

Long-Term Results of Fat Grafting: Clinical and Radiological Study

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ABSTRACT

Material: The present study included two randomized prospective studies (both clinical and histological) on 50 patients (42 females and 8 males) to evaluate the behavior of injected fat subcutaneously through one year follow-up.

Methods: All patients underwent preoperative evaluation by CT scan to measure subcutaneous fat volume and this was repeated after three, six and twelve months from fat injection. Also histological assessment of grafted fat was done in ten patients through true cut biopsy taken one year after fat injection and examined underlight microscope after staining with H&E, Sudan III and oil red O stains. Patients were divided into two groups: Aesthetic and reconstructive and fat injection was done by Coleman structural fat grafting technique.

Results: Showed no statistical significant difference between the two groups as regards the degree of fat resorption at three, six and twelve months postoperatively but there was a higher rate of clinical and pathological complications among the reconstruction group due to less optimum recipient bed as regards scar tissue and more tissue ischemia.

Conclusion: The CT scan proved to be a valuable objective device for estimation of volume of fat graft which enables the surgeon to know exactly the amount of fat needed to be injected in a second session to have patient satisfaction.

INTRODUCTION

Fat grafts are used for soft tissue augmentation of various anatomic regions, most frequently for improvement of facial contours [1]. Most investigators believe that fat, as autologous tissue, can be considered the ideal soft-tissue filler because it is abundant, readily available, inexpensive, host compatible, and can be harvested easily and repeatedly [2].

Resorption of the fat grafts is the main problem, and several different procedures have been described to minimize this phenomenon [1]. The most acceptable explanation for absorption has been

based on Peer's cell survival theory, which states that the number of viable adipocytes at the time of transplantation may correlate with ultimate fat graft survival volume, so the harvested and processed fat must remain viable before implantation [2,3].

In 1994, Coleman first described his technique, which uses a syringe, cannula, and centrifuge for structural fat grafting [2]. The lack of objective measurements of fat graft viability may be one of the reasons why plastic surgeons are still skeptical about fat transplantation [3].

Recent publications show a resorption rate of 20% to 90%, but these statistics are not based on objective measurements [3-5].

To prevent fat resorption, fat grafts should be harvested without deforming adipocytes, and an abundantly vascularized recipient site should be provided [6].

The neovascularization stage for skin grafts begins at the fourth day on well-prepared granulation tissue, and a similar mechanism is also valid for fat grafts [1].

There are numerous publications reporting on histological measures of fat graft viability in animals but there are few such reports in humans because of the invasive evaluation methods, therefore a very limited number of patients have been evaluated and the results of non-invasive methods are merely descriptive as it is difficult to quantify the long term volumetric effects of fat grafts over time [3]. In order to determine fat graft viability, CT scans were used volumetrically to measure fat tissue as it evolves in the treated area over time as it can distinguish fat density from all other tissues with more exact measurements than those of MRI [3].

The objective of this study was to study the behavior of grafted fat by clinical, histological and radiological studies through one year follow-up.

PATIENTS AND METHODS

The present study included two randomized prospective studies both clinical (including radiological) and histological. The study included fifty patients presented to Mansoura University Hospitals and Matareya Teaching Hospital from January 2010 to December 2012. The cases included 8 males and 42 females and their ages ranged between 22 to 68 years. The cases were classified according to the pathology into reconstruction group (24 cases) and aesthetic group (26 cases). In each case the followings were fulfilled: Analysis of the patient's complaint, complete medical examination, preoperative, postoperative and follow-up photographs after three months, six months, and up to one year after fat injection were taken. CT scan was done for each case preoperatively, at three months, six months and one year post operatively to evaluate the volume of subcutaneous fat before and up to one year after fat injection using specific software (VOLUME in a Leonardo VD30B workstation) in Siemens AG, Berlin, Germany CT scanner. All cases were done under general anaesthesia using the Coleman structural fat grafting technique as saline-adrenaline 1:200000 was infiltrated subcutaneously in a volume similar to the volume of fat to be harvested and fat was harvested from several regions including abdomen, thighs, and buttocks using Coleman fat harvesting 2mm blunt tip cannula (Byron Medical Inc., Tucson AZ) connected to a ten mL syringe to obtain -0.5 atmospheric pressure by pulling the plunger slowly. Centrifugation of fat was done at 3000rpm for 3min. to separate fat into 3 layers; upper layer of oil (free fatty acids) to be decanted, middle fat layer and lower fluid layer (also to be decanted). Fat was injected in the form of streaks during withdrawal of Coleman fat injecting blunt style I cannula number 17 (Byron Medical Inc., Tucson AZ) in 3 layers (superficial subdermal, superficial subcutaneous, and deep intramuscular (in case of deep contour defects only). The end point of injec-

tion was slight overcorrection and the procedure was repeated according to the clinical improvement with an interval of six months. Samples for histological evaluation of the fat grafts were obtained by true cut biopsy before and one year after fat grafting and were examined under light microscopy using haematoxylin & eosin, Sudan III and oil red-O stains. In every specimen, each of the following items were evaluated; necrosis, fibrosis, revascularization, cyst formation and calcification. The collected data was revised, coded, tabulated and introduced to PC using Statistical Package for Social Science (SPSS 15.0.1) calculating mean values, standard deviation (SD), and Mann Whitney test (U test) was applied for non parametric variables and Wilcoxon signed rank test was applied to compare between the degree of fat resorption among aesthetic and reconstruction groups (p -value less than 0.05 is significant).

RESULTS

We compared the degree of fat resorption among the patients of each group (aesthetic and reconstructive) at three months, six months, and at twelve months after fat injection separately but we found no statistical significant difference between the three points of time and then we compared the rate of fat resorption between the two groups and we also found no statistical significant difference between the two groups at three, six and twelve months after fat injection (as shown in Table (1)). We noticed fat necrosis in ten cases all of them were in the reconstruction group. There was cyst formation in two cases of aesthetic breast augmentation (Fig. 5) and severe infection occurred in two cases of breast reconstruction after quadrantectomy. This massive infection may be due to skin devitalization by previous irradiation. Skin sloughing was a major complication after fat injection under a full thickness skin graft of the arm and this may be due to subcutaneous dissection to create a pocket for fat injection that led to skin ischemia and sloughing. We found a statistical significant difference among the patients of both groups as regards the rate of complications with the reconstructive group having a higher rate (p -value <0.05) (as shown in Figs. 1, 2).

Table (1): Comparison between the two groups as regards percent of fat resorption at 3, 6 and 12 months after fat injection.

	Group						p	Sig.
	Aesthetic group			Reconstructive group				
	Mean	\pm SD	Median	Mean	\pm SD	Median		
Percent of fat resorption at 3 months	19.0	15.1	15	22.7	15.8	21	.321	NS
Percent of fat resorption at 6 months	28.1	16.1	24	28.7	15.5	27	.756	NS
Percent of fat resorption at 12 months	34.7	16.4	30	36.3	14.8	33	.540	NS

*Mann Whitney test.

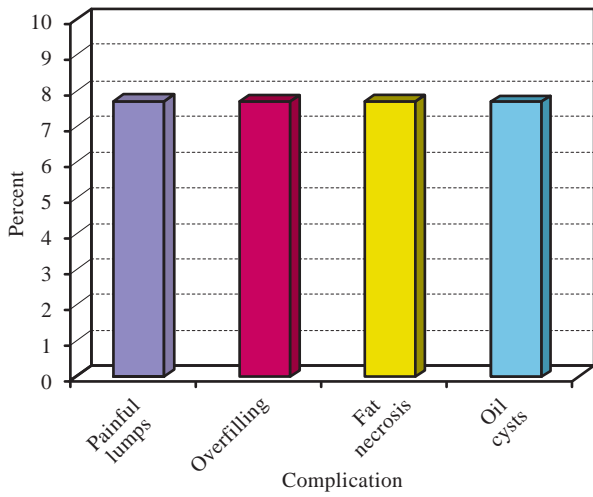


Fig. (1): Description of complications among aesthetic group.

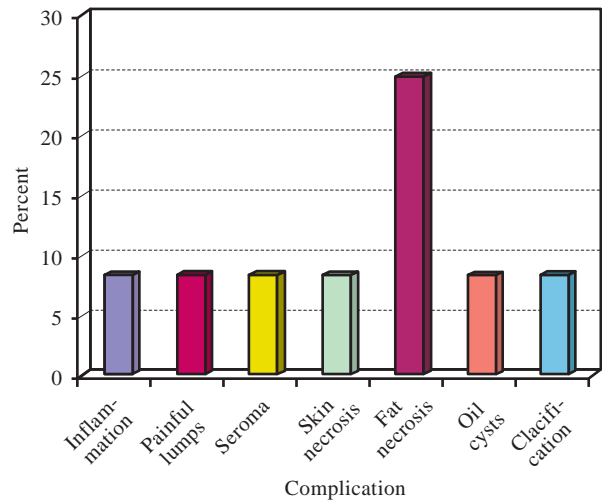


Fig. (2): Description of complications among reconstructive group

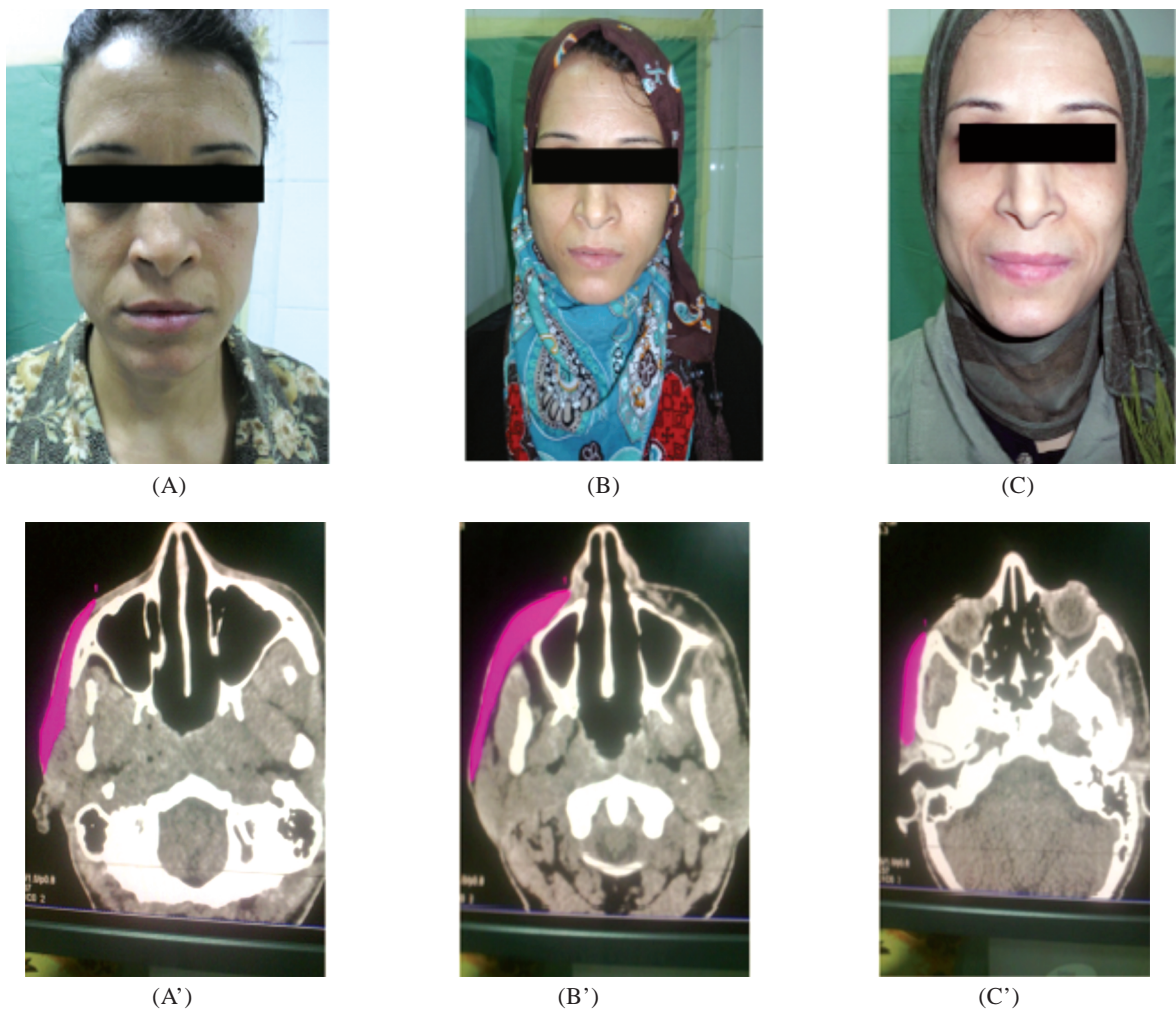


Fig. (3): A 37 year-old female patient with facial lipoatrophy. (A) Preoperative photograph. (A') Preoperative CT scan. (B) 3 months postoperative photograph. (B') CT scan at 3 months postoperatively. (C) 12 months postoperative photograph. (C') CT scan at 12 months postoperatively.

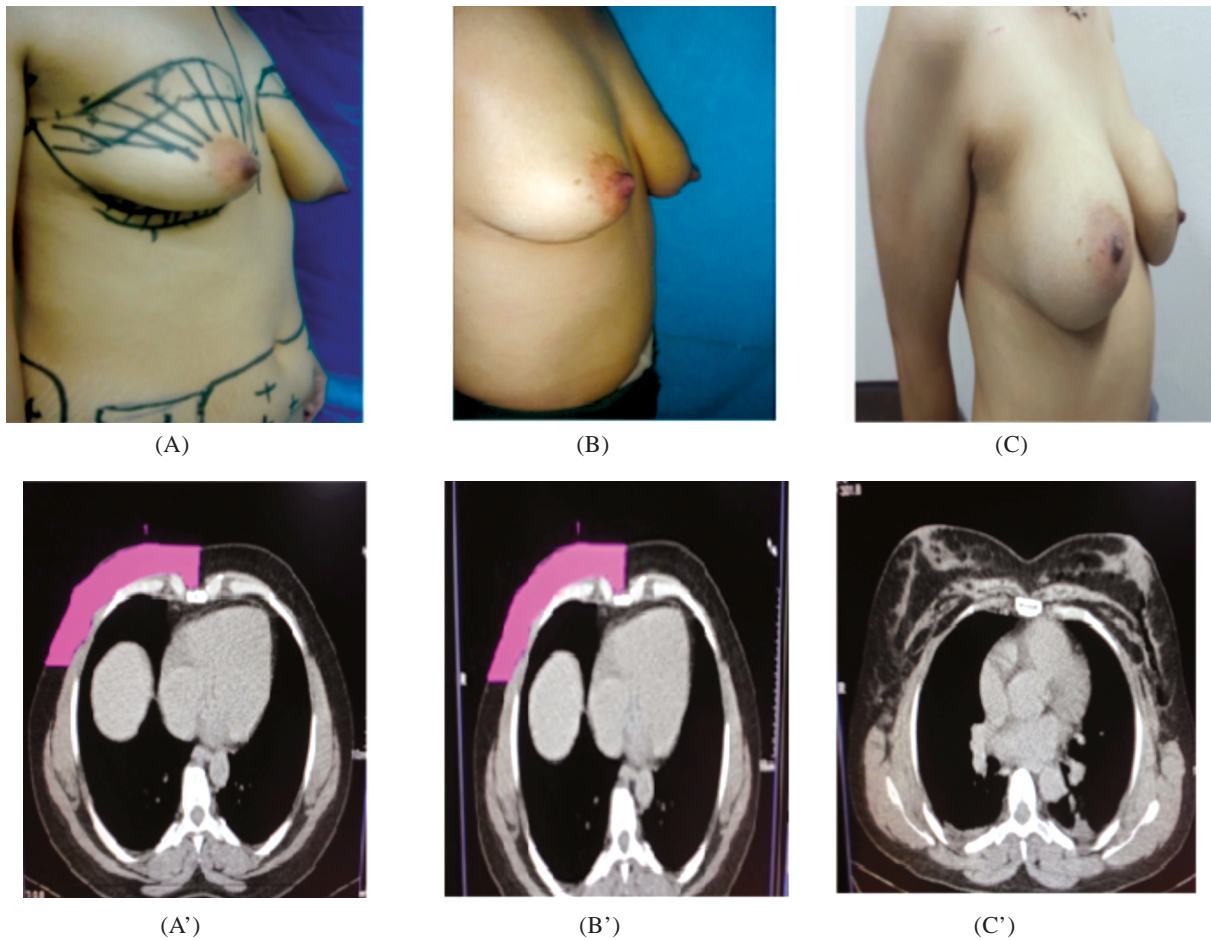


Fig. (4): A 32 year-old female patient with right congenital unilateral hypomastia. (A) Preoperative photograph. (A') Preoperative CT scan. (B) 3 months postoperative photograph. (B') CT scan at 3 months postoperatively, (C) 12 months postoperative photograph after injecting 250cc of fat in the right breast and doing mastopexy of the left breast. (C') CT scan at 12 months postoperatively.

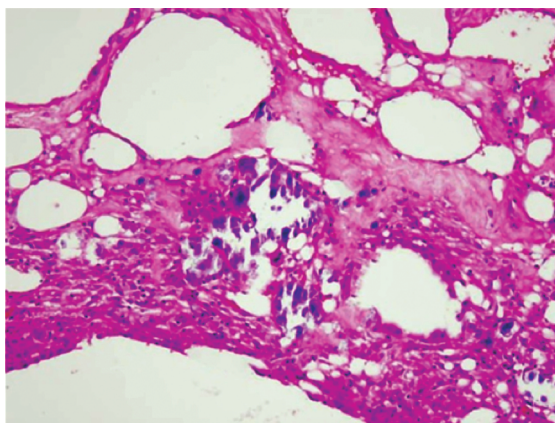


Fig. (5): A light microscopic photograph of subcutaneous fat stained with haematoxylin and eosin (x400 magnification) showing fat cysts and calcification.

DISCUSSION

Fat naturally fulfills many of the characteristics required of a soft tissue filler. It is autologous, nontoxic, biocompatible, easily available in most patients, potentially removable and long lasting [7].

Significant limitations to traditional fat transplantation remain, such as unpredictability and variable rate of graft survival [8]. Early experience noted that graft re-absorption was the main drawback with 50-90% graft loss [9]. But in a comparative study between autologous fat grafts harvested and refined by the Coleman technique of structural fat grafting and another group by conventional liposuction higher viable adipocyte counts with more optimal level of cellular function within fat grafts were found in Coleman technique group [2].

We adopted the structural fat grafting technique by Coleman and conducted two randomized prospective studies both clinical (including CT assessment) and histological on 50 patients with subcutaneous defects and divided them into two groups [aesthetic (26 cases) and reconstruction (24 cases)] aiming to study the behavior of grafted fat by clinical, histological and radiological (CT assessment of fat volume) methods through one year follow-up.

In order to determine subcutaneous fat volume before injection and 3, 6 and 12 months postoperatively we used computed tomography (CT) scan to volumetrically measure fat tissue as it evolved in the treated area as CT scan can distinguish fat density from all other tissues, and was chosen over magnetic resonance imaging (MRI) because CT measurements are more exact than those of MRI [3].

As regards the best donor site for harvesting fat several authors published no statistical significant difference among different donor sites [5,10].

As adipose tissue has recently been identified as a source of processed lipoaspirate cells or adipose derived stem cells [11], The cell concentration of processed lipoaspirate in 6 commonly used donor sites for fat grafting (upper abdomen, lower abdomen, trochanteric region, inner thigh, knee and flank) was evaluated in 25 women [12]. Based on this well-conducted study it was concluded that both lower abdomen and inner thigh have higher concentrations of processed lipoaspirate cells and these areas may be better donor sites of adult adipose-derived stem cells [12]. We harvested fat from several sites including lower abdomen, thighs and buttocks.

Many studies assess the viability of fat grafts harvested with different techniques. Fat grafts harvested with either standard liposuction or syringe aspiration were evaluated [13]. It was concluded that, there is no significant difference in adipocyte viability of fat grafts harvested with standard liposuction compared with syringe aspiration using only the colorimetric assay of cell proliferation [13]. The histology of adipocytes in the fat grafts harvested from different settings of suction force was studied [14]. Based on only histological examination, it was concluded that adipocytes become damaged and disrupted only when as high as -700 mm Hg vacuum is used for collection of lipoaspirate [14]. The potential role of adipose aspirates collected from conventional liposuction as a source of autologous fat grafting and the viability of adipose aspirates was evaluated by viable cell count, glycerol-3-phosphate dehydrogenase assay and histology [15]. It was concluded that although adipose aspirates collected from conventional liposuction maintain normal structure with near the same number of viable adipocytes compared with fresh fatty tissues, but they have a less than optimal level of cellular function and therefore may not survive well after they are transplanted [15].

We adopted the technique of fat harvesting as described by Coleman and known as structural fat grafting in which we used a small 2mm Coleman blunt-tipped aspiration cannula which has the smallest hole that can produce small fat parcels that do not obstruct the fat injection cannula during fat placement and the aspiration cannula was attached to 10ml Luer-lock syringe with gradual withdrawal of the plunger to create maximum negative pressure of -0.5 atmospheric to keep the viability of adipocytes, preadipocytes and adult-derived stem cells.

This atraumatic technique of Coleman was investigated and supported by a comprehensive study to determine the viability of fat grafts harvested and refined with a common technique (conventional liposuction and low force centrifugation) [2], and it was concluded that although fat grafts harvested and refined by both techniques maintain normal histologic structure, the Coleman technique yields a great number of viable adipocytes and sustains a more optimal level of cellular function with fat grafts and should be considered superior to the conventional liposuction as a preferred method of choice for fat graft harvesting [2].

Although some authors [16] concluded that, the use of larger cannulas for syringe aspiration appears to provide more viable adipocytes of fat grafts based on the viable cell count [16], another comprehensive study [17] measuring viable cell count, a cell proliferation assay, an enzyme assay and oil red O stain for histological assessment concluded that, the viability of fat grafts is significantly better when fat graft is harvested by 2mm diameter cannula with a blunt tip and several side holes connected to a 10cc syringe as compared to a 3mm diameter blunt tipped cannula connected to a 60cc syringe [17].

Most surgeons believe that fat grafts harvested with syringe aspiration or conventional liposuction need to be processed in some way in order to limit the blood or oil within the lipoaspirates so that only pure fat as a soft-tissue filler will be used for injection. However, this has become a highly controversial issue and currently there is no agreement among surgeons in terms of which is the best method for processing fat grafts. Three primary methods (Sedimentation by gravity, filtering technique, and centrifugation) have been used clinically to process fat grafts.

We centrifuged the harvested fat at a rate of 3000rpm for 3 minutes. Histologically, significantly

distorted and fractured adipocytes were seen when the centrifugal speed reached 4000rpm [18]. The optimal speed and duration of centrifugation were studied by Kim et al., They found that adipocyte survival rates, evaluated by trypan blue staining, were significantly lower when fat grafts were centrifuged at 1500 and 3000rpm for more than 5min. or centrifuged at 5000rpm for more than 1min. In addition, the ruptured cell membranes, fusion of cells, and irregular cell shape were identified when fat grafts were centrifuged at 5000rpm for 5min. Therefore, they concluded that centrifugation with 3000rpm for 3min is optimal and should be recommended for processing fat grafts [18].

A more comprehensive study was conducted in Japan [19]. It was concluded that excess centrifugation can destroy adipocytes and ADSCs, but appropriate centrifugation concentrates these cells, resulting in enhanced fat graft take. They recommend 1200g as an optimal centrifugal force for processing fat grafts [19]. Interestingly, such a centrifugal force is close to the 1286g generated by the centrifuge from the Coleman's instrument set if one would use this centrifuge to process fat graft. Whether overcorrection would be necessary for fat grafting remain unclear. Since the viable fat grafts are only observed in the peripheral zone approximately 1.5mm from the edge of the grafts and the percentage of graft viability depends on its thickness and geometrical shape [20,21]. Overcorrection for "better" graft survival in the recipient site appears to be without scientific support. In addition, significant overcorrection may increase the incidence of fat necrosis and subsequent calcification or even severe infection. Therefore, significant overcorrection should be avoided at the present time until its necessity and safety can be confirmed by future studies. We just slightly overcorrected the area to be injected by fat. We analyzed the changes of estimated fat volume using Wilcoxon rank sum test for paired data and we found that there was no statistical significant difference between the percentage of fat resorption after 3, 6, and 12 months from fat injection in the aesthetic group (p -value >0.05) and also there was no statistical significant difference between the percentage of fat resorption after 3, 6, and 12 months from fat injection in the reconstruction group (p -value >0.05) indicating that fat injection in non-scarred well vascularized tissues in aesthetic group takes as well as in scarred and less vascularized tissues in reconstruction group. But, the incidence of complications in the reconstruction group was higher than that in the aesthetic group (both clinical and histological). The degree of fat resorption in the aesthetic group mostly was in the mild degree

(0-20% resorption) while the degree of fat resorption in the reconstruction group was mostly in the mild to moderate degree (0-30% resorption). Forty eight percent of patients required a second session of fat grafting aided by the objective measures obtained from CT scan and the subjective clinical assessment of pre and post-operative photographs with much higher degree of patient satisfaction after the second session of fat injection indicating that CT volume assessment of subcutaneous fat is a good valuable objective measurement to obtain good patient satisfaction.

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