Effects of Litter Size on Gestation Length and Plasma Progesterone Concentration in Mongolian Gerbils (*Meriones unguiculatus*)

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Abstract A total of 31 female Mongolian gerbils (19–54 weeks of age at mating) were used for this study. The mean litter size at birth in all females was 4.4 ± 0.3 , ranging 1 to 8 pups. Although maternal age did not affect to litter size and gestation length, litter size did significantly to gestation length. Plasma progesterone (P_4) was measured in serial blood samples collected every 3 days throughout gestation period. The P_4 concentration increased rapidly after mating, reaching a peak level on day 6 of gestation, maintained the high level to day 12 of gestation, then decreased to a moderate level and sustained at the level until day 21. The P_4 concentration decreased to a lowest level on day 24 of gestation (mean gestation length : 25.2 days). Females having a large number of litter size tended to reveal higher level of P_4 during gestation, comparing with that females having a fewer litter size, and significant differences between them were found on days 6 and 9 of gestation. There were significant differences in P_4 levels among 3 groups according to gestation length, resulting in lower P_4 level from females with longer gestation length. Therefore, difference of plasam P_4 level during the gestation is due to litter size.

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In a previous paper¹³⁾, we reported plasma concentration of progesterone (P_4) during gestation and lactation in Mongolian gerbils. The pattern of the P_4 level in pregnant gerbils was quite different from those in mice^{1, 8, 15)} and rats^{3, 10)}. In these animals, blood P_4 level increased rapidly during early gestation and maintained a plateau which was followed by a higher peak, starting at midgestation. The P_4 level declined rapidly in the 2 days before parturition. Bridges³⁾ reported in rats that serum P_4 rose from 5–10 ng/ml on days 1 and 2 of gestation to about 70 ng/ml on days 4–10. Then, a second increase in the level occurred between days 10 and 15 of gestation. Average P_4 level ranged from 100 to 152 ng/ml between days 12 and 20 of gestation. The P_4 level declined significantly on days 20 and 21, and dropped further to about 10 ng/ml on day 22 of gestation³⁾. In

gerbils, however, plasma P_4 level showed a peak in early gestation but not in second half of gestation¹³⁾. A sharp increase in the P_4 level was found in early gestation and it reached a peak on day 6. The higher concentration was maintained to day 12, and then decreased to a moderate level on day 15 and continued at that level until day 21. The P_4 level declined to the lowest level on day 24 of gestation, one day before parturition (mean gestation length : 25.2 days). However the result was obtained with limited number of animals ($n=7$)¹³⁾, effects of the litter size at birth to the gestation length and the plasma P_4 level in the gestation were possible to be in gerbils, as found as some species of polytocous animals. In pregnant rats with 0, 1 or 10 and more conceptuses experimentally adjusted on day 7 of gestation, a direct relation has been found between the number of conceptuses and

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serum P₄ level on days 12, 15, 17, 19 and 21 of gestation¹⁴⁾. Plasma P₄ level in mice with one fetus was significantly lower than in those with 2–5 or 9–14 fetuses⁹⁾. Furthermore, in mice in which the number of fetuses was experimentally adjusted to 1–2, 3–4, or 8–10 on day 7 of gestation, serum concentration of P₄ was directly proportional to litter size²⁰⁾.

An inverse relationship between litter size and gestation length has been reported in several species of polytocous animals, such as mice^{2,4,7)} and rats^{17–19)}. In our previous experiments^{17,18)}, pregnant rats whose conceptuses were reduced surgically to various sizes less than 10 on day 8 of gestation gave birth on day 22 to day 24 of gestation. The regression line of gestation period on litter size in the mothers whose litter sizes were less than 10 was $Y = 23.46 - 0.17X$, where Y was the gestation period and X was the litter size, with a correlation coefficient of -0.602 ($P < 0.01$; $n = 71$)¹⁷⁾.

Therefore, the aims of this study are, first, to confirm the pattern of plasma P₄ level throughout the gestation and to clarify the characteristic features of the P₄ pattern in gerbils, and second, to analyse the relationships between litter size at birth and the P₄ level, gestation length or maternal age.

Materials and Methods

Mongolian gerbils from our colony¹²⁾ were used in this study. Gerbils were maintained at $22 \pm 3^\circ\text{C}$ with automatically controlled lighting from 0500 to 1900 h (14 L : 10 D) and provided with food pellets (LABO MR STOCK, Nihon Nosan Kogyo) and water *ad libitum*. Male gerbils were placed individually into mating cages (20 × 35 × 16 cm, HAM-2, Okazaki Sangyo) for several days. Thereafter, each female (nulliparous or parous), 19 to 54 weeks of age, was placed into the mating cage in the early afternoon. Female gerbils were examined their pregnancy by vaginal smear between 1830 and 1900 h, since gerbils generally mate in the late afternoon. The day when spermatozoa were found in the vaginal smear was designated day 0 of gestation. Since each mated male was removed at the next morning, all the mated females were then housed singly in the cage. The pregnant females were weighed and bled every 3 day,

starting on day 0 of gestation, until they littered. Blood sampling and measurement of plasma P₄ were described previously¹³⁾. In brief, blood was collected between 1800 and 1900 h from a lateral tail vein into a plastic clad hematocrit tube (HEMATO-CLAD HEPARINIZED, Drummond Scientific Co.) and immediately centrifuged at room temperature for 20 min. The centrifuged blood in a tube was stored frozen at -20°C until analysis. The plasma P₄ concentration was measured with a commercially available kit for EIA (Cayman Chemical Co.). The plasma samples were diluted with EIA buffer to 500 or 1,000 times and assayed in duplicate. Each breeding cage was checked daily till delivery. The date of birth and the number of pups were recorded.

The data were given as means $\pm$ SEM. Statistical evaluations were made using Student's t-test and Duncan's new multiple range test after ANOVA. Values of $P < 0.05$ were treated as significant.

Results

Total 31 pairs mated 2.7 ± 0.5 days (range : 0–13 days) after the introduction of females into the mating cages. The mean litter size at birth in all females was 4.4 ± 0.3 (1–8 pups), and the mean gestation length was 25.2 ± 0.1 days (24 days : $n = 4$; 25 days : $n = 18$; 26 days : $n = 9$). The mean body weight gain of 18 females increased gradually from day 0 to day 12 of gestation (1.7 – 2.5 g/3 days), with significantly heavier than that of 3-day before from day 12 of gestation continuously until day 24 (3.7 – 5.4 g/3 days). The maximum value of the body weight gain was 26.5 ± 0.9 g on day 24 of gestation, and the gain abruptly decreased to 9.0 ± 1.0 g on day 25, after parturition. The mean litter size of these females ($n = 18$) was 4.8 ± 0.4 .

Relationships between maternal age, litter size and gestation length were investigated using the data from littered females ($n = 31$). Firstly, to analyse the effect of maternal age on litter size and gestation length, the females were divided into 3 groups based on the age at mating (19–25, 26–32 and 33–54 weeks of age). In spite of the difference of maternal age among the 3 groups, the litter sizes and the gestation lengths were not significantly different from each

other. Secondly, to analyse the effect of gestation length on litter size, the females were classified according to the gestation length (24, 25 and 26 days). Females with the long gestation length (26 days) gave birth significantly small number of pups than the other 2 groups (24 and 25 days). However, the ages at mating were not significantly different among the 3 groups (Table 1). Thirdly, to analyse the effect of litter size on gestation length, the females were divided into 3 groups based on the litter size (1-3, 4-5 and 6-8 pups). The average of litter size in each group was 2.3 ± 0.2 , 4.4 ± 0.1 or 6.6 ± 0.3 , respectively, differing significantly from each other among the 3 groups. The gestation length was negatively related to the litter size, shortening gradually from small (1-3 pups) to large (6-8 pups) litter size. However, age at mating was not significantly different among the groups (Table 2). Since there appeared to be an inverse relationship between litter size and gestation length, data from all the females ($n=31$) were analysed for the relationship between the both parameters. The analysis shows that the gestation period (Y, day) was significantly negatively correlated with the litter size (X) ($r = -0.570$, $P < 0.01$) and equation for the regression line was $Y = 26.08 - 0.21 X$.

Mean plasma P_4 concentrations in all of the females during gestation are presented in Fig. 1. The plasma P_4 level was 59.2 ± 4.5 ng/ml on day 0, when spermatozoa were observed in the vaginal smear. The concentration rose significantly on day 3 of gestation and reached a peak (165.5 ± 9.7 ng/ml) on day 6. The level was maintained to day 12 (168.0 ± 8.9 ng/ml), then decreased on day 15 and kept at a moderate

Table 1. Effect of gestation length on litter size and ages at mating of females in 3 groups

Gestation length (days)	(n)	Litter size	Age at mating (weeks)
24	(4)	5.8 ± 0.6^a	35.3 ± 7.8^a
25	(14)	4.8 ± 0.4^a	32.1 ± 2.4^a
26	(9)	2.9 ± 0.4^b	36.1 ± 3.6^a

^{a,b}: Means ($\pm$ SEM) within a column not sharing a common superscript letter are significantly different ($P < 0.05$).

level until day 21. The concentration decreased further to the lowest level on day 24 of gestation (50.2 ± 5.3 ng/ml). Figure 2 shows that the P_4 concentration of 3 groups divided according to gestation length. The females with the shortest length tended to be high and especially revealed significantly higher level compared with the others on days 3, 6, 9, 18 and 24 of gestation. Figure 3 shows the P_4 concentration of 3 groups classified by litter size. The females having the largest number of pups showed a significantly higher level compared with another groups on days 6 and 9 of gestation. To estimate relationships between P_4 concentration and reproductive factors (maternal age, litter size and gestation length) on each day of gestation, the data obtained from all the females ($n = 31$) were analysed. As the results, P_4 level on day

Table 2. Effect of litter size on gestation length and ages at mating of females in 3 groups

Litter size	(n)	Gestation length (days)	Age at mating (weeks)
1~3 (2.3 ± 0.2) ^a	(9)	25.7 ± 0.2^a	31.2 ± 3.0^a
4~5 (4.4 ± 0.1) ^b	(14)	25.1 ± 0.1^b	36.1 ± 2.9^a
6~8 (6.6 ± 0.3) ^c	(8)	24.6 ± 0.2^c	31.8 ± 4.6^a

^{a,b,c}: Means ($\pm$ SEM) within a column not sharing a common superscript letter are significantly different ($P < 0.05$).

Fig. 1. Plasma progesterone concentration during gestation in gerbils. Values are the mean $\pm$ SEM of 31 animals. Means with no common letters during gestation are significantly different ($P < 0.05$).

Fig. 2. Plasma progesterone concentration during gestation in gerbils. The 3 groups were divided based on gestation length. Values are the mean. The numbers of animals in each group are shown in Table 1. Group differences were significant on days 3, 6, 9, 18 and 24 ($P < 0.05$).

Fig. 3. Plasma progesterone concentration during gestation in gerbils. The 3 groups were divided based on litter size. Values are the mean. The numbers of animals in each group are shown in Table 2. Group differences were significant on days 6 and 9 ($P < 0.05$).

3 of pregnancy was negatively correlated with gestation length ($r = -0.568$, $P < 0.01$) and P₄ level on day 9 of pregnancy was positively correlated with litter size ($r = 0.437$, $P < 0.05$).

Discussion

An inverse relationship between litter size at birth and the gestation length has been reported in rats¹⁷⁻¹⁹⁾ and in mice^{2,4,8)}. Sherwood *et al.*¹⁹⁾ found that 47% of the rats with three conceptuses gave birth about 24 h later than those of rats which had five or more

conceptuses did. Our previous studies indicated the inverse relationship between litter size and gestation length in rats in which the number conceptuses had been adjusted surgically to various sizes on day 8 of gestation. The regression line of gestation period on litter size in mothers whose litter sizes were less than 10 was $Y = 23.46 - 0.17X$, where Y is the gestation length and X is the litter size, with a correlation coefficient of -0.602 ($P < 0.01$, $n = 71$)¹⁷⁾, and $Y = 23.57 - 0.17X$ with a correlation coefficient of 0.752 ($P < 0.05$, $n = 23$)¹⁸⁾. The present study showed in gerbils a regression line, $Y = 26.08 - 0.21X$, which is highly negatively correlated between two parameters with a correlation coefficient of -0.570 ($P < 0.01$, $n = 31$).

We previously reported that the pattern of plasma P₄ concentration during gestation in gerbils was quite different from those of mice^{1,8,15)} and rats^{2,10)}, in which P₄ concentration increased rapidly to make a first peak during early gestation, maintained a plateau and then raised further to make a second higher peak starting around midgestation. On the other hand, in gerbils the P₄ level increased shortly after mating and reached a peak level in the first half of gestation. Therefore, one of the purposes of this study was to confirm the discrepancy. The present study showed that the pattern of P₄ level obtained from a total of 31 female gerbils coincided well with of the previous report based on the data obtained from 7 females¹³⁾. Although the discrepancy in the patterns among the species is not clear at the moment, the time of the formation of the corpus luteum in the ovary and the pattern of the secretion of P₄ from corpus luteum would be different among the species.

In the course of analysis of the P₄ concentration in the previous study¹³⁾, relationships were possible to exist between the level of P₄ and litter size or gestation length. In this extended study, therefore, the 31 littered females were divided into 3 groups classified according to the parameters; maternal age at mating, litter size at birth and gestation length. Although the females divided into 3 groups based on age at mating did not show significant differences in litter size and gestation length with age at mating of the females, decreased reproductive function in aging rodents has

been reported^{7,11,16,21}). The length of gestation was progressively increased from 22.2 days in the 4-month-old group to 23.7 days in the 13-month-old group in rats¹⁶), and 19.0 in 3–7 months old group to 20.8 in 11–12 months old group in mice⁷). However, the aged group (33–54 weeks old) in this study did not show a prolonged gestation length comparing with younger groups (19–25 and 26–32 weeks old). In the old group of this study, however, the age of females at mating was doubtful about a representation of reproductive senescence in gerbils.

A positive relationship between litter size and P_4 concentration during gestation has been established in mice^{1,9,15,20}) and rats^{6,14}). Michael *et al.*¹⁵) and Barkley *et al.*¹) found that P_4 level in the small litter size line in mice was comparable to that of control line until day 10 of gestation. Thereafter, a marked increase of the level at midgestation (day 15) found in the control line was not seen in the small litter size line. Similar results were obtained in which 3 groups of mice were set up by the number of conceptuses (1–2, 3–4 and 8–10 pups) adjusted surgically on day 7²⁰). The P_4 levels on day 15 were significantly different among 3 groups. Females with 1–2 conceptuses had significantly lower P_4 level than females with 3–4 conceptuses and females with 8–10 conceptuses, exhibiting a significantly greater level in the latter than in the other 2 groups. In rats in which the number of conceptuses per rat was adjusted on day 7, P_4 level on day 12 was statistically the same in 4 groups (0, 1, 2–4 and 5–10 conceptuses). However, the levels on day 15 were significantly different from those on day 12 and the values increased significantly in proportion to the number of conceptus¹⁴). Moreover, Golos and Sherwood⁶) reported similar results in rats. The results on the relationship between litter size and the P_4 level were determined during the second peak period which existed in second half of the gestation (after day 12 in rats and after day 10 in mice). Elbaum *et al.*⁵) reported that a significantly positive correlation ($P < 0.01$; $r = 0.97$; $n = 21$) was found between total luteal weight and serum P_4 concentration on day 16 of gestation in rats. In gerbils, on the other hand, the relationship between litter size and plasma P_4 was found in first half of the gestation (until day

12), but not in second half of the gestation (Fig. 3). It is considered that this discrepancy is due to the time of the peak in P_4 level; in second half of the gestation in rats and mice, but in first half in gerbils. The placenta plays an important role in the regulation of corpus luteal activity and the production of P_4 during the second half of gestation in rats^{6,14}). In gerbils, accomplishment of placenta is as early as day 12 of gestation, judging from the result in which the implantation of the fertilized eggs occurs around day 8 of gestation (unpublished data). Therefore, the placenta might not influence to P_4 level in the first half of the gestation. Taking together, the number of corpus lutea should be mainly contributed serum P_4 level in first half of the gestation in gerbils.

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