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Study of the timing of Caesarean section and progesterone administration for routine microbiological decontamination of mice

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Abstract

Caesarean section (C-section) is conducted to decontaminate mice from microorganisms in our facility. In the present study, pregnant mice (C57BL/6J) were administered progesterone on gestational days (GD) 17 and 18 and were C-sectioned on GD 19 (normal schedule), 20 (1 day later than usual), or 21 (2 days later than usual). The fetal survival rates on GD 19 and 20 were similar, whereas on GD 21, it was quite low. In another set of study, pregnant mice (C57BL/6J) were administered progesterone on GD 17 and 18 (normal schedule), 16 and 17 (1 day earlier than usual), or 15 and 16 (2 days earlier than usual). The progesterone administration on GD 16 and 17 were as effective as the normal schedule in maintaining pregnancy until GD 19, whereas administering progesterone on GD 15 and 16 did not maintain pregnancy until GD 19. Administration of single shot of progesterone to pregnant (C57BL/6J) mice on GD 15, 16, 17, or 18 resulted in effective maintenance of pregnancy until GD 19 only in GD 18 group. These findings will allow us to better manage work schedules.

Key words : mouse, C-section, progesterone

Introduction

In our facility, decontamination from microorganisms is usually conducted by *in vitro* fertilization (IVF) and embryo-transfer. Three to four weeks old female mice are most suitable for IVF because the highest number of oocytes can be obtained from them after superovulation (Sugiyama *et al.,* 1992; Xu, 2001). On the other hand, male mice with wider age range can be used for IVF. However, in case that there are not any male mice but only adult female mice, C-section is conducted as another way of decontaminating mice from microorganisms in our facility. Usually, most mouse strains deliver in the early morning on gestation day (GD) 19 (Wolfensohn and Lloyd, 1998). However, some gene-modified mice reared in our facility often delivers on GD18, resulting in premature fetuses. To prevent this (Hall, 1957; Parkes, 1928; Wiest and Forbes, 1964), administered progesterone on GD17 and 18 and C-section were done before noon on GD19. Despite this, fetal immaturity and low resuscitation rates were observed sometimes. This presumably due to delayed implantation and shifting back of fetal developmental stage. Various steroid hormones and genes are involved in delayed implantation (Diao *et al*., 2008; Lee *et al.,* 2003; Paria *et al.,* 1993; Smith *et al.,* 1997; Wu, 1988; Yoshinaga and Adams, 1966). Some of gene-modified mouse strains bred in our facility had abnormalities possibly due to defective mechanisms in

implantation. In light of delayed implantation, we examined whether and how long C-section can be postponed. In addition, we examined the timing of progesterone administration and whether a single shot of progesterone is also effective in maintaining the pregnancy for better management.

Materials and Methods

Animals

We used C57BL/6J strain because most mice reared in our facility are gene-modified and C57BL/6J is the most common background strain for them. The C57BL/6J mice were purchased from CLEA Japan, Inc. (Tokyo, Japan) and were housed in microisolation cage (MBS7115RHMV, 19.1 x 29.2 x 12.7 cm, Allentown, NJ, USA) under a 12:12-h light:dark cycle (dark period: 20:00 to 08:00). The bedding materials were procured from Harlan (TEK-FRESH, Harlan Teklad, WI, USA). Food (CRF-1, Oriental Yeast Co. Ltd., Tokyo, Japan) and filtered water were provided *ad libitum*. All mice were kept in specific pathogen free (SPF) facilities that were free of the following microorganisms: Mouse hepatitis virus, Sendai virus, Ectromelia virus, Lymphocytic choriomeningtis virus, Mouse rotavirus, Mouse parvovirus, Mouse encephalomyelitis virus, Pneumonia virus of mice, Mouse adenovirus, Reovirus thpe 3, Lactate dehydrogenase elevating virus, *Mycoplasma pulmonis*, *Salmonella typhimurium*, *Clostridium piliforme*, *Corynebacterium kutscheri*, *Pasteurella pneumotropica*, Cilia-associated respiratory bacillus, *Escherichia coli* O115a, *Helicobacter hepaticus*, *Pseudomonas aeruginosa*, *Pneumocystis carinii*, *Syphacia obvelata* and *Aspiculuris tetraptera*. All the mice were treated humanely and all experimental procedures were approved by the RIKEN Institutional Animal Care and Use Committee.

Routine C-section protocol

We conducted a series of operations as outlined in Fig. 1a. (1) On the first Wednesday evening, 1 male and 2-3 female mice were housed together in a cage. (2) From first Thursday to Sunday, vaginal plugs were checked every day morning. Plugged female mice were separated from the male mouse, and the day was considered as gestational day (GD) 0. (3) On first Sunday morning, all the female mice were separated from male mouse after plug checks. (4) On GD 17 and 18, the pregnant female mice were administered progesterone (66.7 mg/kg/injection, Luteum Injection, Asuka Pharmaceutical, Tokyo, Japan) subcutaneously. In the case of Thursday-plugged female mice, GD 17 is the third Sunday. (5) On GD 19, the pregnant female mice were sacrificed by cervical dislocation and C-section was carried out.

Experiments

Our first objective was to examine whether C-section could be postponed. Hence, we selected twelve-month-old male mice (n=5) and 10-week-old female mice (n=15) for this study and were mated together. On GD 17 and 18, all the pregnant mice (11) were administered progesterone (normal schedule). The pregnant mice were then divided into 3 groups according to the day of C-section. Group1: GD 19 (normal schedule, n=3), Group 2: GD 20 (1 day later than usual, n=4), and Group 3: GD 21 (2 days later than usual, n=4).

Next, we examined whether progesterone administration could be moved forward. For this study, we selected twelve-month-old male mice (n=5) and 10-week-old female mice (n=15) and were mated together. The pregnant mice (12) were divided into 4 groups according to the day of progesterone administration viz. Group 4: without progesterone administration (natural delivery, n=2), Group 5: GD 17 and 18 (as usual, n=3), Group 6: GD 16 and 17 (1 day forward, n=3), Group 7: GD 15 and 16 (2 days forward, n=4). Finally, we examined whether a single shot of progesterone was effective in maintaining the pregnancy. For this study, we selected twelve-month-old male mice (n=8) and 10-week-old female mice (n=24) were mated together. The pregnant mice (20) were divided into 4 groups according to the day of progesterone administration. Group 8: GD 15 (n=5), Group 9: GD 16 (n=5), Group 10: GD 17 (n=5), Group 11: GD 18 (n=5).

Statistical analysis

Statistical analysis was conducted using Prism 4 (GraphPad software, La Jolla, CA, USA). Statistical analysis of the data was performed using logrank test, logrank trend test, or one-way analysis of variance (ANOVA) with a Tukey's multiple comparison test. The results were considered significant if the probability of error was 5% or less.

Results

All the results are summarized in Table 1. Two of the 4 pregnant mice in Group 3 delivered before C-section on GD21, whereas none of the pregnant mice in Groups 1 and 2 delivered on GD19 and 20 respectively. A significant difference in the delivery rate curves ($p<0.001$, logrank test) was observed. Only 2 of the 15 fetuses excised from the remaining 2 pregnant mice of Group 3 started spontaneous respiration, whereas all the fetuses excised from the pregnant mice of Groups 1 and 2 started spontaneous respiration. There was also a significant difference in the fetal survival rates ($F(2, 6)=855.20$; $p<0.001$; ANOVA). The fetal survival rate in Group 3 was significantly low compared with those of Group1 and 2 ($p<0.05$, Tukey).

All the pregnant mice in Group 4 delivered by 10:00 AM on GD 19. One of the 4 pregnant mice in Group 7 died on GD 17 and another female delivered on GD 19, whereas no pregnant mice in Group 5 or 6 delivered on GD 19. There was a significant difference in the delivery rate curves ($p=0.048$, logrank test). All the fetuses excised from the pregnant mice in Groups 5, 6 and 7 started spontaneous respiration.

Finally, four of the 5 pregnant mice in Group 8 delivered on GD 19 and the remaining one delivered on GD 20. One of the 5 pregnant mice in Group 9 delivered on GD 19 and the remaining 4 mice delivered on GD 20. Two of the 5 pregnant mice of group 10 delivered on GD 19 and the remaining 3 mice delivered on GD 20. On the other hand, none of the 5 pregnant mice in Group 11 delivered on GD 19. The remaining 4 and 1 pregnant mice delivered on GD 20 and 21 respectively. There was a significant trend in the delivery rate curves ($p=0.024$, logrank trend test).

Discussion

In the present study, we examined whether and how long C-section could be postponed in order to resolve the fetal immaturity presumably because of delayed implantation. A marked increase in fetal death was observed when pregnant mice were C-sectioned on GD 21, although performing C-section on GD 19 or 20 did not affect the fetal survival rate. Holinka *et al.,* (1978) reported that the gestation period of the C57BL/6J strain of mice ranges from 18.6 to 20.8 days and is dependent on the age of the pregnant mouse and also the incidence of fetal death at birth increases with gestation length. In addition, Kroc *et al.,* (1959) reported that experimentally prolonged gestation is associated with increased fetal mortality. These reports correspond with our results. Moore (1961) suggested that elevated levels of progesterone causes vasoconstriction and fetocidal ischemia. The prolongation of fetocidal ischemia might have caused the markedly increased incidence of fetal death observed on GD 21 in the present study.

Progesterone levels decline rapidly two days before delivery (Michael *et al.,* 1975; Murr *et al.,* 1974; Virgo and Bellward, 1974), and this withdrawal of progesterone is required to permit parturition (Kroc *et al.,* 1959; Virgo and Bellward, 1974). In the present study, administering progesterone one day earlier (on GD 16 and 17) was effective in maintaining pregnancy until GD 19. This was presumed to be due to the progesterone administration on GD 17 (2 days before the C-section). However, administering a single shot of progesterone on GD 17 was not sufficient to maintain pregnancy until GD 19, whereas a single shot of progesterone on GD18 was effective. These results suggest that a single shot of progesterone at the rate of 66.7 mg/kg, might be somewhat low. Further examination of progesterone doses might resolve the discrepancy.

One-day-longer pregnancy brought by progesterone administration without extreme fetal death might resolve the fetal immaturity presumably because of delayed implantation. In addition, it was revealed that 1-day-earlier administration of progesterone was effective in maintaining pregnancy, and that administering a single shot of progesterone on GD 18 was also sufficient to maintain pregnancy. These findings will also allow us better management of work schedules (Fig. 1b): (1) On the first Wednesday evening, 1 male and 2-3 female mice will be housed together in a cage. (2) On the first Thursday and Friday, checks for vaginal plugs will be performed in the morning. All the plugged female mice will be separated from the male mouse, and the day is considered as GD 0. Plugs will not be checked on the first Saturday or Sunday. (3) On the first Monday morning, all the female mice will be separated from the male mouse after plug checks. The female mice that are found to be plugged on this day will not be operated. (4) The Thursday-plugged female mice will be administered a single shot of progesterone on GD 18 (third Monday) and will be C-sectioned on GD 19 (third Tuesday). The Friday-plugged female mice will be also administered a single shot of progesterone on GD 18 (third Tuesday) and will be C-sectioned on GD 19 (fourth Wednesday). The pregnant mice with unknown plug dates are presumed to have been plugged on the first Saturday or Sunday. They will be administered progesterone on the third Tuesday and the fourth Wednesday. In the case of the Saturday-plugged mice, the third Tuesday and fourth Wednesday correspond to GD 17 and 18, respectively. In the case of the Sunday-plugged mice, the third Tuesday and fourth Wednesday correspond to GD 16 and 17 respectively. (5) The Thursday- and Friday-plugged mice will be C-sectioned on GD 19, the third Tuesday and fourth Wednesday respectively. The pregnant mice with unknown plug dates will be C-sectioned on the fourth Friday, which corresponds to GD 20 and 19 for the Saturday- and Sunday-plugged mice respectively.

In the present study, we examined whether and how long C-section can be postponed in order to resolve the fetal immaturity presumably because of delayed implantation. In addition, we also examined the timing of administering progesterone and whether a single shot of progesterone was also effective in maintaining the pregnancy. Our results showed that 1-day-later Caesarean section did not affect the fetal survival rate and 1-day-earlier administration of progesterone was effective in maintaining pregnancy and also a single shot of progesterone at GD 18 was sufficient to maintain pregnancy. These findings would be useful to maintain mouse strains and allow us better management of work schedules.

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Table 1. Summary of experiments and results

	Day of progesterone administration	Day of CS	Number of natural delivery			Number of C-sectioned mouse			Number of spontaneously-breathing fetus / Total number of fetus (%)†
			GD19	GD20	GD21	GD19	GD20	GD21	
Group 1 (n=3)	GD17, 18	GD19 (normal schedule)	0/3	-	-	3/3	-	-	20/20 (100.0%)
Group 2 (n=4)		GD20 (1 day later than usual)	0/4	0/4	-	-	4/4	-	31/31 (100.0%)
Group 3 (n=4)		GD21 (2 days later than usual)	0/4	0/4	2/4	-	-	2/4	2/15 (13.3%) *
Group 4 (n=2)	no	no	2/2	-	-	-	-	-	-
Group 5 (n=3)	GD17, 18	GD19 (normal schedule)	0/3	-	-	3/3	-	-	20/20 (100.0%)
Group 6 (n=3)	GD16, 17		0/3	-	-	3/3	-	-	24/24 (100.0%)
Group 7 (n=4)	GD15, 16		1/3†2	-	-	2/3	-	-	17/17 (100.0%)
Group 8 (n=5)	GD15	-	4/5	1/5	0/5	-	-	-	-
Group 9 (n=5)	GD16		1/5	4/5	0/5	-	-	-	-
Group 10 (n=5)	GD17		2/5	3/5	0/5	-	-	-	-
Group 11 (n=5)	GD18		0/5	4/5	1/5	-	-	-	-

CS: Caesarean section, GD: gestational day
†, In case of CS
†2, One of 4 pregnant mice died on GD17.
*, $p<0.05$ compared with Group1 and 2.

Figure 1 (a) Current time schedule for C-section

	SUN	MON	TUE	WED	THU	FRI	SAT
1				M	P	P	P
2	P						
3							
4	H (GD17)	H (GD18) H (GD17)	CS (GD19) H (GD18) H (GD17)	CS (GD19) H (GD18) H (GD17)	CS (GD19) H (GD18)	CS (GD19)	

Figure 1 (b) Proposed time schedule for C-section

	SUN	MON	TUE	WED	THU	FRI	SAT
1				M	P	P	
2		P					
3							
4		H (GD18)	CS (GD19) H (GD18) H (GD17) H (GD16)	CS (GD19) H (GD18) H (GD17)		CS (GD20) CS (GD19)	

The operations required for various days are indicated in letters
M: start of mating
P: plugs checks
H: administration of progesterone
GD: gestational day
CS: C-section