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Creation of a rabbit model for intrauterine adhesions using electrothermal injury*

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Abstract: The pathogenesis and therapeutic treatment of intrauterine adhesions (IUAs) remain unsolved, highlighting the need for stable and effective experimental animal models. In this study, uterine electrocoagulation of twenty-one female New Zealand White rabbits was carried out to establish an IUA model. As rabbits have two completely separate uterine horns, each rabbit had its own internal control: one uterine horn was given an electrothermal injury (Group A, n=21), and the contralateral uterine horn received no treatment and served as the control (Group B, n=21). The endometrial morphology, number of endometrial glands, area of endometrial fibrosis, and number of implanted fetuses were compared between the two groups. In Group A, the numbers of endometrial glands on Days 7 and 14 and the number of implanted fetuses were significantly lower than those in Group B (P<0.05, P<0.05, and P<0.01, respectively), while the ratio of the area with endometrial stromal fibrosis to the total endometrial area was significantly increased (P<0.01). These results suggest that this method of electrothermal injury is effective for the establishment of a rabbit IUA model between 7 and 14 d after surgery.

Key words: Intrauterine adhesion; Electrothermal injury; Rabbit model
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1 Introduction

Intrauterine adhesions (IUAs) refer to the partial or complete (Asherman's syndrome) adherence of endometrial surfaces with fibrotic tissue resulting from damage to the basal layer of the endometrium. Such adhesions can cause infertility, menstrual abnormalities, and recurrent pregnancy losses (Kodaman and Arici, 2007; Yu et al., 2008). Asherman's syndrome is being diagnosed with increasing frequency and any uterine surgery can lead to IUAs (March, 2011). The exact mechanism of synechiae formation is unknown and effective preventive treatments are lacking. These issues highlight the need for having stable and effective experimental animal models to enable intensive research on the pathogenesis of IUAs and to explore new treatments, including the application of stem cell therapy (Gargett and Ye, 2012; Alawadhi et al., 2014; Hu et al., 2015; Santamaria et al., 2016).

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Previous studies have proposed various mechanical injury methods to establish models of endometrial injury in rabbits. However, these methods resulted in variable degrees of injury to the endometrium, and the reports included no assessment of fertility after the injury (Li et al., 2011; Khrouf et al., 2012; Liu et al., 2013). Any factor that causes endometrial basal layer damage can lead to the occurrence of IUAs (Deans and Abbott, 2010), including curettage and hysteroscopic surgery (Dalton et al., 2006). Therefore, it is theoretically feasible to use electrothermal injury for establishing a model of IUA in rabbits. In addition, with the widespread clinical application of hysteroscopic surgery, the effect of electrothermal damage to the endometrium is a problem that deserves attention. However, reports of such effects on the endometrium and related animal models of IUAs are rare.

In this study, we used electrothermal injury to construct animal models of IUAs in New Zealand White rabbits. We included fertility assessment in this study as a parameter for evaluating the impact of the injuries in this model. Based on this study, we hope to provide a rabbit model for further investigations of the pathogenesis and therapeutic treatment of IUAs.

2 Materials and methods

2.1 Animals

All experimental procedures were approved by the Animal Experimentation Ethics Committee of the Provincial Hospital Affiliated to Shandong University, Jinan, China. A total of 21 mature female New Zealand White rabbits (Jinan, Shandong, China) weighing 3000–4000 g were housed individually with free access to food and water in a temperature ((23±2) °C) and light (12 h light and 12 h dark) controlled environment from one week before surgery.

2.2 Establishment of animal models

As two completely separate uterine horns are present in rabbits, each rabbit had its own internal control: one uterine horn was given an electrothermal injury (Group A, n=21), and the contralateral uterine horn received no treatment and served as the control (Group B, n=21). Five rabbits were sacrificed at each of three time intervals (Days 7, 14, and 28) after surgery to analyze differences between the injury and the control groups in endometrial morphology, the number of endometrial glands, and the area of endometrial fibrosis; six rabbits were mated on Day 7 after the surgery and sacrificed during pregnancy to quantify the number of implanted fetuses.

All the rabbits were anesthetized intravenously through the marginal ear vein with pelltobarbitalumnatricum (30 mg/kg) and supplemented if necessary. The surgeries were performed by the same researcher under sterile conditions. To minimize bias, the uterine horns were randomly assigned and operated on. After disinfection with povidone iodine, a 5-cm vertical midline abdominal incision was performed and both uterine horns were exposed.

A 0.5-cm tubal midline incision was performed with scissors on one uterine horn (Group A), and then the whole endometrial lining of the uterus was destroyed using an electric scalpel through the tubal incision with 70 W power three times at a speed of 6 mm/s. On the control side, the contralateral uterine horn (Group B) received no treatment (Fig. 1). After flushing the peritoneal cavities, the tubal and abdominal incisions were sutured.

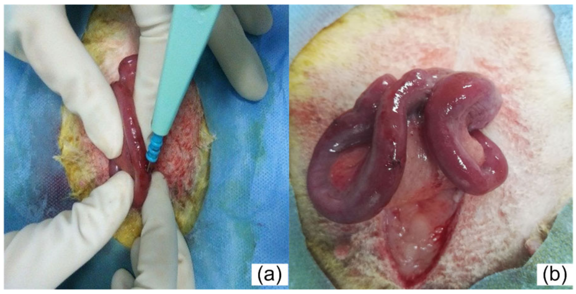

Fig. 1 Surgical procedure
(a) Intrauterine electrocoagulation of the uterine horns with an electric scalpel. (b) Rabbit bicornuate uterus: the left uterine horn was injured by intrauterine electrocoagulation

2.3 Histologic examination

Segments of uteri were fixed in 4% buffered formaldehyde for 24 h, embedded in paraffin, and routinely stained with hematoxylin and eosin (HE) and Masson stains. Histologic evaluation was performed by one experienced pathologist who was blinded to the groups. We randomly selected four high-power fields on each HE slice, counted the number of glands in each field, and calculated the average. Images of four randomly selected high-power fields on each Masson-stained slice were analyzed with ImageJ, and the mean of the ratios between the area of endometrial stromal fibrosis and endometrial area per high-power field was calculated as described by Liu et al. (2013).

2.4 Pregnancy outcome

On Day 7 after surgery, the females were mated with reproductive age male rabbits. Fourteen days after mating, animal euthanasia and laparotomy were performed to compare the numbers of implanted fetuses identified visually in the injury and control groups.

2.5 Statistical analysis

Statistical analysis was performed using SPSS 20. Paired-sample *t*-tests were used for comparison of the number of endometrial glands, the area of endometrial fibrosis, and the number of implanted fetuses in the injury and control groups. A *P*-value of <0.05 was considered to denote a significant difference.

3 Results

3.1 Endometrial morphology in rabbit

Morphological changes in the endometrium were indicated by HE and Masson staining. After HE staining, we observed that the normal endometrium of Group B composed of four to six polypoid protrusions was covered with columnar epithelial cells on the surface. Most endometrial glands were located in the basal and submucosa layers (Figs. 2b, 2d, and 2f). After Masson staining, the endometrial stromal fibers were dyed blue, while the mucosa, blood vessels, endometrial glands, and muscle appeared red (Fig. 3b).

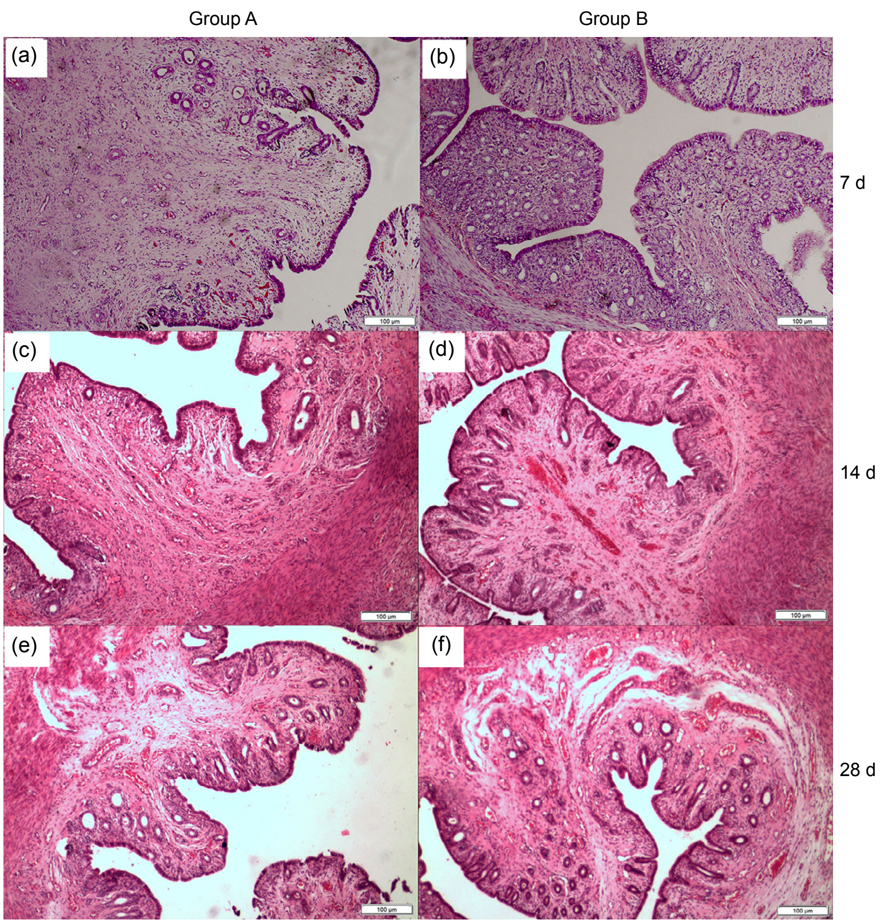

Fig. 2 HE-stained sections of rabbit uterine tissue after electrothermal injury
The rabbit endometrium from Group A: 7 d (a), 14 d (c), and 28 d (e); The normal rabbit endometrium from Group B: 7 d (b), 14 d (d), and 28 d (f). Bar=100 μm

On Days 7 and 14, HE staining showed that the polypoid endometrium in Group A became flat and the surface was partially lined by epithelial cells. Compared with Group B, in Group A there were fewer glands and more congestion, and leukocytic infiltration and focal hemorrhages could be distinguished (Figs. 2a–2d). Moreover, the numbers of endometrial glands on Days 7 and 14 were significantly reduced in Group A (P<0.05; Table 1). On Day 28, histologic reconstruction of the endometrium was largely completed in Group A, and no obvious differences were observed between the two groups (Figs. 2e and 2f; Table 1). Even though no intrauterine synechiae were observed, Masson-stained slices on Day 7 showed that in Group A, the number of endometrial stromal fibers dyed blue had clearly increased (Fig. 3), and the ratios of the area with endometrial stromal fibrosis (blue) to total endometrial area (blue and red) on Day 7 were significantly increased (P<0.01) compared to those in Group B (Table 1).

3.2 Pregnancy outcomes

After the electrothermal injury, the number of implanted fetuses in Group A (2.17±1.94) was lower than that in Group B (4.33±1.75), with P=0.003 (Fig. 4).

4 Discussion

In this study, we designed and tested a rabbit model of IUA using electrothermal injury and demonstrated that electrothermal injury was effective in forming IUAs between 7 and 14 d after surgery.

Currently, the species commonly used for establishing IUA models include the rat (Wang et al., 2016), mouse (Alawadhi et al., 2014; Wang et al., 2016; Zhang et al., 2016), rabbit (Liu et al., 2013; Bazoobandi et al., 2016), and a few primates (Schenker and Yaffe, 1978). The presence of two completely separate uterine horns where 2–7 fetuses can develop on each side makes the rabbit an appropriate model for simultaneous evaluation of control and treated horns within an individual animal. Moreover, rabbits have larger uterine cavities making surgery easier than rats and mice, and they are much less costly to study than primates and larger animals. In addition, rabbits are considered to be in estrus more or less permanently, and ovulation occurs only after mating (Bazoobandi et al., 2016). These characteristics made it possible for all the rabbits in this study to mate and ovulate after the same time interval following the endometrium injury.

Table 1 Number of glands and the ratio of fibrotic area in each group

Group	Number of glands per HPF			Ratio of the fibrosis area on Day 7
	Day 7	Day 14	Day 28	
A	10.95±3.67	14.55±5.66	25.30±10.20	0.80±0.07
B	35.35±10.36	33.15±9.37	32.90±8.91	0.59±0.05
P	0.012*	0.015*	0.110	0.007**

HPF: high-power field. * P<0.05, ** P<0.01, Group A vs. Group B. Data are expressed as mean±SD (n=5)

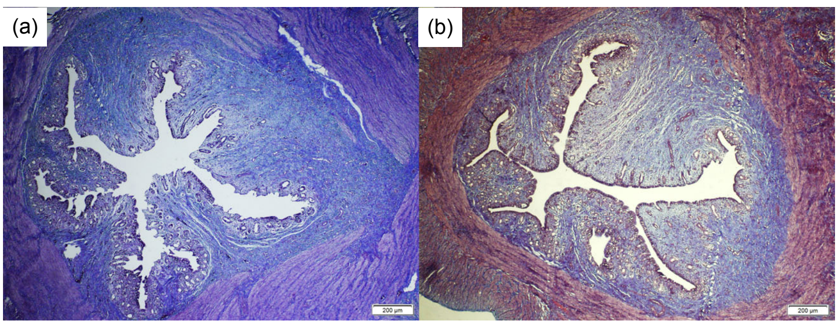

Fig. 3 Masson sections of rabbit uterine tissue after electrothermal injury
Seven days after electrothermal injury, the rabbit endometrium from Group A (a) and the normal rabbit endometrium from Group B (b). The results showed that in Group A, the area of endometrial stromal fibers dyed blue had clearly increased. Bar=200 μm (Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

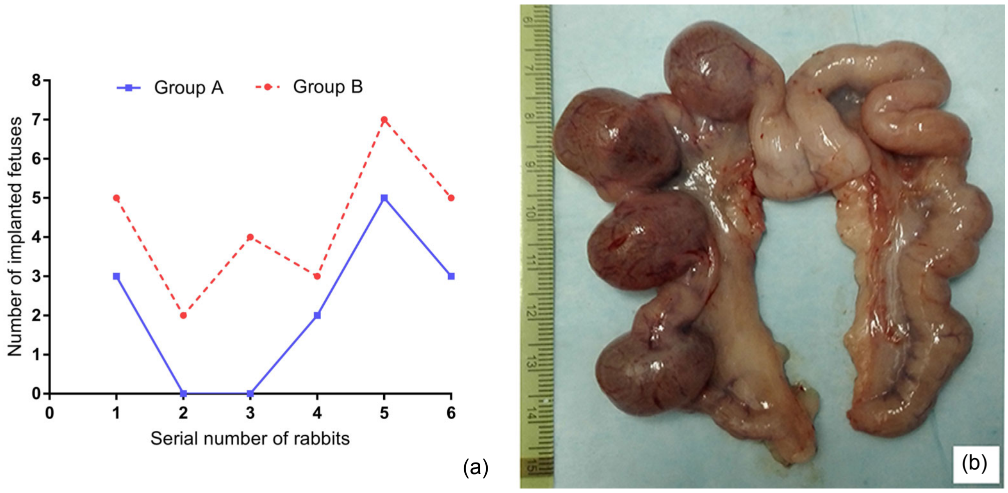

Fig. 4 Number of implanted fetuses in each group

(a) Numbers of implanted fetuses in uterine horns in each rabbit. (b) Uterine horns of one rabbit after electrothermal injury: the right horn was the electrothermal injury side, and the left horn was the control side

In previous studies, a reduction in endometrial thickness and the number of endometrial glands, an increase in fibrotic area, and the presence of adhesions were considered to be indicative of the formation of an IUA animal model (Liu et al., 2013; Alawadhi et al., 2014; Bazoobandi et al., 2016). However, unlike the human endometrium which has a uniform thickness and even surface, the endometrium of rabbits consists of 4–6 plicae, and the irregular and uneven surface makes it difficult to measure the thickness of the endometrium accurately. So in this study we did not include the endometrial thickness in our evaluation of the modeling effect. Preserving fertility after endouterine surgery is of great concern to clinicians and a causal relationship between endometrial surgery and infertility has been established based on retrospective studies (Schenker and Margalioth, 1982; de Ziegler et al., 2010; Cenksoy et al., 2013; Li et al., 2017). Thus, in contrast to previous studies, in addition to endometrial morphology and the number of endometrial glands, we included a fertility assessment in this study as a parameter for evaluating the animal model.

Previous studies have conducted simple endometrial curettage to establish a model of IUA in rabbits, but the surgery resulted in various degrees of injury to the endometrium, and the authors reported a complete regeneration of the endometrium by Day 7 (Khrouf et al., 2012) or Day 28 (Liu et al., 2013) after surgery, with no synechiae observed. So it may be difficult to achieve an ideal effect in establishing a rabbit model of IUA by simple mechanical injury.

Some researchers have established rabbit models of IUAs by combining two types of damage methods. For example, some have performed a two-step procedure including a subcutaneous graft of sponge and its insertion into the uterine horn after three weeks (Polishuk and Schenker, 1973; Schenker and Yaffe, 1978). Li et al. (2011) placed a copper wire in the uterine cavity after curettage and concluded that endometrial injury impeded blastocyst implantation in rabbit. Liu et al. (2013) constructed a rabbit IUA model by placing lipopolysaccharide in the uterine cavity after curettage. However, these methods had the disadvantages of a tedious operating procedure and a long testing period. To develop an easy and effective method for an animal model, in this study we tested electrothermal injury.

In Group A, histopathologic examination on Day 28 showed the absence of fibrosis and a partial regeneration of the endometrium. Partial regeneration might have resulted from the strong endometrium repairing ability of rabbits and the remaining endometrium undamaged by the electric scalpel in the plicate uterine cavities. However, the examination of endometrial morphology on Day 14 and the reduction in implanted fetuses demonstrated that electrothermal injury was effective in causing endometrial destruction and infertility between 7 and 14 d after the surgery. This conclusion is supported by several other observations. Firstly, previous studies that reported IUA animal models showed that no synechiae were still meaningful because they represented a pathogenesis condition in the rabbit similar to IUAs observed in humans, with a negative impact on implantation (Khrouf et al., 2012). Secondly, in clinical practice, not all IUAs caused by curettage or electrothermal damage during hysteroscopic operation include the formation of synechiae. Many patients present only the formation of fibrous scars, a thin endometrium or infertility. So this model can be used to investigate the safety and effectiveness of preventive therapeutics for IUAs between 7 and 14 d after surgery. Moreover, with the widespread clinical application of hysteroscopic surgery, the model could also be meaningful for studies related to the effects of electrothermal damage on the endometrium and infertility caused by damage to the endometrium.

However, there were some limitations to our study. Firstly, the sample size was small and our conclusion needs to be confirmed using larger scale animal experiments and clinical trials. Secondly, in clinical medicine, IUAs can cause infertility, intrauterine growth retardation, and recurrent pregnancy losses (Kodaman and Arici, 2007; Yu et al., 2008). Therefore, the sizes of the embryos and placentas, the number of viable fetuses, and the number of pregnancy losses are all significant parameters for evaluating the effect of injury. Because of the small sample size, these criteria were not included in our study.

In this paper, we propose a model of IUA in rabbits using electrothermal injury. Even though no synechiae were observed, we concluded that electrothermal injury is effective for the formation of IUAs and has a negative impact on spontaneous fertility between 7 and 14 d after the surgery. This will provide an opportunity to perform studies in an animal model to explore new treatments for IUAs and examine their effectiveness and safety.

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Compliance with ethics guidelines

Xin-xin XU, Lian-bao CAO, Zhe WANG, Zhen XU, Bing-qian ZHANG, She-ling WU, Sha-sha QI, Lei YAN, and Zi-jiang CHEN declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

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中文概要

题　目：电热损伤法建立新西兰大白兔宫腔粘连模型

目　的：目前宫腔粘连（Intrauterine adhesion，IUA）发病机制及治疗方法仍未明确和统一，建立一个稳定有效的宫腔粘连动物模型是开展相关研究的前提和基础。本文旨在利用电热损伤法构建兔 IUA 模型，观察和评估该方法的建模效果。

创新点：首次提出利用电热损伤法建立兔宫腔粘连模型，并得出在损伤后 7~14 天内建立的兔 IUA 模型是有效的结论。

方　法：将 21 只成年雌性新西兰大白兔一侧子宫内膜用医用多功能高频电刀电灼损伤模拟宫腔粘连形成（A 组，n=21），另一侧子宫不做处理作为自身对照（B 组，n=21）。分别在损伤后 7、14 和 28 天收集兔双侧子宫组织，行苏木精-伊红染色法（HE）和 Masson 染色观察两侧子宫内膜病理改变，并对两侧子宫内膜的腺体个数和内膜纤维化面积比进行统计学分析和比较。另外，将损伤后 7 天的雌兔与成年雄兔合笼， 14 天后观察比较两侧子宫胚胎个数。

结　论：病理组织学观察显示，损伤后 7 和 14 天，A 组子宫内膜腺体数量较 B 组减少，差异均有统计学意义（P<0.05）；而损伤后 30 天，A 组子宫内膜腺体数量与 B 组相比差异无统计学意义。损伤后 7 天，A 组内膜纤维化面积较 B 组增大，差异有统计学意义（P<0.01）；A 组子宫胚胎个数较 B 组减少，差异有统计学意义（P<0.01）。综上所述，采用电热损伤法建立的兔宫腔粘连模型在损伤后 7~14 天是稳定有效的。

关键词：宫腔粘连；电热损伤；兔模型