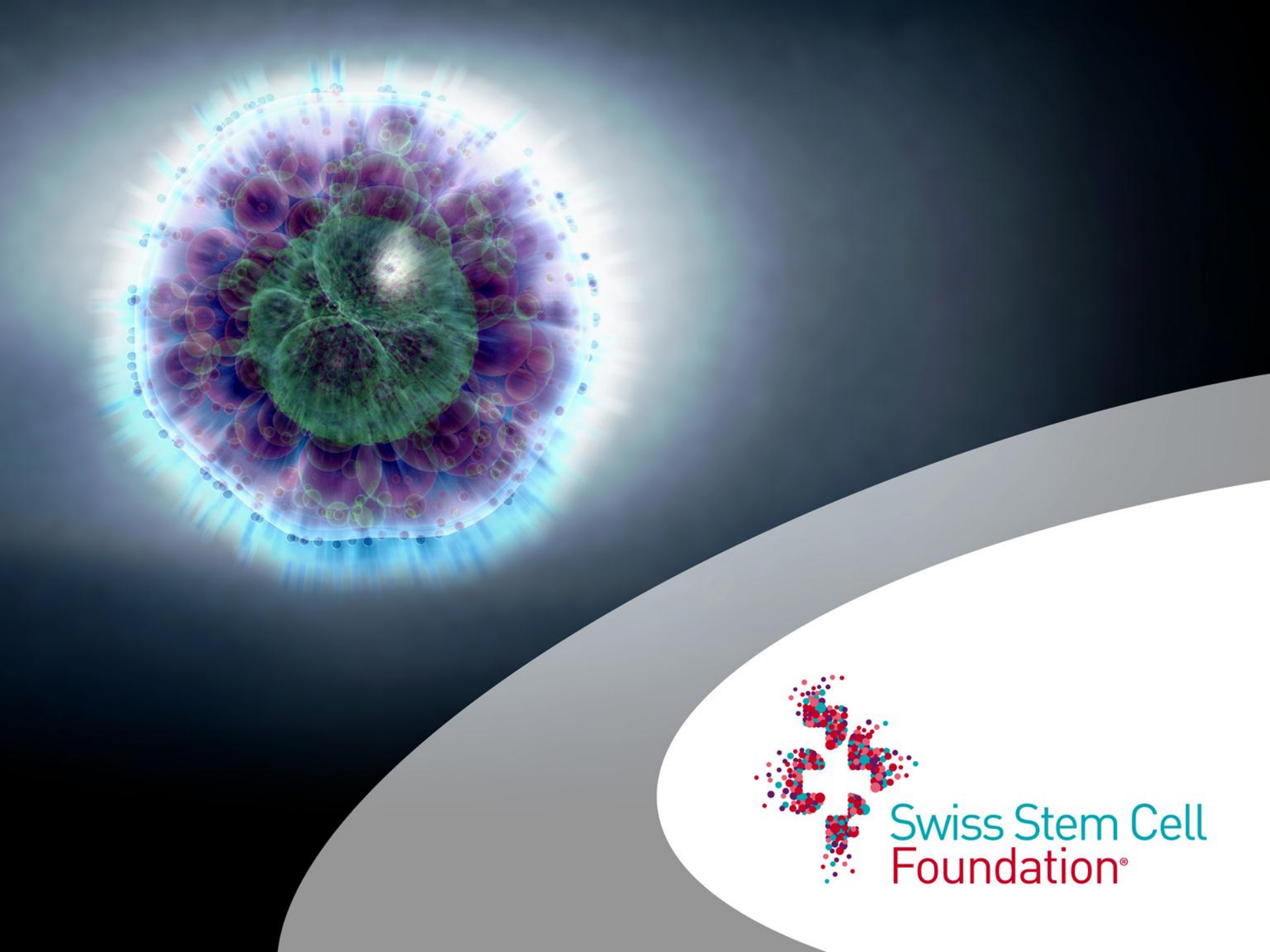
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Inter-variability of adipose tissue samples

iCAST2017

Dr Luca Mariotta / Dr Christian Caprara

Introduction

Introduction

- Adipose tissue is the best candidate from which to obtain stromal/stem cells because:
    - it is an abundant tissue
    - it is easily accessible
    - it is a rich source of Stromal Vascular Fraction (SVF) cells
- Many researchers consider the use of freshly-isolated non-expanded cells as safer and more practical for cell therapies

    for this purpose, the number of recovered cells must be sufficiently high to reach the therapeutic dosage

Introduction

- The aging process is known to affect the regeneration capability of adult tissues.
- Adipose tissue is also affected by age-related changes:

  Adipose tissue-derived mesenchymal cell number, differentiation potential and angiogenic functional capacity are affected by age (Alt et al, 2012; Madonna et al, 2011)
- However, there are opposing results related to mesenchymal cell yield after adipose tissue processing in aged human patients (de Girolamo et al, 2009; Wu et al, 2013; Aust et al, 2004)

Aim of the Study

- Aim of this study is to investigate the influence of age and gender on the yield of SVF cell populations

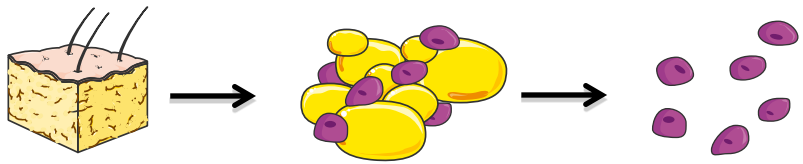
- Question:

  does the subject's age and/or gender influence the SVF cell yield?

Methods

Stromal Vascular Fraction (SVF)

The cellular part of lipoaspirate without adipocytes and extracellular matrix



J Transl Med. 2007 Oct 31;5:55.

"In vitro" and multicolor phenotypic characterization of cell subpopulations identified in fresh human adipose tissue stromal vascular fraction and in the derived mesenchymal stem cells.

Astori G[1], Vignati F, Bardelli S, Tubio M, Gola M, Albertini V, Bambi F, Scali G, Castelli D, Rasini V, Soldati G, Moccetti T.

J Cardiovasc Transl Res. 2011 Apr;4(2):200-10. doi: 10.1007/s12265-011-9257-3. Epub 2011 Feb 15.

Adult human adipose tissue contains several types of multipotent cells.

Tallone T[1], Realini C, Böhmler A, Kornfeld C, Vassalli G, Moccetti T, Bardelli S, Soldati G.

Cryobiology. 2014 Oct;69(2):211-6. doi: 10.1016/j.cryobiol.2014.07.005. Epub 2014 Jul 15.

Frozen adipose-derived mesenchymal stem cells maintain high capability to grow and differentiate.

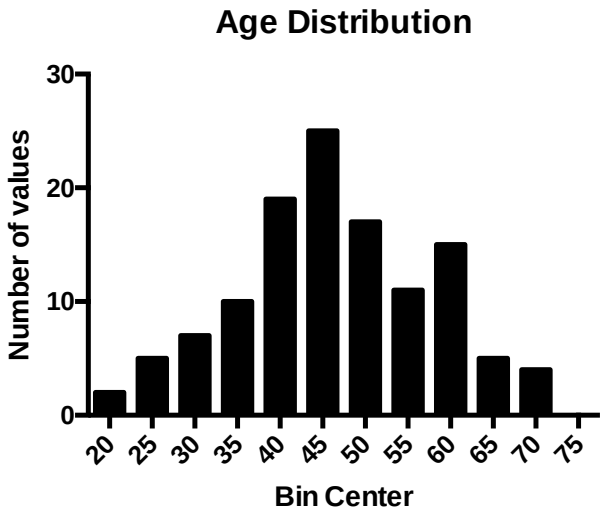
Minonzio G[1], Corazza M[1], Mariotta L[1], Gola M[2], Zanzi M[3], Gandolfi E[4], De Fazio D[5], Soldati G[6].

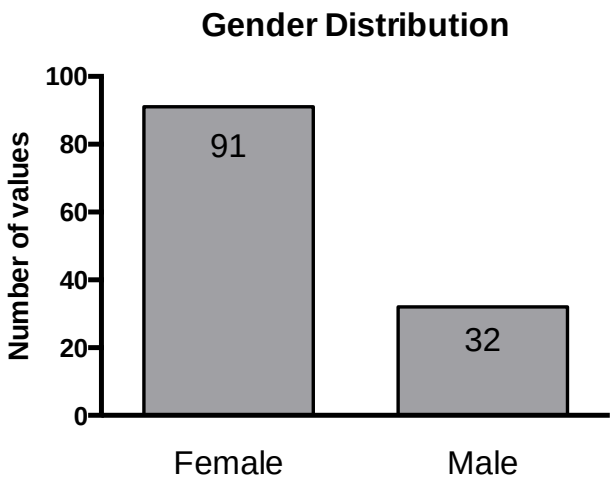
Methods: SVF Isolation

- SVF isolation with enzymatic digestion
- N=123 donors (91 F, 32 M)
- Biases:

  different surgeons, different liposuction techniques, different harvesting anatomical sites, different BMIs

  => accompanying form





Methods: SVF characterization

- Cell count and viability: Nucleocounter (propidium iodide-based automated method)

- SVF cell sub-populations: Navios Flow Cytometer (Immunophenotyping):

swissmedic

CERTIFICATE OF GMP COMPLIANCE

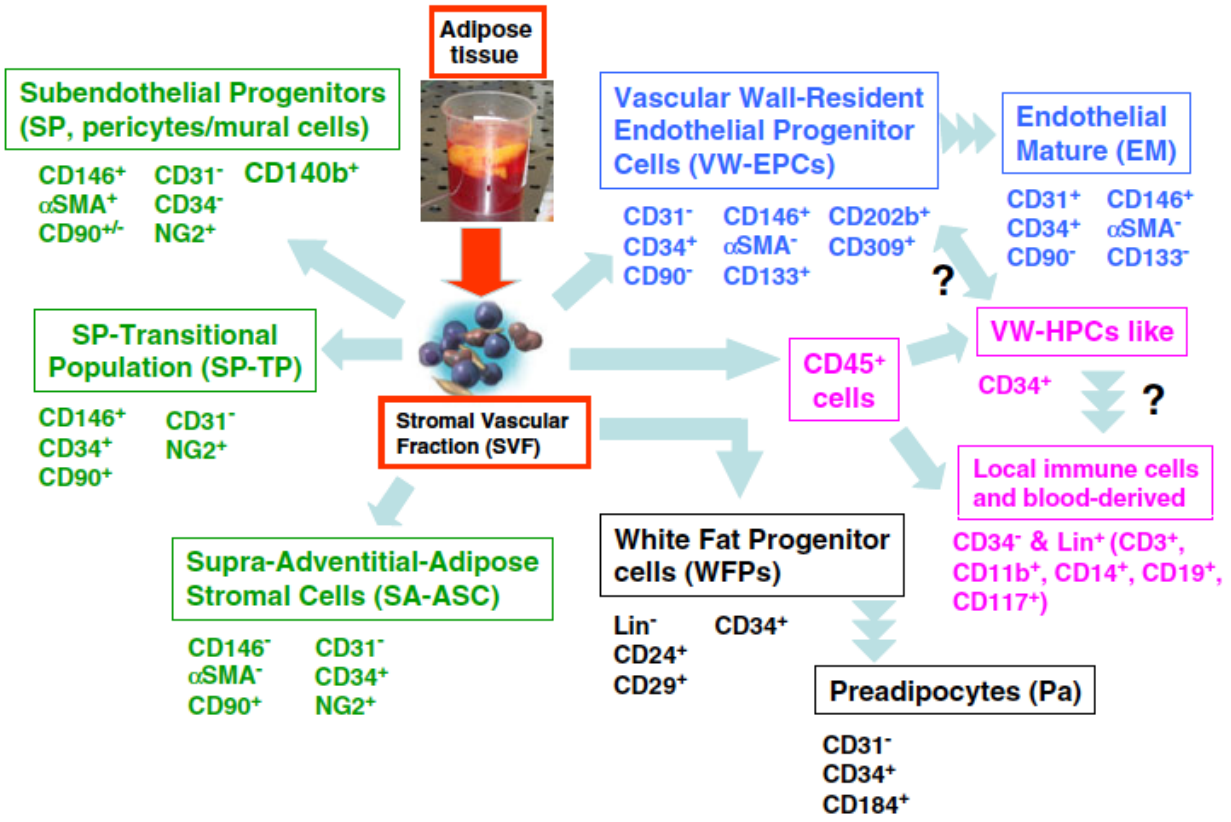
We certify herewith

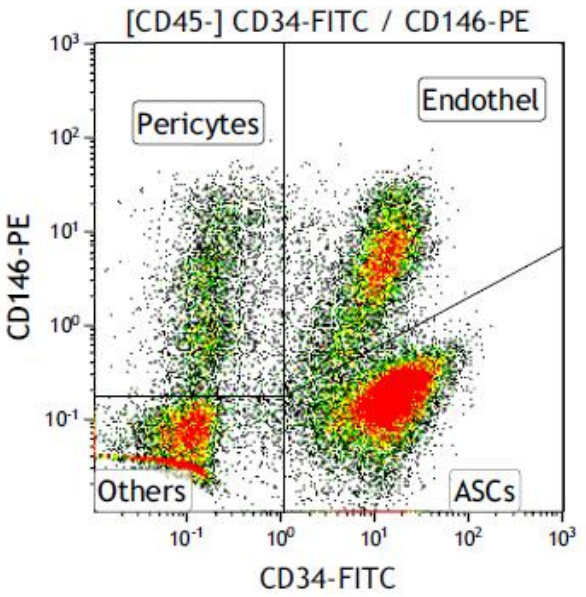
that the company **Swiss Stem Cell Foundation, Via Tesserete 48, 6900 Lugano, Switzerland** with its site **Swiss Stem Cell Foundation, Technoparkstrasse 1, 8005 Zürich, Switzerland,** has been duly authorized to manufacture and distribute transplant products, the licence includes the following product categories:

...logous use

Swiss Stem Cell Foundation

SVF is composed of several types of cells, comprised Adipose-derived mesenchymal Stem Cells (ASCs)

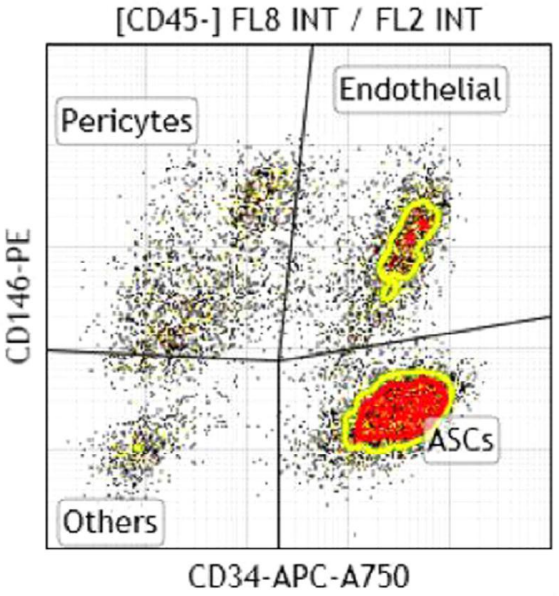




Tallone et al., 2011

Methods: Immunophenotyping

- Identification of nucleated cells: Syto40
- viability: 7-AAD
- SVF stained with:
  - ASCs: CD45- / CD34+ / CD146-
  - Endothelial cells: CD45- / CD34+ / CD146+
- Absolute count: Flow-Count Fluorospheres



Minonzio *et al*, 2014

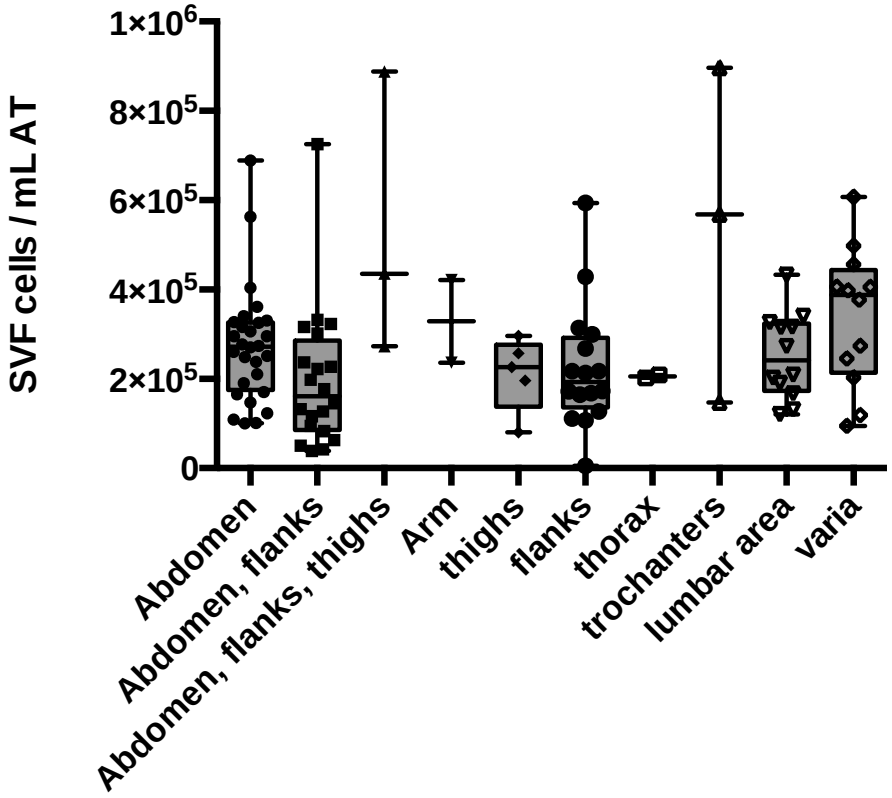
Methods: Statistical Analysis

- Correlation analysis: Pearson correlation coefficients
- Age grouped analysis: ANOVA

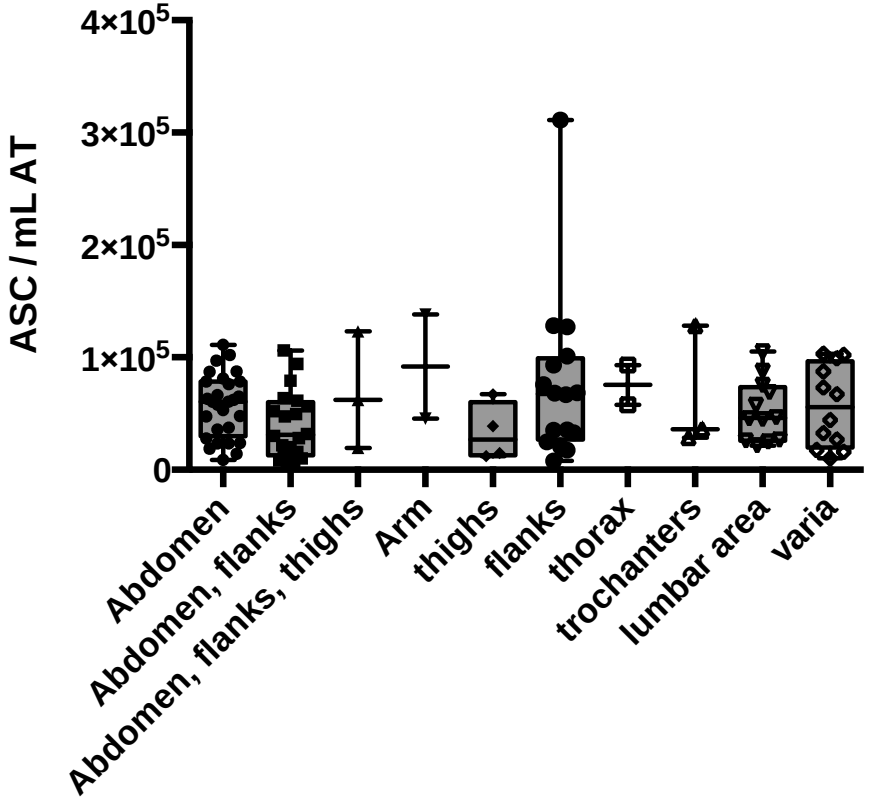
Results

No significant influence of the harvest anatomical site

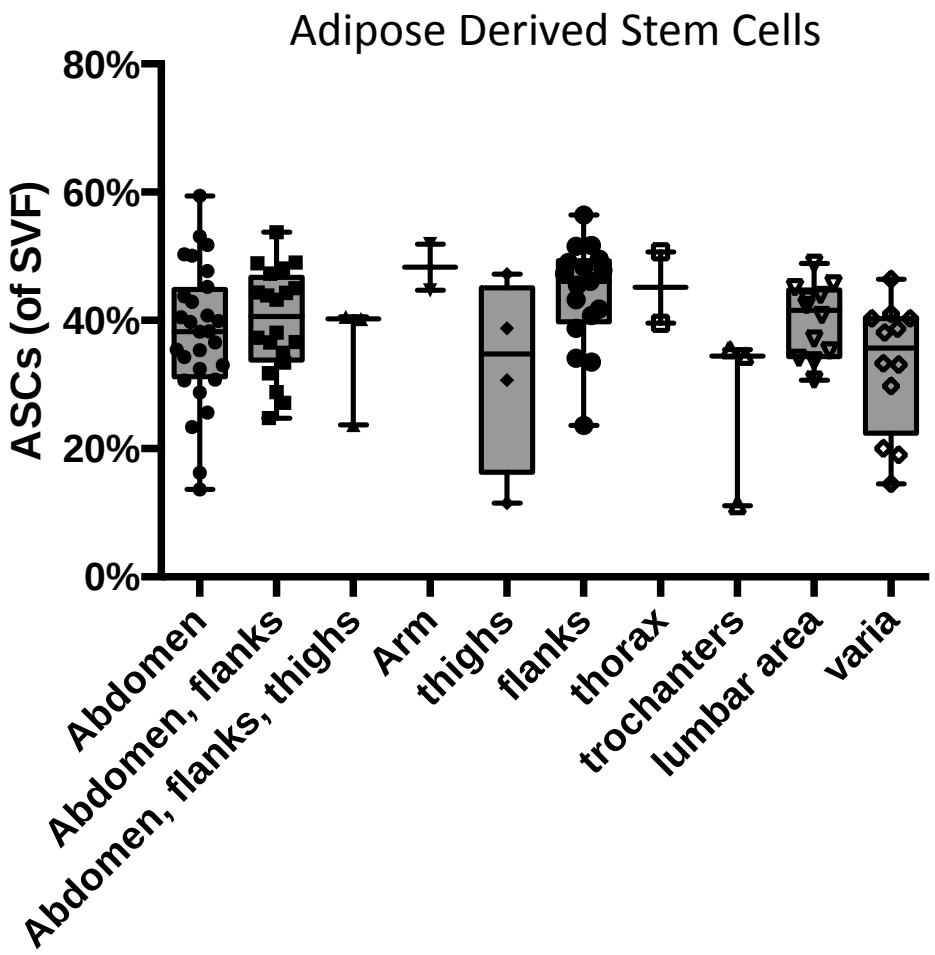
Total Nucleated Cells



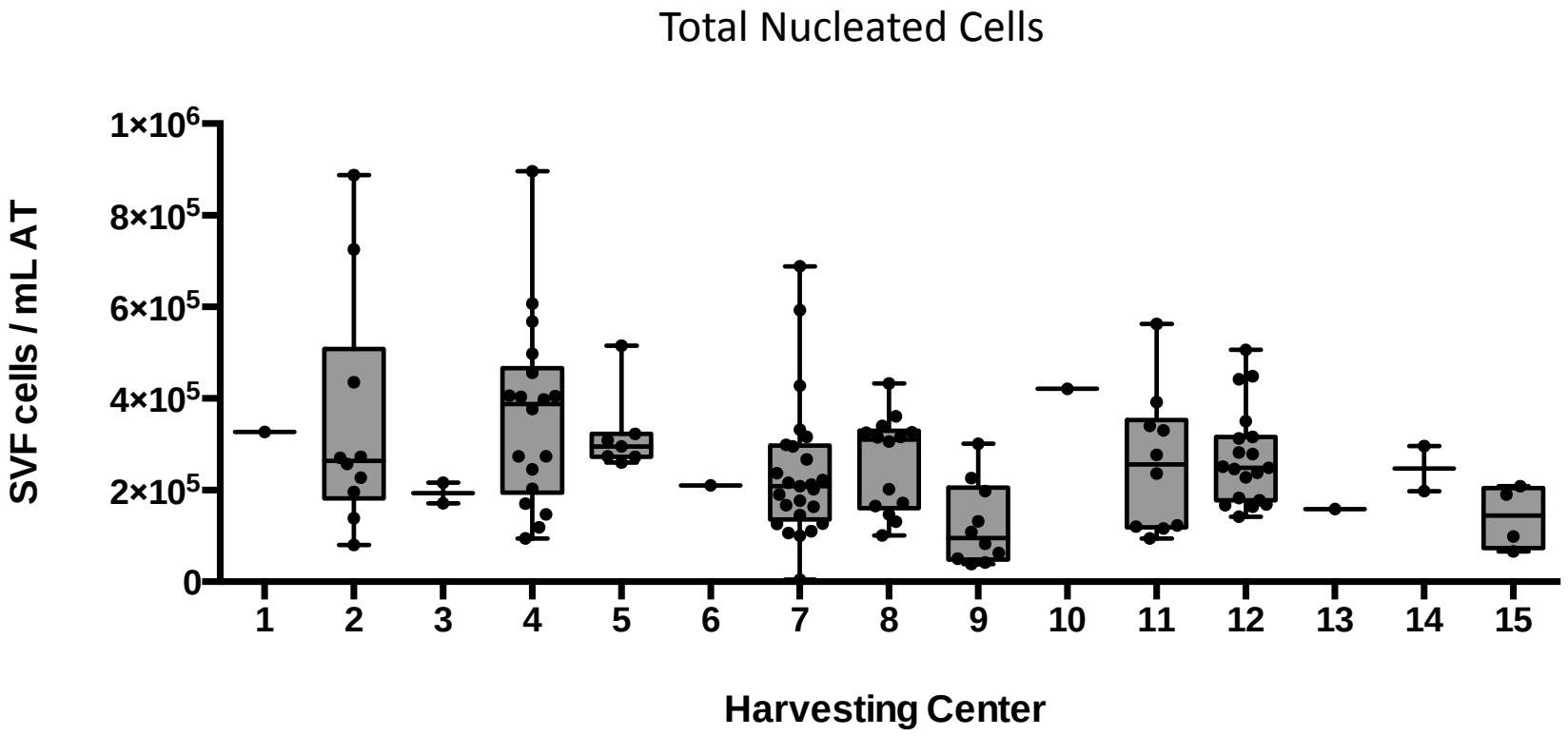
Adipose Derived Stem Cells



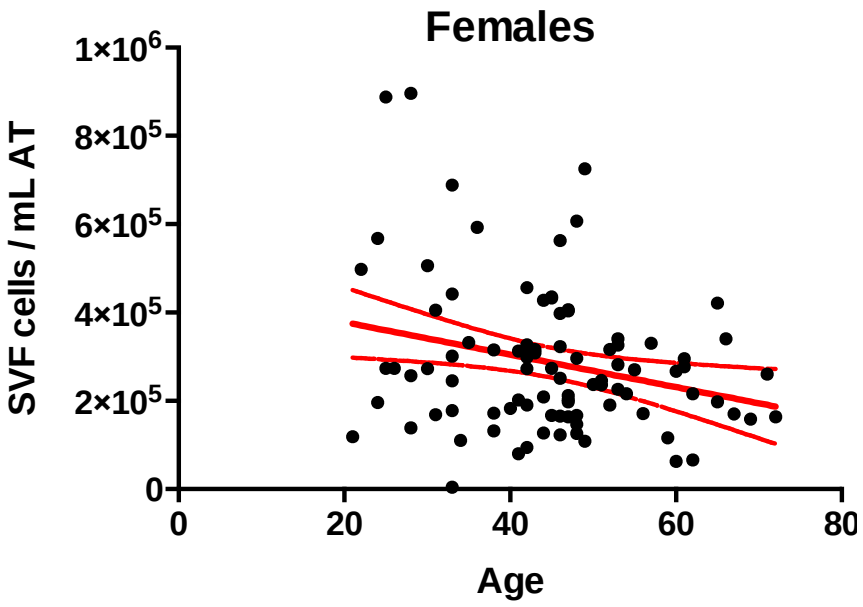
No significant influence of the harvest site



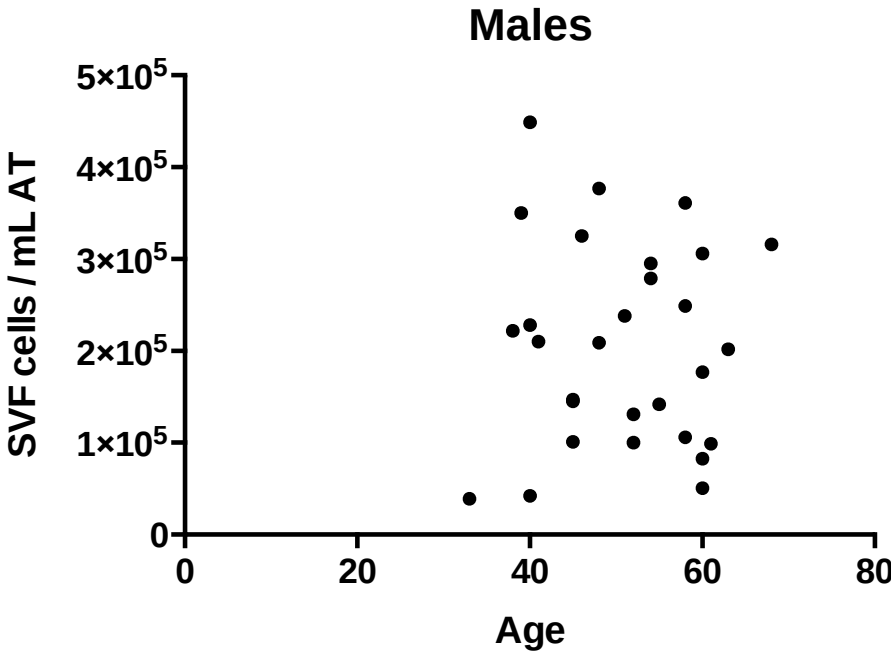
No significant influence of the harvesting center



SVF nucleated cells decrease with age in female donors

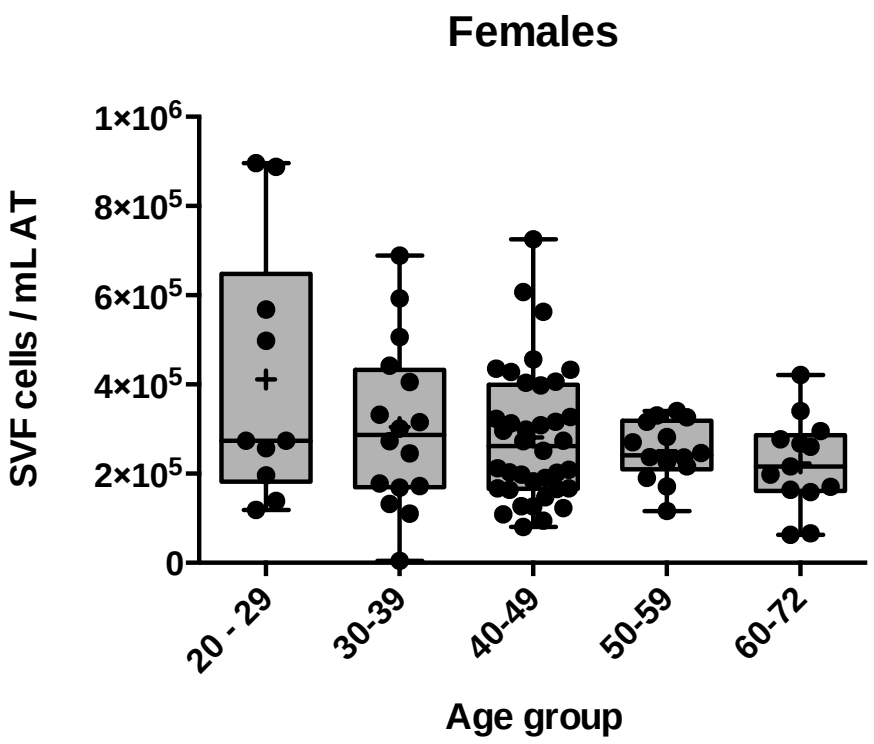


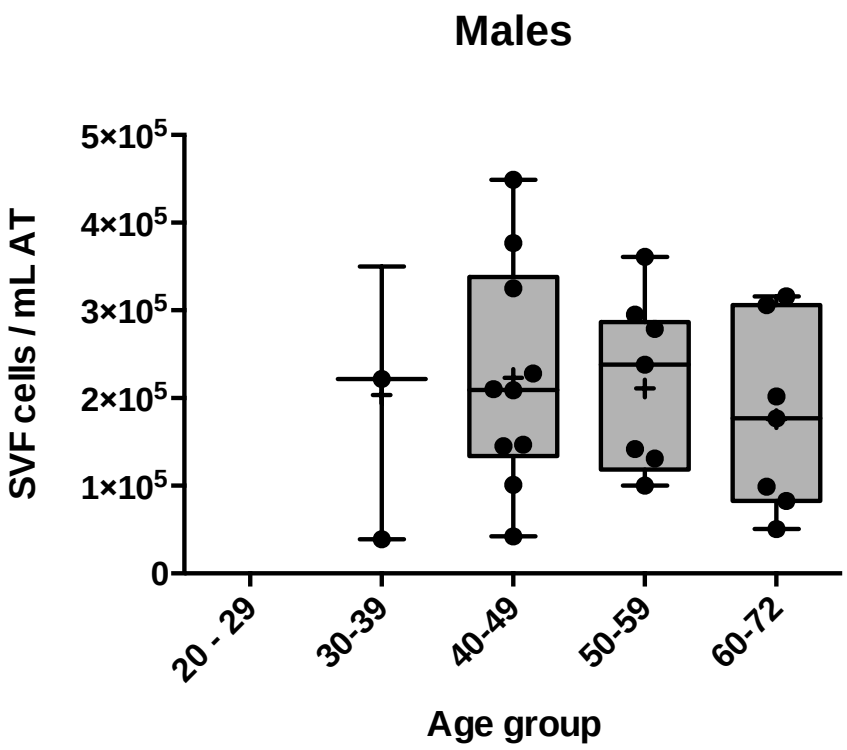
R squared: 0.06831
P (two-tailed): 0.0123
Significant? Yes (*)



R squared: 6.75 e-5
P (two-tailed): 0.9663
Significant? No

SVF nucleated cells decrease with age in female donors

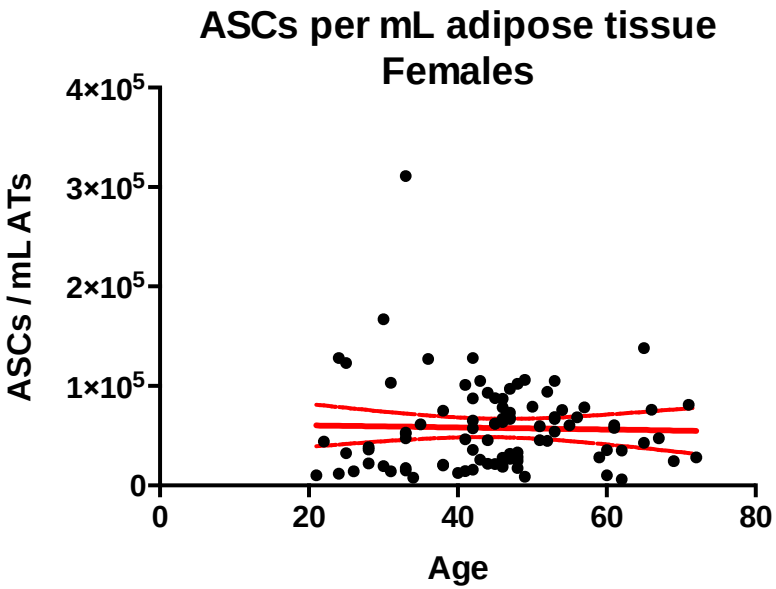


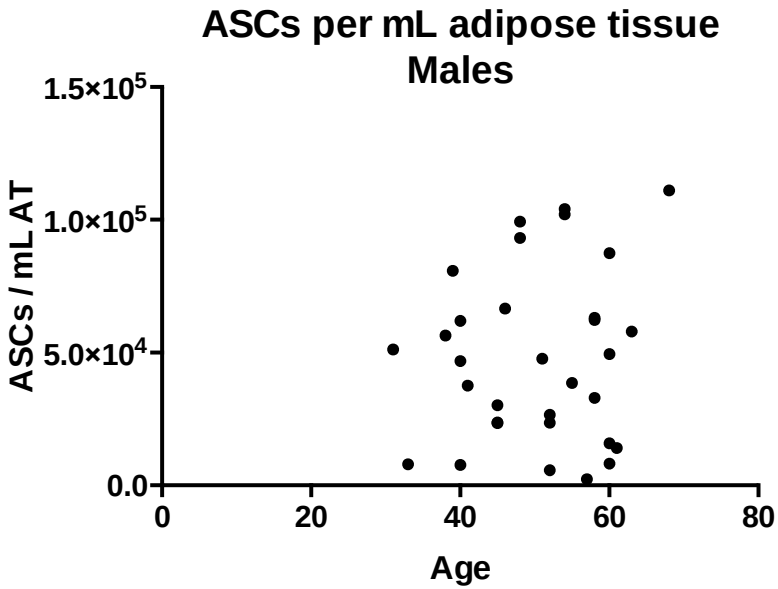


Brown-Forsythe test
P value 0.0078 (**)
Bartlett's test
P value < 0.0001 (****)

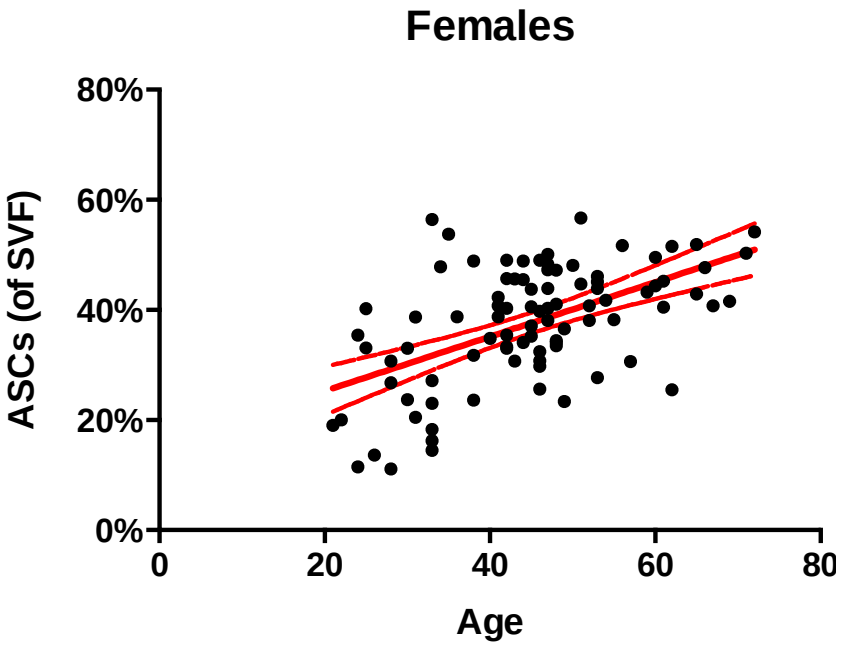
Brown-Forsythe test
P value 0.92
Bartlett's test
N/A

Adipose Derived Stem Cells (ASCs) remain constant with age

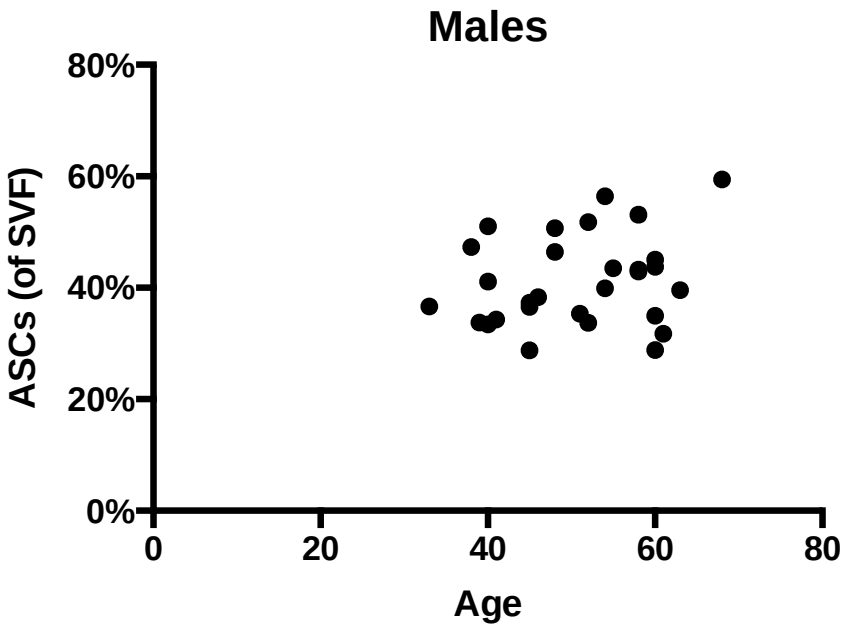




The percentage of ASCs in SVF increases with age in female donors

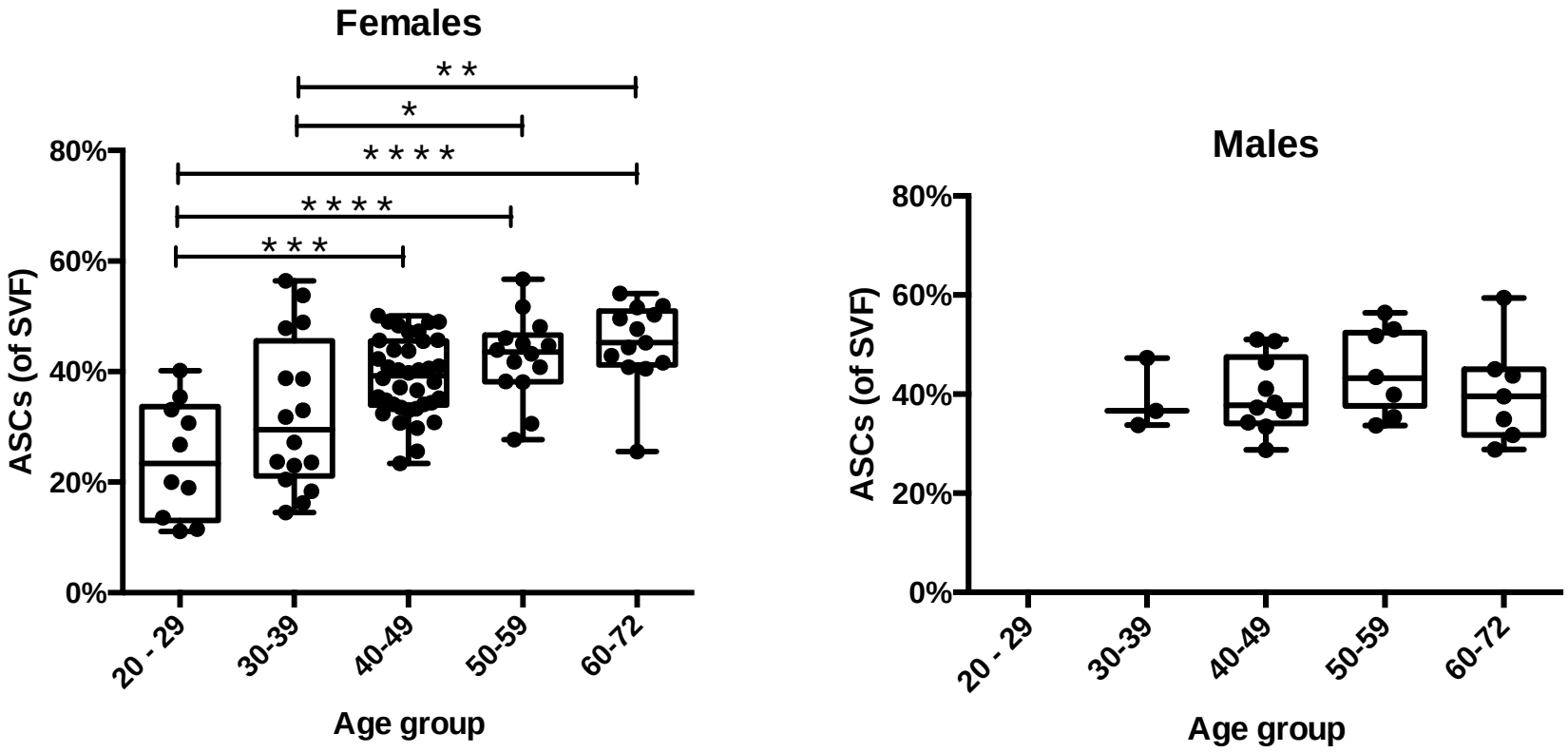


R squared: 0.2993
P (two-tailed): <0.0001
Significant? Yes (****)

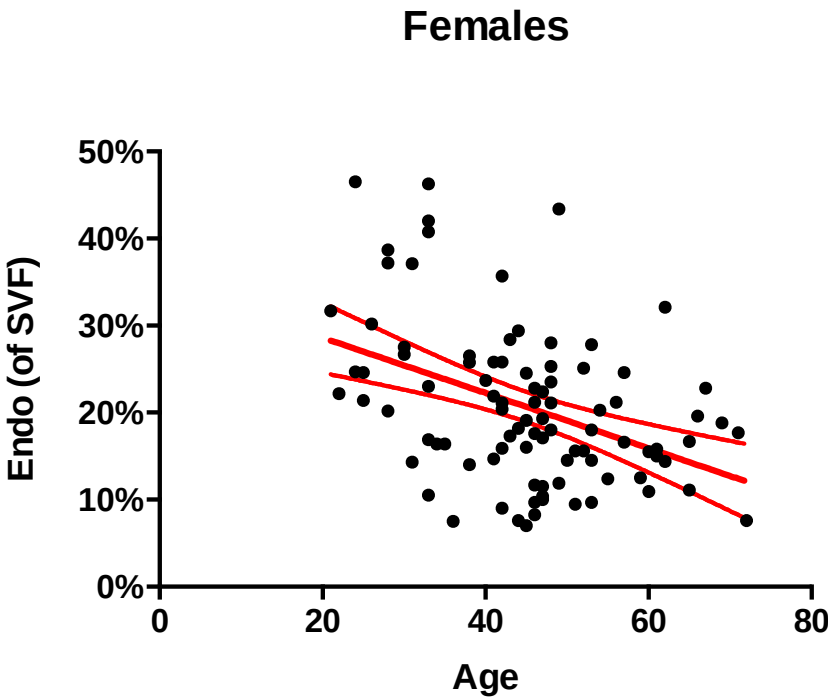


R squared: 0.0554
P (two-tailed): 0.2192
Significant? No

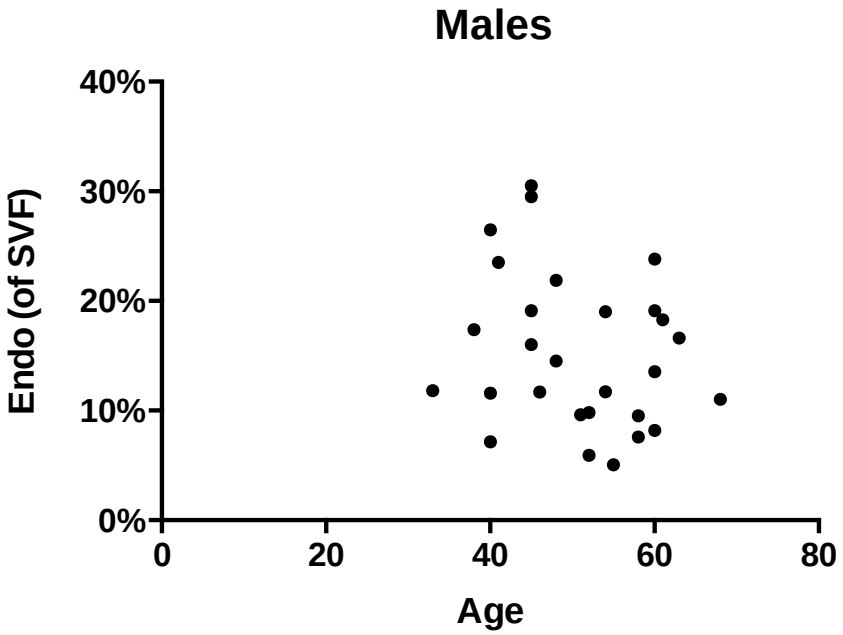
The percentage of ASCs in SVF increases with age in female donors



The percentage of endothelial cells in SVF decreases with age in female donors

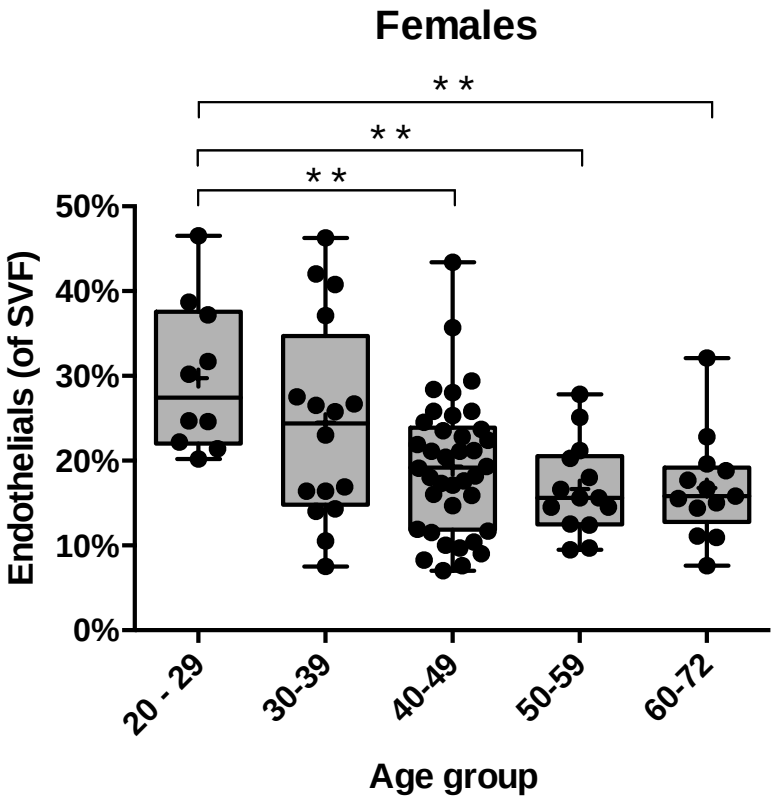


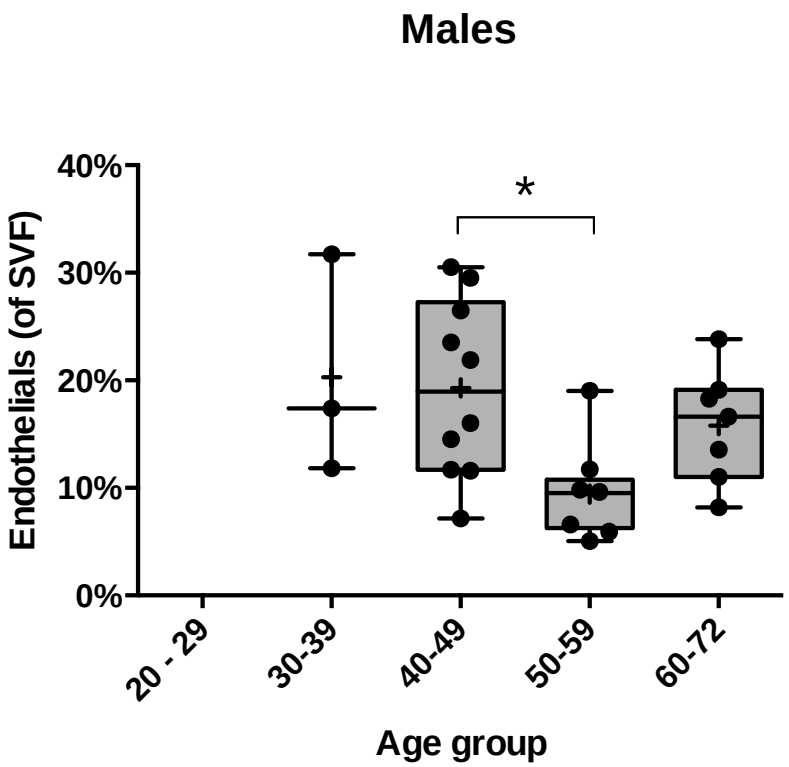
R squared: 0.1727
P (two-tailed): <0.0001
Significant? Yes (****)



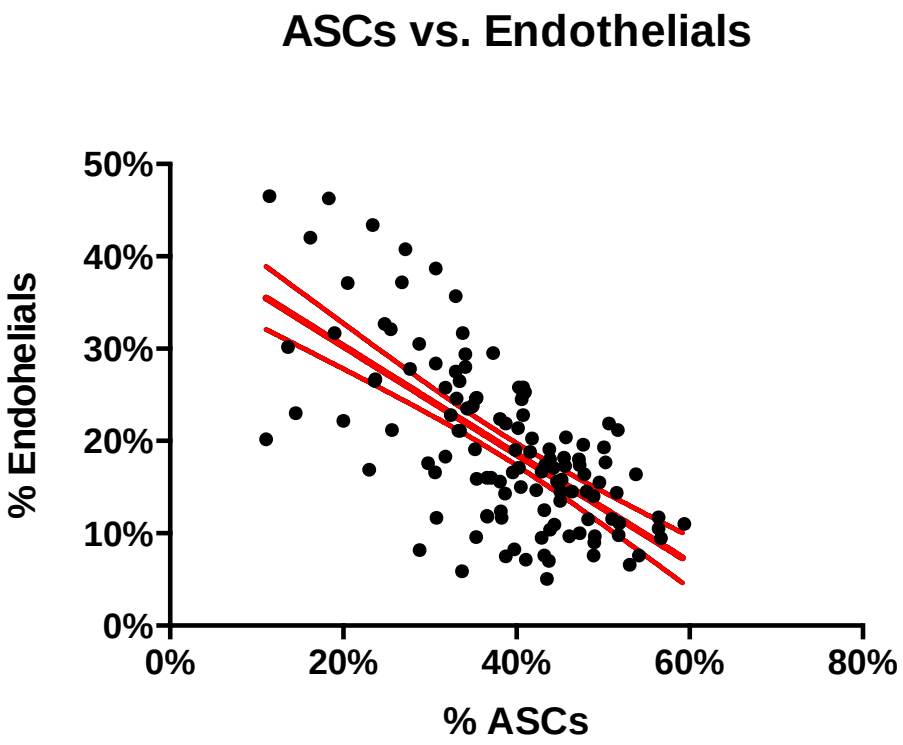
R squared: 0.0526
P (two-tailed): 0.2406
Significant? No

The percentage of endothelial cells in SVF decreases with age in female donors





The percentage of ASCs correlates negatively with the percentage of endothelial cells



R squared: 0.4410
P (two-tailed): <0.0001
Significant? Yes (****)

Summary of the Results

- The amount of nucleated cells in the SVF decreases in females as a function of age
- ASCs represent an increasing fraction of SVF cells as a function of age in female donors
  - concomitantly, the endothelial cell fraction decreases with aging
- More samples are needed to infer about age-dependant correlations male donor

Discussion and Outlook

Discussion

- Is the loss of differentiation ability responsible for the "accumulation" of ASCs in elderly donors? (Caso et al, 2013)
- Is an endothelial dysfunction at the basis of the "loss" of endothelial cells in elderly donors? (Chudek et al, 2006)
- Is there an hormonal influence (e.g estrogen, menopause)?
- Change in the expression of CD146 (discriminator between ASCs and endothelial cells)? How would it be with CD31 marker?

Outlook

- Differences in gene expression between young and elderly donors?
- Differences in differentiation and immunomodulatory potential between young and elderly donors?
- Age-dependant secretome analysis?
- Differences in cell potency?

Thank you for your attention!

Questions?

Special thanks to all collaborating physicians and SSCF team