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Title	Blood Volume Changes In Pregnant and Pseudopregnant Rabbits
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Citation	Journal of the Faculty of Agriculture, Hokkaido University, 57(1), 1-24
Issue Date	1972-07
Doc URL	<a href="https://hdl.handle.net/2115/12864">https://hdl.handle.net/2115/12864</a>
Type	departmental bulletin paper
File Information	57(1)_p1-24.pdf



# BLOOD VOLUME CHANGES IN PREGNANT AND PSEUDOPREGNANT RABBITS

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Received March 19, 1971

In observations of the physiological and pathological states of animals, inspection of the blood is a routine procedure because of the direct or indirect relationships to various conditions or stimuli. Fluctuations in the blood cellular components in the female rabbit during the stages of the reproductive cycle have been reported in relation to physiological and morphological aspects (175-178). The present study deals with fluctuations in the volume of the circulating blood and of its components in the pregnant and pseudo-pregnant rabbit.

## Survey of the Literature

A number of studies on the blood volume of various animals has been reported using blood dilution methods (estimation from changes in concentration of whole blood following infusion of large known volume of fluid), cell volume methods (washout technique and dilutions of labeled cells), plasma volume methods (dilution of test substances) and total blood volume methods (simultaneous cell and plasma volume measurements) (reviewed by BURKE, 30, and GREGERSEN and RAWSON, 76). Most such studies have estimated the average total blood volumes of various species, with methodological discussions, such as in rats (16, 33, 41, 50, 53, 79, 112, 129, 182, 183, 187), in guinea pigs (6, 120, 187), in cats (166, 187), in rabbits (1, 9, 38, 39, 187), in dogs (3, 4, 26, 27, 38, 46, 63, 65, 75, 77, 92, 97, 98, 110, 140, 156, 161), in goats (38, 105, 106), in sheep (69, 84, 91, 124), in swine (8, 32, 84, 85), in cattle (43, 84, 94, 119, 128, 145, 146, 163, 167), and in horses (36, 38, 40, 44, 101, 104, 136).

The absolute total blood volume increases significantly in association with growth, though the blood volume per unit body weight falls steadily with increase in body weight in rats (15, 37, 131, 183). A similar trend has been reported in guinea pigs (37), in rabbits (23, 29, 108), in goats (186),

in sheep (69, 82), in swine (32, 55, 82, 139, 154), and in cattle (82, 135, 186), with a few exceptions noted in rats (125) and dogs (64). Some reports indicate no significant difference in blood volume between the sexes in rats (112), rabbits (29) and dogs (27, 63, 110), while GIBSON and EVANS (62) stated that the blood volume in normal men is greater than that of women, the difference being due to the greater red cell volume in the male.

The blood is an important part of the body composition, and as one of the body fluids has numerous roles in maintaining physiological and biophysical activities. Therefore blood volume, or plasma volume, as one of the body fluid compartments, has been investigated by a number of researchers interested in physiological, immunological, genetic, nutritional and economic considerations—in rats (53, 182, 183), in rabbits (1), in dogs (77), in goats (90), in sheep (83, 90, 117, 133), in swine (54, 55, 83, 154), in cattle (83, 90, 157, 163), and in horses (101).

The blood volume may be affected by environmental conditions. Seasonal fluctuations in the volume were in evidence in cattle (135, 167) and man (13, 57, 121). Changes in the volume were also reported under experimental climatic conditions. Results of these experiments, however, seem to vary with species (rabbit—149, 150; dog—45; cattle—20, 42; buffalo—134; man—13). The volume changed with the atmospheric altitude (rat—60; goat—21). The blood volume in horses was investigated in detail by PERSSON (136) in relation to working capacity.

For clinical purposes or for monitoring blood loss, blood volume has been measured by numerous workers (70, 168). For example, there have been reported effects of the endotoxic and hemorrhagic shocks in rats, rabbits and dogs (47, 52, 72), of anesthetic agents in man (71), of nephrectomy in rats (14), of splenectomy in rats (95, 96) and dogs (141–143, 151), of x-irradiation in rats (96), etc. Studies on thrombo-angiitis obliterans indicated that thyroid activity has an intimate relation to blood volume regulation (107, 158–160, 169).

Many investigations have focused on changes in blood volume during and after pregnancy in women. In brief, the total blood volume increases progressively during pregnancy owing to the marked increment in plasma volume, and the relative increase in the plasma volume is greater than that of red cell mass, resulting in lower hematocrit values. This condition in women has been called anemia, hypervolemia or hydremia of pregnancy (2, 17, 31, 34, 35, 51, 86, 115, 122, 123, 127, 137, 152, 162, 170, 180). However, the evidence for a decrease in plasma volume before term seems to be conflicting. The pregnant women must tolerate a huge loss of blood at delivery.

The difference between the blood volumes before and after delivery has been estimated by many workers, with considerable disparity in the reported patterns of change in blood volume in the early postpartum period. It has been considered that the decrease in blood volume after delivery is greater than the volume of the apparent blood loss, and that some of the blood is removed from the circulation and sequestered elsewhere in the body at delivery. It was, however, established recently that the difference between pre- and postdelivery blood volumes is due to the blood loss associated with labor, and not to sequestration of blood, by complete recovery of blood of lochia and from the placenta. It was also noted that the blood volume returned to the nonpregnant, rather than the prepartum, level ultimately (17, 19, 34, 35, 48, 51, 68, 86, 114, 115, 122, 132, 137, 138, 170, 180). These fluctuations in blood volume have been discussed in relation to many factors such as the hemoglobin, erythrocyte and leucocyte levels in the blood, blood viscosity, extravascular fluid volume, blood pressure, etc. (35, 48, 67, 68, 80, 86, 113, 114, 144, 170, 180).

In spite of these reports on women, studies which treat changes in blood volume during the reproductive cycle are very few in lower animals. In rats, blood volume increased proportionally faster than erythrocyte number during pregnancy, and this caused an anemia of pregnancy due to hemodilution, while blood volume per unit body weight remained constant. After parturition a rapid loss in blood volume was recorded (24). During the lactation period, blood volume increased steadily and polycythemia was evident. The normal hematocrit values of this period showed that erythrocyte and plasma volumes increased proportionally. The blood volume decreased following removal of the young (25).

HEIDENHAIN (87) found that a pregnant rabbit contained  $1/14.9$  of its body weight in blood, while the normal figure was  $1/18$ . SALVESEN (153) mentioned that pregnant rabbits had a larger blood volume absolutely and relatively than nonpregnant ones, and that at one week postpartum the blood volume was restored to the normal value. According to HÖRGER and ZARROW (93), a marked decrease occurs in relative concentration of the cellular components of the blood during the last trimester of pregnancy, though they found no significant change in total cell volume or in total hemoglobin during gestation. Concomitant with the decrease in the blood cellular components, the plasma volume increased 12.3% on the 7th day antepartum. The changes in the blood volume paralleled those of the plasma volume, reaching a maximum increase of 6.3% on the 7th day antepartum. This indicated an anemia of late pregnancy in rabbits due to hemodilution.

In dogs, SPEIGELBERG and GSCHIEDLEN (164) mentioned that a real increase in blood volume, both relative and absolute, occurred in pregnancy. TIETZ, BENJAMIN and ANGLETON (171), however, claimed that anemia was severe and developed at a significantly later time of pregnancy.

In sheep, the blood volume showed a general tendency to rise up to a summit at about 45 days in pregnancy, then to drop to a valley at about 90 days and finally to rise to its greatest height which was at, or within, a few days of term. The first rise was due essentially to an increase in the plasma volume. The corpuscle volume tended to fall. The final rise was also principally a rise in plasma volume. These results were discussed in relation to uterine growth and placental and fetal development (11).

In dairy cows, blood volume increased during pregnancy in proportion to the weight increase and decreased with body weight after parturition (126). The increases in blood and plasma volume during gestation, in the primiparous cow, are attributable in part to the animal's maturing growth, and in part to the gestational process. No anemia developed during pregnancy (146). FELDMAN *et al.* (56) stated also that neither hydremia nor anemia develops in the gestating cow. TURNER and HERMAN (179) found that lactating cows had a larger blood volume per unit body weight than nonlactating cows. This relative increase was maintained at the new level and no anemia developed during lactation (146).

### Materials and Methods

Twenty-two female Japanese native-breed rabbits of 3 to 4.5 kg body weight were used in the present study. They were isolated in individual cages and received water and feed *ad libitum*. They were bred with two bucks. One female became pregnant twice and three pseudopregnant rabbits were bred again later, resulting in two pregnancies and one pseudopregnancy. Thus, a total of fifteen pregnant rabbits and eleven pseudopregnant ones were obtained for the blood volume estimation.

The blood volumes were estimated from the plasma volume and the hematocrit value. The plasma volume was determined by the dilution technique using Evans blue dye (39, 61, 63, 73, 74, 95, 103, 146). The hematocrit value was determined by the Wintrobe method.

The procedures were as follows: 1) A blood sample of about 1.5 ml was taken from the marginal vein of the ear (right side). Hematocrit value was determined, using a Wintrobe tube, by centrifugation for 30 min at 3,000 r.p.m. and the plasma was used for a dye-blank test. 2) 0.8 ml of

0.5% dye solution was injected into the marginal vein of the opposite ear (left side), taking about 30 sec. Exactly 10 min after dye injection, 1.5 ml of blood was taken again from the ear vein on the right side. After reading the hematocrit value, dye-tinged plasma was pipetted off to determine the dye concentration. 3) Each rabbit was weighed immediately after bleeding. 4) 0.2 ml of the dye-blank and dye-tinged plasma were diluted 20 times with distilled water. The dye concentration of the solution was measured in a photoelectric photometer (Hitachi EPU-2) at  $620\text{ m}\mu$  (4). The plasma volume was calculated from the reading of the photometer by a calibration curve plotted from rabbit plasma samples of known dye concentration. 5) Oxalate mixture was used for anticoagulant. The mixture was prepared by dissolving 0.8 g of potassium oxalate and 1.2 g of ammonium oxalate in 100 ml of distilled water. 0.2 ml of this mixture was taken into a small tube and dried at  $55^{\circ}\text{C}$ , thus coating the inner surface of the tube with the oxalate mixture. The drops of blood from a puncture were received into the oxalate-coated cup of the tube and quickly agitated by shaking (88, 102).

Blood samplings were made at 5-days intervals from 5 days before copulation to 2 months after copulation in pregnant does, and to one month after copulation in pseudopregnant does.

## Results

### 1. Changes in body weight

Changes in body weight in pregnant and pseudopregnant rabbits are illustrated in Figure 1. Average body weight in pregnant rabbits was  $3,565 \pm 119.5$  g (mean  $\pm$  standard error) at copulation, and  $3,958 \pm 103.7$  g before delivery. Mean litter size was  $5.8 \pm 0.76$ , ranging from 1 to 10, and birth weight of the fetuses was  $54.17 \pm 4.46$  g on the average. Ratios of fetal birth weight to maternal body weight before delivery were 1.95% to 12.42% (mean of  $7.18 \pm 0.87\%$ ). Increase in body weight was marked during pregnancy and rate of gain in weight was about 11%. Gestational length averaged  $30.5 \pm 0.3$  days. A marked decrease in weight was evident after delivery and the weight decreased gradually during lactation. Average body weight was  $3,708 \pm 89.8$  g at 65 days *post coitum* (*p.c.*).

Body weights in pseudopregnant rabbits showed no marked fluctuation.

### 2. Changes in hematocrit value

The hematocrit values, just before copulation, were  $42.3 \pm 0.37$  in the pregnant group and  $41.7 \pm 0.53$  in the pseudopregnant group. Fluctuations in these does are shown in Figure 2. During pregnancy the values began

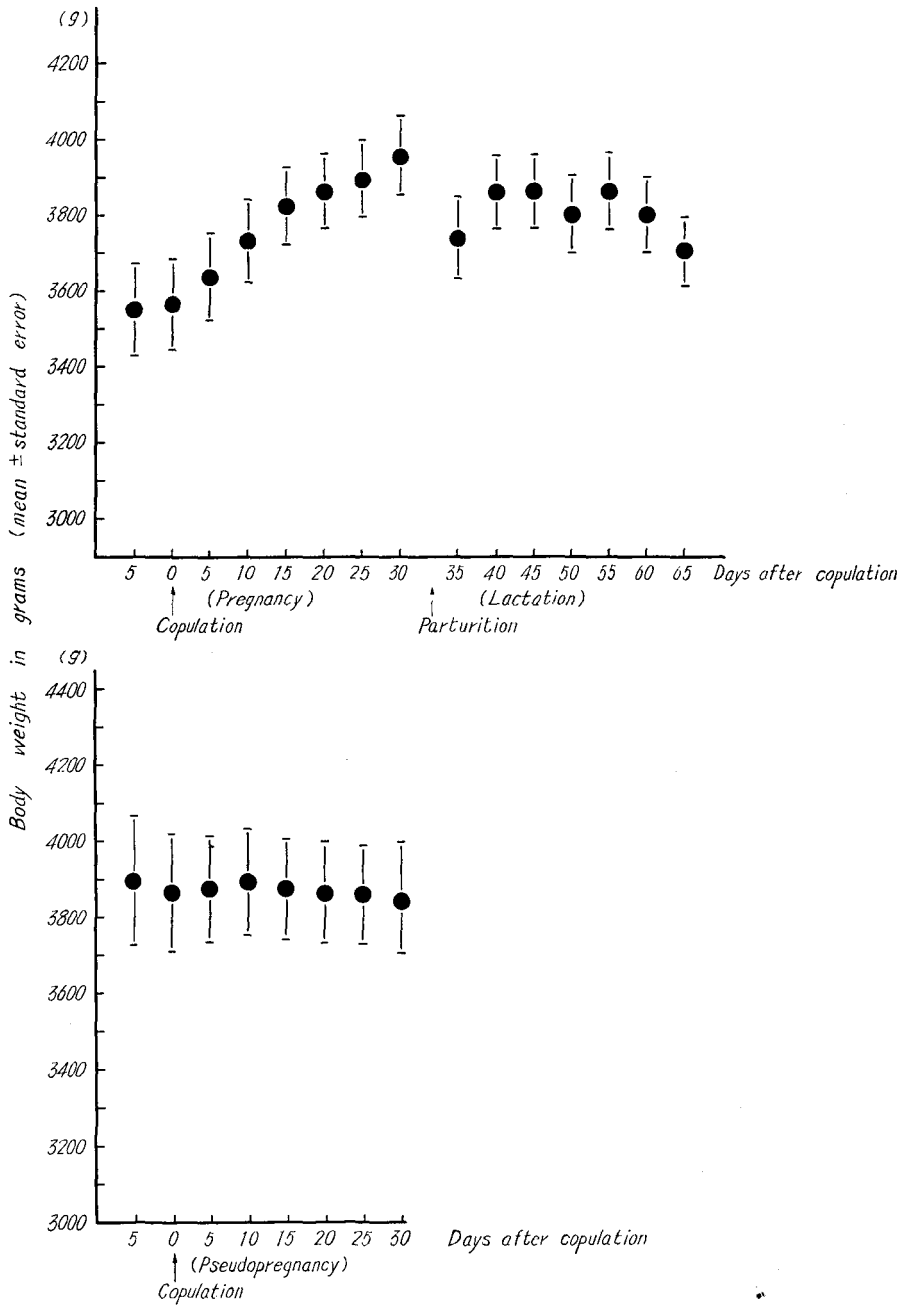


Fig. 1. Fluctuations in the body weight (mean  $\pm$  standard error) during pregnancy, lactation and pseudopregnancy.

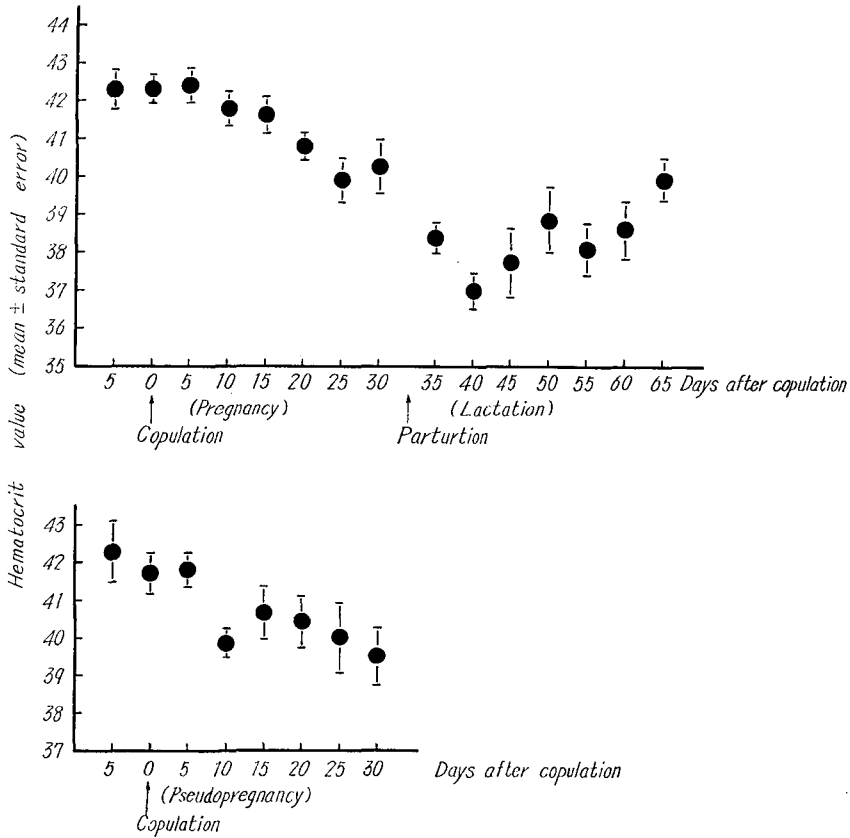


Fig. 2. Fluctuations in the hematocrit value (mean ± standard error) during pregnancy, lactation and pseudopregnancy.

to fall at 10 days *p.c.*, reaching the lowest value ( $39.9 \pm 0.58$ ) at 25 days *p.c.* (about 7% decrement), and then some increment was shown before term. In pseudopregnant does, the values decreased gradually to 30 days *p.c.*, with a temporary sharp fall at 10 days *p.c.* ( $39.8 \pm 0.37$ ). After delivery the values fell remarkably ( $37.0 \pm 0.47$  at 40 days *p.c.*, about 13% below the value before copulation), followed by gradual recovery. The value at 65 days *p.c.* was  $39.9 \pm 0.55$ , and this was still 7% below that before copulation.

### 3. Changes in plasma volume

The changes in plasma volume of the pregnant and pseudopregnant does are shown in Figure 3. Average plasma volumes before copulation were  $122.2 \pm 4.35$  ml in the pregnant does and  $138.6 \pm 9.31$  ml in the pseudopregnant does. The volume increased up to 30 days *p.c.* in both groups, though

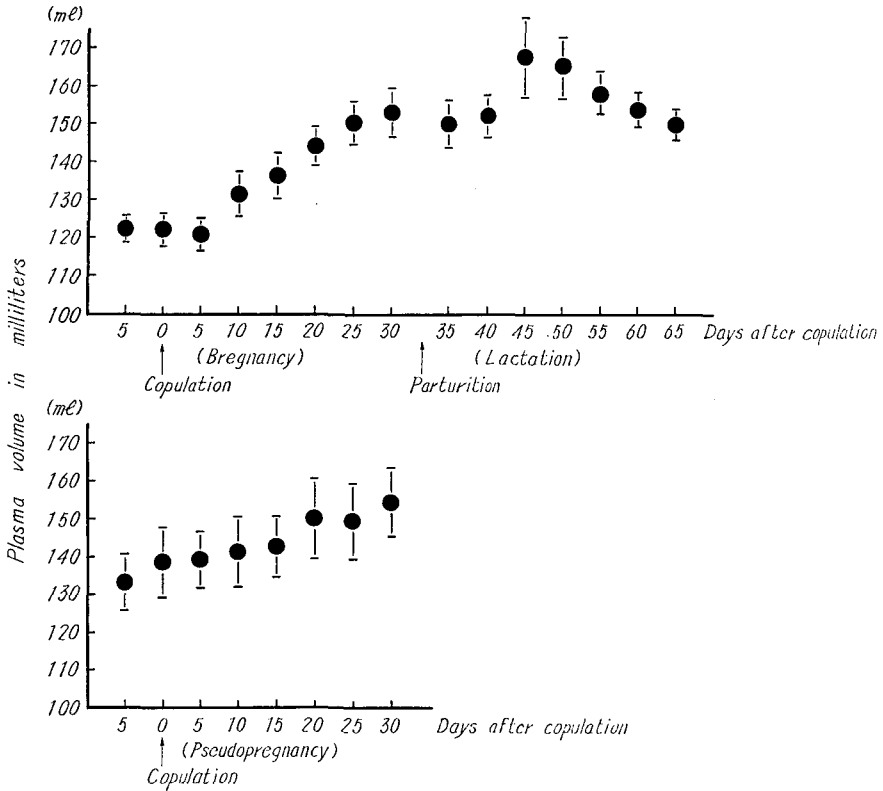


Fig. 3. Fluctuations in the plasma volume (mean  $\pm$  standard error) during pregnancy, lactation and pseudopregnancy.

the increment was more marked in the pregnant does. The increments at 30 days *p.c.* were about 25% in the pregnant does and about 17% in the pseudopregnant does.

The volumes at 35 and 40 days *p.c.* remained at the same level as before term ( $153.3 \pm 6.42$  ml at 30 days *p.c.*). However, the volume increased markedly at 45 days *p.c.* ( $167.7 \pm 10.43$  ml), followed by a gradual decrease to 60 days *p.c.* ( $150.0 \pm 4.09$  ml).

#### 4. Changes in cell volume

Average cell volumes before copulation were  $89.6 \pm 2.74$  ml in pregnant does and  $98.9 \pm 6.05$  ml in pseudopregnant does. Fluctuations of the cell volume during pregnancy and pseudopregnancy are demonstrated in Figure 4. The volume increased gradually from 10 days *p.c.* to term ( $102.8 \pm 5.13$  ml). In pregnant does, increment was about 14% in comparison with the volume

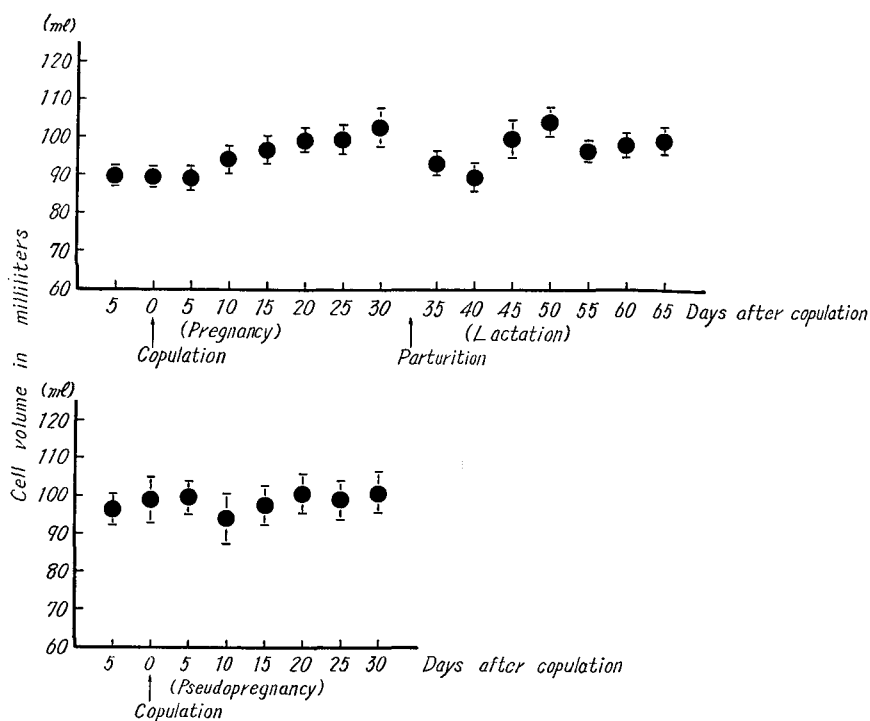


Fig. 4. Fluctuations in the cell volume (mean  $\pm$  standard error) during pregnancy, lactation and pseudopregnancy.

before copulation, but the volume at 30 days *p.c.* in pseudopregnancy was about the same as before copulation. Marked changes in the cell volume occurred after delivery—a decrease at 35 and 40 days *p.c.* and an increase at 45 and 50 days *p.c.*

##### 5. Changes in total blood volume

The fluctuations in the total blood volume are shown in Figure 5. The blood volume increased markedly from 10 days *p.c.* to term, while the volume showed a progressive increase in pseudopregnant does from 10 days *p.c.* to 30 days *p.c.*, also. The mean volumes before copulation were  $212.2 \pm 5.80$  ml in pregnant does, and  $230.1 \pm 10.89$  ml in pseudopregnant does. The volumes at 30 days *p.c.* were  $256.1 \pm 10.85$  ml in pregnant does (21% increment compared to normal state) and  $255.2 \pm 14.26$  ml in pseudopregnant ones (11% increment compared to normal state). The volume decreased to  $243.4 \pm 9.27$  ml at 35 days *p.c.*, following delivery. However, the volume increased markedly to  $267.6 \pm 14.56$  ml at 45 days *p.c.* and  $269.6 \pm 10.44$  ml at 50 days *p.c.*, then decreased gradually to 65 days *p.c.* ( $249.2 \pm 7.52$  ml).

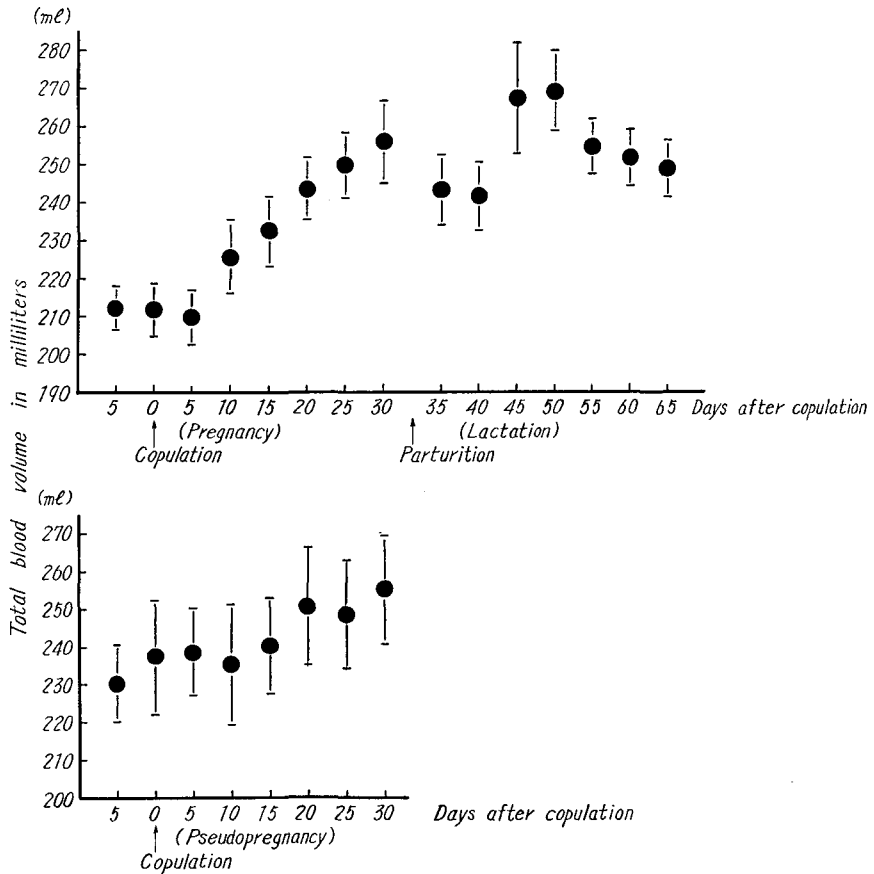


Fig. 5. Fluctuations in the total blood volume (mean  $\pm$  standard error) during pregnancy, lactation and pseudopregnancy.

### Discussion

Results of the present study are summarized in Figure 6, which compares changes in volumes of total blood, plasma and cells. Data calculated per unit body weight are given in Figure 7.

Mean total blood, plasma and cell volumes per kg body weight before copulation were  $60.0 \pm 2.31$  ml,  $34.7 \pm 1.46$  ml and  $25.4 \pm 0.88$  ml for the pregnant group, and  $61.3 \pm 1.36$  ml,  $35.8 \pm 1.57$  ml and  $25.6 \pm 1.23$  ml for the pseudopregnant group. These values were within the range reported by other workers (1, 9, 23, 29, 30, 38, 39, 108, 153, 187), despite the fact that we did not use a correction factor for calculation as described by GREGERSEN (73).

As shown in figures 6 and 7, the fluctuations in the total blood, plasma and cell volumes were similar in pregnant and pseudopregnant does. Ratios of the total blood volumes at 30 days *p.c.* to the volumes just before copulation were 120.9% in the pregnant group and 109.4% in the pseudopregnant group (Table 1). However, ratios per kg body weight were almost the same in the two groups—109.0% in the former and 110.5% in the latter. If total

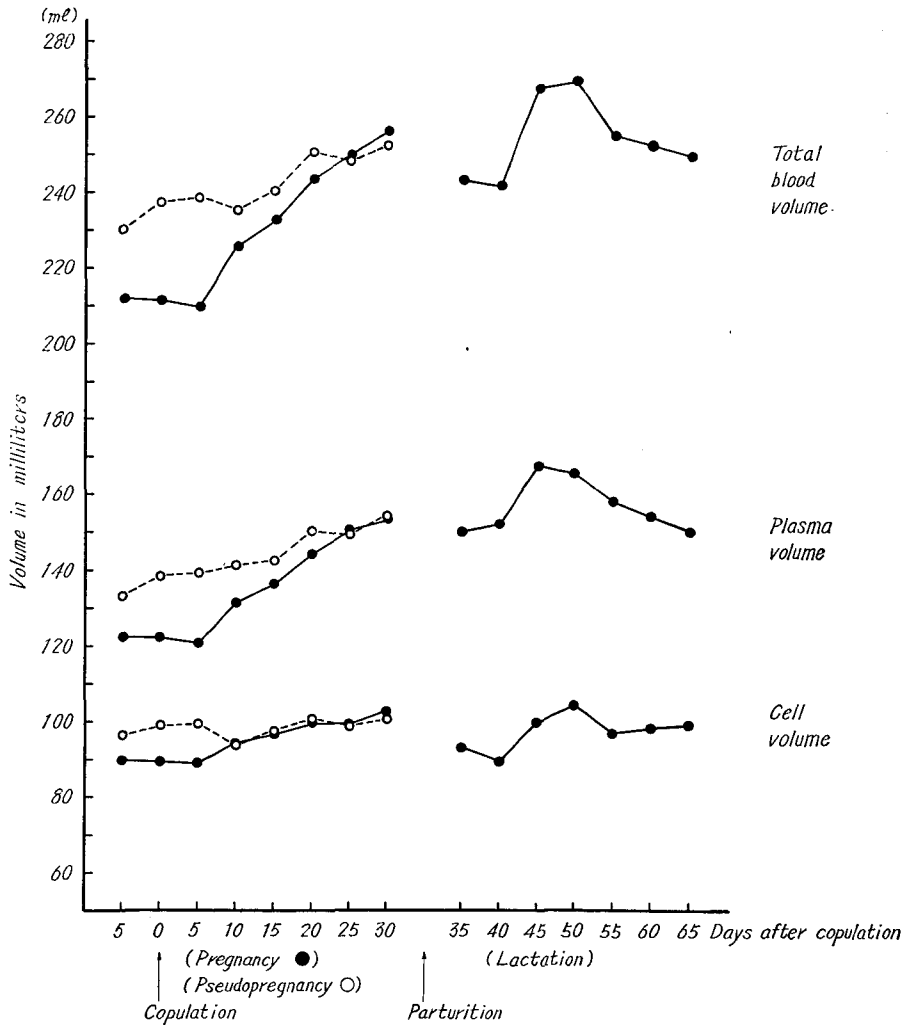
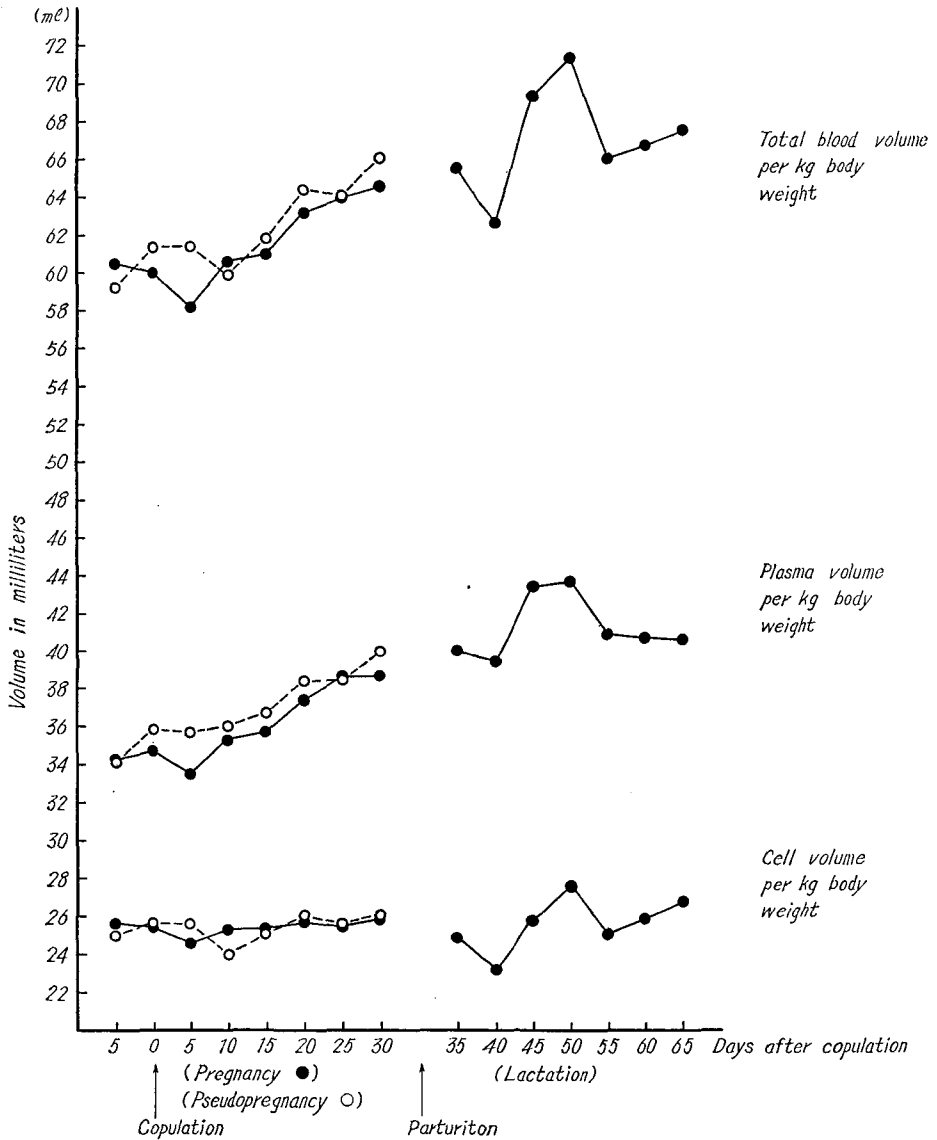


Fig. 6. Comparisons among the Fluctuations in the total blood, plasma and cell volumes during pregnancy, lactation and pseudopregnancy.



**Fig. 7.** Fluctuations in the total blood volume per kg body weight, in the plasma volume per kg body weight and in the cell volume per kg body weight during pregnancy, lactation and pseudopregnancy.

TABLE 1 Increments of the total blood volumes in each rabbit during pregnancy (ratio of the volume at 30 days *p.c.* to that just before copulation) and comparison with the increments between pregnant and pseudopregnant does at 30 days *p.c.*

Absolute volume			Volume per kg body weight			Volume per kg body weight in elimination of the total litter weight	
Pregnant		Pseudo-pregnant	Pregnant		Pseudo-pregnant		
Number of fetus	Ratio (%)	Ratio (%)	Number of fetus	Ratio (%)	Ratio (%)	Number of fetus	Ratio (%)
1	143.4	96.0	1	134.4	111.3	1	137.7
	128.9	97.7		122.4	112.3		124.8
		101.5			98.6		
2	113.1	102.2	2	104.7	93.6	2	107.0
		105.3			99.7		
4	112.1	107.9	4	102.6	115.6	4	106.7
	111.3	111.2		94.0	114.9		99.8
		112.3			109.1		
5	164.7	116.5	5	145.9	140.7	5	157.1
	112.0	119.3		88.6	102.2		95.5
	99.5	134.2		87.8	117.8		89.2
7	113.5		7	109.0		7	120.4
	118.9			110.4			120.8
8	121.8		8	96.4		8	107.4
9	122.2		9	110.1		9	124.3
	102.7			83.9			95.0
10	115.4		10	110.5		10	126.2
	135.1			133.7			142.9
Average	120.9	109.4		109.0	110.5		117.0

litter weights are eliminated from the body weights at 30 days *p.c.*, the ratio in the pregnant group became 117.0%.

Anemia of pregnancy has been demonstrated in rabbits by ZARROW and ZARROW (189), HORGER and ZARROW (93) and TSUTSUMI *et al.* (176, 177). The results of the present study seem to support these observations. It is clear that the anemia is due to a hemodilution, since the plasma volume per kg body weight increased during pregnancy and pseudopregnancy, in spite of the constant level of the cell volume.

Generally, pseudopregnancy is similar to pregnancy with respect to the behavior of does. The hormonal conditions are basically similar between them, though a comparative study showed differences in the release patterns of progesterone and  $20\alpha$ -hydroxy-pregn-4-en-3-one from the ovary (89). HORGER and ZARROW (93) demonstrated that estrogen and progesterone may play important roles in the fluid metabolism of pregnant rabbits. FRIEDLANDER *et al.* (58) reported that the average blood volume in bilaterally ovariectomized women showed a reduction of about 25% from the normal value. In the cat, a striking reduction in blood volume followed the removal of both ovaries (59). Hypophysectomized rats established a new level of total circulating red cell volume by the 258th postoperative day, which was 45% lower than in normal rats of the same age (18). Even in the hypophysectomized toad, it was reported that both plasma and blood volumes decreased in spite of no significant alteration in total body water (28). Generally, it is well established that anemia follows after removal of the hypophysis, in terms of the red blood cell count (181), and that estrogen produces anemia by an inhibiting effect on erythropoiesis (see TSUTSUMI *et al.* 176).

WITTEN and BRADBURG (188) reported that there was a lowering of the erythrocyte count, hemoglobin level and hematocrit 4 to 10 days after estrogen was administered in women, and postulated that the blood volume had increased sufficiently to account for this. The role of progesterone in the water retention of pregnant mice was reported by DEWAR (49). Subcutaneous injection of estradiol affected uterine weight and its water content in rats (10). The administration of estrogen and progesterone in an optimum ratio caused extensive vascular changes in the uterus of castrated rabbits (66). REYNOLDS (148) studies extensively the uterine physiology in relation to the uterine circulation, and TSUTSUMI and HAFEZ (173) showed marked vascular changes in the endometrium of pseudopregnant rabbits.

Thus, the total blood volume appears to be increased in the pseudopregnant rabbits mainly by hormonal effects, including changes in the uterus. TSUTSUMI *et al.* (176) reported that the red blood cell count in rabbits decreased significantly during pseudopregnancy. This phenomenon was also indicated (as decrease in hematocrit value) during pseudopregnancy in the present study. The plasma and blood volumes per unit body weight increased gradually to 30 days *p.c.* over the period of pseudopregnancy, though the cell volume remained rather constant through pseudopregnancy to 30 days *p.c.* TSUTSUMI and HAFEZ (173) recognized that in some pseudopregnant rabbits the syncytium in the endometrial epithelium remained until 20 days *p.c.*, and TSUTSUMI and HACHINOHE (172) observed that the average time

required from ovulation to the appearance of the normal estrous condition in the vaginal mucus was  $28.0 \pm 9.7$  days in pseudopregnant rabbits. HILLIARD *et al.* (89) observed a dramatic increase in  $20 \alpha$ -hydroxy-pregn-4-en-3-one ( $20 \alpha$ -OH) secretion from the rabbit ovary at the end of pseudopregnancy (days 15 through 21), while the decline in progesterone output began about 15 days *p.c.*  $20 \alpha$ -OH content then decreased to about 28 days *p.c.* Therefore it appears that some effects, which may be acting on the regulation of the plasma and blood volumes, remain for some period after the end of pseudopregnancy.

It was noted here that an unequivocal sharp fall in the hematocrit value occurred at 10 days *p.c.* in pseudopregnant rabbits. This seemed to be due to a reduction in cell volume, though the plasma volume tended to increase. The authors reported previously that a sharp and temporary reduction in red blood cells was recognized on the 12th or 13th day in pseudopregnancy (176), and that a temporary fall in afternoon body temperature was recorded on the 11th day of pseudopregnancy (175). Strong sexual behavior, culminating in mating and ovulation, was noted by HUGHES and MYERS (99) at about either the 6th or 12th day of pseudopregnancy. From these facts, it seems reasonable to consider that some important physiological changes occur around the 10th day of pseudopregnancy, though their exact nature is not clear.

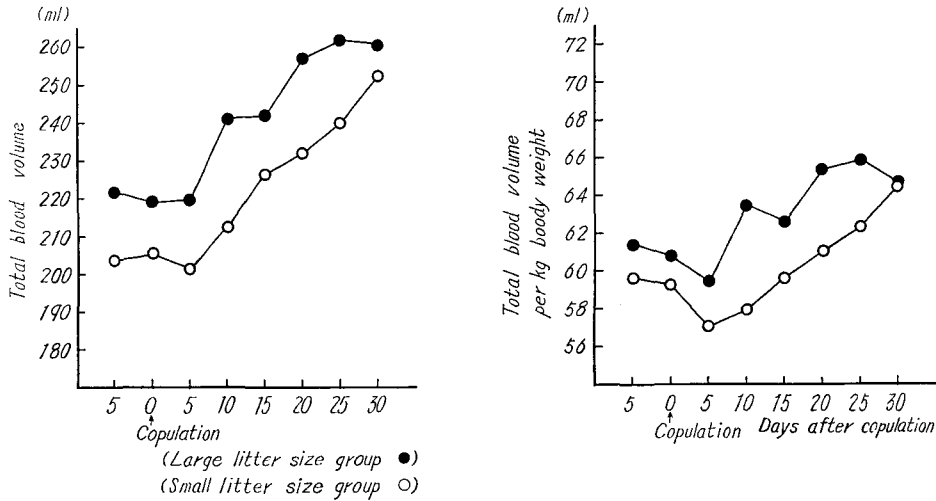
As shown in table 1, discrepancy in the ratio of total blood volume at 30 days *p.c.* to that just before copulation between pregnant and pseudopregnant groups was about 6.5% (117.0%–110.5%). This value may be due to some specific effect of pregnancy. NEWCOMER (130) stated that the anemia of pregnancy in the rat developed primarily under the influence of the placenta and partially or indirectly under the influence of the fetus, while TIETZ *et al.* (171) claimed that removal of the uterus from dogs did not materially affect the development of the anemia which occurred between 7 and 9 weeks after estrus in normal dogs.

It was indicated recently that uterine blood flow increased markedly during pregnancy (22, 78, 111, 116). In the last month of pregnancy in the sheep, about 84% of the uterine venous flow came from the placenta and only 3% from the myometrium, and the remaining 13% came from a region of the endometrium (118). However, the changes in the blood volume in sheep uterus started in early pregnancy before the fetus became of any considerable size. By 40 days the fetus weighed about 4 g and the placenta including the cotyledons weighed only 40 g, though the plasma volume increased about 18% above the original volume (11). Changes in blood volume

in the rabbit uterus during pregnancy were reported by BARCROFT and ROTHSCHILD (12). In the resting condition the genital organs contained less than 2 ml of blood. They became appreciably more vascular from the 5th or 6th days onwards, and about half-way through pregnancy contained about 10 ml of blood. Up to this point the embryos were of negligible weight. By the 20th day the embryos weighed only about 5 g and the generative tract of the mother contained about 15 ml of blood. The maximal quantity of blood, about 30 ml, seemed to occur about 28 to 29 days and then the quantity fell rapidly before parturition. Such a phenomenon in early pregnancy was confirmed in the present study, also. This seems reasonable because physiological conditions in early pregnancy are very similar to conditions in pseudopregnancy. The body weight in pregnant does showed an increase at 5 days *p.c.*, while the body weight in pseudopregnant ones remained unchanged. This led to falls in the blood, plasma and cell volumes per unit body weight in pregnant rabbits at 5 days *p.c.*, though their absolute volumes remained unchanged.

In the present study the anemic condition was seen during the period from 20 days *p.c.* to term. The authors reported previously a marked decrease in the red blood cell count beginning the 19th day of pregnancy (176). The hematocrit value at 30 days *p.c.* in pregnant does showed a slight increase, and this phenomenon was recognized in the red blood cell count, also. It is interesting that demarcation of the gestation period in rabbits according to fluctuations in these volumes coincides with stages denoted by changes in the vaginal mucus during pregnancy (174).

It was considered also that the changes in the blood volume during pregnancy might be partly due to litter size. Table 1 shows the increments at 30 days *p.c.* in both pregnant and pseudopregnant rabbits. Changes in blood volume in pregnant rabbits are illustrated in Figure 8, in which the rabbits are divided into two groups—a small litter size group (1 to 5) and a large litter size group (7 to 10). In comparing the blood volume per kg body weight between these two groups, significant differences were shown in the average volumes at 10, 20 and 25 days *p.c.* ( $P < 0.01$ ), though the volumes were the same in both groups at 30 days *p.c.* The highest values were attained at 25 days *p.c.* in the latter group and at 30 days *p.c.* in the former group. There was no significant difference in the increments of blood volume between the two groups at 30 days *p.c.*, when the total litter weights were subtracted from their mothers' body weights (Table 1). From these results it may be speculated that the increase in blood volume per unit body weight may be faster in rabbits of large litter size than in those of



**Fig. 8.** Changes in the absolute and relative total blood volumes in the large litter size group (7-10 in litter size) and the small litter size group (1-5) during pregnancy.

small litter size; and that the volume may attain equilibrium faster in rabbits of large litter size than in those having small litters.

After parturition the hematocrit value fell markedly, and the absolute volumes of blood, plasma and cells decreased, also. The blood and plasma volumes per unit body weight, however, increased slightly at 35 days *p.c.*, while the cell volume per unit body weight decreased. At 40 days *p.c.* marked falls in the hematocrit value and blood volume per unit body weight were noticed. This was due to decrease in cell volume per unit body weight. Following this period, the highest values in the blood, plasma and cell volumes were achieved at 45 and 50 days *p.c.*, though the hematocrit values were not so high. This means that plasma volume increased relatively more than cell volume did. It was reported by HAMMOND and MARSHALL (81) that the normal involution of the uterus after parturition lasted only about 4 to 8 days in the nursing rabbit. 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. Thus milk secretion in the mother may be heaviest in the period around 45 and 50 days *p.c.* In general, blood and plasma volumes remained significantly high during lactation, as reported in rats (25, 100) and cattle (146, 147). In the present study the hematocrit value did not return to the normal level during lactation, up to one month after parturition, though a gradual increase was shown during this period,

as in dogs (7) and cattle (109, 164). REYNOLDS (147) claimed that the hematocrit values remained in or near the control range in lactating cows. However, it is concluded that blood and plasma volumes, both absolute and relative, remain in high level during lactation in rabbits.

### Summary

Blood volume in fifteen pregnant and eleven pseudopregnant rabbits was estimated by the dilution technique using Evans blue dye. Blood volume estimation were made at 5-days intervals from 5 days before copulation to 2 months after copulation in pregnant rabbits and to one month after copulation in pseudopregnant rabbits.

In general, fluctuations in the hematocrit values, and in the blood, plasma and cell volumes, were similar between pregnant and pseudopregnant rabbits. It was postulated that the fluctuations in these volumes are mainly due to hormonal mechanisms.

Effects of pregnancy accounted partly for the fluctuations in these volumes. There was a tendency for the blood volume per unit body weight to increase faster in rabbits of large litter size than in those of small litter size, and it appeared that the volume stabilized faster in the rabbits of large litter size than in those with small litters.

Anemia of pregnancy was evident especially during the last trimester of pregnancy and for about one month after parturition. The blood and plasma volumes remained high during lactation, in both absolute and relative terms.

### Acknowledgment

The authors wish to express their appreciation to Professor WILLIAM J. MELLETT, Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, Massachusetts, for reading the manuscript and offering his valuable suggestions. They would like to express their sincere thanks to Mr. K. KATO for his technical assistance, also.

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