Journal of Plastic, Reconstructive & Aesthetic Surgery 108 (2025) 176–182

Optimization of autologous adipose tissue transplantation: The impact of patient-specific and intraoperative factors on cellular viability before and after cryopreservation

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Received 17 April 2025; Accepted 21 June 2025

KEYWORDS
Autologous fat grafting;
Lipofilling;
Cryopreservation;
Cell viability;
Fat graft optimization;
Fat graft survival

Summary *Background:* Autologous adipose tissue transplantation is a versatile surgical procedure used for aesthetic, reconstructive, and regenerative purposes. Cryopreservation of adipose tissue collected during liposuction reduces the need for repeated fat collection, minimizing patient discomfort while preserving volumetric and regenerative properties.

There are currently no studies in the literature correlating cellular viability, before and after thawing, with patient-specific variables and collection techniques.

This study investigates patient-specific factors affecting cellular viability before and after thawing to refine protocols for patient selection and tissue collection.

Materials and methods: A retrospective analysis of 55 patients from the University Hospital Policlinico of Modena was conducted. Intraoperative factors (disinfectant used, type of anesthesia, operators, associated procedure, duration of surgery, harvesting area, quantity collected) and clinical variables (age, sex, smoking status, comorbidities, antiblastic therapy, hormonal therapy, radiation therapy, immunological therapy) were analyzed. Cell count and metabolic activity tests were performed pre- and post-cryopreservation.

Results: Hormonal therapy significantly correlates with reduced cellular metabolic activity and viability, both pre- and post-thawing.

This study was presented at the congress of the Italian Society of Plastic, Reconstructive and Aesthetic Surgery in October 2024.

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https://doi.org/10.1016/j.bjps.2025.06.049

Conclusions: The findings underscore the need to evaluate adjuvant therapies when planning adipose tissue collection. Optimizing timing and preparation can improve graft viability, reducing resorption rates and enhancing volumizing and regenerative effects.

Preoperative planning must account for the timing of hormonal and other therapies to ensure effective autologous adipose transplants with improved outcomes

Clinical background and significance of fat grafting

Autologous adipose tissue transplantation is an established practice in plastic and reconstructive surgery, used to correct volume deficiencies, improve the quality and trophism of skin and subcutaneous tissue, reshape body contours, and improve the aesthetic appearance of various body areas.[1]

The technique is based on harvesting fat from a donor site, purifying it through various techniques, and injecting it into a recipient site. At first developed for cosmetic purposes, this procedure has gained increasing relevance in reconstructive applications, such as correcting post-traumatic or post-surgical deformities, including breast reconstruction after mastectomy, but also in the more modern field of regenerative medicine.[2]

Historically, autologous fat transplantation has undergone significant evolution. The first applications of fat transfer date back to the late 19th century, when surgeons used small fat grafts to correct aesthetic defects and scars.[3] However, early techniques often resulted in high fat resorption rates and unpredictable clinical outcomes. With the advent of liposuction in the 1970s and advancements in fat processing, the procedure has seen considerable improvements in terms of safety and efficacy.

Currently, research efforts focus on optimizing the cellular viability of transplanted adipose tissue. Long-term survival of adipocytes is a critical factor for the success of the procedure, and various elements can influence this process, including surgical technique, processing of the harvested fat, recipient site and the patient's physiological conditions.

Emerging role of cryopreservation and stem cells

An emerging area of investigation is the role of mesenchymal stem cells found within adipose tissue, which appear to contribute to tissue regeneration and long-term volume stability.

The recent innovation of cryopreservation of adipose tissue collected during liposuction represents an interesting opportunity for modern plastic surgery, as it permits the storage of harvested fat for future use.[4] This possibility allows a reduction in the number of fat collection surgeries when multiple sessions are required (resulting in less discomfort for the patients), potentially enabling its on-demand use after thawing from the bank, while preserving to a large extent its volumetric and regenerative properties, as currently under investigation.

Identified gap in current knowledge and study objectives

While previous studies have primarily focused on assessing the general viability of adipose tissue after cryopreservation, little is known about how patient-specific characteristics and intraoperative variables may influence this viability. Understanding these correlations is essential, as they could help optimize the quality of stored fat, reduce the need for repeated harvesting procedures, and ultimately improve graft retention and clinical outcomes. This study aims to bridge this gap by systematically evaluating the impact of these factors on the cellular viability of fresh and cryopreserved autologous adipose tissue.

Currently, there are no studies available in the literature that correlate cellular viability, before and after defrosting, with patient-specific variables and collection techniques.

Patient selection protocols and shared guidelines for sample collection could optimize clinical outcomes and improve fat viability.

Materials and methods

Study design and patient selection

This study is a retrospective analysis of prospectively collected adipose tissue samples. Fat tissue harvesting and viability testing were performed prospectively as part of an ongoing protocol. The samples were then cryopreserved and stored under controlled conditions for subsequent retrospective analysis of cellular viability. This approach allowed us to systematically evaluate both fresh and frozen-thawed samples with respect to patient-specific and intraoperative factors.

We conducted a retrospective analysis of 55 patients who underwent autologous adipose tissue collection for cryopreservation from October 2023 to June 2024. All patients provided written informed consent for the use of their adipose tissue samples for research purposes.

A comprehensive set of variables was collected to thoroughly investigate potential factors influencing adipose tissue viability.

The patient-specific clinical variables analyzed were gender, age, BMI, smoking status, patient comorbidities, history of chemotherapy prior to adipose tissue collection and the cessation time before collection, use of hormone therapy, immunotherapy, or radiotherapy.

Comorbidities and treatments (such as chemotherapy and hormone therapy) were defined based on active or recent exposure within six, between six and twelve, more than twelve months prior to the surgical procedure. Data regarding these variables were extracted from patient medical records to ensure accuracy and consistency, rather than relying on patient self-reporting.

The intraoperative variables analyzed included the disinfectant used for the sterile field, the type of anesthesia administered, the number of surgical teams involved, and the complexity of the surgery.

For instance, the number of surgical teams involved may impact the duration and coordination of the procedure, potentially affecting tissue ischemia time and handling, which in turn can influence cellular viability. Similarly, the type of disinfectant used could alter the risk of contamination or cellular toxicity, thereby affecting the quality and survival of the harvested fat. Including these variables allows for a more detailed analysis of intraoperative factors that may contribute to variability in graft outcomes associated with the adipose tissue collection, the duration of the procedure, and how many and which tissue collection sites were used.

Cell viability analysis

To measure cellular metabolic activity, we used the MTT assay, a standard colorimetric test used to assess the metabolic activity of biological samples. MTT stands for 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide, which is reduced to formazan by metabolically active cells, particularly by the mitochondrial enzyme succinate dehydrogenase, giving the substance a blue/purple color. The test is performed in replicate on a multi-well plate. Four exemplary adipose tissue samples (approximately 1 ml) are weighed using an analytical balance (Analytical Plus OHAUS Model AP250 DE). Then, 1 ml of MTT (SIGMA Aldrich) is added to each of the four wells with 1 ml of adipose tissue, and the plate is incubated in a Hera Cell 150 incubator at 37 °C and 5% CO2 (Thermo Scientific) for a total time of 3 h. After 3 h, the MTT solution is removed by aspiration. Since formazan is insoluble and precipitates, it is solubilized by adding DMSO (CRYO ON Alchimia) to the tissue in a 1:1 ratio, and then everything is transferred into a cuvette. After 20 min of adding DMSO, the reading is taken using a spectrophotometer set to a wavelength of 570 nm (Beckman Coulter TM DU® 530 Life Science UV/VIS Spectrophotometer). The amount of formazan produced, as well as the intensity of the purple color, is directly proportional to the cellular metabolic activity of the analyzed samples. Specifically, the metabolic activity of the tissue is calculated by estimating the average percentage viability index (Iv%), obtained from the ratio between the optical density (OD) and the weight of each sample (in grams), using the formula Iv% = OD/grams.

Technical replicates were performed for each sample in the cell viability assay, and the results were averaged to calculate the final percentage of viable cells (Iv%).

The Iv% formula used in this study is based on established cell viability assessment methods commonly reported in the literature. While this specific calculation has not been explicitly validated in previous studies, it aligns with standard viability metrics and provides a consistent measure for comparing samples.

Harvesting technique

The patient is placed in the supine position, and the procedure is performed under general anesthesia. A sterile field is prepared using chlorhexidine or betadine. The adipose tissue harvesting areas are infiltrated with a solution consisting of one vial of adrenaline diluted in 500 cc of saline solution. The adipose tissue is manually harvested using 2 mm blunt Sorensen cannulas connected to 20 cc syringes.

The volume of adipose tissue harvested per patient ranged from 90 ml to 553 ml.

The fat is decantated and washed with lactated Ringer's solution using a closed-circuit system.

The purified adipose tissue is then stored in sterile bags, which are sent to the Skin and Tissue Bank in Cesena.

The duration between harvesting and cryopreservation ranged from 2 to 12 h, and all samples were stored at 4 °C during this interval to minimize cell degradation before cryopreservation.

Cryopreservation and thawing procedures

Once the tissue has been aliquoted, the freezing liquid is added, and the aliquots are brought to a temperature of −150 °C with a programmed descent planner (certified with a freezing curve).

The aliquots are finally stored in liquid nitrogen tanks at −196 °C.

The thawing of the tissue for release takes place at the laboratories of the Cesena Skin Bank (RER) under a laminar flow hood in a GMP class B room by two highly specialized and qualified laboratory technicians.

Once the suitability for transplantation of the thawed adipose tissue has been obtained, the thawing of the requested aliquots is carried out, according to the thawing protocol immediately before the scheduled date for the regraft. The LAL TEST is carried out on the tissue for bacterial endotoxins.

On the day of the infiltration, the Lipobank specialist will bring the thawed tissue, transported at a controlled temperature of 4 degrees, in a double sterile bag, and ready for grafting to the surgical clinic.

Statistical analysis

Statistical analysis was performed using STATA® software version 17 (StataCorp. 2021. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC.). Descriptive statistics were presented for baseline demographic clinical characteristics for the entire patients. Continuous variables were presented as mean, standard deviation (SD), minimum (min), and maximum (max) and compared between subgroups using Unpaired Student's t-test; Analysis of variance (ANOVA) was used to evaluate the differences of the parameters under

examination for variables with three or more categories, we performed a post hoc test using Tukey-Kramer's method for pairwise comparison of subgroups; while categorical variables were presented as frequency (N, percentage [%]) and compared using Pearson's chi-squared test. A p-value < 0.05 was considered significant.

This study adheres to the STROBE guidelines for observational studies.

Results

Vitality of fresh and thawed adipose tissue after 3 months

We analyzed the vitality of fresh adipose tissue and that of thawed tissue three months post-collection using the MTT assay.

No statistically significant differences were found between fresh and thawed tissue in terms of cellular vitality (p-value = 0.858). The average cell viability index in both samples was above 92%.

While a mean cell viability of 92% was observed, there is currently no universally established threshold that defines a clinically meaningful drop in adipose tissue viability. Given the very recent nature of this topic, no established clinical thresholds are currently available. Therefore, we arbitrarily considered cell viability below 80% as indicative of a potentially negative impact on graft success, and our findings indicate that the viability values measured are within a range considered favorable for clinical efficacy.

The results are summarized in Table 1.

Analysis of intraoperative variables

The intraoperative variables studied included the type of anesthesia used during tissue collection (general vs. local with sedation), operating time ($<$90 min, 90–180 min, $>$180 min), the disinfectant used to prepare the surgical field (betadine vs. chlorhexidine), the number of teams involved in the procedure (one, two, or three), and the complexity of the procedure associated with adipose tissue collection (low vs. high complexity). None of these variables showed a statistically significant difference in cellular vitality between fresh and thawed adipose tissue.

Further, we examined both the number and specific locations used for adipose tissue harvesting. The sites analyzed included the abdominal and inner knee area, flanks, supratrochanteric, and subcostal regions. No statistically significant differences in cellular vitality were found between fresh and thawed adipose tissue for any of these variables.

Table 1 Supplementary Digital Content 2. A table that shows Vitality of Fresh and Thawed Adipose Tissue after 3 Months.

Variable	N (%)	Mean ± SD	p-value
MTT			
Fresh	55	92.9 ± 5 (78–100)	0.858
Thawed	55	93 ± 5 (83–100)	

The results are summarized in Table 2.

Analysis of clinical variables

The patient-specific clinical variables analyzed included age ($<$40, 40–60, $>$60), gender, smoking status (current, former, never), and comorbidities (none, mild, severe). No statistically significant differences in fat vitality between fresh and thawed tissue at three months for any of these variables were found.

Moreover, we examined any oncological treatments undergone by the patients. In detail, we investigated correlations between adipose tissue vitality and prior neoadjuvant chemotherapy or chemotherapy before tissue collection, including the time elapsed since the last treatment ($>$12 months, 6–12 months, $<$6 months), as well as the use of hormone therapy, immunotherapy, or radiotherapy.

We found significant differences in the mean vitality index of adipose tissue (both fresh, p-value = 0.046, and thawed, p-value = 0.049) associated with prior hormone therapy. Specifically, the metabolic activity of cells obtained via liposuction was reduced in patients who had undergone adjuvant hormone therapy.

The hormone therapy taken by our patients included either exemestane, tamoxifen, letrozole, or anastrozole.

On the other hand, no statistically significant correlations were identified between adipose tissue vitality (fresh or thawed) and the administration of chemotherapy, immunotherapy, or radiotherapy.

The results are summarized in Table 3.

Discussion

Preliminary results of our study offer new perspectives on cryopreserved autologous fat transplantation. The cellular vitality of fresh adipose tissue and tissue thawed three months after collection shows no statistically significant differences. With vitality remaining above 92% in both cases, cryopreservation proves effective in preserving adipose tissue's biological properties, highlighting the remarkable potential of this fat grafting method.

The correlations between operational variables and adipocyte survival post-transplant during a standard fat graft operation have already been studied.[5]

Our analysis revealed that none of the intraoperative variables studied, such as the type of anesthesia, operating time, or procedure complexity, significantly affected the vitality of cryopreserved adipose tissue after thawing. This suggests that, in terms of maintaining cellular vitality after thawing, operative details do not play a crucial role.

Moreover, we found that patient-specific variables such as age, gender, smoking status, and comorbidities did not show any significant impact on the cellular vitality of fresh or thawed adipose tissue. This implies that cryopreserved fat transplantation can be used in a wide range of patient populations without the need to personalize treatment based on these factors.

An interesting aspect of our study is the lack of a significant correlation between cellular vitality and oncological treatments such as chemotherapy, immunotherapy, or radiotherapy.

Table 2 Supplementary Digital Content 2. A table that shows Analysis of Intraoperative Variables.

Variable	N (%)	MTT Fresh (Mean ± SD)	p- value	MTT 3 Months (Mean ± SD)	p-value
OPERATING TIME					
< 90	21 (38.2)	93.0 ± 5.8 (78–100)	0.605	93.4 ± 5.2 (83–100)	0.576
90-180	19 (34.5)	92.1 ± 5.3 (84–100)		91.9 ± 5.7 (84–100)	
> 180	15 (27.3)	94 ± 4.4 (86–99)		93.5 ± 4 (86–98)	
ANESTHESIA					
Local	24 (43.6)	93.2 ± 5.7 (78–100)	0.759	93.6 ± 5.1 (83–100)	0.376
General	31 (56.4)	92.8 ± 4.9 (84–100)		92.4 ± 5.0 (84–100)	
DISINFECTANT					
Betadine	30 (54.5)	92.7 ± 5.4 (78–99)	0.721	92.8 ± 4.9 (83–99)	0.882
Chlorhexidine	25 (45.4)	93.3 ± 5.1 (84–100)		93.0 ± 5.4 (84–100)	
N° EQUIPE					
1	36	92.4 ± 5.6 (78–100)	0.481	92.6 ± 5.5 (83–100)	0.648
2	18	93.9 ± 4.5 (85–99)		93.3 ± 4.4 (85–99)	
3	1	95.0		95.0	
OPERATING COMPLEXITY					
Low complexity	25 (45.4)	93.1 ± 5.7 (78–100)	0.838	93.4 ± 5.2 (83–100)	0.500
High complexity	30 (54.5)	92.9 ± 4.9 (84–100)		92.5 ± 5.0 (84–100)	
N° OF TISSUE HARVESTING					
1	9 (16.4)	94.4 ± 5.5 (85–100)	0.840	94.0 ± 5.2 (85–99)	0.712
2	17 (30.9)	92.9 ± 5.7 (78–100)		93.5 ± 4.9 (83–100)	
3	22 (40.0)	92.9 ± 5.0 (84–100)		92.5 ± 5.2 (84–100)	
4	6 (10.9)	92.0 ± 5.5 (86–98)		92.1 ± 5.7 (85–99)	
5	1 (1.8)	89		87	
FLANK					
No	36	92.4 ± 5.6 (78–100)	0.481	92.6 ± 5.5 (83–100)	0.648
Yes	18	93.9 ± 4.5 (85–99)		93.3 ± 4.4 (85–99)	
TROCHANTERIC					
No	25 (45.4)	93.1 ± 5.7 (78–100)	0.838	93.4 ± 5.2 (83–100)	0.500
Yes	30 (54.5)	92.9 ± 4.9 (84–100)		92.5 ± 5.0 (84–100)	
INNER KNEE					
No	32 (58.2)	93.1 ± 5.0 (84–100)	0.797	93.1 ± 5.1 (84–100)	0.777
Yes	23 (41.8)	92.8 ± 5.6 (78–100)		92.7 ± 5.2 (83–100)	
SUBCOSTAL					
No	14 (63.6)	93.1 ± 5.3 (84–100)	0.290	92.7 ± 5.2 (84–99)	0.436
Yes	4 (18.2)	88.7 ± 8.1 (78–96)		89.5 ± 5.9 (83–95)	

A particularly significant finding of this study is the impact of adjuvant hormone therapy on the viability of autologous adipose tissue. Unlike other patient-related or intraoperative variables, hormone therapy emerged as a consistent negative predictor of cellular vitality, affecting both freshly harvested and cryopreserved fat. This reduction in cell viability suggests that systemic hormonal modulation may interfere with the regenerative and volumetric potential of adipose grafts, raising important clinical implications for patient selection, graft planning, and postoperative expectations.

This effect could be attributed to hormonal changes that negatively affect the cellular metabolism of adipose tissue, an aspect that has been studied in a fat grafting mice model[6] and in human cells in vitro,[7] but warrants further investigation, especially in oncological patients.

The decrease in fat vitality in patients who underwent hormone therapy highlights the importance of considering the oncological treatment profile, particularly in patients undergoing reconstruction after mastectomy. Understanding how systemic treatments influence cellular survival post-transplant could be crucial in optimizing treatment protocols in this patient population. For this reason, it would be advisable to harvest adipose tissue for future use during the mastectomy, before the subsequent initiation of hormone therapy.

Study limitations

Preliminary data reported are encouraging for a clinical improvement of the fat graft procedures. One limitation of this study is the small sample size (55 patients) but justified by the recent introduction of the method and the limited use on a large scale. Another drawback is the relatively short follow-up after cryo-preservation (three months) but compatible with the standard timing of treatment.

We did not analyze dose- or duration-dependency of hormone therapy effects on fat viability, nor did we investigate whether the observed impact is related to systemic inflammation or vascular changes. Regarding the consistency of the effect between different hormone agents

Table 3 Supplementary Digital Content 2. A table that shows Analysis of Clinical Variables.

Variable	N (%)	MTT Fresh (Mean ± SD)	p-value	MTT 3 Months (Mean ± SD)	p- value
GENDER					
F	54 (98.2%)	93.0 ± 5.3 (78-100)	0.797	92.9 ± 5.1 (83- 100)	0.777
M	1 (1.8%)	95.0		95.0	
SMOKING STATUS					
Never	41 (74.5)	93.0 ± 4.8 (84–100)	0.417	92.6 ± 4.9 (84–99)	0.239
Smoker	6 (10.9)	95.2 ± 4.4 (88–100)		96.2 ± 3.9 (91–100)	
Former	8 (14.5)	91.4 ± 7.5 (78–99)		91.8 ± 6.1 (83–98)	
COMORBIDITIES					
None	48 (87.3)	92.8 ± 5.2 (78–100)	0.0522	92.7 ± 5.0 (83–100)	0.068
Yes	3 (5.4)	89.0 ± 4.6 (84–93)		89.7 ± 5.5 (84–95)	
Severe	4 (7.3)	98.2 ± 1.2 (97–100)		98.0 ± 1.4 (96–99)	
AGE					
< 40	5^9	95.4 ± 5.9 (85–100)	0.369	95.0 ± 5.8 (85–100)	0.477
40-60	36 (65,4)	95.2 ± 5.0 (84–100)		93.1 ± 4.9 (84–100)	
> 60	14 (25,4)	91.4 ± 5.6 (78–99)		91.8 ± 5.0 (83–99)	
NEOADJUVANT CHEMOTHERAPY					
No	32 (58.2)	93.1 ± 5.0 (84–100)	0.797	93.1 ± 5.1 (84–100)	0.777
Si	23 (41.8)	92.8 ± 5.6 (78–100)		92.7 ± 5.2 (83–100)	
CHT SUSPENSION DURATION					
> 12 months	14 (63.6)	93.1 ± 5.3 (84–100)	0.290	92.7 ± 5.2 (84–99)	0.436
6–12 months	4 (18.2)	88.7 ± 8.1 (78–96)		89.5 ± 5.9 (83–95)	
< 6 months	4 (18.2)	95.0 ± 3.6 (91–99)		94.0 ± 3.2 (90–97)	
IMMUNOTHERAPY					
No	43 (78.2)	93.1 ± 4.8 (84–100)	0.713	93.0 ± 4.8 (84–100)	0.745
Yes	12 (21.8)	92.5 ± 6.7 (78–100)		92.5 ± 6.0 (83–100)	
RADIOTHERAPY					
No	30 (54.5)	92.9 ± 5.4 (84–100)	0.959	92.6 ± 5.2 (84–99)	0.606
Yes	25 (45.4)	93.0 ± 5.1 (78–100)		93.3 ± 5.0 (83–100)	
HORMONE THERAPY					
No	32 (58.2)	94.2 ± 4.9(85–100)	0.046	94.0 ± 4.7 (85–100)	0.049
Yes	23 (41.8)	91.3 ± 5.3(78–99)		91.0 ± 5.2 (83–100)	

(e.g., tamoxifen vs. aromatase inhibitors), this aspect was not specifically analyzed in our study and could represent an interesting topic for future investigations.

This finding regarding hormone therapy should be considered preliminary and hypothesis-generating, given the limited sample size (n = 23) of this subgroup. Furthermore, potential confounding factors such as age, BMI, or cancer stage may contribute to the observed association and should be evaluated in future studies; future studies should also extend the tissue preservation interval to assess whether cellular vitality remains stable over longer periods for further uses.

Conclusion

In conclusion, cryopreservation of adipose tissue appears to be a valid technique, maintaining high levels of cellular vitality. Hormone therapy significantly affects the vitality of adipose tissue, both fresh and thawed. These results provide a promising outlook for the use of cryopreserved fat in clinical practice and suggest future directions to further improve fat grafting protocols and fat harvesting timing.

In light of our results and the high percentage of patients requiring hormone therapy, in order to optimize surgical timing, it is advisable to establish shared guidelines for harvesting fat during the mastectomy rather than in subsequent surgical stages.

Ethical approval

Not required.

Funding

None.

Conflicts of interest

None declared.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bjps.2025.06.049.

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