Adipose tissue represents an important, easily available source of mesenchymal stem cells (ADASc) for regenerative medicine. These cells are abundant in the stromal vascular fraction (SVF) and they share many characteristics of bone marrow-derived stem cells, including a significant ability for multilineage differentiation. An intriguing challenge is posed by the choice of the optimal site and collecting technique according to subject characteristics. In this work, Coleman’s abdominal liposuction or dorsal resection have been employed, and SVF yield was evaluated in terms of cell recovery and vitality, taking into account the effect of gender, age and body mass index (BMI).

A total number of 37 informed subjects (14 males and 23 females) were enrolled for adipose tissue recovery. In order to evaluate the effect of age, BMI, gender and recovery technique on SVF yield and vitality, an analysis of covariance model (ANCOVA) was applied, considering collection technique and gender as fixed factors, and age and BMI as covariates, possibly aging as confounding variables. ANCOVA results indicate that Coleman’s liposuction technique leads to higher recovery rates of stromal vascular fraction with respect to resection, taking into account gender, body mass index and age of subject. The high recovery rates should be confirmed by the SVF selection by plating, typization, as well as differentiation potentiality for stem cell regenerative therapy.

## 1 Introduction

Adipose tissue represents an important, easily available source of mesenchymal stem cells (ADASc) for regenerative medicine. These cells are abundant in the stromal vascular fraction (SVF) and they share many characteristics of bone marrow-derived stem cells, including a significant ability for multilineage differentiation[1]. An intriguing challenge is posed by the choice of the optimal site and collecting technique according to subject characteristics. In this work, Coleman’s abdominal liposuction [2] or dorsal resection have been employed, and SVF yield was evaluated in terms of cell recovery and vitality, taking into account the effect of gender, age and body mass index (BMI).

## 2 Experimental methods

<table>
<thead>
<tr>
<th>Recovery Technique</th>
<th>Subjects</th>
<th>Age Mean ± st dev</th>
<th>BMI Mean ± st dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASPIRATION</td>
<td>9</td>
<td>31.6±19.19</td>
<td>23.5±5.56</td>
</tr>
<tr>
<td>RESECTION</td>
<td>28</td>
<td>58.3±18.8</td>
<td>27.7±5.34</td>
</tr>
</tbody>
</table>

A total number of 37 informed subjects (14 males and 23 females) were enrolled for adipose tissue recovery. Suction harvesting of sub-umbilical subcutaneous fat tissue was manually performed by negative pressure using a 10-mL syringe, with a blunt tip cannula and processed following Coleman’s technique [2].
Resected fat samples were obtained during surgery for herniated disc disease (standard microdiscectomy): adipose tissue was gently resected with scissors from the subcutaneous tissues. Age, BMI and harvesting technique are summarized in Table 1. In the operating room adipose samples were suspended in PBS with penicillin/streptomycin 1%, put into a sterile box, forwarded to the laboratory at the temperature of 4°C and processed following the GMP guidelines. Adipose tissue samples (about 3 mL) were digested with 0.05% collagenase in PBS plus penicillin 100 IU/mL and streptomycin 100 µg/mL for two hours. SVF was centrifuged and washed twice with PBS medium, as reported by Zuk et al. [3]. Harvested adipose stromal cells were counted under the microscope, and their vitality was assessed by the trypan blue exclusion technique in Burker chamber. SVF yield was calculated, and the cell number and live cell number yield for volume of treated tissue was estimated. In order to evaluate the effect of age, BMI, gender and recovery technique on SVF yield and vitality, an analysis of covariance model (ANCOVA) was applied, considering collection technique and gender as fixed factors, and age and BMI as covariates, possibly aging as confounding variables. Significance level was set at alpha=0.05.

3 Results and discussion

After the first hour of adipose tissue digestion (Fig. 1(a)), the samples appeared partially digested, whereas SVF was isolated from the extracellular matrix within two hours (Fig. 1(b)). ANCOVA results for differences in SVF recovery according to collection technique are reported in Table 2: the techniques appear strongly different in terms of cell yield, since lipoaspiration gave better yield results than resection (almost 8 times higher). Vitality results are similar in both of the groups.

**Table 2:** SVF yield (million SVF cells/mL of treated tissue), SVF vitality (%) and live SVF yield (live SVFcell millions/mL of treated tissue). Different letters indicate a P<0.001 difference between techniques within the same parameter.

<table>
<thead>
<tr>
<th>Recovery Technique</th>
<th>SVF yield mean±SEM</th>
<th>SVF vitality mean±SEM</th>
<th>live SVF yield mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASPIRATION</td>
<td>20.8±3.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.7±4.24</td>
<td>17.1±2.91&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>RESECTION</td>
<td>2.7±1.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>77.9±2.20</td>
<td>2.2±1.52&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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ANCOVA also indicates that neither gender nor covariates (age and BMI) influence the output variables. A similar collection technique effect in was reported by von Heimburg et al [4], on a sample of 20 subjects: after SVF isolation, cells were plated and cultured for 24 hours; adherent cells were trypsinized, counted, with 80,000-350,000 cells/g tissue yield. In our trial, cells were counted soon after digestion: the higher quantitative result reported in the present study can be due to high heterogeneous cell population. Vitality can be influenced by tissue processing (collagenase concentration, digestion timing), as reported by Bakker et al. [5]: the lack of significance in vitality between groups could be due to the same digestion conditions employed for all samples.

Conclusions

Coleman’s liposuction technique leads to higher recovery rates of stromal vascular fraction with respect to resection, taking into account gender, body mass index and age of subject. The high recovery rates should be confirmed by the SVF selection by plating, typization, as well as differentiation potentiality for stem cell regenerative therapy.

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References