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Objective: We aimed to investigate whether mouse mothers born prematurely give birth to preterm pups.

Methods: Sexually mature female C57BL/6 mice were used. To create the pPROM model, the fetal membranes of the right uterus were pierced using sterile 21- or 26-gauge needles on gestational day 15.5.

Results: The mean gestational duration of the first-generation pPROM model was 17.5 days. The mean gestational duration for the second-generation female mice born prematurely from the pPROM model was 19.8 days. The gestational duration between the first and second generations was significantly different ($P<0.01$). The average pup weight was greater in the third-generation mice than in the second-generation mice.

Conclusion: Mice born to pPROM model mice did not give birth to preterm pups. It appears that mother born prematurely through iatrogenic pPROM does not have preterm birth.

Key Words: Cervix, Iatrogenic prelabor premature rupture of membrane, Offspring, Prelabor premature rupture of membrane, Preterm birth

Introduction

Preterm birth is defined as delivery at 20-37 weeks’ gestation. Every year, 15 million babies are born prematurely worldwide. The preterm birth rate in Korea has increased by approximately 1.5 times from 5.2% in 2007 to 7.6% in 2017. In addition, the average maternal age in Korea is increasing annually. In 2017, the average age of mothers with a gestational period <37 weeks was 33.2 years, 0.7 years more than that of mothers with a gestational period of 37-41 weeks. Preterm birth is associated with increased infant morbidity and mortality rates. Even if the preterm babies survive, they are at high risk of experiencing damage to the nervous and respiratory systems. The preterm birth has various causes, such as microbial–induced inflammation, reduced progesterone activity, and uterine or cervical disease.

Prelabor premature rupture of the membranes (pPROMs), a cause of preterm birth, accounts for 25–30% of the obstetric precursors of preterm birth. The fetal membrane consists of the amniotic membrane, chorionic membrane, amnion epithelium, and other such membranes and plays an important role in protecting against microorganism access through the vagina and external shock while the fetus is growing in the uterus. pPROM is caused by environmental and genetic factors, such as cigarette smoking, anemia, malnutrition, and collagen vascular disorder or by uterine anomalies, cervical insufficiency, and ascending bacterial infection. In addition, iatrogenic pPROM that results from an invasive prenatal diagnostic can occur after amniocentesis, fetoscopy and surgery. The risk of pPROM after
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amniocentesis is 1–2%. The risk of pPROM after the fetoscopy is associated with the degree of fetal membrane damage. Despite these low risks, mothers are reluctant to take tests during pregnancy.

A previous study reported that women who were born spontaneously preterm were more likely to give birth to preterm babies. Conversely, a cohort analysis of 38,700 women revealed that women born prematurely are not at significant risk of experiencing preterm birth. Recent studies have reported that there is controversy regarding the significance of a family history of preterm birth. Mothers who delivered early because of pPROM are concerned that their children will subsequently have preterm babies. Thus, we aimed to determine whether a child born to a pPROM mother would later have preterm offspring. However, there are ethical concerns regarding conducting such experiments with women, and it is difficult to control individual differences among women. Therefore, the previously developed pPROM mouse model was used as an alternative in this study.

Here, we investigated the ability of the pPROM mouse models developed by sterile rupture of the fetal membranes (small and large ruptures) to deliver pups.

Methods

1. Animal model and treatment

All animal experiments were approved by the Institutional Animal Care and Use Committee for the Care and Use of Laboratory Animals. We used sexually mature C57BL/6 mice. Water and food were supplied ad libitum. The experimental room was maintained under controlled light conditions (12 hours light/12 hours dark) and temperature (22–24°C).

The pPROM mouse model was created, as previously described. Pregnant C57BL/6 mice were purchased from Japan SLC Inc. (Hamamatsu, Japan). On day 15.5 of gestation, the mice were insufflated with isoflurane, and the right abdomen was opened. The fetal membranes of the right uterus were pierced with sterile needles (21- or 26-gauge). The first and second gestational sacs were pierced 4 and 8 times, respectively, with 21- and 26-gauge needles. Membrane rupture was confirmed by the flow of amniotic fluid out of the uterus. The subcutaneous tissues were sutured using Vicryl sutures, and the skin was sutured with black silk thread. The two mice were housed in one cage.

Preterm birth was defined as delivery within 19 days of gestation. A pPROM mouse gave birth to premature pups; these pups and were weaned at 4 weeks after birth. The 8-week-old female mice born prematurely from the pPROM model and 10-week-old normal male mice that were purchased commercially (Orient Bio Inc., Seongnam, Korea) were bred at a ratio of 2:1 per cage. Day 0.5 was defined as the day on which the presence of the vaginal plug was confirmed in the morning. The pregnant mice were housed one per cage. The length and weight of the pups were measured on birth. The length was measured from the head to the back using calipers, while the weight was measured using a weighing scale.

2. Statistical analyses

Data were expressed as mean±standard deviation (SD) and were normally distributed. The results of each experiment were analyzed using independent t-test and Mann–Whitney U-tests. P-values <0.05 or 0.01 indicated a significant difference between the values compared. The statistical analysis was performed using SPSS version 13 software (IBM Corp., Armonk, NY, USA).

Results

On day 15.5 of gestation, the gestational sacs of the mice were pierced with a 21- or 26-gauge needle to create the pPROM models. The mice gave birth to pups in the morning of day 17.5 of pregnancy. Three female and five male pups were born to the pPROM model created using a 21-gauge needle. From the pPROM model created using a 26-gauge needle, five female and four male pups were born. All second-generation mice, a total of 17 pups, were born prematurely at 17.5 days of pregnancy. Of the 17 second-generation mice, a total of 8 female mice were selected and tested for cross-breeding. Consequently, all third-generation mice were born at a mean 19.8 (SD, 0.7) days of gestation: thus, all third-generation mice were born at full term (Fig. 1). The difference in the gestational period between the pPROM model and second-generation mice born from the pPROM model was statistically significant (P<0.01), and the
The first-generation pPROM mice using a 21-gauge needle did not differ significantly. The first-generation pPROM mouse model created using a 26-gauge needle delivered five female pups, and 35 pups were born to these female mice. The mean (SD) weight of the 35 pups was 1.8 g (0.2 g) (Fig. 3B). The mean weight of the second-generation pPROM mice was significantly lower than that of the third-generation pPROM mice for which a 26-gauge needle was used (P<0.01).

The mean length of the second-generation pups born to the first-generation mothers whose gestational sacs were pierced using a 21-gauge needle was 25.5±2.1 mm for and that of pups born to the first-generation mothers whose gestational sacs were pierced using 26-gauge needle was 24.6±3.0 mm (Table 1). The mean body length of the preterm pups was not significantly different between the two groups. The mean (SD) weight of the pups born to the first-generation mothers whose gestational sacs were pierced using a 21-gauge needle was 1.4 g (0.1 g) for and that of pups born to the first-generation mothers whose gestational sacs were pierced using a 26-gauge needle was 1.2 g (0.1 g) (Table 1). The mean weight of the preterm pups born to the pPROM model created using a 26-gauge needle was smaller than that of those born to the pPROM model created using a 21-gauge needle (P<0.01). Two of the three preterm female pups born to the pPROM model created using a 21-gauge needle and four of the five preterm female pups born to the pPROM model created using a 26-gauge needle showed symptoms of hair loss on the head and back.

The weight of the second- and third-generation mice was then compared according to the needle gauge. Three female pups were born from the first-generation pPROM model created using a 21-gauge needle, and 23 pups were born to these female mice. The mean (SD) weight of the 23 pups was 1.5 g (0.2 g) (Fig. 3A). The weight of the second- and third-generation pPROM mice using a 21-gauge needle did not differ significantly. The first-generation pPROM mouse model created using a 26-gauge needle delivered five female pups, and 35 pups were born to these female mice. The mean (SD) weight of the 35 pups was 1.8 g (0.2 g) (Fig. 3B). The mean weight of the second-generation pPROM mice was significantly lower than that of the third-generation pPROM mice for which a 26-gauge needle was used (P<0.01).

Fig. 1. Genogram of the prelabor premature rupture of the membranes (pPROM) mouse model.

Fig. 2. Difference in the gestational period between the first-generation mice (prelabor premature rupture of membranes model) and their offspring. *P<0.01 (using the independent t-tests).
preterm is defined as the gestational period being shorter than the normal gestational period for the particular strain. Murray et al. reported that the mean gestational time for C57BL6/J mice is approximately 19.0 days. In this experiment, the pPROM mouse models gave birth at 17.5 days’ gestation; thus, the pPROM mice in this study are preterm birth models. In this study, mice born to pPROM model mice did not give birth to preterm pups. It appears that mothers born prematurely through iatrogenic pPROM such as amniocentesis, fetoscopy, intrauterine procedures and fetal surgery does not have preterm birth. Some studies reported on preterm birth in the next generation. Previous population cohort studies showed that mothers who were born prematurely were more likely to experience preterm birth.

Discussion

The pPROM model mice were born prematurely. However, the second-generation female mice born from the pPROM model showed a normal gestational period. Among the second-generation female mice, a greater number of pups was born to the model mice created using a 26-gauge needle, and the mean weight was lower than that of the pups born to the model created using a 21-gauge needle. The mean weight of the second-generation pups tended to be lower than that of the third-generation pups, and the second-generation pups of the pPROM model created using a 26-gauge needle weighed significantly less than the third-generation pups.

Since the gestational period varies among the murine strains, Table 1. Characteristics of the Study Subjects in the Prelabor Premature Rupture of Membrane-based Preterm Birth Mouse Model

|                          | First-generation | 21-G needle | 26-G needle | P-value | Second-generation | 21-G needle | 26-G needle | P-value |
|--------------------------|-----------------|-------------|-------------|---------|-----------------|-------------|-------------|---------|
| Number of maternal mice  | 1               | 1           |             |         | 3               | 5           |             |         |
| Maternal weight (g)      | 24.3            | 25.5        |             |         | 26.8±1.1        | 26.9±0.7    |             | 0.899   |
| Gestational period (days)| 17.5            | 17.5        |             |         | 19.8±0.6        | 19.7±0.8    |             | 0.818   |
| Mean number of pups per female mouse | 8 | 9 | - |         | 7.7±2.5 | 7.0±2.4 |             | 0.725   |
| Mean length of live pups (mm) | 25.5±2.1 | 24.6±3.0 | 0.514       |         | 24.9±2.2 | 22.3±2.3 |             | 0.001*  |
| Mean weight of live pups (g) | 1.4±0.1 | 1.2±0.1 | 0.011*      |         | 1.5±0.2 | 1.8±0.2 |             | 0.626   |
| Survival rate (%)        | 100             | 100         |             |         | 100             | 100         |             |         |

Data are presented as mean±standard deviation unless otherwise indicated. Abbreviation: G, gauge. *Statistically significant (P<0.01).

Fig. 3. Weight of the second-generation mice (preterm pups) versus that of their offspring. (A) Offspring of the prelabor premature rupture of the membranes (pPROM) model created using a 21-gauge needle, (B) offspring of the pPROM model created using a 26-gauge needle. *P<0.01 (using the Mann-Whitney U-test).

preterm is defined as the gestational period being shorter than the normal gestational period for the particular strain. Murray et al. reported that the mean gestational time for C57BL6/J mice is approximately 19.0 days. In this experiment, the pPROM mouse models gave birth at 17.5 days’ gestation; thus, the pPROM mice in this study are preterm birth models. In this study, mice born to pPROM model mice did not give birth to preterm pups. It appears that mother born prematurely through iatrogenic pPROM such as amniocentesis, fetoscopy, intrauterine procedures and fetal surgery does not have preterm birth.

Some studies reported on preterm birth in the next generation. Previous population cohort studies showed that mothers who were born prematurely were more likely to experience preterm birth.
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However, these studies did not include several risk factors, such as maternal body mass index, hypertension, or diabetes mellitus. The credibility of previous human data studies was a concern as it is difficult to consider the various causes underlying preterm birth. In addition, preterm birth was not classified into different types, such as pPROM, spontaneous preterm birth, and medically indicated preterm birth. In contrast, our study was conducted with experimental mice; therefore, only a few environmental factors affected the experimental conditions.

Pups born to the pPROM model created using a 26-gauge needle weighed considerably less than those born to the pPROM model created using a 21-gauge needle. In general, in any species, including humans, multiple infants born in a single delivery weighed less than single infants. Therefore, it is natural that the pups born to the pPROM mouse model created using a 26-gauge needle, which delivered a greater number of more pups, weighed less. The difference in weight between the second- and third-generation pups was associated with the gestational period. The third-generation pups had remained in the mother’s uterus for 1.0–4.0 days more than the second-generation pups; therefore, they weighed more.

Six of the eight female pups born prematurely showed signs of hair loss. All the experiments were conducted using specific pathogen-free mice; therefore, hair loss was not due to an infectious skin disease. In mouse strains, such as C57BL/6, hair loss is known to be caused by abnormal excessive barbering due to mice being housed in a cage. Barbering-induced alopecia was also considered to be an indicator of a poor prognosis of preterm birth, potentially leading to brain injury or development of a stressful environment. Additional research is required because the precise relationship between preterm birth and hair loss associated with brain injury has not been elucidated to date.

Our research was based on the reliable pPROM-induced preterm birth mouse model described by Mogami et al. Many known preterm birth mouse models, including those created by exposure to infection, progesterone withdrawal, and administration of thrombin injection, are available. However, the previous models could not give birth to live pups consistently and reliably because the administered drugs or materials were toxic to the fetuses or the pups were delivered too early and could not survive. Generally, the models give birth within 24 hours after drug administration; a high mortality rate is noted among most pups born to premature mice; thus, it is difficult to study the next generation of mice. In contrast, all pups born to the pPROM models assessed in this study survived. Therefore, the current pPROM mouse model may be optimal for observing and comparing preterm birth across multiple generations. Hereafter, more detailed data on pups born prematurely would be aid in increasing our understanding of the outcomes of preterm offspring in the light of human-mimicking comparative animal experiments.

A limitation of this study is that there were only two replicates of the pPROM model. We ensured that the number of pPROM models to be sacrificed was as small as possible because the focus was on identifying the birth conditions of preterm pups born to the pPROM model. Also, additional experiments must be conducted to assess the behavior, function, detailed anatomical structure, including that of the brain, lungs, heart, and other vital organs, and growth of the pups. It would be interesting to conduct a study investigating the reasons underlying the slight delay in preterm birth in the pPROM model compared with other infection-related models in the future. In conclusion, mice born to pPROM model mice did not give birth to preterm pups. It appears that mother born prematurely through iatrogenic pPROM does not have preterm birth.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Acknowledgements

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