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EARLY HISTOPATHOLOGICAL COMPARISON OF SINGLE VERSUS DOUBLE-LAYER CLOSURE OF UTERINE INCISION DURING CESAREAN: A CONTROLLED STUDY IN RATS

SEZARYEN SIRASINDA UTERUSUN TEK YA DA ÇİFT KAT KAPATILMASININ ERKEN HİSTOPATOLOJİK KARŞILAŞTIRILMASI: RATLARDA KONTROLLÜ ÇALIŞMA

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ABSTRACT

Objective: Long-term complications of cesarean delivery are numerous and are increasing due to the high cesarean rate. The long-term complications are attributed to the different suturing methods. The single-layer locked (SLL) and double-layer unlocked (DLU) are the most compared. This study aims to compare the early histopathological properties of these two suture techniques in an experimental animal study.

Material and Method: Ten pregnant Wistar-Albino rats had cesarean at term. The right uterine horns were repaired using the SLL suture technique, and the left ones were repaired using the DLU technique. The control subjects were the six uterine horns of three pregnant rats delivered vaginally. Each animal underwent a hysterectomy on day 15 of pregnancy, and each uterus was an experimental unit. The mean thickness of the endometrium and myometrium of the intervention groups with histopathological features and controls were compared.

Result: In SLL and DLU groups, the endometrium (276.6±123.1 vs. 258.4±125.9 μm; p=0.748) and myometrium (531.7±189.2 vs. 505.9±182.1 μm; p=0.612) in scarred areas were similar. In non-scarred areas, the endometrium was measured as 239.9±68.9, 256.5±127.2, and 316.4±98.6 μm in SLL, DLU, and control group, respectively (p=0.347). The myometrium of non-scarred areas

ÖZET

Amaç: Sezaryenin birçok uzun dönem komplikasyonu vardır. Yüksek sezaryen oranları nedeniyle bunlar artmaktadır. Uzun dönem komplikasyonların farklı sütür teknikleri ile ilişkisi olduğu düşünülmektedir. Tek kat kilitlenerek (SLL) ve çift kat kilitlenmeden (DLU) en çok karşılaştırılan yöntemlerdir. Bu çalışmanın amacı iki farklı sütür tekniğinin erken dönem histopatolojik sonuçlarını bir deneysel hayvan modelinde karşılaştırmaktır.

Gereç ve Yöntem: On Wistar-Albino rata miadında sezaryen yapıldı. Sağ uterus SLL, sol uterus DLU olarak kapatıldı. Kontrol grubunda ise normal doğum yapmış üç ratın altı uterusu bulunmaktaydı. Doğumdan 15 gün sonra tüm hayvanlara histerektomi yapıldı ve her bir uterus deney ünitesi olarak kullanıldı. Çalışma ve kontrol grupları arasında endometrium ve myometrium ortalama kalınlıkları ile histopatolojik özellikler karşılaştırıldı.

Bulgular: SLL ve DLU gruplarında endometrium (276,6±123,1 vs 258,4±125,9 μm; p=0,748) ve myometrium (531,7±189,2 vs 505,9±182,1 μm; p=0,612) skarlı alanda benzer kalınlığa sahipti. Skarsız alanda SLL, DLU ve kontrol gruplarında endometrium kalınlığı sırasıyla 239,9±68,9; 256,5±127,2, ve 316,4±98,6 μm olarak ölçüldü ve benzer olarak değerlendirildi (p=0,347). Benzer şekilde skar olmayan alanda SLL, DLU ve kontrol gruplarında myometrium kalınlığı da benzerdi (387,2±119,9;

was also similar in all groups evaluated (387.2±119.9; 364.5±154.1 and 234.0±49.1 µm; p=0.265). The histopathological features of uteri repaired with two different techniques were also similar.

Conclusion: The early histopathological properties of all uteri examined were similar after repairing with SLL or DLU techniques. These results indicate that the repair process does not differ in the early stages, regardless of the uterine closure technique used.

Keywords: Surgical technique, uterine closure, cesarean section, pregnancy, histopathology

364,5±154,1 ve 234,0±49,1 µm; p=0,265). İki farklı teknikle kapatılan uterus örneklerinin histopatolojik özellikleri de benzerdi.

Sonuç: SLL ve DLU teknikleri ile uterus kapatıldıktan sonra uterusların erken dönem histopatolojik özellikleri benzer olarak değerlendirildi. Bu sonuçlara göre uterus nasıl kapatılırsa kapatılsın erken dönemde iyileşme sürecinin etkilenmediğine işaret etmektedir.

Anahtar Kelimeler: Cerrahi teknik, uterusun kapatılması, sezaryen, gebelik, histopatoloji

INTRODUCTION

In 1916, Professor Edwin Bradford CRAGIN first proposed the idea of "once cesarean, always cesarean." With the introduction of fetal monitoring in the delivery wards, the cesarean section (C/S) rate increased incrementally from the mid-1970s. Today, nearly 1.2 million babies are born annually in Turkiye, and more than half are delivered by C/S, corresponding to 74 operations every hour (1). However, while Turkiye is one of the world leaders in C/S rates, only a few countries have reached the World Health Organization's goal of 10-15% since 1985.

By the mid-1990s, the trial of labor after cesarean (TOLAC) trend gained popularity, and total C/S rates decreased. However, this trend could survive only one decade due to fear of the catastrophic outcomes of uterine rupture, which is not more than 1%. Therefore, nowadays, healthcare providers and patients stand aloof from TOLAC (2). From a historical perspective, the uterine incision has shifted from the vertical to the low transverse; suture material has moved from the chromic catgut to polyglactin 910, and the closure technique has changed from the double-layer to single-layer. The change in choice for suture material and closure technique almost coincided with the popularity of TOLAC. Consequently, the investigators questioned the possible relation between the uterine rupture during TOLAC and the uterine closure technique. In the last two decades, many attempts have been made to find the proper way for uterine closure in C/S (3-18). In this experimental animal study, we aimed to investigate the early histopathological properties of the uterus in rats exposed to the two most preferred uterine closure techniques (single-layer locked *versus* double-layer unlocked) in C/S.

MATERIAL and METHODS

Virgine, female Wistar albino rats, aged three months and weighted 200-250 g, were included in the study. Each animal was housed in a separate cage in a heat-stabilized room at 22-24°C from the first day of pregnancy diagnosis. A standard laboratory diet and water were allowed *ad libitum*. The weight of each rat was measured daily,

and its general condition was monitored. The rats were divided into study and control groups according to their order in the experiment. After C/S was administered to the first three rats, the rest were allowed to give birth spontaneously. In other words, rats numbered 1, 2, 3, 5, 6, 7, 9, 10, 11, and 13 were allocated to the study groups, and 4, 8, and 12 were allocated to the control group.

The average gestation period in rats is 22-23 days and C/S was not performed before day 20 of pregnancy. The rats were anesthetized by the injection of 40 mg/kg ketamine hydrochloride (Ketalar® 500 mg, Pfizer PFE İlaçları, İstanbul, Turkiye) and 5 mg/kg xylazine hydrochloride (Xylazin Bio® % 2, Bioveta, Czechia) into the peritoneum. A midline vertical incision was performed to access the abdomen. Each uterine horn was examined before the incision. An incision 1.5-2 cm in length was made on the anti-mesenteric side of the uterus. All embryos were removed with their adjacent tissues and maternal hemostasis was secured. The number of fetuses in each uterine horn was recorded. The uterine incisions were closed with multifilament 4/0 polyglactin 910 (Pegalak®, Doğsan, Trabzon, Turkiye) on both uteri. An indicator, a non-absorbable suture, was placed 0.5 cm above the upper border of the uterine incision with 4/0 polypropylene (Propilen®, Doğsan, Trabzon, Turkiye). On the right side single-layer, continuous, locked suture technique was applied to close the uterus, and on the left side double-layer, the continuous, unlocked technique was employed. For prophylaxis of surgical site infection, 60 mg of ampicillin (Ampisid® 250 mg, Mustafa Nevzat İlaç Sanayii, Istanbul, Turkiye) was injected into the peritoneum and then the abdominal incision was closed using 3/0 polyglactin 910 (Pegalak®, Doğsan, Trabzon, Turkiye). The skin incision was closed using a separate stitch of 4/0 polypropylene. The animal was kept under a heater until it fully recovered from anesthesia. Rats were determined to be hemodynamically stable and housed in their cage. The antibiotic prophylaxis continued for two more days with an intraperitoneal injection of 30 mg of ampicillin. Exclusion criteria were that the rat died before the day of the hysterectomy, or the specimen was of poor quality. The delivered mice naturally formed the controls.

All animals underwent hysterectomy on the postpartum 15th day. First, the anesthesia was administered as stated previously, then the abdominal cavity was opened. Each uterus specimen was preserved and labeled in a 10% formaldehyde solution. Samples were embedded in paraffin blocks and 5 µm sections were cut using a rotary microtome, after which an automated system stained them with hematoxylin-eosin and Masson's trichrome stain (Leica ST 5020, Leica Biosystems Nussloch, Germany). The histopathological examinations were performed by one expert. The quantitative outcome parameters on hematoxylin & eosin staining are the thickness of the endometrial lining, muscle layer, and whole uterus on the uterine incision (scarred area) and the opposite of the incision (non-scared area) in C/S samples or controls. Uterine thickness was determined by adding up the endometrial and myometrial thicknesses. The qualitative outcomes with hematoxylin & eosin staining were wound healing, inflammation, collagen deposition, gland density, hemosiderin-laden macrophages, and residual suture material. Collagen formation was stained with Masson's trichrome. The images were transferred to Aperio ImageScope [v12.3.3.5048], and measurements were obtained using a computer-assisted image analyzer. First, three measurements were obtained for each quantitative criterion, and the mean value was calculated. Then, the statistical analysis was performed using the mean values.

The sample size was calculated according to the resource equation in conditions where the standard deviation information was scant, or the effect size specification was problematic (19). The normality of data was checked with the Kolmogorov-Smirnov test. The homogeneity of variances was confirmed by using the Levene test. The normality assumption met one-way ANOVA, or student's t-test was used for comparing groups. For all statistical tests, $p<0.05$ was accepted as significant. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) 17.0 for Windows.

This study was performed in compliance with the Declaration of Helsinki. The experiments complied with the Turkish Inspectorate of Animal Experiments' guidelines for using and caring for laboratory animals and were approved by the Local Ethics Committee of Laboratory Animal Use and Committee of the University of Cukurova (Date: 10.12.2020, No: 9). The ARRIVE guidelines for animal research are followed (20).

RESULTS

Thirteen pairs of the uterus have been obtained. Of these uteri, 10 single-locked, 10 double-locked, and six control were prepared for pathological examination. The descriptive properties of animals are depicted in Table 1.

Compared to controls, two weeks after C/S, the endometrial layer of the repaired uterus become thinner. However, the thickness of the endometrium was similar regardless of the suture technique used. While the endometrial layer got thinner compared to controls after C/S, the myometrium and uterus got thicker. However, these differences still did not reach a significant level. Furthermore, the thickness of the myometrium and the total thickness of the uterus were similar. The measurements obtained at the microscope are presented in detail in Table 2. Figure 1 depicts one sample from each group.

The macroscopic appearance of uteruses in both suturing techniques was similar. Wound integrity was formed in almost all uteruses in two weeks except for one uterus in the single-layer locked group. Collagen deposition was observed in the majority of specimens in both groups. Endometrial gland density also appeared to be normal in both groups. While there was more lymphocytic infiltration in the single-layer locked group, it did not reach a statistically significant level. In eight samples from each group, hemosiderin-laden macrophages were present. Remnant sutures were present in nearly all specimens at the end of two weeks. Tissue healing and collagen deposition were complete before the suture materials were entirely lost. The histopathological features of the scarred uteruses are displayed in Table 3.

Table 1: Descriptive data of experimental animals in all groups

	Cesarean (n=10)	Normal (n=3)
	Mean±SD (Median)	Mean±SD (Median)
Gestational age at delivery (days)	21.8±0.9 (21)	24.3±0.6 (24)
Weight on the first day of pregnancy (g)	231.5±29.7 (234)	223.0±12.8 (223)
Weight on the day of delivery (g)	278.9±25.6 (275)	267.3±18.9 (263)
Weight on the day of hysterectomy (g)	215.3±18.5 (214)	250.0±29.1 (257)
Litter (#)	9.1±0.9 (9)	9.0±1 (9)

SD: Standard deviation, #: Number

Table 2: Comparison of the thickness of endometrium, myometrium and uterus among the groups

	Endometrium		Myometrium		Uterus	
	Non-scarred (µm)	Scarred (µm)	Non-scarred (µm)	Scarred (µm)	Non-scarred (µm)	Scarred (µm)
Single-layer locked (n=10)						
Mean±SD (Median)	239.9±68.9 (238.8)	276.6±123.1 (280.3)	387.2±119.9 (392.1)	531.7±189.2 (536.4)	627.2±159.5 (669.2)	802.3±135.1 (799.4)
Double-layer unlocked (n=10)						
Mean±SD (Median)	256.5±127.2 (250.5)	258.4±125.9 (292.4)	364.5±154.1 (385.7)	505.9±182.1 (509.5)	620.9±234.4 (631.4)	786.4±248.4 (770.2)
Control (n=6)						
Mean±SD (Median)	316.4±98.6 (306.9)	-	234.0±49.1 (225.9)	-	550.4±144.2 (532.9)	-
p[a]	0.347	0.748	0.265	0.612	0.408	0.767

SD: Standard deviation, [a]: One way ANOVA or student's t test was performed
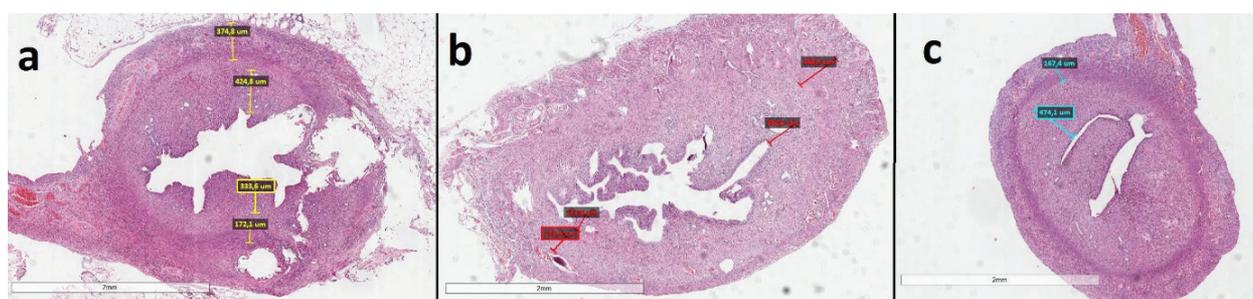

Figure 1: From left to right, images a, b, and c depict a sample from single-layer locked, double-layer unlocked, and control groups, respectively.

DISCUSSION

The basics of wound healing consist of five phases: hemostasis (first few minutes), inflammation (ends within three days), epithelization or migration (ends within two days), fibroplasia (the fibroblasts produce collagen; it reaches its maximum at the fifth day and continues for at least six weeks) and maturation (the main action is the collagen remodeling which takes approximately six months). The synthesis of collagen fibers depends on the events occurring in the injury's first week. The interaction between the collagen fibers determines wound strength. However, uterine wound healing in C/S shows some uniquity. The high input of blood supply decreases significantly and rapidly; the size of the human uterus diminishes and becomes non-palpable in the true pelvis by the 9th day of puerperium, and the endocrine environment shifts to the non-pregnant status. In humans, the involution, as a summary of these steps, is generally completed within two to three weeks. The uterine involution is faster than the wound healing. The rapid shrinkage of the uterus makes the sutures hang in the space within a few weeks.

Most recent publications use radiologically measured thickness as a determinator of tissue healing. However, the radiological examination did not definitively determine tissue structure, niche-related symptoms, and uterine rupture resistance in the subsequent pregnancy. Rozenberg et al. have stated that transabdominal ultrasonography measurement of the thickness of the lower uterine segment before the delivery does not lower maternal and perinatal adverse results (21). The methodology of measurement is another issue, Bolten et al. have demonstrated that the combined use of transvaginal ultrasound and magnetic resonance imaging is a reproducible tool for examination of the lower uterine segment following C/S, but the transabdominal approach is not (22). Therefore, the data needs to be more consistent. Some observations depicted a thicker lower uterine segment, and less niche occurs with a double layer; others did not (5,12,13,16). Our results showed that the suturing techniques did not differ between tissue thickness and histopathological features. The wound integrity and adequate collagen deposition were obtained in both groups. On the other hand, the residual sutures are still present in the majority.

Table 3: The histopathological features of the uteruses in the study groups

	Single-layer locked (n=10)	Double-layer unlocked (n=10)	p
Macroscopic appearance			
Normal	8	8	0.999
Inflamed, necrotic	2	2	
Wound integrity			
Present	9	10	0.305
Absent	1	0	
Collagen			
Present	8	7	0.606
Absent	2	3	
Gland density			
Normal	8	6	0.329
Absent	2	4	
Inflammatory lymphocytes			
Present	9	6	0.121
Absent	1	4	
Hemosiderin-laden macrophages			
Present	8	8	0.999
Absent	2	2	
Residual suture			
Present	9	7	0.264
Absent	1	3	

SD: Standard deviation

The uterine wound healing after C/S has been evaluated using different parameters. These are measuring residual myometrial thickness and/or demonstration of uterine niche in symptomatic or asymptomatic patients radiologically (transabdominal and/or transvaginal ultrasound, magnetic resonance imaging, hysterogram, sonohysterography); direct observation during the next C/S (tissue thickness, uterine rupture, or dehiscence); uterine rupture during TOLAC and development of abnormal placental invasion. To our knowledge, any study investigating the risk of scar pregnancies regarding the suture technique has not been published yet. Bujold et al. have retrospectively evaluated the risk of uterine rupture during the TOLAC and found that the risk was nearly fourfold after single-layer (23). A meta-analysis of 12 trials has been published by Roberge et al. The authors have stated that the risk of uterine rupture at TOLAC was similar between single-layer versus double-layer and single-layer unlocked versus double-layer. However, the risk of uterine rupture is five times higher in a single-layer locked than in a double-layer (7).

On the other hand, Hesselman et al. compared 2589 patients with single-layer to 5002 patients with double-layer. This large's study outcomes signify no difference in uterine rupture at TOLAC regarding the suture technique (14). Sumigama et al. have stated that the risk of placenta accreta is six times higher when the uterus is closed continuously instead of interrupted sutures (15). This is the only study evaluating suturing techniques' role in the development of placenta accreta.

Studies evaluating the histopathological appearance of uterine wounds are focused on the long-term consequences of the C/S scar (24-26). However, none of them made a comparison regarding the layer count of the uterine suture. Lapointe-Milot et al. compared the histopathological features of the uterus after single-layer *versus* double-layer uterine closure in a sheep model (27). Due to placental features, the uterus was closed while the placenta was still in place. The early stages of wound healing would not be comparable to humans due to this dissimilarity.

According to our observations in this study, we believe uterine wound healing is not solely related to suturing technique. The number of possible suturing techniques takes into account many parameters. For single-layer, there are six possibilities: continuous, locked ± endometrial layer (2); continuous, unlocked ± endometrial layer (2); interrupted ± endometrial layer (2). Three more options accounted for the outer layer of the double-layer suturing. These options are continuous, locked (1); continuous, unlocked (1); interrupted (1). In sum, six times three, there are 18 possibilities for double-layer suturing. Therefore, designing such a study does not seem rational. Additionally, other factors may contribute more than the suturing technique including the site of uterine incision, time of C/S, medical conditions contributing to wound healing like anemia, maternal obesity, premature rupture of membranes, having multiple C/S, post-partum endometritis, hypoalbuminemia, diabetes, and preeclampsia (28). The effects of different uterine closure techniques on the rates of infertility, abortion, preterm delivery, premature rupture of membranes, and bleeding in the subsequent pregnancy may also be the subjects of future investigations. Another future research area should be the surgical technique for multiple C/S. To date, the majority of published data focus on the primary C/S. However, many women experience repetitive C/S, and numerous uterine surgeries increase the risk of uterine rupture and placenta accreta spectrum.

CONCLUSION

To conclude, single-layer locked and double-layer unlocked sutures have similar histopathological features in the early wound healing period. Therefore, while awaiting the studies seeking long-term histopathological and biomechanical outcomes, both techniques seem safe and available.

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