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ORIGINAL ARTICLE

AUTOLOGOUS FAT REINJECTION AFTER PRESERVATION AT −2 TO −18°C: A CLINICAL STUDY OF 973 PATIENTS

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Summary

Objective: To study the efficacy of autologous fat transplantation after preservation at −2 to −18°C in a domestic refrigerator.
Patients and methods: This study was conducted in a private clinic from December 2017 to December 2021. A total of 973 female patients were included. Under a full aseptic technique, fat was harvested using a 4 mm suction blunt cannula with three longitudinal slits. Three 50 mL syringes of fat were obtained before transferring it to 10 mL syringes; then, the fat was centrifuged at 3500 rpm for 1 min and injected through an 18 gauge blunt cannula. In addition, 50 mL of fat without centrifugation was stored in a domestic refrigerator for 3 weeks to be reinjected after fast thawing for 20 min, and then centrifuged to be ready for the second session. All participants had follow-up visits at 3 weeks, 6 months, and 1 year.
Results: Participants ranged in age from 18 to 65 years. The abdomen was the fat donor site in 63.3% of the patients, while round-face style augmentation was performed in 48.3% of the patients. An assessment at 3 weeks revealed that 84.1% of patients required a second session. After 6 months, on an assessment using a 10-point scale, patients who received a single session and a second session scored 6.05 and 7.46, respectively. At 1 year, the assessment scores were 5.65 and 7.12 for those with a single and second session, respectively, and 60% of patients were fully satisfied.
Conclusion: Autologous fat preserved in a domestic refrigerator for 3 weeks is a safe, cheap, and tolerated filler for facial augmentation.

Key words: autologous fat; cryopreserved; fat grafting; augmentation

Introduction

The story of fat transplantation began in 1889 when Van der Meulen attempted the fat auto-transplantation procedure, and then in 1893 Neuber made the first true adipose graft (1, 2). Many trials were conducted until 1986, when the studies of Coleman led to a radical breakthrough. Coleman modified and corrected the method and results of this process and proposed an atraumatic protocol for the treatment of adipose tissue. He reported that the technique was mandatory for successful fat grafting (3, 4).

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In 1993, Klein completed the story by proposing a new method called the tumescent technique, in which a fluid solution (saline solution, lidocaine, sodium bicarbonate, and epinephrine) was used to infiltrate the donor site, which was named Klein's solution. This improved the safety of large-volume liposuction because it eliminated the need for general anaesthesia and reduced haemorrhage (5).

From then on, fat transplantation became extremely popular and started to be performed across the globe. The reason for its popularity is because adipose tissue is close to the ideal filler due to it being readily available, easily obtainable, inexpensive, versatile with low donor site morbidity, repeatable, and biocompatible (6). However, unpredictable absorption of the autologous fat grafting results in repeated harvesting procedures, with some authors finding that approximately 25–70% of the total implanted volume is absorbed (7), while others have found a 20–80% reduction from the original volume (8). Moreover, repeated procedures may be costly and increase the risk of side effects. A possible solution to these problems could be cryopreservation.

Yehuda *et al*. (10) found that fat can be frozen in a domestic refrigerator at −18°C for up to 7 months; however, its histological parameters, weight, and volume were significantly inferior to those of fresh fat. Therefore, the authors suggested refraining from using fat that has been frozen for 7 months or longer.

Furthermore, Lee LQ Pu *et al*. found that the histology of autologous fat grafts harvested using Coleman's technique and preserved with their preferred cryopreservation method is similar to that of fresh fat grafts, with nearly the same number of viable adipocytes (11).

Moreover, Wolter *et al*. (12) demonstrated that the simple storage of fat in a freezer at −20 and −80°C led to reinjection of nonviable tissue unless a cryoprotected agent was added. In addition, Li *et al*. (13) noted that cryopreservation at −20, −80, and −196°C for up to 4 weeks had no differences in terms of cell viability nor in the use of cryopreservation.

Additionally, Shoshan *et al.* (14) revealed that minimum harmful effects were observed in a fat sample preserved at −20°C without controlling the cooling process and even without a cryopreservative agent. They observed viable adipocytes after preservation at this temperature.

However, some authors stated that adipocytes were destroyed after short-term freezing at −20°C, which explained the higher rate of volume absorption after the reinjection of cryopreserved fat (15).

In fact, these results are contradictory and confusing, and cryopreserved fat can be altered by many conditions, such as the fat harvesting technique, processing, storage, injected area, selected donor site, and thawing rate (14, 15). Therefore, we aimed to study the efficacy of autologous fat transplantation after preservation at −2 to −18°C in a domestic refrigerator, to assess the level of patient satisfaction after injection, and to monitor the side effects.

Methods

We conducted a case-series study in a private clinic between December 2017 and December 2021. This study was approved by the Medical Research Ethics Committee of the Maysan Health Directorate (Maysan Governorate, Iraq). A total of 973 patients were enrolled in this trial, all of whom were female. A full history was taken from each patient regarding their age, occupation, marital status, any chronic diseases, medication consumption, and pregnancy.

Then, a general physical examination was performed followed by a local examination of the donor site (abdomen, thigh, or flanks) to assess the quantity and quality of fat and skin. The selection of the donor site was based on it having a sufficient quantity of fat. Areas with a fibro-fatty texture and lax skin were excluded.

Moreover, all patients were fully informed about the nature of the procedure and any possible complications. Written consent was obtained from all patients. They were advised to stop taking aspirin and its derivatives prior to undergoing the procedure. Photographs were taken with a Sony camera (W300©) before and after the procedure with the patient's permission.

Under a full aseptic technique, tumescent anaesthesia was infiltrated at the donor site using a 50 mL syringe manually, and fat tissue was harvested using a 4 mm suction blunt cannula with three longitudinal slits. Manual aspiration under a low negative pressure was performed. Three 50 mL syringes were placed vertically for 15 min to drain the blood and fluid; then, the fat was transferred to 10 mL syringes and was centrifuged at 3500 rpm for 1 min to obtain pure adipose tissue. The fat was injected through an 18 gauge blunt cannula. Each patient received 60–80 mL in the first session followed by a gentle massage.

In addition, approximately 50 mL of fat without centrifugation was labelled and stored in a domestic refrigerator (temperature −2 to −18°C) for 3 weeks to be reinjected after being thawed for 20 min. It was centrifuged to be ready for the second session, which was performed 3 weeks after the first.

All participants were had a follow-up assessment at 3 weeks to check whether they needed a second session, and then a second assessment was performed at 6 months both clinically and using photos. Lastly, a final assessment was performed 1 year after the first session clinically and/or using photos.

The baseline and follow-up assessments were performed using a 10-point scale score. Patients' postoperative satisfaction was measured using a questionnaire, which was given to them at the end of each sitting and graded from fully satisfied to moderate, poor satisfaction, and not satisfied. Systemic antibiotics and acetaminophen were prescribed to each patient postoperatively.

Data are presented as mean ± SD and were analyzed using Chi square tests; p values below 0.05 were considered significant. They were processed using the statistical package SPSS version 26.

Results

This section presents the results of the study. A total of 973 patients were included in this clinical study, all of whom were female, and their ages ranged from 18 to 65 years with a mean ± SD of 31.8 ± 9 years. Patients in the third decade of their life were the most common age group followed by the fourth decade, as seen in Figure 1.

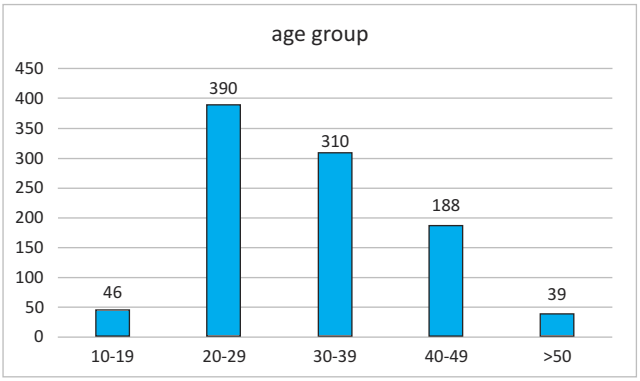

Figure 1. Patients' age distribution.

The abdomen was selected as a fat donor site in 616 (63.3%) patients, followed by the flanks in 328 (33.7%) and the thighs in 29 (2.9%) patients. The desired face shape was chosen after a discussion with the patient; specifically, a round face (or moon face) was selected by 470 patients (48.3%), followed by cheek and jaw contouring by 362 patients (37.2%) and jawline contouring only by 141 patients (14.4%).

Assessment at 3 weeks

The assessments at 3 weeks revealed that 155 patients (15.9%; 81 patients with the round face style, 61 with cheek and jaw contouring, and 15 with jaw contouring) only required one transplantation session, whereas 818 patients (84.1%) required a second session using frozen fat (385 round face, 298 cheek and jaw, and 125 with jaw only).

Only nine (1%) patients (four round face, four cheek and jaw, and one jaw only) received a third session, which was performed 6 months after the first session with fresh fat.

Assessment at 6 months

At the 6-month follow-up assessment, the mean score on a 10-point satisfaction scale for those who received a single and a second session was 6.05 and 7.46, respectively. Of those who received a single session, 151 (15.5%) were fully satisfied, 649 (66.7%) were moderately satisfied, and 169 (66.7%) were poorly satisfied. For those with two sessions, 512 (62.5%) were fully satisfied, 220 (26.9%) were moderately satisfied, and 81 (9.9%) were poorly satisfied (Table 1).

Table 1. Patients' satisfaction at 6 months.

Satisfaction	Full	Moderate	Poor	Not satisfied
Single session	151 (15.5%)	649 (66.7%)	169 (17.3%)	4 (0.4%)
Two sessions	512 (62.5%)	220 (26.9%)	81 (9.9%)	5 (1%)
p value	0.001*	0.0001*	0.0001*	0.317*
***p value is significant (< 0.05)**				

Assessment at 1 year.

The satisfaction scale score was 5.65 and 7.12 for those with a single and a second session, respectively. Patients' satisfaction after 1 year for both groups was as follows: 585 (60.1%), 276 (28.3%), and 103 (10.5%) patients indicated having full, moderate, and poor satisfaction, respectively, as presented in Table 2. The percentage of autologous fat absorption after 1 year had a mean ± SD of 25 ± 17.3% (Table 3).

Table 2. Patients' satisfaction at 1 year.

Satisfaction	Full	Moderate	Poor	Not satisfied	Total
Both groups	585 (60.1%)	276 (28.3%)	103 (10.5%)	9 (0.9%)	973 (100%)

Table 3. Number of patients and percentage of fat absorption according to the site of fat injection.

	Round face	Cheek and jaw contouring	Jaw line contouring only	Number of satisfied patients*	Total
1st session	470	362	141	155	973
2nd session	385	298	125	809	818
3rd session	4	4	1	9	9
Percentage of fat absorption after 1 year	30.8% ± 15.4%**	21.6% ± 18.5%**	13.7% ± 12.3%**		25% ± 17.3%***
* Number of patients who were satisfied and did not wish to undergo another session ** Mean and standard deviation *** Sum of mean and standard deviation					

Few adverse effects were reported, with oedema, bruising, and swelling occurring in less than 10% of patients. Furthermore, contour irregularities were observed in 6.6% of patients. Moreover, lumpiness or dimpled skin due to fat necrosis developed approximately 2–3 months after the fat transplant in 5.2% of patients and was treated with intralesional steroids. No patients developed an infection, and no differences existed in the side-effect profile between those with one session and those with more than one session.

Patients' levels of satisfaction with both the first and second sessions were statistically significant. Before and after photos for those who indicated that they were fully satisfied are presented in Figures 2–6:

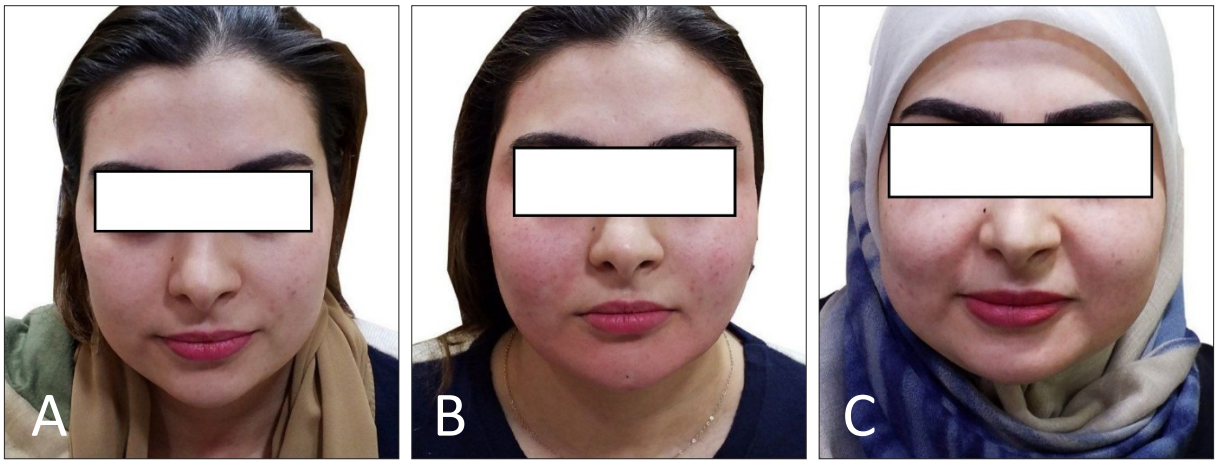

Figure 2. A 25-year-old lady treated with the round face technique: (A) before autologous fat transplantation; (B) after the procedure; and (C) after 3 months.

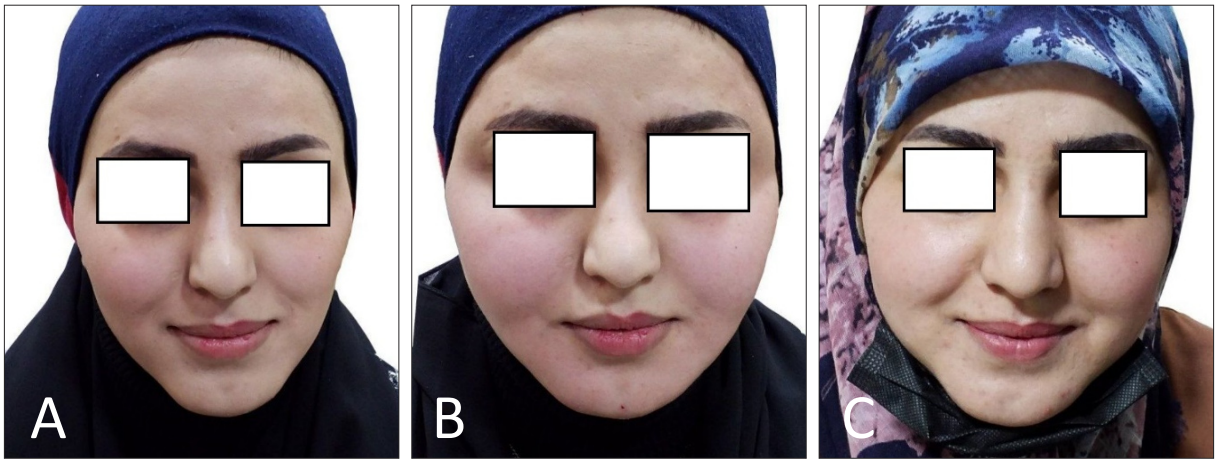

Figure 3. A 22-year-old lady treated with the round face technique: (A) before autologous fat transplantation; (B) after the procedure; and (C) after 6 months.

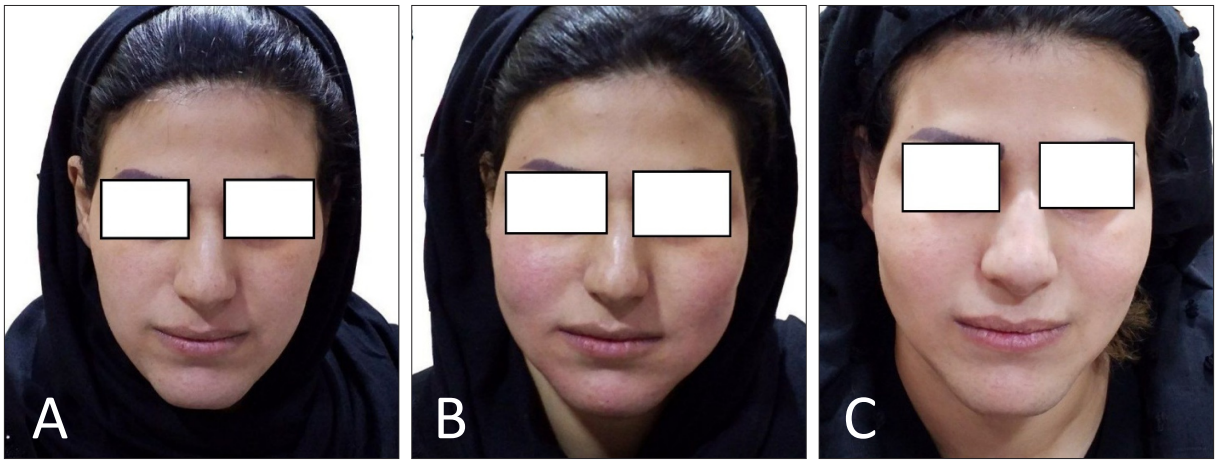

Figure 4. A 33-year-old lady treated with the round face technique: (A) before autologous fat transplantation; (B) after the procedure; and (C) after 1 year.

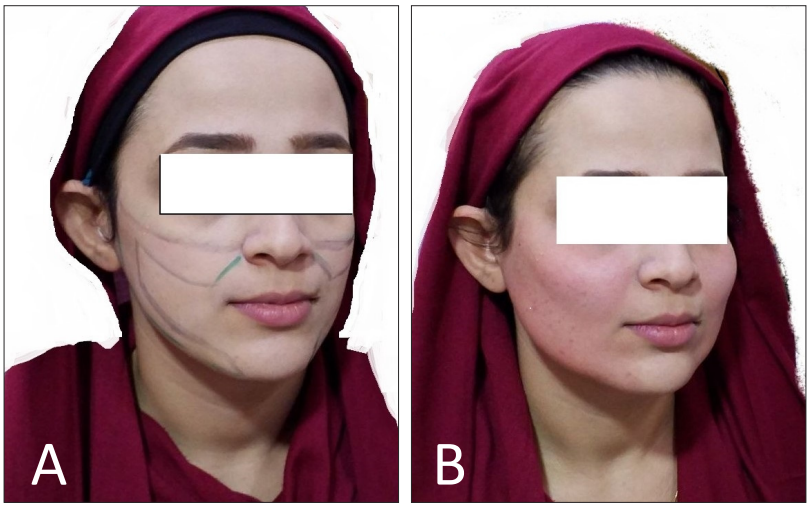

Figure 5. A 22-year-old lady who received cheek and jaw contouring: (A) before autologous fat transplantation; and (B) after the procedure.

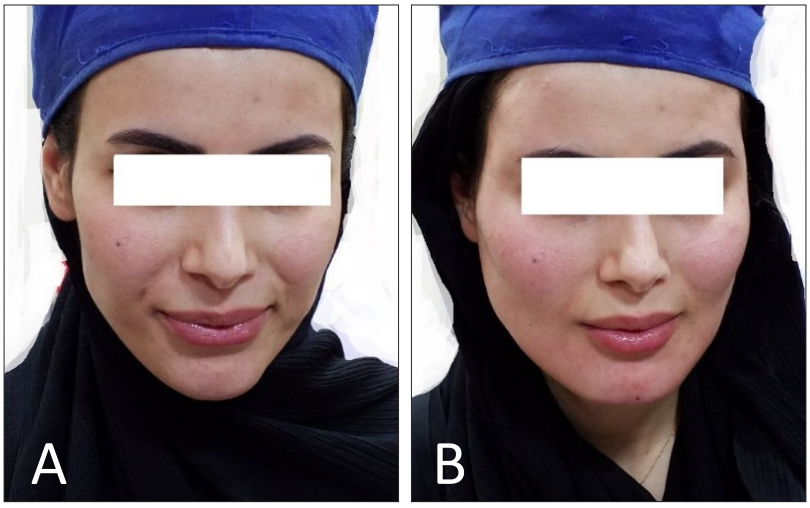

Figure 6. A 26-year-old lady who received chin and cheek contouring: (A) before autologous fat transplantation; and (B) after the procedure.

Discussion

In recent years, the incidence of autologous fat transplantation for aesthetic purposes has increased dramatically. However, due to the high rate of fat absorption and its sequelae overcorrection and reinjection, cryopreserved fat has become indispensable for both surgeons and patients for reducing patient discomfort, cost, and time (1, 8, 9).

Most case series have found that the best sites to harvesting fat from are the abdomen, flanks, thighs, and medial knee (16); however, Ullmann *et al*. (17) reported no difference among the sites. Our study coincides with the majority of published studies in finding the abdomen, flanks, and thighs to be the optimal harvest sites.

Although the optimal cannula size for aspiration still lacks a consensus, nearly all authors, including Ozsoy *et al*. (18) and Rubino *et al*. (19), have reported that a large-bore cannula is optimal, especially ≥5 mm. Furthermore, other researchers have found that a multiperforated cannula helps to reduce pressure on each hole and decreases damage during fat collection (20, 21). We made a similar finding regarding the 4 mm cannula with three slits, which was used in this study.

Pu *et al*. (22) reported that negative-pressure liposuction causes massive destruction of adipocytes, with approximately 90% of grafting cells being functionally impaired in comparison with fresh fatty tissue samples and syringe aspiration. Our study is in parallel with these findings. A lipoaspirated sample contains blood, collagen fibers, and debris, and these elements cause inflammation in addition to the acceleration of the degradation of transplanted fat by blood (23). Our study also indicated that sedimentation of the harvested fat is crucial for obtaining a large number of vital and intact adipocytes (24, 25).

A conflict exists regarding the use of a centrifuge, which is used to obtain the highest possible concentration of stem cells and angiogenic growth factors (26). Ferraro *et al*. (27) found that centrifugation with a force greater than 5000 rpm resulted in cell damage, and they suggested that centrifugation with 1300 rpm for 5 min was more effective. By contrast, Kurita *et al*. (24) recommended 1200 rpm for 3 min, while Coleman suggested that aspirated fat should be spun in a syringe at 3000 rpm for 3 min to obtain good results (28), In our study, we used 3500 rpm for 1 min, which was near Coleman's suggestion.

Furthermore, Ozsoy *et al*. (29) observed that adipose tissue retains greater vitality when infiltrated with a cannula with a diameter of 2.5 mm; however, Erdim *et al*. (30) found no significant difference in cell vitality with a different needle gauge. Our study agrees with most studies that have advised the use of an injected blunt cannula of 1 to 2 mm (31, 32).

Notably, not study has mentioned the benefit of massage following fat injection. We believe that this is essential for blood circulation and redistribution (symmetry) through moving the fat from one area to another. Moreover, the main problem in autologous fat transplantation is absorption. While this varies, most authors have reported an absorption rate of 25–70% (6, 7). As we also found the same rate, we performed overcorrection by approximately 25–50% in all cases.

In addition, previous studies on the viability of frozen fat tissue have reported contradictory results. Some authors (12, 13) have found that few harmful effects were generated on fat tissue frozen at −20°C without cryopreservative agents, and viable fat cells were found after preservation at this temperature. Furthermore, Pu and Cui and their colleagues (11, 33, 34) have concluded that controlled slow cooling and then fast thawing in a 37°C water bath leads to the best results.

Yahuda *et al*. (10) highlighted the capability to use fat after it has been frozen for up to 7 months. Based on the present study, we suggest fat reinjection after freezing for up to three weeks at −2 to −18°C, since we observed that preservation for more than 3 weeks greatly diminished the viability of cells (Figure 5). By contrast, Mazur (35), Walter (13), and Mascatella (36) have suggested that the injection of frozen fat as an inert filler should be avoided.

Additionally, the results of studies on thawing have been confusing, Butterwick *et al*. (37) found that freezing at −40°C and then slow thawing over a couple of hours at room temperature provided good results. Our study agrees with Pu and Cui and their colleagues (11, 32, 33), who have reported that slow cooling and fast thawing lead to best results. Figure 7 is showing the difference between freshly thawed fat and the unviable whitish mucoid fat thawed after 6 weeks.

Figure 7. Difference between freshly thawed fat after 3 weeks (bottom) and the unviable whitish mucoid fat (top) thawed after 6 weeks.

To the best of our knowledge, this is the first study with a large sample size, long follow-up, and consistent results to minimize the number of fat harvesting sessions to one and store fat in an ordinary domestic freezer for up to 3 weeks. In conclusion, autologous fat preserved in a domestic refrigerator at −3 to −18°C for 3 weeks is a safe, cheap, and clinically tolerated filler for patients who seek face augmentation with few side effects.

Limitations of the study

Histological and immunohistochemical examinations of the fat sample were performed before and after freezing to reveal any histological and/or immunological changes.

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Funding

Not applicable.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Adherence to Ethical Standards

This study was approved by the Medical Research Ethics Committee, College of Medicine, University of Mosul, Iraq (ref no.: UOM/COM/MERC/22-23) (10).

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