

Original Article

Examination of histopathological changes of scalpel, monopolar, bipolar, and thermocautery applications in rat experimental circumcision model

Ahmet Ali Tuncer¹, Mehmet Fatih Bozkurt², Altınay Bayraktaroğlu¹, Halit Buğra Koca³, İsmet Doğan⁴, Salih Çetinkursun¹, Didem Baskın Embleton¹

Departments of ¹Pediatric Surgery, ³Biochemistry, ⁴Biostatistics, Medical Faculty, Afyon Kocatepe University, Afyonkarahisar, Turkey; ²Department of Pathology, Veterinary Medicine Faculty, Afyon Kocatepe University, Afyonkarahisar, Turkey

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Abstract: This study endeavors to analyze the effects of thermocautery, bipolar cautery, monopolar cautery, and the scalpel to show that the thermocautery is a safe device to be used in circumcision. Twenty-four rats were assigned to 4 different groups: the scalpel, thermocautery, bipolar cautery, and monopolar cautery groups. Circumcisions were performed using the scalpel, thermocautery, bipolar cautery, or monopolar cautery devices. The circumcised foreskin was removed for histopathological analysis. The circumcision techniques were compared in terms of the depth of injury on the prepuce. Wound healing on the 5th day on the circumcision plane was evaluated by using a grading scale from 0-4 and by comparing re-epithelization, granulation tissue, and collagen deposition. Blood samples were taken 1st hour after the operation and the 5th day, prior to the necropsy. The totals of the oxidant/anti-oxidant levels were determined. For statistical analyses, the SPSS packet program was used. Statistical significance was determined as a *P* value <0.05. The least trauma was with the scalpel which was comparable with the thermocautery in regard to depth of injury, epithelization, granulation tissue formation, and collagen buildup. Thermocautery group showed superior collagen proliferation compared with the monopolar cautery group, while being superior in epithelization and injury depth when compared with the bipolar cautery group. The use of thermocautery for circumcision has shown to be safe and resulted in better wound healing in rats with no apparent complications, and, if used in the human population, it may be a safe and effective technique.

Keywords: Circumcision, foreskin, cautery, wound healing, Sprague-Dawley rat

Introduction

Circumcision is the surgical removal of the prepuce. This operation has been carried out for hundreds of years and is done for cultural or medical reasons [1]. Every year, approximately 20 million males undergo circumcision [2]. Given the high numbers, the general global trend has been in favor of performing circumcisions in an outpatient setting with local anesthesia. Circumcision can be done by using surgical instruments (scissors and/or scalpel) and clamps (Gomco clamp, Ali's clamp, Tara clamp, Mogen clamp, etc.), or it can be done with equipment that uses electrical energy (monopolar cautery, bipolar cautery, thermocautery) [3-6]. The thermocautery technique is rapidly gaining popularity as an alternative method because it shortens the procedure and

decreases occurrence of bleeding problems with its use.

However, there are not enough studies in the literature that examine the effects of thermocautery applied to the prepuce, either in animal models or in the human population [4]. In the study presented here, the effects of thermocautery, bipolar cautery, monopolar cautery, and the scalpel were compared to show that thermocautery technique can be used safely in circumcision.

Materials and methods

Ethics

The experiment in this study was performed in accordance with the guidelines for animal

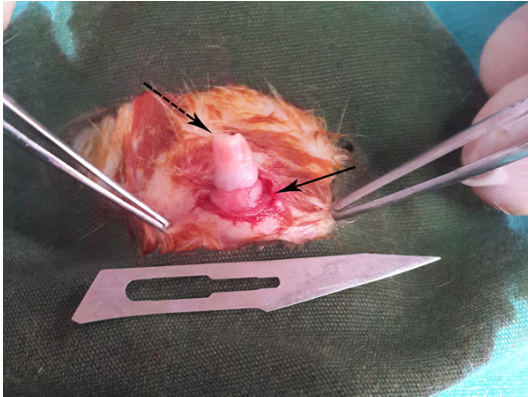


Figure 1. End of circumcision. The dashed arrow shows the glans penis, and the bold arrow shows the circumcision line.

research from the National Institutes of Health and was approved by Afyon Kocatepe University Local Ethics Committee for Animal Experiments (Number: 49533702/39 Date: 20.03.2014).

Animals and surgery

In the present study, the male Sprague-Dawley rats (ranging from 250-300 g in weight) were used instead of other species, as they tend to have a better developed scrotum and foreskin. Rats were included in the experiment when they had intact circadian rhythm within their natural environment. Twenty-four rats were assigned to 4 different groups: the scalpel, thermocautery, bipolar cautery, and monopolar cautery groups. All surgical operations were performed under intramuscular anesthesia, using 50 mg/kg ketamine (Ketasol 10%; Richter Pharma AG, Wels, Austria) and 10 mg/kg xylazine (Alfazyne 2%; Alfasan International BV, Woerden, Netherlands). Following anesthesia, circumcision was performed on the rats (**Figure 1**). The foreskin was suspended with clamps, a forceps was placed on the foreskin, and the circumcision was subsequently performed by cutting the foreskin with a scalpel (Broche no. 11 surgical blades; Nurteks Ltd., Istanbul, Turkey), monopolar cautery (CONMED Sabre 2400; CONMED Electrosurgery, Utica, New York, USA), bipolar cautery (CONMED Sabre 2400; CONMED Electrosurgery, Utica, New York, USA), or thermocautery (Thermo-Med TM 806; Termomedikal, Adana, Turkey). Monopolar and bipolar devices were set to deliver 15 watts of energy. The thermocautery device was set to 250°C during the experiment.

Bleeding was controlled by pressure with gauze for the scalpel technique. No bleeding control was needed with the cautery techniques. The circumcised foreskins were removed for histopathological analysis. Blood samples were taken to be biochemically examined one hour after the procedure. The circumcision line was dressed once a day with antiseptic solution. Five days later, the animals were anesthetized again to obtain blood samples, and the penis was excised to histopathologically examine the healing line of the circumcision.

Histopathological analysis

On the 1st day, the foreskins and, on the 5th day, the penises were placed in a 10% neutral buffer formaldehyde solution following the operative process. The tissues were fixed for 48 hours. The tissues were then sliced vertically and maintained in cassettes. An automatic tissue tracing device was used (Leica TP1020; Leica Microsystems, Nussloch, Germany) to form paraffin blocks, following treatment by alcohol and xylol. Next, 5-micron slices were acquired by means of a microtome device. All of the slices were then dyed with the hematoxylin and eosin (H&E) staining method [7]. The stained preparations were examined under light microscopy (Zeiss Axio Lab.A1; Carl Zeiss International, Jena, Germany), and microscopic images were captured with a microscope camera (Zeiss Axio Cam ICc 5; Carl Zeiss International, Jena, Germany). ZEN 2 software was used to analyze the histopathology of the lesions (ZEN 2 ZEN Imaging Software; Carl Zeiss Microscopy GmbH, Jena, Germany).

Following the circumcision of the rats, two different evaluations were made: the comparison of the depth of injury during the different circumcision techniques and the healing of the circumcision line. To compare the depth of injury in the circumcised foreskin, the deepest point of edema or necrosis, taken as the base, was measured microscopically. To determine the differences in wound healing on the 5th day, the circumcision plane on the penis was evaluated by using a grading scale from 0-4, comparing re-epithelialization, granulation tissue, and collagen deposition [8].

Biochemical analysis

Blood samples were taken the 1st hour after the operation and the 5th day prior to the necropsy.

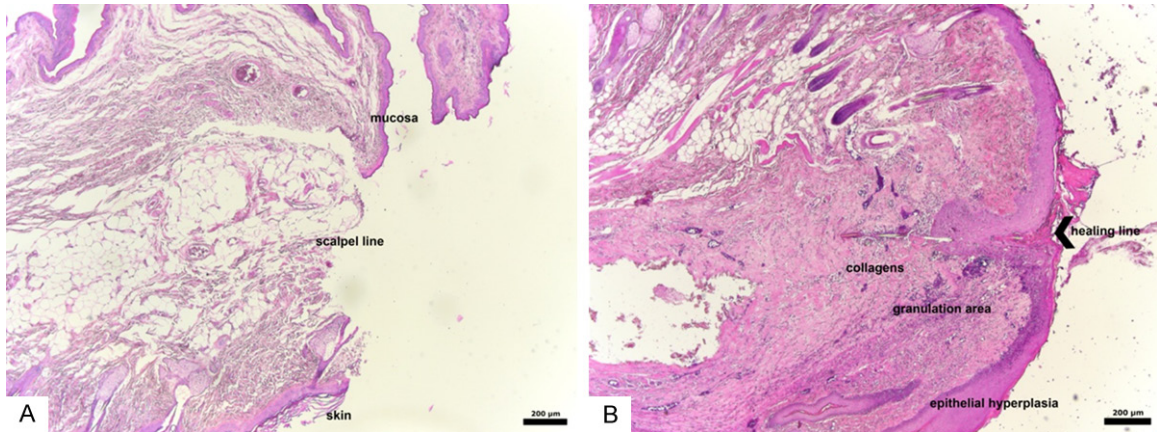


Figure 2. Scalpel group. A: Histopathological appearance of the incision line after the use of scalpel. There is no coagulation necrosis. Only epithelial tissue loss is observed. B: Histopathological appearance of the wound edges on the 5th day. Epithelization and collagen maturation are complete after cutting with the scalpel. (Bar =200 µm, 5×, H&E).

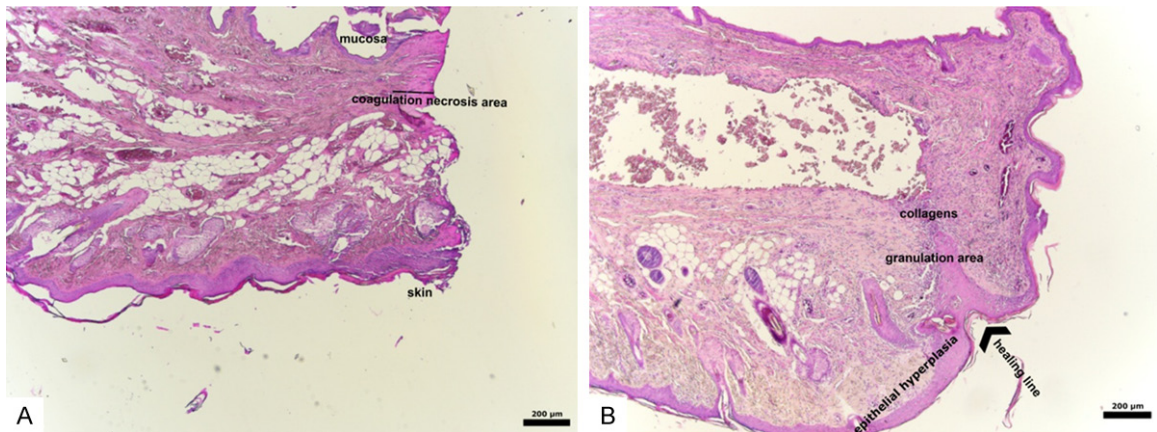


Figure 3. Thermocautery group. A: Histopathological appearance of the incision line after the use of thermocautery. There is slight coagulation necrosis. B: Histopathological appearance of the wound edges on the 5th day. Epithelization and collagen maturation are nearly complete after cutting with the thermocautery. (Bar =200 µm, 5×, H&E).

The totals of the oxidant/anti-oxidant levels were determined.

Statistical analysis and evaluation

The descriptive quantitative data statistics and arithmetic mean \pm were expressed as their standard deviations. In order to make the variable data between the groups more visually apparent, box-plot graphs were drawn. To accentuate the more apparent inter-group variables of interest, the Kruskal-Wallis test was used, and, for the post hoc analysis, the Dunn multiple comparison test was used. To determine if the variables were normally distributed in groups, Shapiro-Wilkand, Kolmogorov-Smirnov normality tests were used. Variables

were not distributed normally, so the Kruskal-Wallis test was used for the comparison of the groups. The results of the tests, mean \pm SD and *P* values were shown in tables. For statistical analysis, the Statistical Package for the Social Sciences (SPSS version 20.0; IBM, Armonk, NY, USA) program was used. Statistical significance was determined as *P*<0.05.

Results

There was no bleeding or penile necrosis in the penises of the rats in any of the techniques.

Histopathological analysis

In the histopathological study of the foreskin, the examination of the foreskin, obtained after

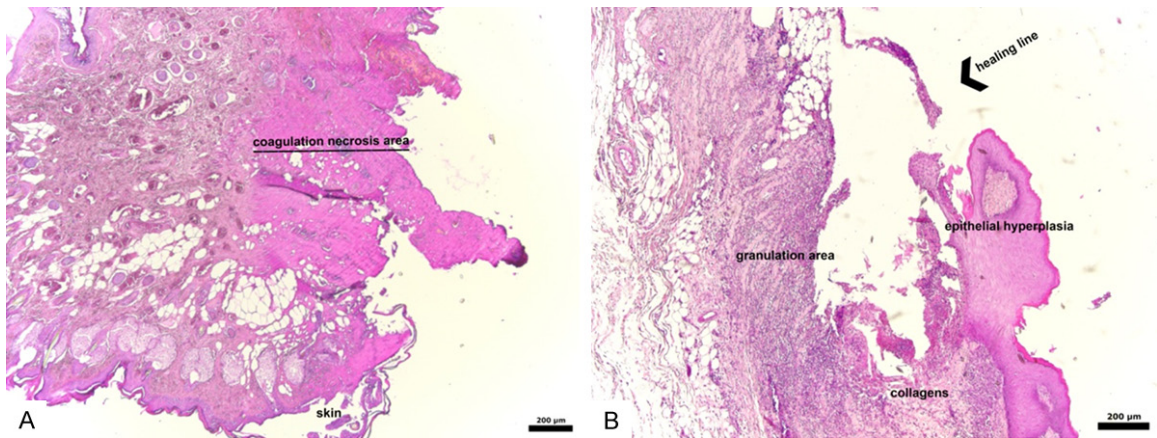


Figure 4. Bipolar cautery group. A: Coagulation necrosis was the thickest after the use of bipolar cautery. B: Histopathological appearance of the lesion caused by bipolar cautery shows less epithelial hyperplasia on the 5th day. Wound edges are still separated, collagen is immature, and acute inflammatory cells are still present in the granulation tissue. (Bar =200 µm, 5×, H&E).

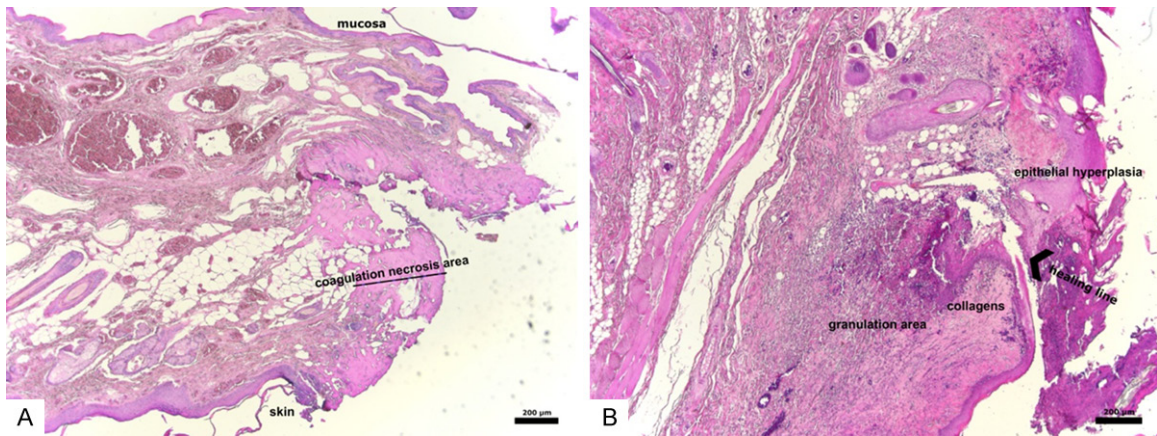


Figure 5. Monopolar cautery group. A: Coagulation necrosis was mild after the use of monopolar cautery. B: Histopathological appearance of the lesion caused by monopolar cautery shows mild epithelial hyperplasia on the 5th day. Wound edges are still separated, collagen is immature, and acute inflammatory cells are still present in the granulation tissue, which is similar to the lesion caused by bipolar cautery. (Bar =200 µm, 5×, H&E).

circumcision with a scalpel, showed a line of hyperemia and a 200 µm deep edema along the line of the incision. There was no presence of coagulation necrosis (**Figure 2A**). Hyperemia at the excision line was also present in groups where monopolar cautery, bipolar cautery, and thermocautery were used. Also, at the excision line, burn-dependent epidermis, mucosal epithelium, and regional connective tissue coagulation necrosis were seen (**Figures 3A, 4A, 5A**). The depth of injury was measured as follows: scalpel < thermocautery < monopolar cautery < bipolar cautery. According to statistical analysis, while the scalpel group showed statistically acceptable levels of damage present in comparison to the bipolar cautery and monopolar

cautery groups, there was no significant difference between the scalpel group and the thermocautery group. There was significantly less damage present in the thermocautery group, compared to the bipolar cautery group ($P=0.016$) (**Figure 6** and **Table 1**).

On the 5th day, the circumcision line was healed in all groups macroscopically. While there was no significant statistical difference between the scalpel and the thermocautery groups in respect to epithelization, the monopolar and bipolar cautery groups had significantly less epithelization (thermocautery $P=0.533$; bipolar cautery $P=0.003$; and monopolar cautery $P=0.006$). Epithelization was significantly greater

Circumcision model in rat

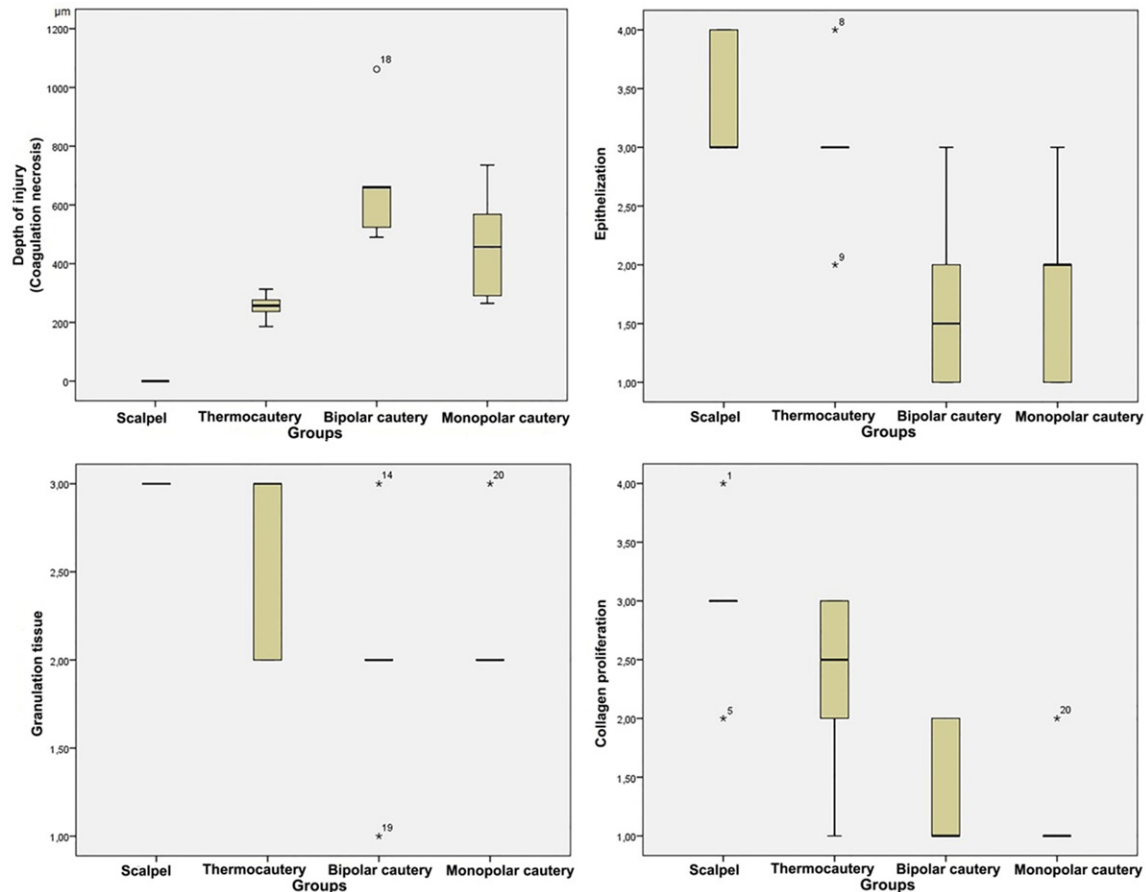


Figure 6. Comparison of the effects of scalpel, thermocautery, monopolar cautery, and bipolar cautery on depth of injury, epithelization, granulation tissue, and collagen proliferation. The depth of injury is the least with scalpel (left upper); scalpel and thermocautery use resulted in better epithelization (right upper) and better formation of granulation tissue (left lower); and collagen deposition is better in scalpel and thermocautery groups (right lower).

in the thermocautery group, compared with the monopolar cautery and bipolar cautery groups (monopolar cautery $P=0.033$ and bipolar cautery $P=0.018$) (**Figures 2B, 3B, 4B, 5B, 6** and **Table 1**).

Formation of granulation tissue in the scalpel group showed more statistically significant granulation when compared to the bipolar cautery and monopolar cautery, but the statistical difference was not important when compared with the thermocautery group (bipolar cautery $P=0.003$; monopolar cautery $P=0.008$; and thermocautery $P=0.288$). Although there was no meaningful difference for the thermocautery group, when compared with the bipolar and monopolar cautery groups, granulation in the thermocautery group was more prominent than in the other cautery groups (**Figures 2B, 3B, 4B, 5B, 6** and **Table 1**).

Collagen proliferation in the scalpel group was found to have statistically higher levels compared with the bipolar cautery and monopolar cautery groups, but no difference was found in the thermocautery group (bipolar cautery $P=0.003$; monopolar cautery $P=0.001$; and thermocautery $P=0.279$). While more statistically significant levels of collagen were present in the thermocautery group, compared to the monopolar cautery group, there was no statistically significant difference seen when compared to the bipolar cautery group (monopolar cautery $P=0.026$ and bipolar cautery $P=0.60$) (**Figures 2B, 3B, 4B, 5B, 6** and **Table 1**).

Biochemical analysis

There was no statistical difference in the biochemical parameters for the 1st hour and the 5th day samples.

Table 1. Table shows the mean \pm SD of the depth of injury, epithelization, collagen deposition, and granulation in study groups. Kruskal-Wallis test showed the difference between the groups ($P < 0.05$ in all groups). The groups that caused the difference were identified by using the Dunn pairwise comparison test

Groups		Depth of injury (Coagulation necrosis) (μm)	Epithelization*	Collagen deposition*	Formation of granulation tissue*
Scalpel		0.00 \pm 0.00	3.33 \pm 0.51	3.00 \pm 0.63	3.00 \pm 0.00
Thermocautery		254.7 \pm 41.47	3.00 \pm 0.63	2.33 \pm 0.81	2.66 \pm 0.51
Bipolar cautery		679.60 \pm 227.53	1.66 \pm 0.81	1.33 \pm 0.51	2.00 \pm 0.63
Monopolar cautery		462.33 \pm 182.69	1.83 \pm 0.75	1.16 \pm 0.40	2.16 \pm 0.40
P value of Kruskal-Wallis test		0.005	0.004	0.002	0.009
P value of Dunn pairwise comparison test	P_{T-P_S}	0.059	0.533	0.279	0.288
	P_{T-P_B}	0.016	0.018	0.060	0.061
	P_{T-P_M}	0.142	0.033	0.026	0.111
	P_{B-P_M}	0.312	0.813	0.729	0.782
	P_{S-P_M}	0.001	0.006	0.001	0.008
	P_{S-P_B}	0.000	0.003	0.003	0.003

P_S : P value of scalpel; P_T : P value of thermocautery; P_B : P value of bipolar cautery; P_M : P value of monopolar cautery. *The values reflect the grading scale (0-4) that we used to evaluate the changes.

Discussion

Male Sprague-Dawley rats were preferred in the study due to the better development of their foreskin and scrotum compared with other rat species. A search of the literature did not reveal any similar experimental models on rats for circumcision.

The most commonly seen postoperative complication of circumcision is bleeding, which is encountered in 0.1-35% of cases [9]. To stop bleeding in the human patients during circumcision, various techniques, such as free ties, cautery, and thermocautery, may be used. Furthermore, thermocautery and bipolar diathermy scissors circumcision (cutting) techniques also serve to achieve hemostasis [10-12]. It is debated whether monopolar cautery may be responsible for penile tissue and nerve damage that may result in penile necrosis [13, 14]. On the other hand, thermocautery makes use of electrical energy and transforms it into heat energy, thereby preventing the electrical flow through the penile shaft during the circumcision; furthermore, it coagulates while cutting which is an effective means of hemostasis. Also, the patient does not require pads with thermocautery. It can be used in patients with implantable cardiac devices because the patient is not subjected to electrical flow, and it is the method of choice during local dermatological excisions [15, 16]. Moreover, there are many commercially available thermocautery

devices that can adjust the heat according to the tissue types.

Studies have shown that the optimum hemostasis can be achieved at 100-400°C. With thermocautery, 350-900°C of heat can be achieved in vitro. However, the peak value of heat in vivo is 50% less. Indeed, this value can be less in a bloody environment [15]. The Thermo-Med TM 806R device uses 1.9 watts of energy, and its maximum heating capacity is 430°C. It was set to 250°C during the experiment.

Monopolar and bipolar cautery devices were set at a common energy value of 15 watts in order to minimize tissue damage and to avoid macroscopic burns in the incision line. The only damage seen in the scalpel group was a 200 μm deep edema. Coagulation necrosis was observed in all cautery groups, the bipolar cautery being the deepest and thermocautery being the most superficial. According to the literature, the presence of coagulation necrosis may delay wound healing or may even result in the amputation of tissue. There are many studies which report circumcisions, especially those done with monopolar cautery, that have resulted in penile necrosis [13, 14]. Moreover, there are studies comparing bipolar cautery and monopolar cautery that suggest higher complication rates with monopolar cautery, but not to the point of being statistically significant compared to bipolar cautery [6].

Thermocautery and bipolar diathermy scissors techniques are both being used to perform circumcision with fast hemostasis [11]. Clinical studies demonstrate that both techniques are safer and faster than the traditional surgical circumcision and that they achieve better hemostasis; thus, they are increasingly preferred [11, 17]. Karaman and colleagues proved in their study that simple thermocautery can be successfully used in the circumcision of hemophilic patients [5]. Furthermore, it was shown that thermocautery can be used safely by experienced personnel in countries where mass circumcisions are carried out often due to religious or cultural reasons [18]. However, in such clinical studies, it is hard to find data about wound healing results with histopathological analysis.

A wound is defined as a surgical or traumatic insult which undermines the integrity of the tissue. Wound healing is affected by age, corticosteroid use, diet, cytotoxic drugs, and many other factors. However, the most important factor in all of these variables is the mechanism of wound formation, and this variable directly affects healing. Circumcision is a surgical wound, and we endeavored to compare wound formation and healing by using different circumcision techniques. Wound healing is comprised of overlapping phases of inflammation, proliferation, and maturation. Epithelization is completed within 24 hours with keratinocyte migration and proliferation. Later, the wound area is increasingly occupied by granulation tissue. Collagen, which makes up the main constituent of connective tissue, is laid down as type I and later becomes type III collagen. The formation of the collagen fibers during days 4-6 results in a rapid rise of tensile strength [19].

In this study, the 5th day was chosen for inflammation comes to an end and the proliferation stage starts. During this phase, epithelization, collagen buildup, and levels of granulation tissue were compared in the various approaches to circumcision. From the point of view of injury depth, epithelization, collagen buildup, and granulation tissue did not reveal any statistically significant differences in the scalpel and thermocautery groups.

Conclusion

The results from the thermocautery group showed superior collagen proliferation, com-

pared with the monopolar cautery group, while being superior in epithelization and injury depth when compared with the bipolar cautery group. The application of thermocautery has revealed comparable results with the scalpel group in regard to depth of damage, epithelization, granulation tissue formation, and collagen buildup compared with the monopolar cautery and bipolar cautery techniques. Thermocautery for circumcision has been shown to be safe in rats, and when used in the human population, it may be a safe and effective technique.

Acknowledgements

Ethics committee approval: Ethics committee approval was received for this study from the Afyon Kocatepe University Local Ethics Committee for Animal Experiments (Number: 49533702/39 Date: 20.03.2014). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Ahmet Ali Tuncer, Department of Pediatric Surgery, Medical Faculty, Afyon Kocatepe University, 03000, Afyonkarahisar, Turkey. Tel: +90 444 03 04; Fax: +90 272 246 33 22; E-mail: drtaali@yahoo.com

References

- [1] Tuncer AA and Tuncer N. Evaluation of two different circumcision techniques and complications associated with these techniques implemented by local circumcisers in Yuksekova. *Van Tıp Dergisi* 2015; 22: 273-237.
- [2] Malone P and Steinbrecher H. Medical aspects of male circumcision. *BMJ* 2007; 335: 1206.
- [3] Senel F and Misirlioglu F. 1623 pain after circumcision: comparison of conventional and plastic clamp circumcisions. *Arch Dis Child* 2012; 97: 459.
- [4] Hay SA. The use of thermal cautery for male circumcision. *JKAU Med Sci* 2009; 16: 89-93.
- [5] Karaman MI, Zulfikar B, Caskurlu T and Ergenekon E. Circumcision in hemophilia: a cost-effective method using a novel device. *J Pediatr Surg* 2004; 39: 1562-1564.
- [6] Harty NJ, Nelson CP, Cendron M, Turner S and Borer JG. The impact of electrocautery method on post-operative bleeding complications after non-newborn circumcision and revision circumcision. *J Pediatr Urol* 2013; 9: 634-637.

- [7] Luna LG. Manual of histologic staining methods of the armed forces institute of pathology. New York: McGraw-Hill; 1968.
- [8] Simonetti O, Lucarini G, Cirioni O, Zizzi A, Orlando F, Provinciali M, Di Primio R, Giacometti A and Offidani A. Delayed wound healing in aged skin rat models after thermal injury is associated with an increased MMP-9, K6 and CD44 expression. Burns 2013; 39: 776-787.
- [9] Kaplan GW. Complications of circumcision. Urol Clin North Am 1983; 10: 543-549.
- [10] Peters RT and Fisher R. Paediatric circumcision using bipolar diathermy. Ann R Coll Surg Engl 2009; 91: 435-441.
- [11] Méndez-Gallart R, Estévez E, Bautista A, Rodríguez P, Taboada P, Armas AL, Pradillos JM and Varela R. Bipolar scissors circumcision is a safe, fast, and bloodless procedure in children. J Pediatr Surg 2009; 44: 2048-2053.
- [12] Kazem MM, Mehdi AZ, Golrasteh KZ and Behzad FZ. Comparative evaluation of two techniques of hemostasis in neonatal circumcision using the plastibell device. J Pediatr Urol 2010; 6: 258-260.
- [13] Uzun G, Ozdemir Y, Eroglu M and Mutluoglu M. Electrocautery-induced gangrene of the glans penis in a child following circumcision. BMJ Case Rep 2012; 2012.
- [14] Aminsharifi A, Afsar F and Tourchi A. Delayed glans necrosis after circumcision: role of testosterone in salvaging glans. Indian J Pediatr 2013; 80: 791-793.
- [15] Lane JE, O'Brien EM and Kent DE. Optimization of thermocautery in excisional dermatologic surgery. Dermatol Surg 2006; 32: 669-675.
- [16] Voutsalath MA, Bichakjian CK, Pelosi F, Blum D, Johnson TM and Farrehi PM. Electrosurgery and implantable electronic devices: review and implications for office-based procedures. Dermatol Surg 2011; 37: 889-899.
- [17] Saracoglu M, Ozturk H, Zengin T and Kerman HS. Comparison of thermal cautery-assisted circumcision with the conventional technique. Human Andrology 2014; 4: 34-37.
- [18] Arslan D, Kalkan M, Yazgan H, Ünüvar U and Şahin C. Collective circumcision performed in Sudan: evaluation in terms of early complications and alternative practice. Urology 2013; 81: 864-868.
- [19] Harper D, Young A and McNaugh CE. The physiology of wound healing. Surgery 2014; 32: 445-450.