count decreased slightly after copulation during several days at the beginning of the pseudopregnancy and pregnancy. This change may be due to some hormonal changes following ovulation, though its mechanism is obscure. While blastocyst attachment was not observed histologically during 8 days after copulation (HAFEZ and TSUTSUMI, 35), body weights of the pregnant rabbits showed no

change during the time from copulation to about 8 days *post coitum* (TSUTSUMI *et al.*, 115).

During pseudopregnancy a sharp and temporary fall was noted in the morning count on the 12th or 13th day in the present study. TSUTSUMI *et al.* (116) recognized a temporary decrease in afternoon body temperature on the 11th day of pseudopregnancy. HUGHES and MYERS (45) stated that behavior with a strong sexual component, culminating in mating and ovulation, was observed at about either the 6th or 12th days of pseudopregnancy. Although the physiological meaning of these phenomena are obscure, some changes may occur involving the physiological conditions in about the 12th day of pseudopregnancy.

Anemia during pregnancy is a well known phenomenon in women and various animals: rats (BEARD and MYERS, 6; BOND, 10; MITCHELL and MILLER, 67; NEWCOMER, 74; SCOTT, 86; VAN DONK *et al.*, 123), dogs (ANDERSEN and GEE, 4; TIETZ, JR. *et al.*, 108), goats (ROY *et al.*, 85), cows (HIRAGA *et al.*, 40) and buffaloes (SOLIMAN and SELIM, 90). In general, it is accepted that the anemia of pregnancy is due to hemodilution in which blood volume increases proportionally faster than erythrocytes. ZARROW and ZARROW (131) described how rabbits developed a normochromic slightly macrocytic type of anemia, with a 20% drop in erythrocytes, and with the onset of the anemia the rabbits developed a definite water retention. HORGER and ZARROW (44) also reported that the anemia of late pregnancy in the rabbit was due to hemodilution since both the blood and plasma volume were increased. The present study showed the marked decrease in the erythrocytes beginning the 19th day of pregnancy, and the time of days in pregnancy were grouped in three stages; the former half of pregnancy during the 1st-18th day, the 19th-26th day, and the 27th to before delivery. In the second stage the level of the red blood cell count remained lowest. It was noted by TSUTSUMI and MATSUMOTO (113) that scanty vaginal mucus was obtainable during the 5th to 18th day of pregnancy and reddish brown, opaque mucus which contained numerous erythrocytes appeared suddenly and abundantly in the vaginal lumen on about the 19th day of pregnancy. This phenomenon suggests that uterine bleeding may occur and this may in some degree cause the decrease in the erythrocyte count of blood.

The third stage of the pregnancy (before parturition) in the present study showed a slight increase of the erythrocyte count, though ZARROW and ZARROW (131) and HORGER and ZARROW (44) did not note such a phenomenon. However, many authors recognized a definite increase at parturition in cows (HIRAGA *et al.*, 40; MIYAKE *et al.*, 68; MURAKAMI, 70; STRAUB *et al.*, 96), though SOLIMAN and SELIM (90) did not mention such increase

in buffaloes.

After parturition a marked decrease in the red blood cell count was noted in one animal out of 7 in the present study. This doe showed a normal appearance in nursing and other behavior. The anemia of this animal may be due to some wound caused by parturition, and there is a definite possibility in a low percentage of usual rabbit breeding. The count of this animal showed a marked increase until the 10th day after parturition, reaching the level in the other 6 rabbits. By HAMMOND and MARSHALL (38) the normal involution of uterus after parturition was quite short and lasted only about 4 to 8 days in the nursing rabbit. Thus this doe achieved uterine involution before the 10th day of puerperium.

In the other 6 animals the level of the red blood cell count began to fall gradually after the 8th day in puerperium and reached the lowest level during the 15th-22nd day. The doe's young open their eyes at about the 11th day after birth, and begin to eat solid food together with their mother after about the 20th day. This probably means that milk secretion is the heaviest burden to mother from about the 15th to 22nd day. Anemia during lactation was reported in cows (HIRAGA *et al.*, 40; ISHII, 46), in mares (HANSEN *et al.*, 39), and in rats and guinea pigs (KAWACHI, 53). The level of the count increased gradually after the 23rd day to reach the normal level on the 44th day in the present study. The effect of lactation seemed to disappear after this period.

SUMMARY

The number of erythrocytes in blood of eight adult female rabbits was recorded on consecutive days, and studied in relation to the estrous cycle, pseudopregnancy, pregnancy and puerperium.

The correlation between fluctuations in red blood cell count and the changes in the estrous cycle, as established by inspection of the vaginal mucus and vaginal smears, was rather poor, though the average length of time between troughs in the curves by the red blood cell counts (5.56 ± 1.56 days) was similar to the interval between estrous cycles (5.87 ± 2.65 days).

No variation associated with copulation or ovulation was recognizable in the counts at 2-hour intervals, beginning just before copulation at 8 a.m. to the 14th hour at 10 p.m.

During pseudopregnancy, a temporary fall in the morning counts on the 12th or 13th day was marked, though such fall was not found in the afternoon count.

In pregnant animals, the mean red blood cell count decreased gradually after copulation, and marked decrease began on the 18th or 19th day of

pregnancy. During the 19th to 26th day, the level of the erythrocyte counts was the lowest in all the pregnancy. The decrease of this period exceeded 10% of the mean before copulation. Thereafter until parturition, the counts increased slightly.

The erythrocyte count decreased slowly after parturition in the six female animals and the lowest level in the count was achieved during the 15th to 22nd day in puerperium. After this period the count increased gradually until about the 33rd day to reach a normal level. However, the counts of another rabbit showed a marked decrease after parturition, and this anemia did not rise to the level of the other six does until the 11th day in puerperium. This doe seemed normal in her nursing behavior. The latter suggests that severely anemic rabbits will be found in a low percentage of the usual rabbit breeding.

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