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EXPERIMENTAL STUDY

The effects of the recipient site on fat graft survival in a murine model

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Abstract

Background Fat grafting is a practice with prior successful outcomes that is frequently used to improve tissue regeneration as well as to add volume to the subcutaneous tissue. The literature review in this study provided references to the information that the survival of a fat graft is related to the duration of its stay and the recipient site to which it is transplanted. Our study aimed to examine the effects of the recipient site of fat grafting on graft survival.

Methods This study was conducted using a prospective and experimental design. Our sample consisted of three groups, each including 9 Wistar albino rats. Skin incision was made in the right inguinal region of the rats in group 1 (control group), and the incision was closed without any dissection to the fat pad. The fat pads extracted from the right inguinal region of the rats in groups 2 and 3 were transformed into chopped fat grafts by the fine cutting method. Micropipettes (Isolab 100 µL, Germany) were used during the fat graft transfer process. Grafts were placed subcutaneously on the right inguinal region in group 2 and on the sternum in group 3. The wounds of the incision areas in all groups were closed with simple sutures. Three months later, the fat pads in group 1 and grafts in groups 2 and 3 were removed for histopathological and stereological evaluations.

Results Our study revealed a statistically significant decrease in the volume of the adipose tissue placed in group 3, compared to groups 1 and 2 ($p \leq 0.01$). However, no statistically significant difference ($p > 0.05$) was observed in the comparison of the volume of adipose tissue placed in the rats in group 1 and those in group 2. In group 3, an intense cellular infiltration developed in the fat grafts placed in the subcutaneous tissue above the sternum, the fat cells could not maintain their normal structure, and there were areas of dense cystic cells and calcification.

Conclusions It was concluded that recipient site differences are effective in maintaining the volume and survival of fat grafts. It is recommended that the volume and survival of the fat graft be supported by effective therapeutic interventions following the application of the fat graft.

Level of evidence: Not ratable

Keywords Fat graft · Survival · Recipient site

Introduction

Fat grafting is a practice with prior successful outcomes that is frequently used to improve tissue regeneration as well as to add volume to the subcutaneous tissue [1–3]. The literature review in this study provided references to the information that the survival of a fat graft is related to the duration of its stay and the recipient site to which it is transplanted [4, 5]. This is due to the phagocytizing of free fats and dead cells [6]. Macrophages in the fat graft model are thought to play a key role in inflammation and tissue regeneration [7]. However, sufficient evidence could not be obtained to support the view that the survival of a fat graft varies according to the recipient site to which it is transplanted [2]. Current evidence emphasizes that fat graft survival is reduced due to reasons such as the rapid injection of the fat product [8], low postoperative angiogenesis and growth factor release [9, 10], and lack of a microenvironment that supports tissue repair and regeneration [11, 12].

In addition to the low survival rates observed after fat grafting in areas with a low capacity of fat [13], the possibility that high-volume fat transfer may occasionally result in necrosis is an important detail [14, 15]. In both cases, it is clear that fat

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grafting is performed with a technique where adverse outcomes are unpredictable. In this context, it is very important for practitioners to consider the site of transplantation while calculating the survival rate of the fat graft. It was determined that fat cysts and calcification may develop in areas where the fat graft is necrotic [16]. It has been reported that these calcifications occur at the micro or macro level after fat grafting, in time periods ranging from 3 months to 2.5 years [17], and their incidence varies between 3.9 and 29% [18–20].

Fat grafting is a well-known and preferred surgical procedure in plastic surgery and aesthetic interventions around the world. However, complications that may occur after the intervention can lead to significant and serious problems and may impose significant burdens on patients. As a matter of fact, sebaceous cysts, which are a common side effect after fat grafting, are associated with prolonged inflammation and progressive calcification, whereas they may become permanent without shrinking at all and cause further pain [4]. Furthermore, the necrosis of the transplanted tissue, including the fat graft, may lead to irreversible tissue damage. This tissue damage can cause physical, economic, social, and psychological devastation in the later life of patients. On the other hand, there are studies in the literature stating that the reliability of therapeutic interventions such as fat grafting is very high [3, 12]. For this reason, it is very important to examine in detail and define the factors thought to be effective in the survival of fat grafts. This study aimed to examine the effects of the recipient site to which fat grafting is applied on the survival of the graft.

Materials and methods

Research design and laboratory rat selection

This study was conducted using a prospective and experimental design. Twenty-seven Wistar albino male rats, each weighing approximately 250 g, were used in our study. The rats to be used in our study were obtained from the Adıyaman University Experimental Animals Production, Application and Research Center. A total of 27 rats were divided into 3 groups as group 1 (control), group 2 (experimental), and group 3 (experimental), with 9 rats in each group. Each rat was placed in an individual cage. Throughout the treatment period, the rats were kept at a 22 ± 2 °C ambient temperature, 50 ± 10% relative humidity, and 12/12 light/dark cycle. The rats were fed ad libitum with standard rat chow (Bil-Bay), and their drinking water (~50 mL/day/rat) was replaced daily.

Fat graft model in rats

During the procedure, intraperitoneal anesthesia was administered to all rats. For this purpose, the rats were injected with ketamine at a dose of 90 mg/kg and xylazine at 10 mg/kg. They were first placed in the supine position, after which the inguinal fat pad was removed from the right inguinal region of the rats. The incision made in the right inguinal region of the rats in group 1 (control group) was closed without any intervention/dissection to the fat pad. The same surgeon transformed the fat pad into the fat graft using the fine cutting method with the help of a surgical scalpel blade no. 20. The fat graft of each rat in group 2 was re-transplanted into their right inguinal region, and the incision site was closed. The fat graft of each rat in group 3 was transplanted subcutaneously on the anterior sternum wall in a fan-like pattern to ensure even distribution/layering under the skin, and the resulting incision site was closed. Due to the low volume of the fat graft transplanted to each rat (0.05 ml), micropipettes (Isolab 100 µL, Germany) were used during the transplant process. Primary 4/0 prolene sutures were used to close the 1-cm incision wound in the sternum and/or right inguinal region of all rats. The incision sites of the rats were regularly dressed in the first week, and the rats were monitored for 3 subsequent months. Three months after the operation, the fat grafts in the inguinal region of the group 2 rats and the subcutaneous region of the sternum of the group 3 rats were compared to the inguinal fat pad in the right groin of the group 1 rats. In our study, the fat tissue volumes of the animals in all groups were measured using Archimedes' principle, also known as the "fluid displacement technique," in a measuring cylinder [21]. The results of the statistical analyses with regard to the volumetric measures revealed no statistically significant difference between groups 1 and 2.

Histological analysis

Histopathological analysis was performed in the laboratories of Adıyaman University Faculty of Medicine, Department of Histology and Embryology. Tissue samples extracted from sacrificed animals were first subjected to a 1-week post-fixation process in 10% formaldehyde solution; then, routine tissue processing procedures were performed to conduct analyses using a light microscope. Sections were taken using a Thermo Shandon Finesse ME microtome (Thermo Fisher Scientific, Cheshire, UK) from paraffin blocks prepared by histological tissue processing (consisting of graded alcohol series, xylene and paraffin series) with an automated tissue processing device (Leica TP1020, Nussloch, Germany). While the sections extracted for volumetric analyses were stained using the hematoxylin–eosin method, the sections prepared for histopathological scoring and evaluations were stained with Masson's trichrome and examined under a light microscope.

Stereological analysis

Stereological methods are used to reveal quantitative data of two-dimensional images and three-dimensional structures [22]. For this study, volumetric calculations were estimated using the Cavalieri principle, which is the most commonly used stereological method [23]. Sections of 1/2 of 7-µm thickness were taken from the tissue blocks of each fat graft, in accordance with the systematic random sampling procedure. The excised sections were stained with hematoxylin–eosin, after which their images were taken under a light microscope for volumetric examinations. All images of the sections were projected onto a computer screen and photographed with a 4-lens digital color camera attached to a Carl Zeiss Axiocam ERc5 model (Carl Zeiss Microscopy GmbH 07745 Jena, Germany) light microscope. The 1/2 area sampling method was used while taking the photographs. Adipose tissue, connective tissue area, and vessel volumes on all excised sections were calculated in accordance with the Cavalieri method. A dotted planimeter, which is a device used for estimating the area of a shape, was used while calculating the volume values, and the unit area of each dot was set to be 25,600 µm^2. Volumetric values were calculated in the ImageJ (Image Processing and Analysis in JAVA, NIH, USA) program, via the dot counting method, by counting the regular grid test points (points corresponding to adipose tissue, connective tissue, and vascular regions) randomly placed on the cross-sectional images taken from the tissues (Fig. 1).

The density of the dots on the dotted planimeter was determined to obtain an appropriate coefficient of error (CE) and coefficient of variation (CV) [24]. After counting the total grid test points corresponding to the respective areas on the dotted planimeter placed on the screenshots of the sections, the following formulae were used to calculate the volumetric values.

$$Vref = \sum Pi \times t$$

$$Pi = P(a)$$

$$\sum V = V1 + V2 + V3 + \cdots$$

"*Vref*" represents the total or reference volume of the relevant structure; "$\sum Pi$" represents total number of dots per cross-sectional area; "$P(a)$" represents the area represented by a dot on the dotted planimeter; "t" represents the average thickness of the section, and $\varepsilon V\varepsilon$ represents the volume value of an entire section [25]. After applying the same formula

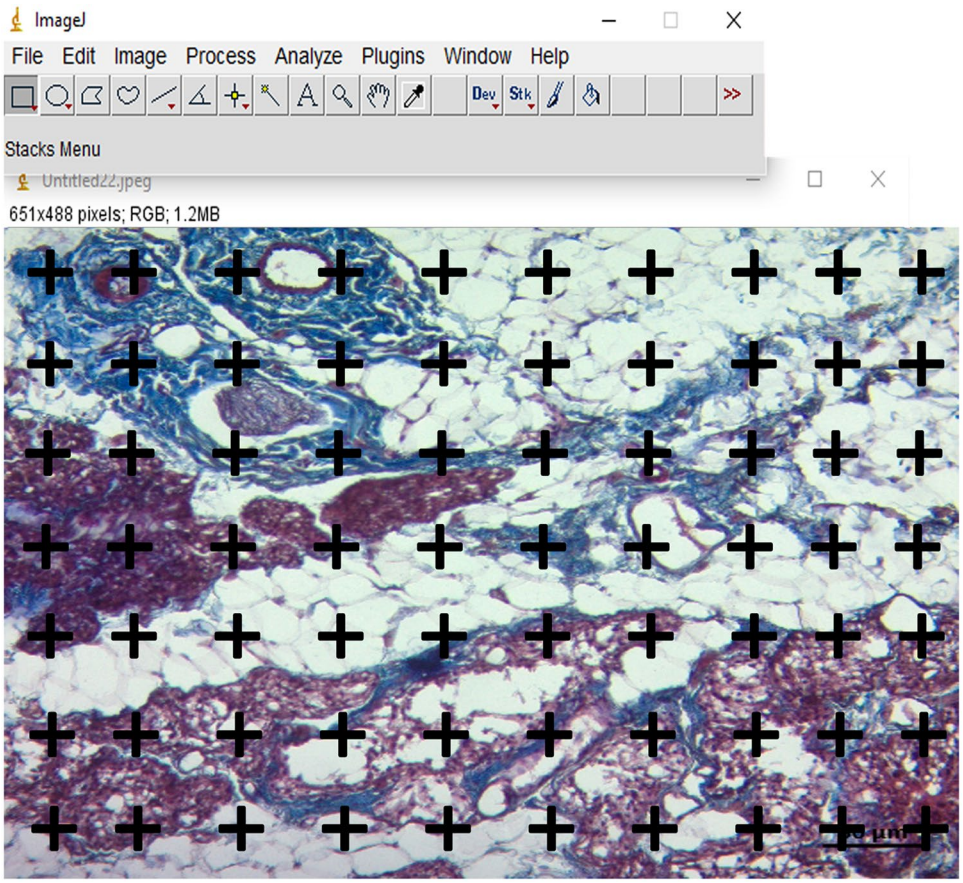

Fig. 1 ImageJ program that was used in the volumetric analyses

for all sections, the volume values were summed to calculate the total volume.

Histopathological scoring

For this study, findings such as infiltration area, cyst formation, and calcification formation in tissues were evaluated semi-quantitatively using Abramov's histological scoring system [25]. Evaluation was made on a scale designated as 0 (none), 1 (little), 2 (moderate), or 3 (intensive) [26]. Each parameter was evaluated independently by a blinded expert histologist.

Statistical analysis

The numerical data of the groups were statistically analyzed further using the SPSS software (SPSS version 25, SPSS Inc., Chicago, IL, USA). The data are expressed as mean ± standard deviation. As a result of the normality (Shapiro–Wilk) and homogeneity tests applied to the data of the groups, it was determined that all groups had a normal distribution. Differences between the groups were evaluated using one-way ANOVA and Tukey's tests. Paired groups were compared using paired-samples *t* test. For all statistical analyses, $p < 0.05$ was considered statistically significant.

Ethical aspect

Prior to beginning the study, the necessary legal approvals were obtained from the Adıyaman University Local Ethics Committee on Animal Experiments.

Results

Histological analysis results

Results on adipose tissue, connective tissue, and vascular volumes

The results obtained by the stereological analyses of adipose tissue, connective tissue, and vessel volumes in the fat grafts of the groups using the Cavalieri method, CE values, and CV values are displayed in Table 1.

Considering the adipose tissue volume analyses of the adipose graft tissues in all groups in our study calculated by the Cavalieri method, a statistically significant decrease was observed in group 3 compared to groups 1 and 2 ($p \leq 0.01$). No statistically significant difference ($p > 0.05$) was observed between groups 1 and 2. In the connective tissue volume analyses of the fat graft tissues in all three groups, no statistically significant difference was observed between the groups ($p > 0.05$). In the statistical analyses of the vascular volumetric values of the fat graft tissues in all groups, it was determined that the vascular volumes in group 3 showed a significant decrease compared to group 1 ($p < 0.05$). No statistically significant difference ($p > 0.05$) was observed between the vascular volumes of groups 1 and 2.

Table 1 Fat tissue, connective tissue, and vascular volumes in fat grafts belonging to all groups ($cm.^3$) (mean ± standard deviation), coefficient of error (CE), and coefficient of variation (CV) values

Groups	Adipose tissue	Connective tissue	Vessels
Group 1	0.0045 ± 0.00039 [a]	0.0019 ± 0.00016	0.0004 ± 0.00005 [c]
CE	0,13	0.16	0.11
CV	0.22	0.26	0.21
Group 2	0.0037 ± 0.00027 [b]	0.0017 ± 0.00015	0.0003 ± 0.00004
CE	0.14	0.14	0,12
CV	0.19	0.21	0.23
Group 3	0.0014 ± 0.00006	0.0015 ± 0.00018	0.0002 ± 0.00001
CE	0.15	0.14	0.12
CV	0.22	0.23	0.22

[a]The highly significant difference between group 1 and group 3 ($p \leq 0.01$)

[b]The highly significant difference between group 2 and group 3 ($p \leq 0.01$)

[c]The significant difference between group 1 and group 3 ($p \leq 0.05$)

Histopathological scoring results

For the objective of this study, the results were evaluated semi-quantitatively using Abramov's histological scoring system. Findings such as cellular infiltration, cyst formation, and calcification formation in tissues were evaluated at the end of histopathological scoring. Since a normal tissue structure was dominant in group 1, and no pathological findings were observed (0 none). In group 2, many cystic cells and areas of calcification were detected in addition to healthy adipocyte cells with a normal appearance. Significant pathological findings such as cystic cells and calcification areas were observed in the tissue samples belonging to group 3 (2–3 moderate-intense). Additionally, there was intense cellular infiltration in the tissue samples of group 3 (3 intense). The scoring results are shown in Table 2.

Histopathological analysis results

In the examinations of the sections belonging to all groups, no abnormality was found in terms of shape and structure in group 1, but round-shaped, intact, and often nucleated adipocytes whose membrane structure was greatly preserved were observed (Fig. 2a and b). In Group 2, anucleated, damaged cystic cells and cells with impaired membrane structures (Fig. 2d) were observed (Fig. 2c), besides healthy cells with a normal appearance. In group 2, calcification areas were

Table 2 Evaluation results of histopathological scoring performed on tissue samples belonging to the groups (mean ± standard deviation)

Groups	Cellular infiltration	Cyst	Calcification
Group 1	0.8 ± 0.374 [a, b]	0.4 ± 0.244 [a, b]	0.4 ± 0.245 [c]
Group 2	2 ± 0.316	1.5 ± 0.223	1.2 ± 0.307 [d]
Group 3	2.5 ± 0.244	2.2 ± 0.4200	2.3 ± 0.210

[a]The significant difference between group 1 and group 2 ($p < 0.05$)

[b]The significant difference between group 1 and group 3 ($p \leq 0.01$)

[c]The highly significant difference between group 1 and group 3 ($p \leq 0.01$)

[d]The significant difference between group 2 and group 3 ($p < 0.05$)

also observed in addition to cysts (Fig. 2c). In group 3, it was observed that the fat cells transplanted with SF could not fully preserve their normal structure, their membrane structure deteriorated, and the cell borders could not be determined clearly (Fig. 2f). Denser cystic cells and areas of calcification were observed in group 3 compared to group 2 (Fig. 2e).

Discussion

In this study, we transformed fat pads extracted from the right groin of rats into fat grafts to examine whether the recipient site where the fat graft was transplanted had any effect on graft survival. Some authors have described the surgical technique to be a safer method with regard to the removal of adipose tissue, despite the risk of disrupting the viability of cells. On the other hand, some authors have claimed that the ultrasonic-assisted fat cell isolation method is safe, and the energy that is used does not damage fat cells [27, 28]. There is no study in the literature that proves or claims that the fine-cutting method damages fat cells. This

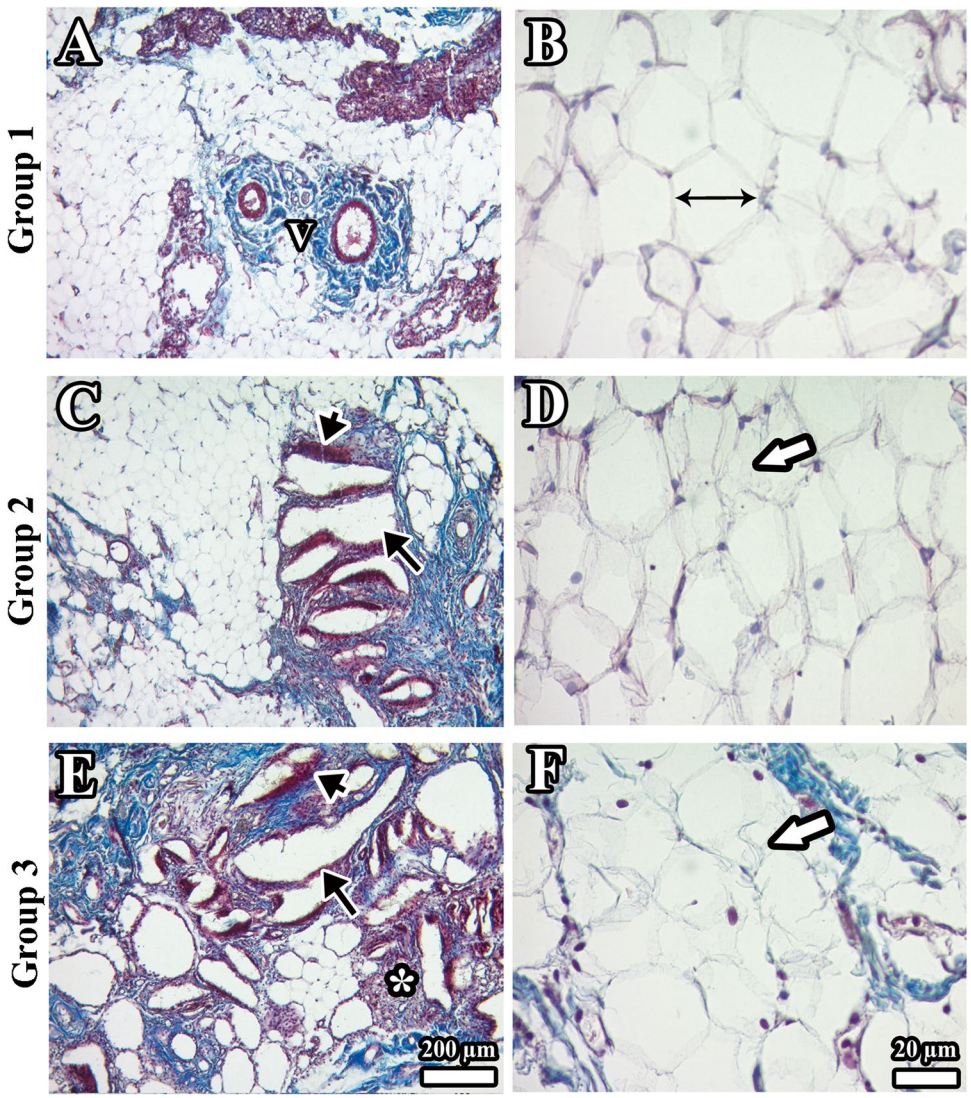

Fig. 2 **a–f** Histopathological assessment of the fat graft tissues of all groups (Masson trichrome staining). Damar (V); degenerated fat cells (white arrow); cystic fat cells (black arrow); calcification areas (arrowhead); cellular infiltration areas (*)

is why we preferred using the fine-cutting method for removing fat pads. Later, we transplanted the fat graft in the right inguinal region of some rats (group 2) where the fat pad was extracted followed by transformation into fat grafts. We placed the fat grafts of rats in group 3 in the subcutaneous tissue above the sternum. We removed the fat grafts transplanted to both groups after 3 months and compared them to the fat pads in the right groin of the control group (group 1).

In the adipose tissue volume analyses of all three groups, a statistically significant decrease was observed in group 3 compared to groups 1 and 2 ($p \leq 0.01$). However, no statistically significant difference ($p > 0.05$) was observed between groups 1 and 2. Therefore, when the fat tissues taken from the right groin were placed back in the same place as fat grafts, no volume loss was observed. On the other hand, dense cellular infiltration was detected in the fat grafts placed in the subcutaneous tissue above the sternum. Furthermore, in group 3, it was observed that the fat cells could not maintain their normal structure, their membrane structure was disrupted, the cell borders could not be distinguished clearly, and there were areas of dense cystic cells and calcification. The literature review in this study revealed that macrophages phagocytize lipids and debris after fat grafting. On the other hand, if macrophages stay in the environment of the graft for a long time, they solidify and cause the development of fibrosis [4, 29]. The excessive accumulation of macrophages after fat grafting in one of the experimental groups of our study (group 3) may have caused a cystic appearance.

Anucleated, damaged cystic cells, calcification areas, and cells with impaired membrane structures were observed besides healthy cells with a normal appearance in the fat graft, which was re-placed in the right inguinal region within the scope of our study. The histopathological examinations of group 2 revealed healthy cells with normal appearance besides degenerated adipocytes. Therefore, we can deduce that cell degeneration, the formation of cysts, and calcifications are related to the effect of the recipient site where the fat graft is transferred and the deterioration of the structure in cells whose vascular system fails to develop and cannot be nourished, followed by the transformation of macrophages into fibrosis. Previous studies have reported that inflammatory processes occur in the first week after fat grafting. In this context, there is TNF (tumor necrosis factor)-α expression on the graft, and hence, dead cells and pathogens are cleared by neutrophils and macrophages [30, 31]. These processes are reminiscent of the healing stages of the wound. A study examining the breakdown of adipose tissue revealed that the rate of volume loss following fat grafting, which results in apoptosis caused by inadequate nutrient and oxygen intake, is over 50% at 12 weeks [2]. The probability of revascularization and graft survival increases [32] due to fat density, vasculogenic cytokines, and progenitor cells in the proliferation of adipocytes, but the probability of fibrosis decreases [33].

Fat graft survival is affected by microenvironmental conditions. These conditions may lead the fat graft to experience ischemia due to insufficient nutrition and be exposed to mechanical compression [34–36]. While ischemic adipose tissue cells may be replaced by new cells at the end of 3 months, they may also transform into cystic cells [37, 38]. Adipose tissue is rich in capillary blood vessels. When expanded adipocytes die with the effect of partial ischemia, deformities may occur in adipose tissue [27, 28]. The site above the sternum to which we transplanted the fat graft in our study represents a region that is largely destitute of a vascular system and does not contain a good circulation because of this. The fat graft, which could not get nutrition due to these microenvironmental conditions, may have degenerated. The differences observed in groups 2 and 3 in our study revealed that the survival of the fat graft was affected by the transplantation site. Nevertheless, to improve survival after grafting, regeneration as observed in group 2 clearly demonstrates the need for more evidence and new plans. In this context, it was noted that the number of vasculogenic progenitor cells reached the highest level on the fourteenth day in the circulation, and stem cell therapy increased the number of vasculogenic progenitor cells of rats by 2.6-fold and 1.7-fold per cm^3 [33].

The viability of fat grafts has been tested in several recent studies. Accordingly, it has been noted that stem cells produced from adipose tissue [39], maternal stem cell lyophilizate [40], platelet-rich plasma (PRP) [41], and hyaluronidase [42] fat grafts increase the adherence. These therapeutic interventions can maintain and improve graft viability by various mechanisms, including increased revascularization, accelerated preadipocyte differentiation, and decreased apoptosis. Stem cell therapy is recognized as a therapeutic intervention that improves fat graft survival. Furthermore, macrophages are the most abundant inflammatory cells observed in a graft's microenvironment after fat grafting, and they are involved in vessel formation, whereas they also take an active role in the transportation of blood-derived stem cells [2]. The apoptosis of adipocytes due to nutrient and oxygen deficiency after grafting suggests low vascularization. In this context, contributing to the production of macrophages on an ideal level and preventing fibrosis caused by overproduction may be a solution that will improve the survival of fat grafts. Additionally, the ability of PRP therapy to facilitate the secretion of the vascular endothelial growth factor (VEGF) and increase the number of capillaries is an important therapeutic effect. Similarly, hyaluronidase is thought to increase angiogenesis. The fact that our study was carried out on rats, the fact that the follow-up was carried out 3 months after grafting, and the failure to follow-up all stages of the graft were considered limitations.

Conclusions

The findings of our study are morphologic in the context of examining the effects of the transplant site on the maintenance of volume and survival of fat grafts, and they are a rich source of information in terms of displaying cellular differentiation. In accordance with the results reached in our study, the changing viability of the fat graft depending on the transplantation site and the transformation of the fat graft into cysts and calcifications due to lack of nutrients and oxygen, resulting in apoptosis and cell degeneration, is due to the failure of the formation of a vascular system because of the lack of an ideal microenvironment. We recommend that future studies be designed with these outcomes in mind.

Funding No funding was received for this study.

Data availability The datasets generated and/or analyzed during this study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate The study was approved by the Institutional Scientific and Animal Research Ethics Committee of Adıyaman University. This study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals.

Informed consent Informed consent was not applicable to this study because the sample did not include human subjects.

Consent for publication Not applicable.

Conflict of interest Fatma Nilay Tutak and Elfide Gizem Bakirhan declare no conflict of interest.

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