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Effect of different surgical methods in the mouse embryo transfer: Electrosurgery versus cold surgical technique effects on repeated use of surrogate mothers, pregnancy rate and post-surgical behavior

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Abstract

Using the cold surgical technique (CST) is the most common practice to accomplish embryo transfer (ET). However, it can lead to uncontrolled bleeding and mortality in laboratory animals. Electrosurgery technique (EST) has provided the opportunity to prevent such complications. This study was aimed to evaluate CST versus EST in terms of repeated use of surrogate mothers, litter size, implantation rate and post-surgical behavior. Virgin female NMRI mice were allocated into two different surgical groups (n = 40): 1) CST-ET (control) and 2) EST-ET. Results showed that the first ET in EST-ET and CST-ET groups did not affect litter size, pregnancy rate and survival of surrogate mothers. Following the second and the third ETs, litter size was significantly affected through CST compared to EST, pregnancy rate and survival of surrogate mothers. Litter size, pregnancy rate and surrogate mothers survival rate did not show any significant reduction following the first and the second ETs in EST group. On the other hand, the third ET showed dramatic reduction for all aforementioned parameters regardless of the chosen surgical method for ET. Mice in EST-ET group did not show any significant change in their behavior indicating reduced well-being during the first 24 hr following the first, the second and the third ETs compared to CST-ET group. In conclusion, using EST for ET in mouse made it feasible to reuse surrogate mothers with minimum animal mortality; this could be pivotal with regard to reproductive and animal welfare aspects and research costs. Also, the results indicated that bleeding has severe diverse effects on ET efficiency.

Introduction

Embryo transfer (ET) in laboratory animals, especially in mice, has played a pivotal role as one of the required steps for revitalization of cryopreserved strains, production of transgenic mice and to study pre-implantation embryo development, or even to overcome fertility problems in mutants. Therefore, an improvement in ET technique could be crucial to enhance the efficacy of all the mentioned practices.[1,2]

Using cold surgical technique (CST) is probably the most frequent surgical practice performed for ET in the murine model. The first ET in mouse had been reported by Tarkowski using CST.[3]

Performing CST for ET has some complications in laboratory animals especially for the beginners. Using cold surgical instruments may lead to hemorrhage impairing animal survival following the surgical practice.[4] One of the limitations in laboratory animal's surgical practice would be the failure in blood transfusion; thus, hemorrhage causing by cold surgical instruments may lead to animal mortality.[5] The incisions made with the scalpel/scissors may lead to profound bleeding, which may obscure the operating field, resulting in wastage of operating time and animal mortality in the severe bleeding.[5]

Using heat to stop bleeding or achieve other medical purposes has a long history and the earliest documents referring to it date back to Albucasis in 980 BC describing

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the use of hot iron in a concept similar to electrocautery to control bleeding in patients.[6,7]

Applying the electrical flow with frequencies above 10.00 kHz leads to heat generation in biological tissues; therefore, this electrical current does not stimulate neuromuscular system.[6] On the other hand, the skin has poor conducting properties for the electrical flow of energy. Thanks to this exclusive feature of the skin and with the benefit of electronic and medical engineering knowledge, in the 1920s, a device was designed by Harvard physicist William Bovie to adapt the electrical current, above 10.00 kHz, for improvement in the delicate surgery procedures and to reduce the post-surgical complications. Superficial, deep coagulation or cutting of the skin is the most prominent feature for the electrosurgery technique (EST).[6]

Repeated use of a surrogate female mouse for ET was reported by Kolbe *et al.*, by the way, some aspects of this concept are still controversial.[2] For instance, in the hands of beginners, performing ET surgery by means of cold surgical instruments may lead to loss of animals due to profound bleeding. Making an improvement in ET technique consequently may lead to a reduction in the animal death following the uncontrolled bleeding during the surgical practice, which has great importance in terms of animal ethics as well as statistical variance decrease due to reduction of the impact of animal change during a study. The expected gain of electrosurgery in mouse ET procedure may encompass reduced blood loss, dry and rapid separation of tissue and more animal survival following ET.[8]

To the current knowledge, there is no published report regarding potential benefits of EST for mouse ET. Therefore, this study was designed to evaluate the potential benefits of EST on mouse ET yield, maximum repeated use of surrogate mothers, post-surgical complications and the possible changes in animals' behavior after surgical practice.

Materials and Methods

Embryo transfer was performed by two different surgical techniques, electrosurgery called EST and conventional surgery with cold surgical instruments called CST. Following the weaning of the first litter, the second ET and following the weaning of the second litter, the third ET was performed to the same oviduct. The experiment design is described in Table 1. In order to evaluate the role of blood volume in the body of surrogate mothers during the 36 days before ET and its effect on the ET efficiency, a control group was considered as follows: 36 days before ET, 1.00 mL of blood was taken from the female tail vein; the blood collection was performed in a period of 5 days each day collecting ≥ 0.20 mL sample. The 36-day period was considered as a summation of the pregnancy duration and lactation. The surgical methods, electrosurgery and cold surgery methods, were applied in control group. More information can be found in Table 1. Unless otherwise stated, all chemicals used were obtained from Sigma-Aldrich (St. Louis, USA) and all solutions were prepared using water for injection obtained from Razi Vaccine and Serum Research Institute, Karaj, Iran. The present study was approved by institutional ethics committee of the Razi Vaccine and Serum Research Institute, Karaj, Iran (license number: 2-18-18-038-960595).

Table 1. Experimental groups of the present study.

Group	Females used	Age (week)
EST-ET^1	40	8
EST-ET^2	40	13-14
EST-ET^3	38	16-17
CST-ET^1	40	8
CST-ET^2	29	13-14
CST-ET^3	14	16-17
EST-control	40	8
CST-control	40	8

EST-ET: Embryo transfer by electrosurgery technique; CST-ET: Embryo transfer by cold surgical technique. The numerical superscript index (1, 2, and 3) in each group refers to the rounds of embryo transfer.

Animal housing. All the animals (NMRI mice) used in this study were bred in the Department of Laboratory Animal Research, Breeding and Production, Razi Vaccine and Serum Research Institute (Karaj, Iran). All the animals were housed under standard laboratory conditions (temperature: 21.00 ± 2.00 ˚C; relative humidity: 40.00%-55.00%; photoperiod: 12 hr light: 12 hr dark) in Makrolon® cages. All the animals were fed with standard breeding diet and tap water *ad libitum*. The NMRI female mice were used as the embryo donors.[8]

Super-ovulation, natural matting and pseudo-pregnancy. The embryo donors were super-ovulated at eight weeks of age (20.00 - 25.00 g weight) by intra-peritoneal (IP) injection with 5.00 IU of equine chorionic gonadotropin (Folligon; Intervet MSD Animal Health, Pune, India) and, 48 hr later, with 5.00 IU of human chorionic gonadotropin (Chorulon; Intervet MSD Animal Health) and then mated with NMRI male mice; two females of 8-17 weeks age (20.00 - 35.00 g weight) were placed in a cage with a NMRI male (8-10 weeks age and 20.00 - 30.00g weight) having approved reproductive performance. Following successful mating and detection of vaginal copulation plug, embryo donors were sacrificed by cervical dislocation to isolate oviducts at 1.50 days post-coitus (d.p.c). Two-cell embryos were flushed and stored on warming plate using synthetic oviduct fluid (SOF) supplemented with HEPES; the HEPES-SOF was prepared according to the formulation described previously.[9,10] To prepare pseudo-pregnant recipient females, the NMRI females were mated naturally with vasectomized males and the following morning identified by vaginal plug

control, a clump of coagulated proteins from the male seminal fluid. The seminal secretions produced by a sterile male are required for the uterus to become receptive to the transferred embryos. To prepare a recipient, two females of 8-17 weeks age were placed with a vasectomized male in the afternoon. The day of vaginal plug detection was considered to be 0.50 d.p.c. The vasectomized males were produced via the same electrosurgical device; in fact, it does not require any additional instruments than required for ET. The detailed procedure to produce vasectomized males was described formerly by Dadashpour Davachi.[11]

Anesthesia. To induce anesthesia, intraperitoneal injection of 87.5 mg kg^{-1} ketamine (Alfasan, Woerden, The Netherlands) and 12.5 mg kg^{-1} xylazine (Alfasan), diluted in physiological saline solution in 1.00 mL syringe with 27G needle was used.[1] The mice were left individually in the cage on a warm stage. The absence of rear foot reflex was checked by toe pinch after unconsciousness and eye ointment was applied to avoid the eye dryness and to check for the absence of palpebral reflex.[1]

Electrosurgery unit and the animal preparation. The electrosurgery unit (Bovie machine) used in this study was KLS Martin ME 80 (Martin, Munich, Germany). This unit has three main parts: 1) Mono-polar electrodes (Surgi-Pen) attached to a needle electrode with 0.40 mm outer diameter for making an incision in the skin, 2) Rubber neutral electrode (8.00 × 16.00 cm) for children, single contact surface 120 cm^2, and 3) Mono-polar double-pedal foot switch. Some serious precautions should be considered during electrosurgery practice using. To avoid animal burning, it is necessary to ensure that the animal abdomen skin is fully connected to the rubber neutral electrode; therefore, before putting the mouse on the rubber neutral electrode, it is needed to cover the abdomen skin with water base lubricant.

Embryo transfer. After ensuring about the proper anesthesia, an incision with needle electrode for EST-ET/scissor for CST-ET was made on the shaved skin and the body wall, on the right side near the ovary to pull out the reproductive tract. The ovarian bursa, a transparent tissue membrane covering the ovary, was ruptured and twelve 2-cell-stage embryos with normal morphology were transferred via the ovarian infundibulum into the ipsilateral ampulla of the oviduct. Then, the reproductive tract was gently placed back into the abdominal cavity and then the peritoneum and skin were sutured. The whole procedure was conducted on a warmed plate under a laminar flow hood and the recipient females were placed in their cage after awakening. Reproductive performance of the treatment groups was recorded for pregnancy rate and litter size at birth.

Behavioral screening. The behavior screening was performed as described before[2] with minor modifications. To assess any distress or discomfort due to the ET, females were also monitored for changes in their behavior. Embryo transfers took place in the morning at approximately 9:00 am. Behavior was observed hourly starting after the ET at 10:00 until 17:00; from 17:00 until 9:00 on the next day a camera was used to record a video for further screening. Several parameters were evaluated after operation for the mice using score sheets including grooming, body posture when awake and during sleep, locomotion, activity, food intake (food was offered in a Petri dish on the floor to facilitate intake) and nest building. Behavior was assessed in comparison with the behavior of NMRI mice only subjected to the anesthesia without ET (referred as normal) of corresponding age and sex by an experienced animal technician who did not know the treatment group of mice. Normal behavior and appearance were noted with zero points and obvious changes in behavior and appearance were noted with one point for each of the listed parameters. Points were summarized and compared between experimental groups.[2]

Statistical analysis. Litter size and scoring points were compared by analysis of variance (ANOVA). Pregnancy rate was compared analyzed by the Pearson chi-square test using SPSS software (version 10.0; SPSS Inc., Chicago, USA). The litter size and scoring points were compared using one-way ANOVA in SigmaStat (version 2.03; Systat Software Inc., San Jose, USA). A *p*-value less than 0.05 (typically ≤ 0.05) is statistically significant.

Results

A swollen ampulla was considered as an approval check point for the successful pseudo-pregnancy in all ETs. Embryo transfers in reused surrogate mothers by both surgical practices, EST and CST, did not show any defects compared to the procedure in virgin females. No adhesions or scars of the ovarian fat pad and the ovarian bursa were observed following the first, the second and the third ETs and the ovarian infundibulum was easily accessible for the first, the second and the third transfer. The recorded data showed that there was no significant reduction in litter size following the first ET in both groups, EST-ET and CST-ET; while, the litter size was significantly ($p < 0.05$) reduced in CST group compared to EST for the second and the third ETs (Table 2). Following the first ET, there were no reductions in terms of litter size in EST and CST groups; while, a significant reduction was recorded in EST-control and CST-control. Litter size in CST-ET group following the second ET showed a dramatic reduction compared to EST-ET group. On the other hand, litter size in CST-ET group following the second ET was reduced dramatically compared to CST-ET group after first ET.

Table 2. Effects of embryo transfer method on litter size, pregnancy rate and surrogate mother survival.

Recorded parameter	1st embryo transfer group				2nd embryo transfer group		3rd embryo transfer group	
	EST	CST	EST-control	CST-control	EST	CST	EST	CST
Litter size	9.10 ± 2.90^{*a}	9.30 ± 3.10^{*a}	6.10 ± 2.10$^{\dagger b}$	6.30 ± 2.30$^{\dagger b}$	9.20 ± 3.20^{*a}	4.10 ± 1.20$^{\dagger c}$	4.20 ± 1.20^{*c}	2.20 ± 1.10$^{\dagger d}$
Pregnancy rate (%)	95.70	94.50	80.10	81.00	95.20	71.10	45.20	20.10
Mothers' survival (%)	100	85	100	84	100	65	60	20

EST: Electrosurgery technique; CST: Cold surgical technique.

*† indicate the significant differences within each embryo transfer groups ($p < 0.05$).

^{abcd} Different superscripts indicate the significant differences between embryo transfer groups ($p < 0.05$).

Table 3. Behavior scores following embryo transfer.

Behaviors	1st embryo transfer group				2nd embryo transfer group		3rd embryo transfer group	
	EST	CST	EST-control	CST-control	EST	CST	EST	CST
Coat cleaning	6.10 ± 1.10^{*a}	6.30 ± 1.30^{*a}	6.20 ± 1.20^{*a}	6.20 ± 1.10^{*a}	6.50 ± 1.50^{*a}	6.20 ± 1.10^{*a}	6.10 ± 1.40^{*a}	6.40 ± 1.20^{*a}
Movements	6.30 ± 1.20^{*a}	6.50 ± 1.10^{*a}	6.20 ± 1.30^{*a}	6.10 ± 1.20^{*a}	3.50 ± 1.30$^{\dagger b}$	3.40 ± 1.40$^{\dagger b}$	1.10 ± 0.60$^{\dagger c}$	1.20 ± 0.50$^{\dagger c}$
Food intake	6.30 ± 1.40^{*a}	6.10 ± 1.50^{*a}	4.20 ± 1.20$^{\dagger b}$	4.10 ± 1.30$^{\dagger b}$	6.40 ± 1.10^{*a}	3.30 ± 1.20$^{\dagger b}$	2.00 ± 0.60^{*b}	2.20 ± 0.60^{*b}
Nest building	3.30 ± 1.50^{*a}	3.30 ± 1.30^{*a}	3.10 ± 1.10^{*,a}	3.20 ± 1.20^{*a}	3.10 ± 1.30^{*a}	3.20 ± 1.50^{*a}	1.30 ± 0.40^{*b}	1.30 ± 0.60^{*b}

EST: Electrosurgery technique; CST: Cold surgical technique.

*† indicate the significant differences within embryo transfer groups ($p < 0.05$).

^{abc} Different superscripts indicate the significant differences between embryo transfer groups ($p < 0.05$).

Following the second and the third ETs, pregnancy rate showed a significant reduction in CST-ET group compared to EST-ET group. In addition, in the EST-ET group, the recorded data showed a significant reduction in pregnancy rate following the third ET (45.20%) compared to the first (95.70%) and the second (95.20%) ETs. The results can be interpreted as follows: with the increase in the age of surrogate mothers in the third round of ET and the significant reduction in food intake as a consequence of animal aging, the pregnancy rate and litter size were also reduced following the third ET. On the other hand, reduction in litter size per se (Table 2) following the second ET, led to a dramatic reduction in food intake and body weight that is consequently can lead to a reduction in pregnancy rate following the third ET.

Table 3 represents behavior scores following the embryo practice. There were not any significant reductions in the coat cleaning, movements and nest building; while, there were great reductions in food intake following the second and the third ETs in CST-ET groups compared to the EST-ET groups.

The recorded data showed that the rate of surrogate mothers survival five days after surgical practice was reduced significantly in CST-ET groups (85, 84, 65 and 20%) following ETs compared to EST-ET ones (100, 100, 100 and 60.00%).

Discussion

The possible explanation for the reduction in litter size following the second and the third ETs in CST group could be due to gradual blood loss as the consequence of the chosen surgical practice (EST versus CST). The results of first ET showed no significant reduction in litter size regardless of the preferred surgical methods, which would be the grate approval for the importance of the blood volume 36 days before ET practice. Comparing the results of first and second ETs in CST-ET groups, the importance of blood volume in a period of 36 days before ET dramatically increased. It seems that due to the intact blood volume of virgin female mice in both groups, EST-ET and CST-ET groups, in the first round of ET, the litter size and pregnancy rate did not show significant difference. The amount of bleeding occurred in CST groups was significantly increased compared to EST groups. Consequently, the concentration of blood luteinizing hormone (LH) reduced in body and uterine blood flow (UBF). As previously reported, the reduced concentration of LH can lead to dramatic reduction in conception and litter size.[12] The entire substances essential for fetal development are carried by maternal blood and taken up by the pregnant uterus. The amount of substrates reaching the fetuses depends on several factors including their concentration in maternal blood and UBF. Therefore, the consequent reduction in the blood volume after each round of ET may lead to a drastic reduction in UBF and the pivotal factors carried to the uterus by the systemic blood circulation.[12,13]

The recorded data from EST-control and CST-control showed significant reduction in litter size as a consequence of blood loss. This is in agreement with findings of Senger *et al*., and Père *et al.*[13,14] This reduction in litter size was consequently led to reductions in food intake and pregnancy rate. These findings confirmed that the litter size would be the crucial factor affecting food intake following mouse parturition. On the other hand, reductions in food intake following second and third ETs in CST-ET groups are in agreement with Wagner *et al*., which showed that litter size has a strong effect on behavior, eating patterns and body weight.[15]

The reduced survival rate of surrogate mothers in CST-ET groups compared to EST-ET groups led to a hypothesis as follows: This finding indicated that the minor or profound bleeding would lead to animal weakness and higher animal mortality following the surgical practice. In a study conducted by Dadashpour Davachi, it has been shown that vasectomy in male mouse using electrosurgery machine can lead to a higher survival rate compared to CST.[11] On the other hand, the reduced mothers survival following the third ET in EST-ET could be due to the aging of the animals.

In conclusion, from the results it can be stated that electrosurgery could serve as the best surgical technique to perform ET in murine model. Also, to achieve better results, it is strongly suggested that the surrogate mothers be used only for two rounds of ET.

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Conflict of interest

The author has no conflict of interest to declare.

References

1. Bermejo-Alvarez P, Park KE, Telugu BP. Utero-tubal embryo transfer and vasectomy in the mouse model. J Vis Exp 2014; (84): e51214.doi: 10.3791/51214.
2. Kolbe T, Palme R, Touma C, et al. Repeated use of surrogate mothers for embryo transfer in the mouse. Biol Reprod 2012; 86(1): 1-6.
3. Tarkowski AK. Experimental studies on regulation in the development of isolated blastomeres of mouse eggs. Acta Theriol 1959; 3(11): 191-287.
4. Scott JE, Swanson EA, Cooley J, et al. Healing of canine skin incisions made with monopolar electrosurgery versus scalpel blade. Vet Surg 2017; 46(4): 520-529.
5. Peneva M, Gjorgjeska A, Gjorgova ST, et al. Evaluation of pain following the use of scalpel versus electro-surgery for skin incisions in the facial regions. Pril (Makedon Akad Nauk Umet Odd Med Nauki) 2018; 39(2-3): 107-112.
6. Aminimoghaddam S, Pahlevani R, Kazemi M. Electro-surgery and clinical applications of electrosurgical devices in gynecologic procedures. Med J Islam Repub Iran 2018; 32: 90.doi: 10.14196/mjiri.32.90.
7. Al-Benna S. Albucasis, a tenth-century scholar, physician and surgeon: His role in the history of plastic and re-constructive surgery. Eur J Plast Surg 2011; 35: 379-387.
8. Charoenkwan K, Iheozor-Ejiofor Z, Rerkasem K, et al. Scalpel versus electrosurgery for major abdominal incisions. Cochrane Database Syst Rev 2017; 6(6): CD005987. doi: 10.1002/14651858.CD005987.
9. Dadashpour Davachi N, Zare Shahneh A, Kohram H, et al. Differential influence of ampullary and isthmic derived epithelial cells on zona pellucida hardening and in vitro fertilization in ovine. Reprod Biol 2016; 16(1): 61-69.
10. Dadashpour Davachi N, Fallahi R, Dirandeh E, et al. Effects of co-incubation with conspecific ampulla oviductal epithelial cells and media composition on cryotolerance and developmental competence of in vitro matured sheep oocytes. Theriogenology 2018; 120: 10-15.
11. Dadashpour Davachi N. Vasectomy in mouse model using electrosurgery machine. Arch Razi Inst 2019; 74(2): 191-195.
12. Johnson CA. Reproductive disorders. In: Willard MD, Tvedten H, (Eds). Small animal clinical diagnosis by laboratory methods. 5th ed. Saint Louis, USA: WB Saunders 2012; 294-303.
13. Senger PL, Lose ED, Ulberg LC. Reduced blood supply to the uterus as a cause for early embryonic death in the mouse. J Exp Zool 1967; 165(3): 337-343.
14. Père MC, Etienne M. Uterine blood flow in sows: effects of pregnancy stage and litter size. Reprod Nutr Dev 2000; 40(4): 369-382.
15. Wagner LA, Menalled L, Goumeniouk AD, et al. Huntington Disease. In: McArthur RA, Borsini F, (Eds). Animal and translational models for CNS drug discovery. 1st ed. San Diego, USA: Academic Press 2008; 207-266.