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SHORT COMMUNICATION

A new animal model for uterine torsion and uterine ischemia-reperfusion studies, but not fetal hypoxia studies

Halef Dogan[1] | Necati Timurkaan[2] | Nevzat Saat[3] | Ibrahim Seker[4] | Ali Risvanli[1]

[1]Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, University of Firat, Elazig, Turkey

[2]Department of Pathology, Faculty of Veterinary Medicine, University of Firat, Elazig, Turkey

[3]Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, University of Balikesir, Balikesir, Turkey

[4]Department of Zootechny, Faculty of Veterinary Medicine, University of Firat, Elazig, Turkey

Correspondence
Ali Risvanli, Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, University of Firat, Elazig, Turkey.
Email: arisvanli@firat.edu.tr

Abstract

The aim of the present study was to develop a new animal model for use in uterine torsion, uterine ischemia-reperfusion, and fetal hypoxia studies in rats. A total of 14 pregnant rats on their 18th-19th gestational days were used. The animals were randomly divided into two groups: those undergoing the shame operation (group 1), and those in which a 360 uterine torsion was performed using a novel technique, which was corrected 6 hours later (group 2). Subsequently, seven female and seven male rat pups aged 1 month were separated from the mothers in each group. The female rats were monitored until puberty via measuring the vaginal apertures. The 1-month old male rats and the female rats on reaching puberty were decapitated and histopathological tests were performed on the dissected organs, including the cerebral, visceral and genital organs. At the end of the study, no differences were observed between the groups with regard to abortions, offspring death rates and congenital abnormalities. It was observed that the time to reach puberty in female rats born from mothers with uterine torsion was longer, but the difference was statistically insignificant. No microscopic lesions were detected in the cerebral, visceral or genital organs of the offspring. Accordingly, it was concluded that offspring of mothers with the uterine torsion were not affected, at least in the short term. It was generally concluded that this animal model is appropriate for use in uterine torsion and ischemia-reperfusion studies, but is not appropriate for fetal hypoxia studies.

KEYWORDS
fetal hypoxia, ischemia-reperfusion, pregnancy, rat, uterine torsion

1 | INTRODUCTION

Uterine torsion, observed commonly in ruminants, especially in cows, is the twisting of the uterus along its long axis. It may be observed in varying degrees; however, it is generally between 45° and 360°. For cows, uterine torsion is a frequent cause of difficult birth and is reported to be observed at a rate of 7% among all difficult birth cases.[1,2] The fetus within the uterus may be exposed to hypoxia or anoxia during the torsion as a result of the stenosis of the umbilical cord. During the correction of the torsion, also known as detorsion, some damage is also expected. Ischemia-reperfusion damage is observed as a result of torsion and detorsion.[3]

It has been suggested that the mammalian fetus or newborn is more resistant to anoxia or hypoxia compared to adults of the same

species. In the case of anoxia in the fetus or newborn, anaerobic metabolism is switched on and the need for energy is reduced[4,5] and blood flow is directed toward important organs such as the cerebral, cardiac and adrenal organs. However, in the case of long-term anoxia, persistent cerebral damage has been demonstrated in dogs, rhesus monkeys, and guinea pigs, and other associated injuries have also been demonstrated.[4,5]

Studies on the formation of experimental uterine torsion on laboratory animals are rare. However, Erlwanger et al[6] reported a 270° uterine torsion in the right cornu of a rat of the Sprague-Dawley breed.

The aim of the present study was to develop a new animal model for use in uterine torsion, uterine ischemia-reperfusion, and fetal hypoxia studies in rats.

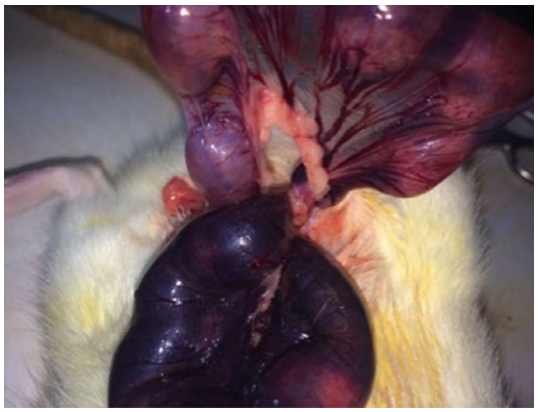

FIGURE 1 Uterine torsion in a rat with a gestational age of approximately 18 days

2 | MATERIALS AND METHODS

In this study, a total of 14 female, pregnant, 3- to 4-month-old Sprague-Dawley rats, weighing 200-250 g, were used on their 18th-19th gestational days. Ethical committee approval was obtained from the Local Ethics Committee of Fırat University Laboratory Animals Department (17.12.2014 - 2014/128).

The animals were grouped as follows:

Group 1: rats at 18-19 gestational days undergoing the shame operation (n = 7);

Group 2: 360° of torsion was formed in the uteri of pregnant rats on their 18th-19th gestational days, and the torsion was corrected 6 hours later (n = 7).

Vaginal irrigations were performed as described by Risvanli et al.[7]

Ether anesthesia was performed during the operations. Under anesthesia, the pregnant rats on their 18th-19th gestational days underwent a laparotomy operation following routine procedures. Subsequently, the right cornu uteri of the animals were passed through the hole formed in the avascular region of the ligamentum lata uteri of the left cornu at the level of vesica urinaria. The right cornu was then passed through the same hole for the second time, which resulted in a 360° torsion (Figure 1). Following this procedure, the abdominal space of the animals was closed up using appropriate suturing material. The animals underwent a second laparotomy under anesthesia at the post-operative 6th hour, and the torsion was corrected.

Following the operations, the animals were monitored at parturition, and any abortions, the number of newborns, survival rate at ablactation, and any congenital abnormalities were recorded. At 1 month of age, seven female and seven male rat pups were separated from each group for the study. The time to reach puberty for the female rats was monitored by observing the vaginal opening. The 1-month-old male rats and the female rats at puberty were decapitated and histopathological tests were performed on the dissected organs, including the cerebral, visceral, and genital organs. Tissue samples from these organs were placed into 10% buffered formaldehyde solution for fixation.

The brain, liver, kidneys, lungs, spleen, heart, testes, ovary, and uteri of each rat in the experimental groups were collected and fixed in 10% neutral formalin solution. Paraffin blocks were prepared following routine protocols. For each rat, sections were taken from the paraffin blocks and stained with hematoxylin and eosin (H&E) for examination under a light microscope.

The Mann-Whitney *U* test was used for comparison of two independent groups in the analyses of vaginal aperture data. The chi-square test and the Fisher's exact test were used for comparison of abortion rates between the groups, and the chi-square test and the Pearson chi-square test were used for comparison of survival rates of the offspring. SPSS version 11.5 was used for the statistical analyses.

TABLE 1 Rates of abortion and offspring survival according to groups

	Abortion				Survival rate			
	Frequency		%		Frequency		%	
Groups	+	−	+	−	Live	Death	Live	Death
Group 1 (n = 7)	0[a]	7	0	100.0	34[a]	32	51.5	48.5
Group 2 (n = 7)	4[b]	3	57.1	42.9	2[b]	12	14.3	85.7
P	*				*			

[a,b]The difference between the values demonstrated by different letters within the same column is significant.
*$P < 0.01$.

TABLE 2 Day of vaginal opening of the female offspring

	Group 1 (day) (n = 7)	Group 2 (day) (n = 5)
Vaginal opening	54.86 ± 1.16	60.80 ± 6.71

The difference between mean values was not statistically significant ($P < 0.05$).

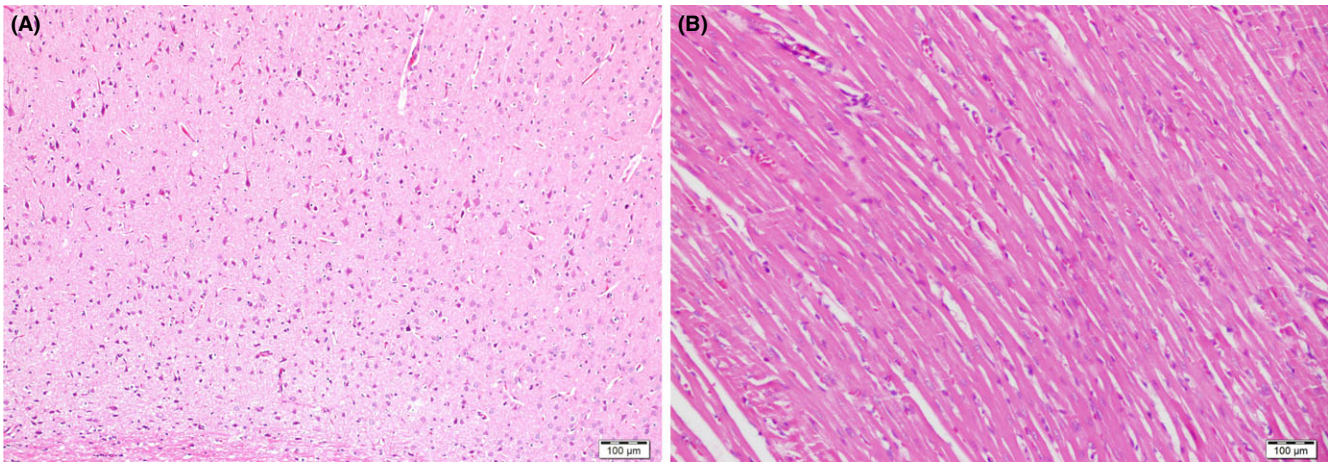

FIGURE 2 Microscopic pictures of the study. A, Photomicrograph showing normal brain histology in a male rat of experimental group. B, Heart of a female rat of experimental group showing normal histological appearance. Staining with hematoxylin and eosin

3 | RESULTS AND DISCUSSION

The clinical observations are presented in Table 1. No abortions were observed in group 1 (no uterine torsion; $P < 0.01$). Comparisons of survival rates of the offspring at ablactation revealed that the lowest mortality (48.5%) was observed in group 1 (Table 1, $P < 0.01$). No congenital abnormality was observed in either group.

It was observed at the end of the study that two female offspring of mothers with uterine torsion had died before puberty. Data on vaginal openings are summarized in Table 2. Statistical analyses revealed no difference between the groups ($P < 0.05$).

Histopathological results (H&E stained sections) of the sampled tissues showed that there were no significant microscopic lesions in the liver, kidneys, heart, lungs, spleen, uterus, ovary, testes, and brain in all rats, with all tissue samples appearing normal (Figure 2).

Uterine torsion is a pregnancy pathology commonly observed in ruminants, especially in cows. A literature search found little information on the effects of hypoxia/anoxia exposure in the offspring of animals with uterine torsion. Although low milk yields have been reported in such offspring, the pathogenesis has not been clearly described. Tissue damage observed as a result of torsion/detorsion is similar to that observed as a result of ischemia-reperfusion. Reactive oxygen species have a role in the pathogenesis of ischemia-reperfusion damage. These reactive oxygen species are formed continuously within cells as a result of biochemical reactions and external factors. Reactive oxygen species damage the cell wall and react with lipids, proteins, and nucleic acids that are released from the cell wall.[8]

In general, resistance to anoxia in the fetus and the survival rate decrease with progression of the pregnancy. Blockage or distorsion of the umbilical cord causes anoxia, which reduces the cerebral functions of the fetus and minimizes the damage. In a previously reported animal model, hypoxic and ischemic damage was induced via a delayed cesarean section. In this model, the umbilical cords of the fetuses were blocked by clamping at a time close to the time of delivery, and circulation and oxygenation were blocked.[9,10] Another method used for the same purpose is ligation of arteria uterine media alone or with arteria ovarica.[11] In these models, chronic hypoxia was induced as a result of malnutrition of the fetus and chronic placental hypoperfusion. In such models, the hypoxia or anoxia is systemic rather than confined to a targeted organ.

Hypoxia harms cerebral function at every stage of life from the early fetal period to old age. It impairs intracellular Ca^{2+} homeostasis and leads to many pathologies such as neuronal cell damage, neurodegeneration, and cell death. The resulting damage varies according to the duration of exposure to ischemia and related hypoxia, and to fetal age.[12] In the present study, the survival rate of offspring of rats with torsion/detorsion uteri was reduced; however, among the survivors in this group, no histopathological differences in cerebral, visceral, and genital organs were observed compared to those observed in the control group.

To date, no other study of hypoxia/anoxia in offspring of laboratory animals induced by torsion/detorsion in the mothers has been reported.

In conclusion, offspring of mothers with uterine torsion experimentally induced in advanced pregnancy were observed to have lower long-term survival rates; however, they were not affected in the short term. Additionally, no differences were observed between the groups with regard to abortion and offspring death rates. Nor were there any differences in the histopathological observations of cerebral, visceral, and genital organs of the survivors compared to those observed in the control group without uterine torsion. It was concluded that this animal model is appropriate for use in uterine torsion and ischemia-reperfusion studies, but would not to be appropriate for fetal hypoxia studies.

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

HD, AR, and NS conceived and designed the study; NT and IS carried out experimental work and data analysis. All authors contributed to revising the manuscript. All authors gave final approval for publication.

ORCID

Ali Risvanli http://orcid.org/0000-0001-5653-0025

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