VARIATIONS IN BODY TEMPERATURE ASSOCIATED WITH REPRODUCTIVE STATE IN THE FEMALE RABBIT

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It is impossible to judge precisely the reproductive state of female rabbits from their appearance or behavior. The present study was undertaken to investigate the possibility that recording of body temperature would facilitate such judgment, as in the human female.

There are many reports on the normal body temperature in various domestic animals: horse (FUKANO, 28; NUSSBAUMER, 67), cattle (ALCAIDE, 2; ARGAÑOSA *et al.*, 4; BEAKLY and FINDLAY, 6; BHATTACHARYA *et al.*, 7; BLIGH, 8, 9; BONADONNA *et al.*, 11; GAALAAS, 29; HEWITT, 33; HUTCHISON and MABON, 37; KENDALL, 44; KING, 47; KRISS, 51; MIYAKE, 62; REGAN and RICHARDSON, 75; SIMMONS *et al.*, 88; WRENN *et al.*, 106), sheep (CLAWSON, 16; MENDEL and RAGHAVON, 59; VEERARAGHAVAN and MENDEL, 98), goat (APPLEMAN and DELOUCHE, 3; DAMANT, 17; MIYAKE, 62), swine (MENSALVAS and PALAO, 60; PALMER, 70), dog (FRIEDMAN and BONNETT, 26), and rabbit (BOCK, 10; FREUND, 25; FROTHINGHAM and MINOT, 27; INOUE, 39; ISENSCHMID and KREHL, 41; KRAUSS, 50; LAZARUS-BARLOW, 53; LEE, 54; MOORE, 63; PURDY, 74; SATO *et al.*, 84; SCOTT and SIMON, 86; SIMPSON and GALBRAITH, 89; VON BORMANN *et al.*, 99; WHITE, 100; WINTERNITZ and PRATT, 101). Many of these data relate to such factors as site of temperature sensing, diurnal variation, environmental temperature and humidity, sex and body size, feeding and growth, season, locality, breed difference, etc.

Since VAN DE VELDE (97) reported that there is a cyclic variation in body temperature during the menstrual cycle, subsequent research has confirmed its existence in women, and the phenomenon is considered to be related to ovarian function. RUBENSTEIN and his associates (78, 79, 80) demonstrated that the correlation between the body temperature, which is measured before rising from bed in the morning, and gonad function takes the following course: During the phase of follicle development and increasing estrogen production, the

temperature tends to drop progressively. The low point in the temperature curve is reached when the follicle matures, that is, just before ovulation. Even the minimal progesterone production which occurs preovulatively suffices to counteract, in part, the temperature-depressing action of estrogen, and to cause an initial temperature rise beginning a few hours before ovulation. The temperature rise continues after ovulation. In the event of pregnancy, corpus luteum function naturally persists and the temperature rise of the postovulative phase is maintained. The results of these basic observations were re-investigated endocrinologically by many researchers (BUXTON and ATKINSON, 12; BUXTON and ENGLE, 13; DAVIS and FUGO, 18; ISRAEL and SCHNELLER, 42; MAGALLON and MASTER, 55; MASUKO, 56; PALMER, 69; ROTHCHILD and BARNES, 76; ROTHCHILD and RAPPORT, 77).

On the whole, though the cause of the temperature variation is not entirely clear, it seems that the body temperature variation in normal women is due to direct action of sex hormones on the area of the hypothalamus which regulates body temperature.

In farm animals, also, measurements of the body temperature have been reconsidered in relation to reproductive phenomena, such as estrus, ovulation, pregnancy and parturition. However, reports have been concentrated in the area of cattle reproduction. Body temperature variations in the cow during the estrous cycle and pregnancy were observed by BANE and RAJAKOSKI (5), FALLON (22, 23), HEWITT (33), KAEMMERER (43), NII (66), SATO *et al.* (83), SMIRNOVA (90), WEISZ (102), and WRENN *et al.* (103, 104). Some attempts have been made to use body temperature to predict the time of parturition in the cow (EWBANK, 21; HIGASA *et al.*, 34; KITAJIMA, 49; OKAMOTO, 68; PORTERFIELD and OLSON, 73). The thermogenic influence of sex hormones was reported by FERNEY (24) and WRENN *et al.* (105). FALLON (23) and SATO *et al.* (83) discussed the relationship between body temperature and fertility in the cow.

In the horse, CHIEFFI *et al.* (15) reported variations in body temperature during the breeding season and INOUE *et al.* (40) described changes in temperature at delivery. PARER (71) observed fluctuations of body temperature during the estrous cycle of the goat, and similar observations were made in swine by SCHILLING and RÖSTEL (85) and by SAITO and INABA (82). WEISZ (102), also, mentioned the temperature fall prior to delivery in the mare, sheep, goat, swine and dog.

In rabbits, body temperature has been studied in reference to ovulation which was induced by copulation or by injection of gonadotropin. YAMAGUCHI (107) recorded a temporary rise of the temperature after coitus, followed

by a fall during the 2nd to 3rd hour after coitus. This lower temperature continued until the 9th hour *post coitum* and then it showed a gradual rise. IKEDA (38) obtained similar results and concluded that ovulation occurs, in most cases, from 0.5–2 hours prior to the beginning of the latter rise in temperature. However, PISCHINGER (72) claimed that no rise in body temperature was observed after ovulation in some females. Referring to hormonal treatments, it is generally agreed that estrogen loweres the temperature and progesterone raises it (AKAMATSU, 1; KITAKAWA, 48; MATSUMOTO, 57; YAMAGUCHI, 107); though NIEBURGS *et al.* mentioned that temperature fluctuations in the female rabbit were less than those in rats and guinea pigs which had received progesterone or estrogen. Consecutive observations in the rabbit during pseudopregnancy and pregnancy have been reported only by DONNET *et al.* (20), but their account is very brief.

MATERIALS AND METHODS

Thirteen adult female and two adult male rabbits, Japanese native white breed, ranging in body 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*.

Body temperature was recorded every day at 8 to 8 : 30 a.m. and at 4 to 4 : 30 p.m. The temperature recordings were obtained by inserting an applicator of thermister with a reading accuracy of ±0.05°C through the anus to a depth of 10 cm, and by keeping for 2 minutes in the place. Room temperature in the morning and afternoon was checked every day, also.

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 in vaginal pH reported by TSUTSUMI (91), simultaneously with the temperature measurement, during three periods: January 24 to February 18, May 24 to June 13, and July 13 to July 26, 1966. From July 27 to November 15, 1966, the temperature was observed in pregnant and pseudopregnant rabbits.

RESULTS

1. Diurnal Variation

From 9 o'clock in the morning on July 8 to noon the next day, body temperature in ten animals was recorded at three-hour intervals. The results are illustrated in Figure 1. Room temperature remained nearly constant during these hours, averaging 19.8°C. Mean body temperature was 39.45°C. The mean temperature at 9 a.m. the first day was the lowest (39.20C°); then it

rose gradually to reach a high-temperature plateau from 6 p.m. (39.61°C) to 9 p.m. (39.60°C). In general, body temperature remained at a high level from 3 p.m. to 3 a.m. the next day. After 3 a.m. the temperature fell to another low point at 9 a.m.

A comparison of the body temperature in the morning (at about 8 a.m.) to that in the afternoon (at about 4 p.m.) during the first three periods of observation showed an average morning temperature of 39.24°C, and an average afternoon temperature of 39.47°C. The difference of 0.23°C was highly significant. However, higher afternoon temperatures were found in only 84 per cent of the animals, with higher morning temperatures being observed in 14 per cent. And no difference was recognizable between morning and afternoon temperatures in the remaining 2 per cent.

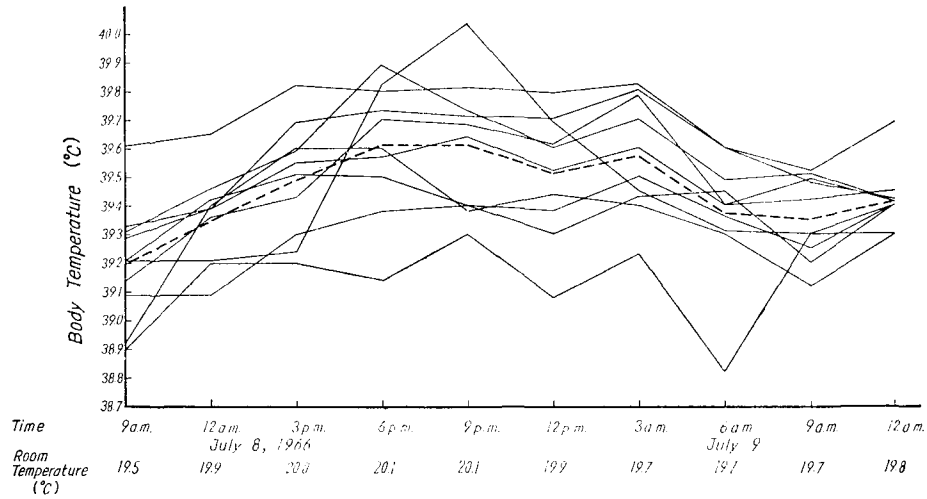

Fig. 1. Temperature curve of rabbits, indicating diurnal variation.
--- Average of 10 rabbits.

2. Estrous cycle variation

Estrous cycle was checked by observations of the vaginal mucus and by vaginal smear. Body temperatures were traced every day in the morning and afternoon. It seemed there was no clear correspondence between the body temperature curve and the estrous cycle; however, the body temperature curve for each doe showed some cyclic variations in both morning and afternoon temperatures. Since the afternoon temperature curve seemed to be more irregular than the morning temperature curve, the latter was especially noted in the present study. As an example, the results of observations on one animal are demonstrated in Figure 2. The morning temperature curve seemed to

exhibit a wave-like cycle. Average length of time between troughs of the morning temperature curves of all females was 5.62±1.63 (S.D.) days and average length of estrous cycles judged by the vaginal mucus and smear was 5.87±2.65 days. There was no significant difference between these values.

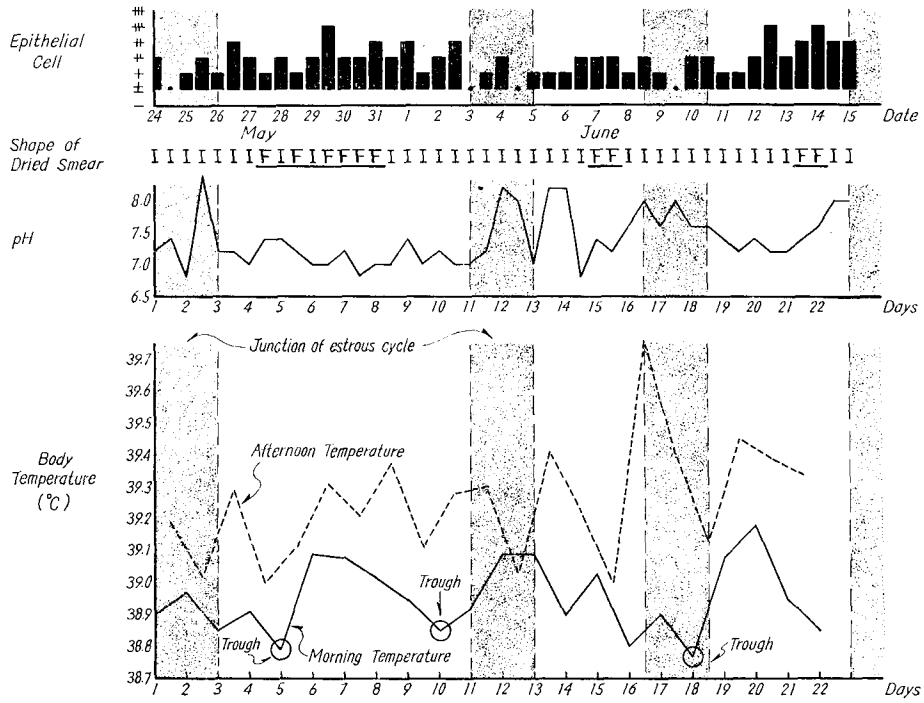

Fig. 2. Illustration of cyclic fluctuations in the vaginal mucus and smear, of pH, and of body temperature variations in one animal. The vestibule was flushed with physiological saline and the flushing placed on two slides. One slide was used for observation of the number of epithelial cells and the another for the shape of dried smear. The smear, F type shows fern- and chrysanthemum-like figures, and I type shows polygonal and other irregular figures.

3. Variation after copulation

The body temperatures of eight females were recorded at 2-hour intervals, beginning just before copulation at 8 a.m. and continuing until the 14th hour at 10 p.m. The results are given in Figure 3. No variation associated with copulation or ovulation was recognizable. Mean body temperature was lowest at 10 a.m., rising gradually to the highest level at 6–10 p.m. This tendency of fluctuation in the body temperature seemed to be the same as the diurnal variation noted previously.

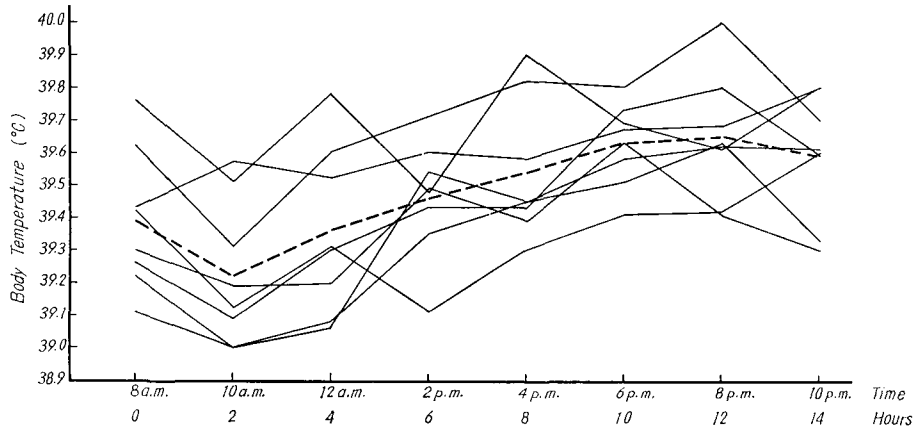

Fig. 3. Changes of body temperature after copulation to 14 hours later.
--- Average of 8 females.

4. Variation during pseudopregnancy

Five females which had been mated with bucks were identified as being pseudopregnant. Variations of the body temperature in these animals are presented in Figure 4. Comparison was made of the body temperature during the 10 days before copulation to that in pseudopregnancy, during the 2nd to 15th day after copulation. Means of the morning body temperatures were 39.30°C before copulation and 39.41°C during pseudopregnancy, and this difference was highly significat (0.1 per cent level). However, no difference was recognizable in the mean afternoon temperatures between the period before copulation (39.57°C) and that of pseudopregnancy (39.59°C). Mean body temperatures, including all morning and afternoon temperatures, were 39.43°C before copulation and 39.50°C in pseudopregnancy. The difference between them was small, but was significant at the 0.1 per cent level, also. Thus it is concluded that the body temperature in pseudopregnancy is highr than that in the normal state.

In general, the morning body temperature rose gradually to reach its highest level between the 5th and 15th days after copulation. On the 16th and 17th days the temperature dropped markedly, and then rose sharply but temporarily on the following day (both morning and afternoon temperatures). There was a significant difference at the 0.1 per cent level among the following three periods: 13th to 15th day, 16th and 17th day, and 18th and 19th day of pseudopregnancy. Mean morning temperatures in each period were 39.47°C in the first, 39.27°C in the second and 39.53°C in the third.

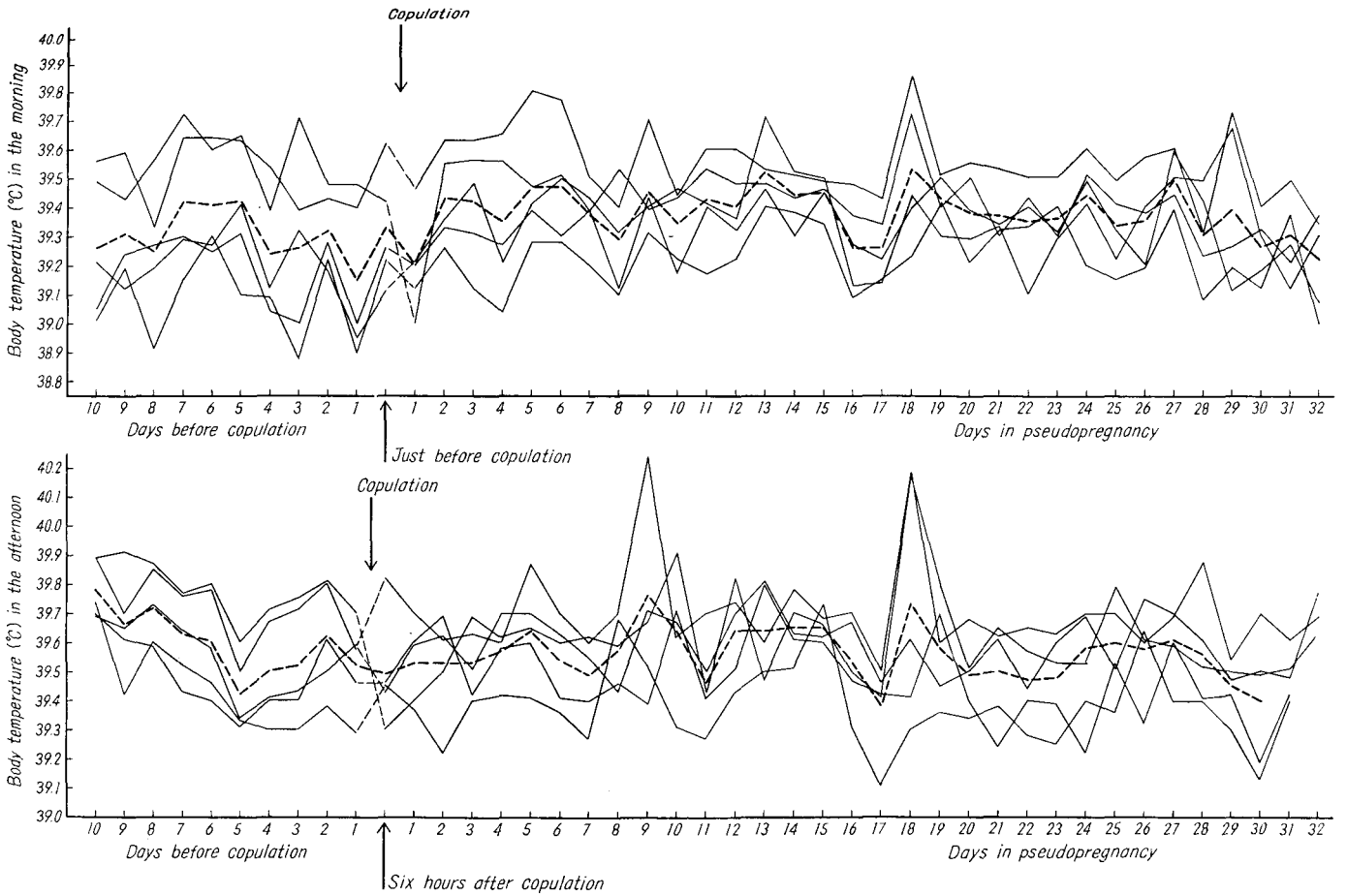

Fig. 4. Variations in body temperature in five pseudopregnant rabbits.
--- Average temperature.

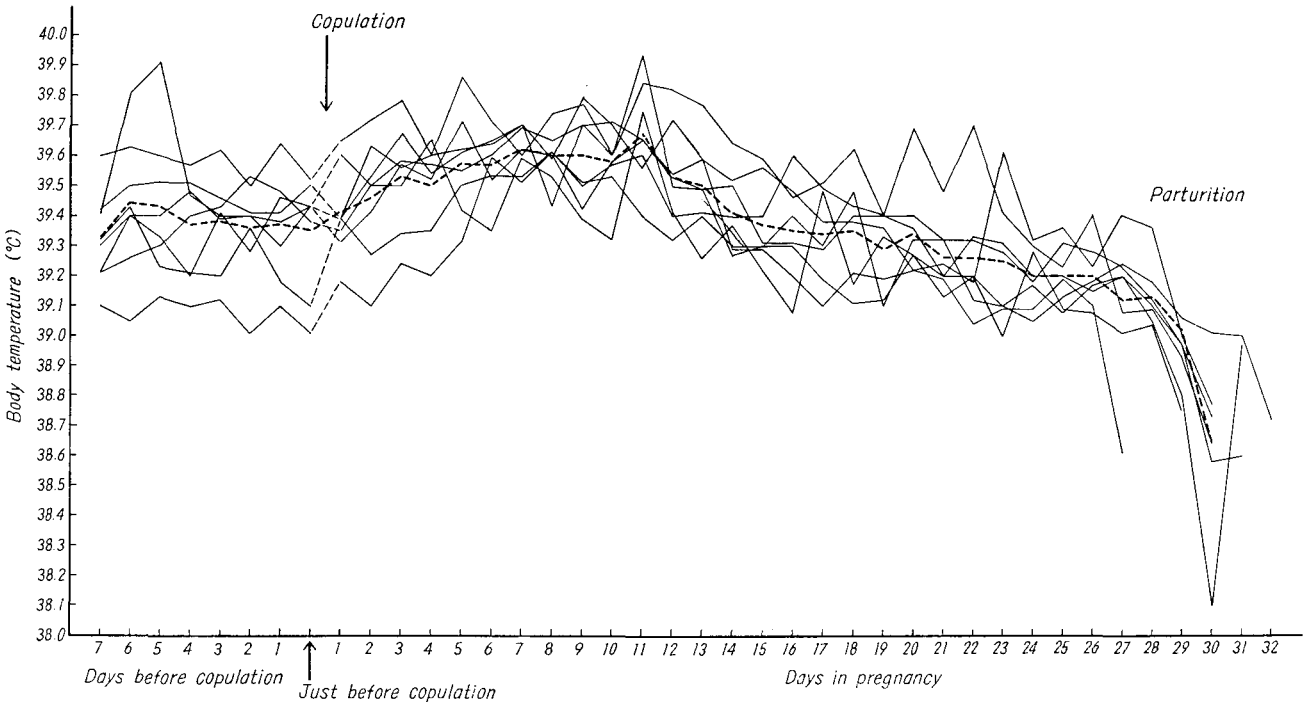

Fig. 5. Changes in morning body temperatures during pregnancy. The highest values were found from the 7th to 11th day of pregnancy.

--- Average temperature in 8 females.

5. Variation during pregnancy

Since the variation in the afternoon body temperature was somewhat irregular and showed no difference between normal state and pseudopregnancy, consideration was given thereafter only to morning body temperature. Changes in the morning body temperature during pregnancy in 8 females are shown in Figure 5.

Although the temperature was very variable, a pattern of change during pregnancy was recognizable, on the whole. The mean temperature rose gradually after copulation to reach the highest level at the 7th day of pregnancy. During the 7th to 11th day, the temperature remained at the highest level seen throughout pregnancy, and the mean temperature for this period was 39.61°C. Normal body temperature during the 7 days before copulation was 39.38°C, on the average. During the 11th to 16th day it dropped continuously, and after the 16th to 28th day it continued to decrease, although the rate of decline was less. Mean temperature during the 24th to 28th day was 39.19°C, with the exclusion of one female which delivered on the 28th day. From the 28th day to parturition, the temperature fell sharply and the mean temperature on the 30th day was 38.64°C.

The mean temperatures among three periods—the 7 days before copulation, the 3rd to 13th days and the 15th to 26th days after copulation—were compared. The difference among them was highly significant ($P<0.001$). In a comparison between the mean temperature before copulation (39.38°C in average) and that in the last half of pregnancy (39.28°C), the difference was highly significant, also.

6. Variation before and after parturition

It was noted above that the body temperature fell markedly at the end of pregnancy. The lowest temperatures were attained one day prior to parturition in 6 of the 8 females and two days prior to parturition in the other 2 rabbits. Mean temperatures were 38.72°C one day prior, 38.84°C two days prior, and 39.07°C three days prior to parturition.

After parturition, the body temperature rose sharply in all animals, and the mean temperatures were 39.52°C on the first day and 39.57°C on the second day. Variations in temperature before and after parturition are illustrated in Figure 6.

7. Variation after parturition to the 54th day

In seven females, the body temperature was measured during the 54 days following parturition. They nursed for 30 days after delivery. The results are shown in Figure 7.

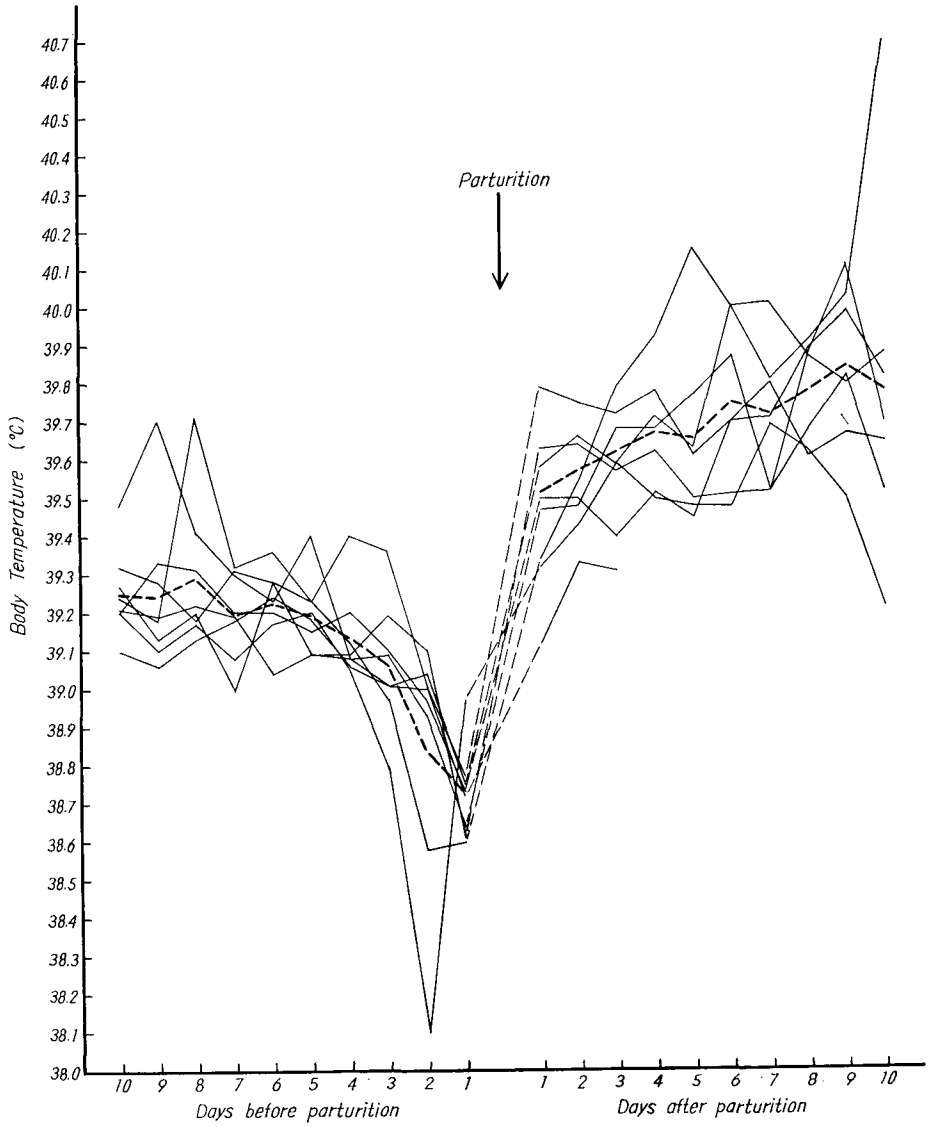

Fig. 6. Variation in body temperature before and after parturition. At the end of pregnancy the temperature falls markedly.
--- Average temperature.

The mean temperature rose gradually to a peak value during the 15th to 20th day after delivery, and then dropped slowly until about the 36th day. After the 36th day it seemed to remain at the normal level. However, this

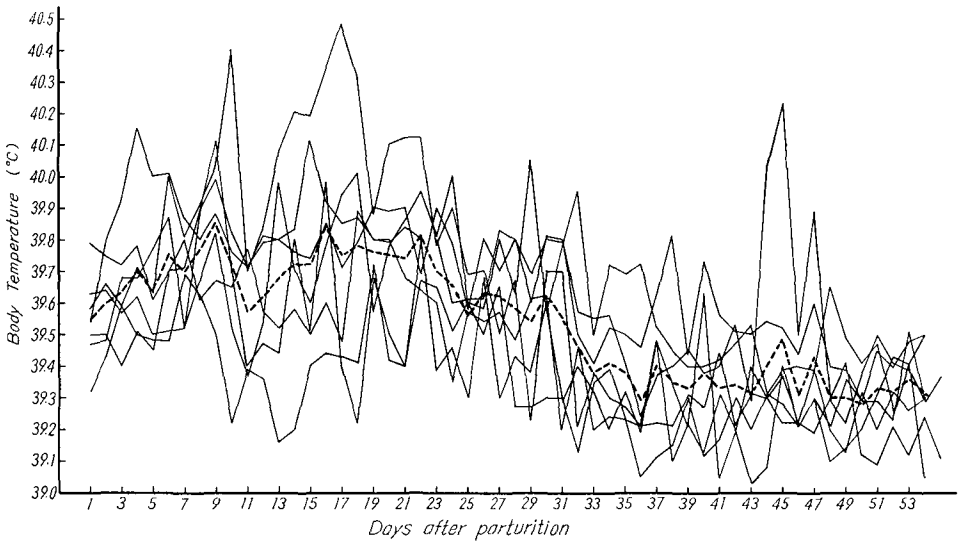

Fig. 7. Changes in morning body temperatures in 7 females for 54 days following parturition.
--- Average temperature.

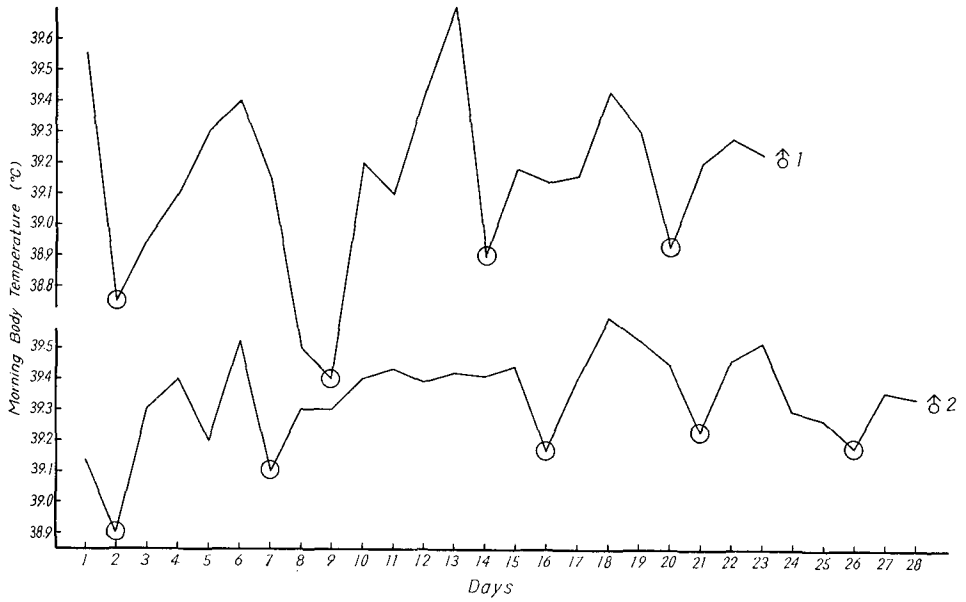

Fig. 8. Cyclic variations in the morning body temperature in adult male rabbits.
○ Trough.

temperature curve appeared to comprise three convex waves, as follows: (1) from delivery to about the 11th day, (2) from about the 11th day to about the 25th day, and (3) from about the 25th day to about the 36th day.

8. Cyclic changes in body temperature of male rabbits

Body temperatures of two male rabbits were recorded every day during 27 days from January 24 to February 20, 1966, for the purpose of comparison with females. The results are presented in Figure 8. Body temperature curves for each rabbit clearly showed cyclic waves. Intervals between troughs were 7, 5 and 6 days in one male, and 5, 9, 5 and 5 days in the other. These cyclic variations appeared to be independent of those in the females.

DISCUSSION

It is important to establish the presence or absence of a diurnal variation in body temperature so that the information may be considered in studies on the effects of some experimental procedures. SIMPSON and GALBRAITH (89) showed a curve of diurnal variation in the rabbit, which attained the maximum temperature in the evening and the minimum in the early morning. According to LAZARUS-BARLOW (53), the maximum temperature was found at 6 p.m. and the minimum at about 6 a.m., while LEE (54) claimed that there was no characteristic diurnal variation (3-hour-interval recordings), whether the rabbit was on food or was fasting up to 60 hours. In hourly measurements for 25 consecutive hours, rabbits showed a general increase in temperature as the measurements were continued, and this was considered to be due to the repeated stimulus of the hourly measurements (LEE, 54). In the present study, diurnal variation was clearly shown, the maximum temperature being attained at 6 p.m. to 3 a.m. and the minimum at about 9 a.m. This is similar to CARTER's results (14), which showed a distinct rhythm of temperature during the day with the maximum at 7–11 p.m. and the minimum at 7–11 a.m. In general, it is agreed that the body temperature in the morning is lower than that in the afternoon. The mean difference between them was 0.23°C in the present study, and the data for morning and afternoon were considered separately after this difference was established.

TSUTSUMI and MATSUMOTO (94, 95) have reported that the female rabbit exhibits cyclic fluctuations in levels of free vaginal epithelial cells, in crystal patterns of dried vaginal contents, and in vaginal pH. The average length of these cyclic fluctuations was close to 6 days, though it had wide variations. The ovarian follicle development was correlated with these cyclic changes. The mucosa of the female genital tracts also showed cyclic changes corre-

sponding to changes in the ovary and vaginal mucus (TSUTSUMI, 91). HAMILTON (31) observed cyclic variations in the vaginal smear, blood estrogen level and histology of the female reproductive tract in the rabbit. MYERS and POOLE (64) demonstrated that female rabbits living with vasectomized males in large compounds exhibit periods of sexual attraction at regular intervals of about 6 days or multiples thereof. In the present experiments, an attempt was made to correlate the fluctuations in body temperature with stages in the estrous cycle. There were cyclic fluctuations in body temperature in each female; but there was generally a poor correlation between fluctuations in temperature and the estrous cycle, though the average time interval between troughs of the temperature curve was similar to the length of the estrous cycles.

It has been proved by many workers that the body temperature in women is affected by progesterone and estrogen levels of the blood (BUXTON and ENGLE, 13; DAVIS and FUGO, 18; ISRAEL and SCHNELLER, 42; MAGALLON and MASTERS, 55; MASUKO, 56; PALMER, 69; ROTHCHILD and BARNES, 76). Similar results were obtained in researches on the cow (FERNEY, 24; WRENN *et al.*, 103, 104, 105) and on the rabbit (AKAMATSU, 1; KITAKAWA, 48; NIEBURGS *et al.*, 65; YAMAGUCHI, 107). Since the estrous cycle in the female rabbit apparently can take place in the absence of functional corpora lutea (TSUTSUMI and MATSUMOTO, 95), it is surmised that the fluctuation in body temperature may be irregular. However, HAMILTON (31) has reported cyclic variations in blood estrogen level and the vaginal smear. From observations on normal uterine mucosa, TSUTSUMI (91) suggested that progestin may be secreted during some period in the estrous cycle of normal non-pregnant rabbits. Thus some possibility remains of a relationship between fluctuation in body temperature and stages in the estrous cycle.

In women, the basal body temperature curve has been observed for detection of ovulation in relation to fertility and sterility. Similar works have been reported on the cow (BANE and RAJAKOSKI, 5; FALLON, 22, 23; KAEMMERER, 43; KUMARAN and IYA, 51; NII, 66; SMIRNOVA, 90; WRENN *et al.*, 103, 104), on the swine (SCHILLING and RÖSTEL, 85), and on the rabbit (IKEDA, 38; PISCHINGER, 72; YAMAGUCHI, 107). IKEDA (38) and YAMAGUCHI (107) reported that a fluctuation was recognizable in rabbits similar to that in women, while PISCHINGER (72) claimed that no specific fluctuation was observed when ovulation occurred in rabbits. In the present study, in which body temperatures after copulation were recorded at 2-hour intervals, the curve showed a diurnal variation but no correlation with ovulation.

Based on the observations of TSUTSUMI and HACHINOHE (92), pseudo-

pregnancy in rabbits was divided into three stages according to the cellular variation in the vaginal mucus. The first stage was up to the fourth day after coitus, the second was from the 5th to about the 15th day, and the third was the remainder. During the 2nd stage the body temperature remained at a high level, and at the beginning of the third stage the temperature dropped markedly. It was also reported that maximal branching of the folds of the uterine mucosa occurred at 4 to 9 days of pseudopregnancy (TSUTSUMI and HAFEZ, 93). A temporary decrease in afternoon body temperature was noted on the 11th or 12th day of pseudopregnancy. The physiological meaning on this phenomenon is obscure. However, HUGHES and MYERS (36) stated that behaviour with a strong sexual component, culminating in mating and ovulation, was observed at the beginning of pseudopregnancy, and similar behaviour was also observed at about either the 6th or 12th days of pseudopregnancy, or both. DONNET *et al.* (20) also described briefly the fluctuations in body temperature during pseudopregnancy. They mentioned a temporary fall in the temperature immediately after copulation, and stated that the temperature remained stable and high during the 2nd to 14th day. In the present study, fluctuation the day following coitus was not uniform in the pseudopregnant and pregnant rabbits.

DONNET *et al.* (20) also mentioned fluctuation in body temperature during pregnancy. They indicated that a plateau of high temperature continued until the 15th or 16th day and a marked fall in the temperature occurred at the end of pregnancy. However, a plateau of high temperature was recognizable only from about 7 to 11 days *post coitum* in the present study. During this time implantation occurs and the origin of the fetal placenta is established superficially on the placental fold of the uterine mucosa (HAFEZ and TSUTSUMI, 30). MIKHAIL *et al.* (61) reported that the progesterone level in rabbit ovarian-vein blood reached its peak at midpregnancy, declining gradually thereafter with a consistent drop two to three days prior to delivery. It seems curious that the body temperature from 12 to 16 days of pregnancy showed a marked decrease in the present work, in spite of a presumably high level of progesterone in the blood.

SHIBATA (87) recorded the body temperature of the guinea pig every day through pregnancy. He stated that from the beginning to the middle of the gestation period, temperatures were not significantly different from those in normal animals; but the temperatures decreased slowly with the advance of pregnancy. He suggested that this decrease in temperature might be related to fetal growth in the uterus and to the position of the fetus in the abdominal cavity. In the rabbit, growth of the uterine contents during pregnancy was

described by HAMMOND (32). The contents increase markedly from 12 days to the end of pregnancy, and the rate of increase is especially great after 16 days. In the present study, body temperature decreased sharply from 12 to 16 days of pregnancy, and the rate of decrease in temperature was less during the following days. It is interesting to note some correlation between the fluctuations in body temperature and the growth of the uterine contents during the latter half of pregnancy.

Many attempts have been made to predict the time of parturition by recording the body temperature in women (MATSUMOTO and OUKURA, 58), in cows (EWBANK, 21 ; HIGASA *et al.*, 34 ; KITAJIMA, 49 ; OKAMOTO, 68 ; PORTERFIELD and OLSON, 73 ; WRENN *et al.*, 104), in mares (INOUE *et al.*, 40) and in guinea pigs (SHIBATA, 87). A precipitous decline in temperature prior to parturition was observed in these experiments, and this phenomenon was observed in our rabbits, also. This may provide a useful indicator in predicting the time of parturition.

After parturition, in our study, the temperature rose and then fell, and seemed to stabilize at the normal level after the 36th day following delivery. From delivery to the 36th day, two troughs were recognizable in the body temperature curve, which were at the 11th and 25th days. Although the physiological meaning of these two periods is obscure, the following factors may be involved. Young of the doe open their eyes at about the 11th day after birth, and they are fed only by mother's milk during this time. After about the 20th day, the young begin to eat solid food, together with their mother, and they grow more rapidly after this period. This probably means that milk secretion activity, at a high level until about the 20th day, is inhibited thereafter. According to TSUTSUMI *et al.* (96), the body weight of the doe decreases until about 30 days after delivery and then increases gradually to normal after that time.

Recently, KIHLSTRÖM and HORNSTEIN (45, 46) and DEGERMAN and KIHLSTRÖM (19) reported that cyclic variation in the body temperature of the male rabbit was recognizable, and that the mean length of these cycles was very close to that of the cyclic variations in the sexual activity of the male rabbit. HORNSTEIN *et al.* (35) also described cyclic variation in body temperature of castrated rabbits, and this was considered to be independent of the testicular hormones. In the present preliminary observation with two male rabbits, cyclic variation was recognizable, also.

SUMMARY

Body temperature of thirteen adult female rabbits was recorded on con-

secutive days, and studied in relation to reproductive activities, such as estrous cycle, pseudopregnancy, pregnancy and puerperium.

The correlation between fluctuations in body temperature and changes in the estrous cycle, as estimated by inspection of the vaginal mucus and vaginal smears, was rather poor, though the average number of days between troughs of the temperature curve was similar to the interval between estrous cycles.

The body temperature curve in mated females showed only a diurnal variation from copulation to the 14th hour *post coitum*.

During pseudopregnancy, mean body temperature remained at a higher level than that in the normal state. On both the 16th and 17th days, the temperature dropped sharply, followed by a temporary increase.

In pregnant animals, the mean temperature rose gradually after copulation to reach its highest level from the 7th to the 11th day. From the 11th to the 16th day, it decreased markedly, and during days 17 to 28 of pregnancy, the rate of decrease was less, but the temperature continued to decline.

One or two days prior to parturition, the temperature fell sharply. This may provide a useful indicator in predicting the time of parturition.

The temperature rose markedly after parturition to reach a peak during the 15th to 20th day of puerperium. After this period, it dropped slowly until about the 36th day. After the 36th day, the temperature remained at the normal level.

In a preliminary observation, cyclic variation in body temperature was recognizable in two adult male rabbits.

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