Cellular and molecular basis of adipose tissue development: from stem cells to adipocyte physiology

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EAAP 2014
Copenhagen, Denmark
25 - 29 August 2014
65th annual meeting of the European Federation of Animal Science

Outline

- Introduction
- Diversity of adipose tissue cell types
- Origin and development of adipose tissues
- Adipose tissue: a dynamic tissue able to adapt to a variety of environmental and genetic factors
- Conclusion & Perspectives

Why adipose tissue development is a topic of great interest?

- The control of body fat distribution is of the upmost importance

- in human

↑ in the prevalence of obesity in the world,

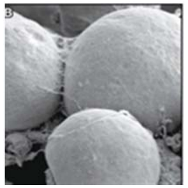
↑ risk of developing metabolic disorders.

- in livestock species

A relationship between the lean-to-fat ratio and production efficiency and meat quality traits.

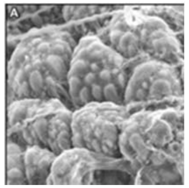
- A significant compartment in the body in term of mass and physiological functions

Two main types of adipose tissues with differences in morphology and functions



White adipose tissue

- Predominates after birth
- Contains white adipocytes (+/- beige adipocytes)
- Important for the storage and release of energy



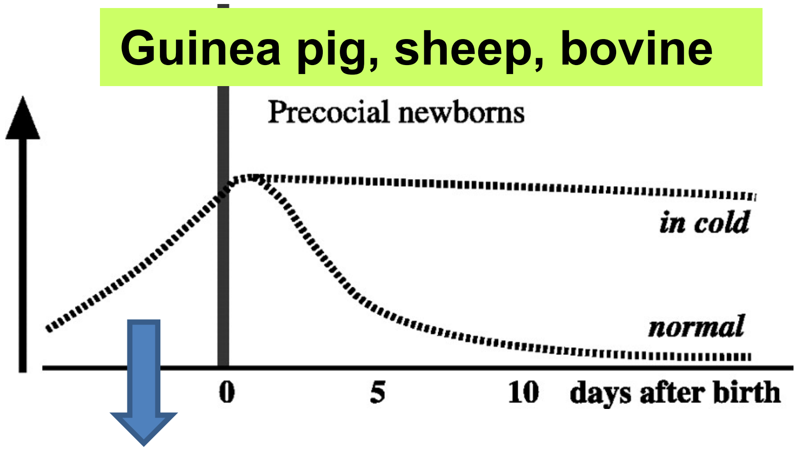
Brown adipose tissue

- Abundant in newborns and hibernating animals
- Contains brown adipocytes
- A thermogenic function

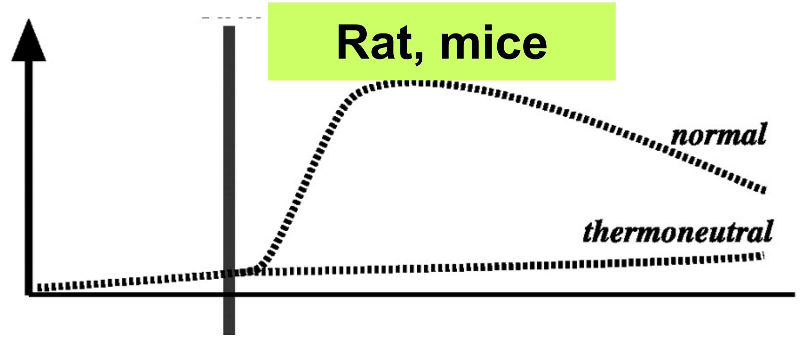
Brown adipose tissue (BAT) in different species

Found in mammals with exceptions: not detected in pigs

Birth



Birth



180 and 260 dpc fetuses

↳ **perirenal adipose tissue is a mix of white and brown adipocytes**

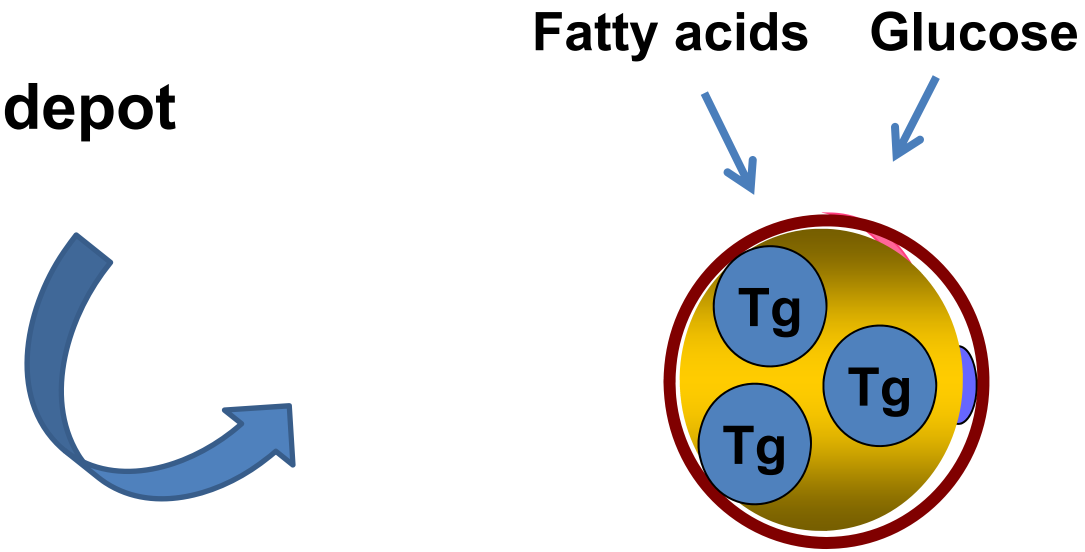
- ❑ **present in adults: hibernating animals, rodents, humans**
- ❑ **BAT → WAT in large mammals ?**

Cannon & Nedergaard, 2004; Taga *et al*., 2012

Functions of white adipose tissue

- An insulating layer (reduction of heat loss through the skin) and a protective function (providing mechanical protection and support around the major organs)

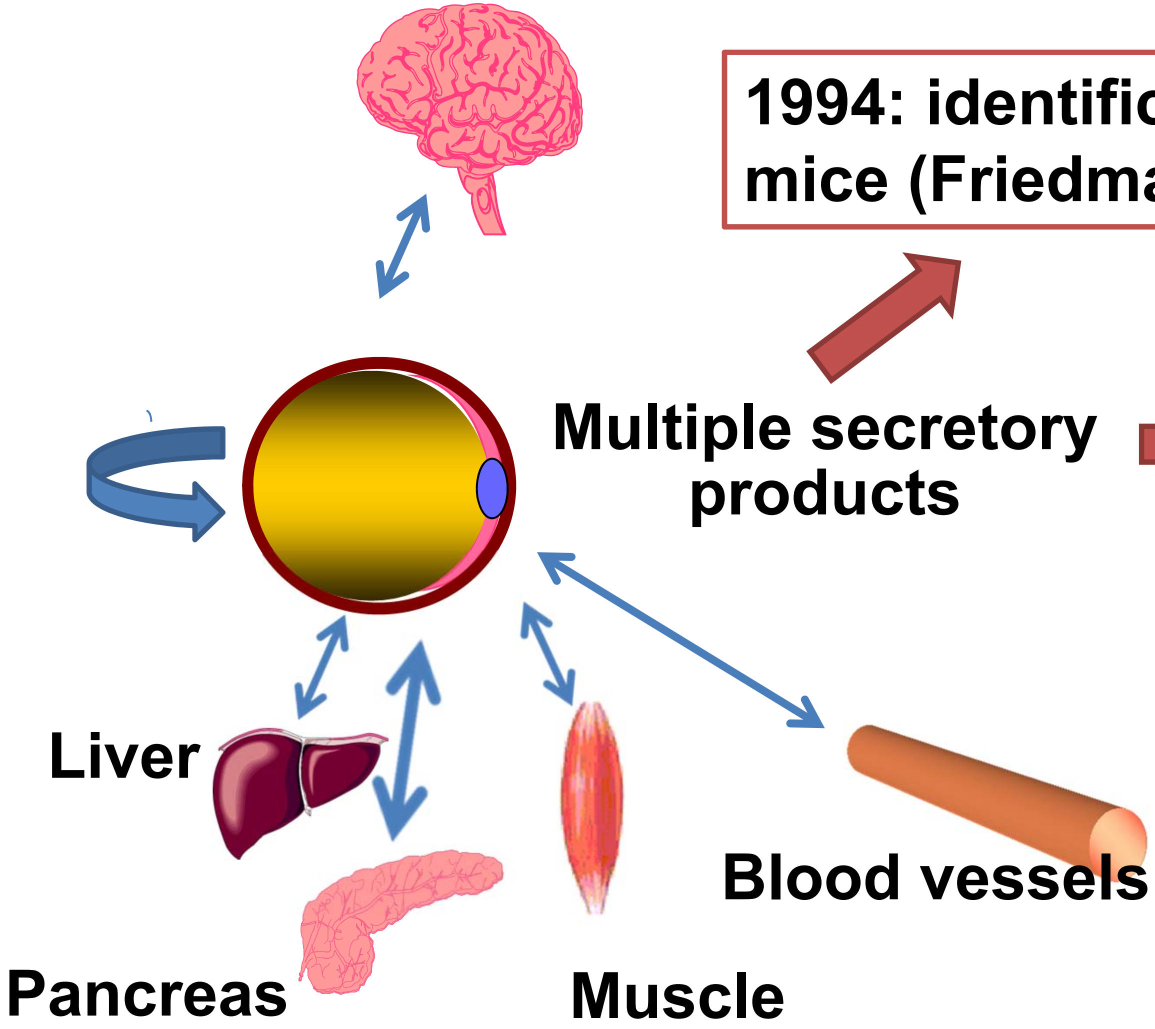
- Energy storage depot



Functions of white adipose tissue

- An insulating layer (reduction of heat loss through the skin) and a protective function (providing mechanical protection and support around the major organs)
- Energy storage depots
- Secretory function

White adipose tissue : a secretory/endocrine organ



1994: identification of leptin in mice (Friedman group)

Adipokines

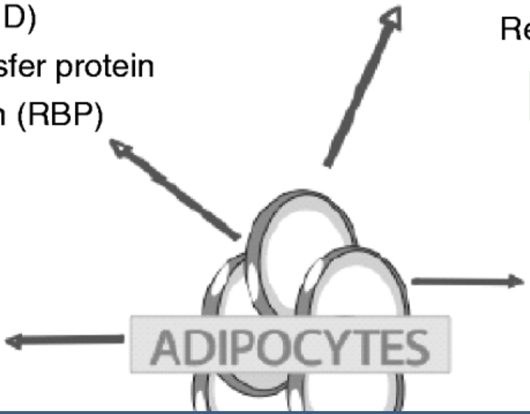
Secretory function

Lipid and lipoprotein metabolism
- Lipoprotein lipase
- Acylating stimulating protein
- Autotaxin (fosfolipase D)
- Cholesteryl ester transfer protein
- Retinol binding protein (RBP)

Energy metabolism

Leptin	Galanin + receptors
Adiponectin	RBP
Resistin	IL-6

Immune system and acute-phase reactants
- TNFα
- IL-6, IL-8, IL-1, IL-1 Ra
- Factor C3, B and D of alternate complement system
- Monocyte chemoattractant protein-1
- α1-Acid glycoprotein



Food intake
- Leptin
- Adiponectin

The discovery and characterization of proteins secreted by white adipose tissue is still ongoing.

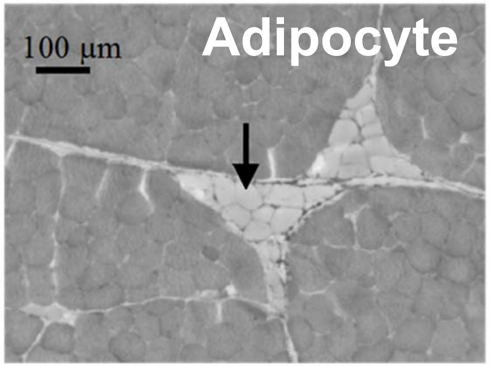
- Angiopoietin-2
- Angiotensinogen

- Plasminogen activactor inhibitor-1
- MMP-2 and -9
- TIMP-1 and -2

Several white adipose depots in the organism

- Large depots with large numbers of cells of different sizes that are located subcutaneously, viscerally and between muscles

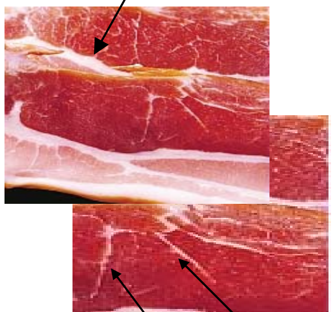
- Small groups of cells located between muscle fiber bundles



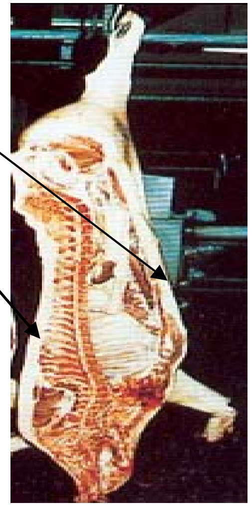
Several white adipose depots in the pig

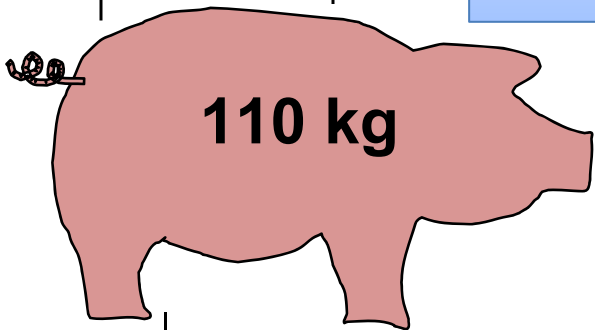
= 1 to 35-40% of body mass
Variation with age, genotype and nutrition

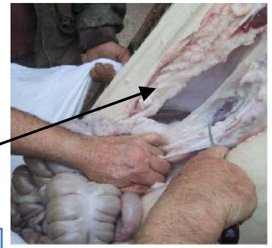
Intermuscular AT (25-33%)



Subcutaneous AT (65-75%)







Intramuscular AT (1-2%)

Visceral AT (5%) (perirenal)

Dumont & Février, 1957; Girard et al., 1998; Monziols et al., 2005

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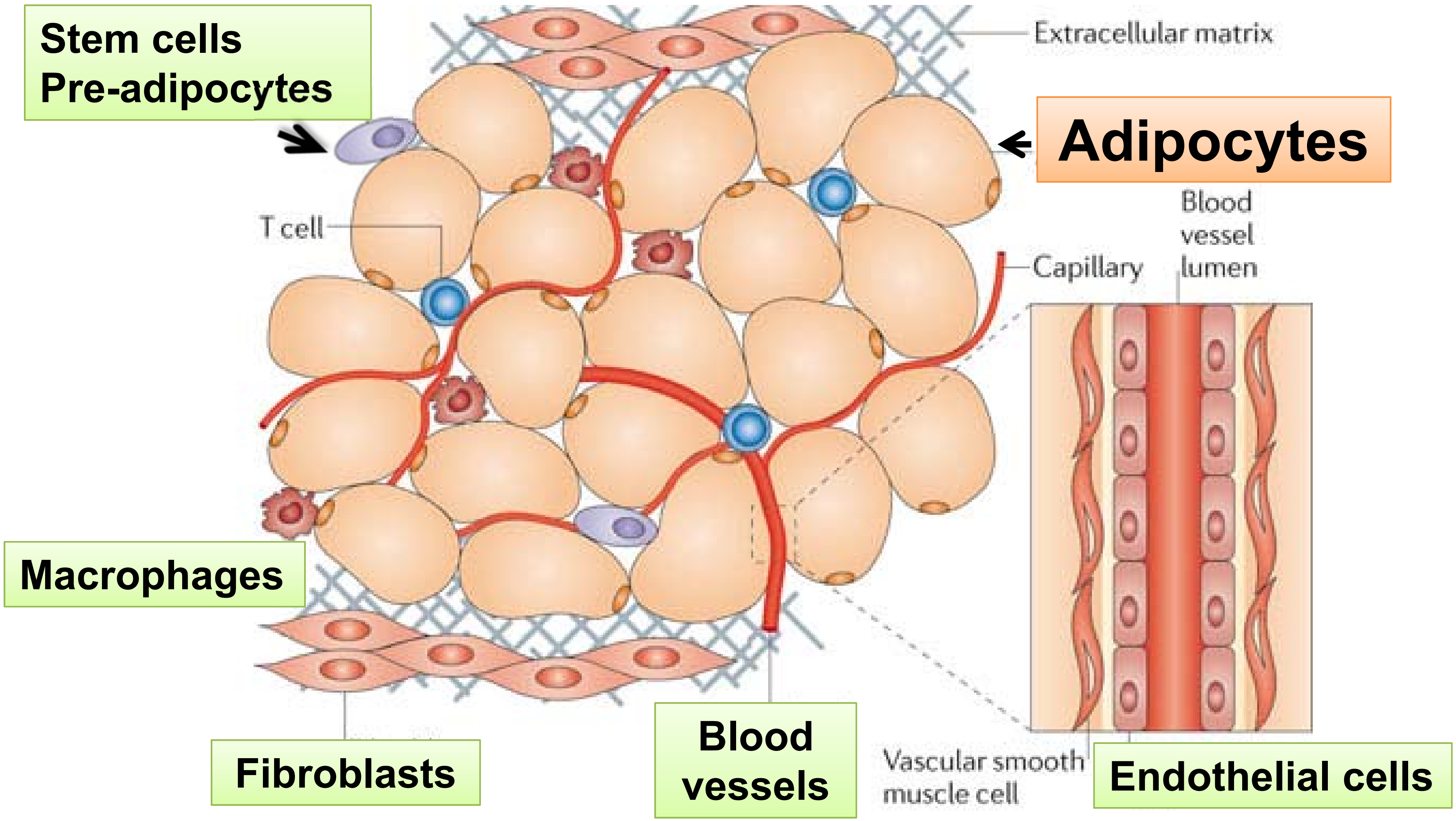
White adipose tissue contains many cell types

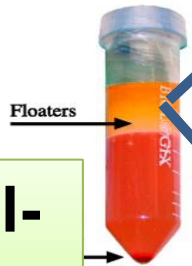


Adapted from Ouchi *et al*., 2011

Features of mature adipocytes compared with stromal-vascular cells *in vitro*

Adipose tissue

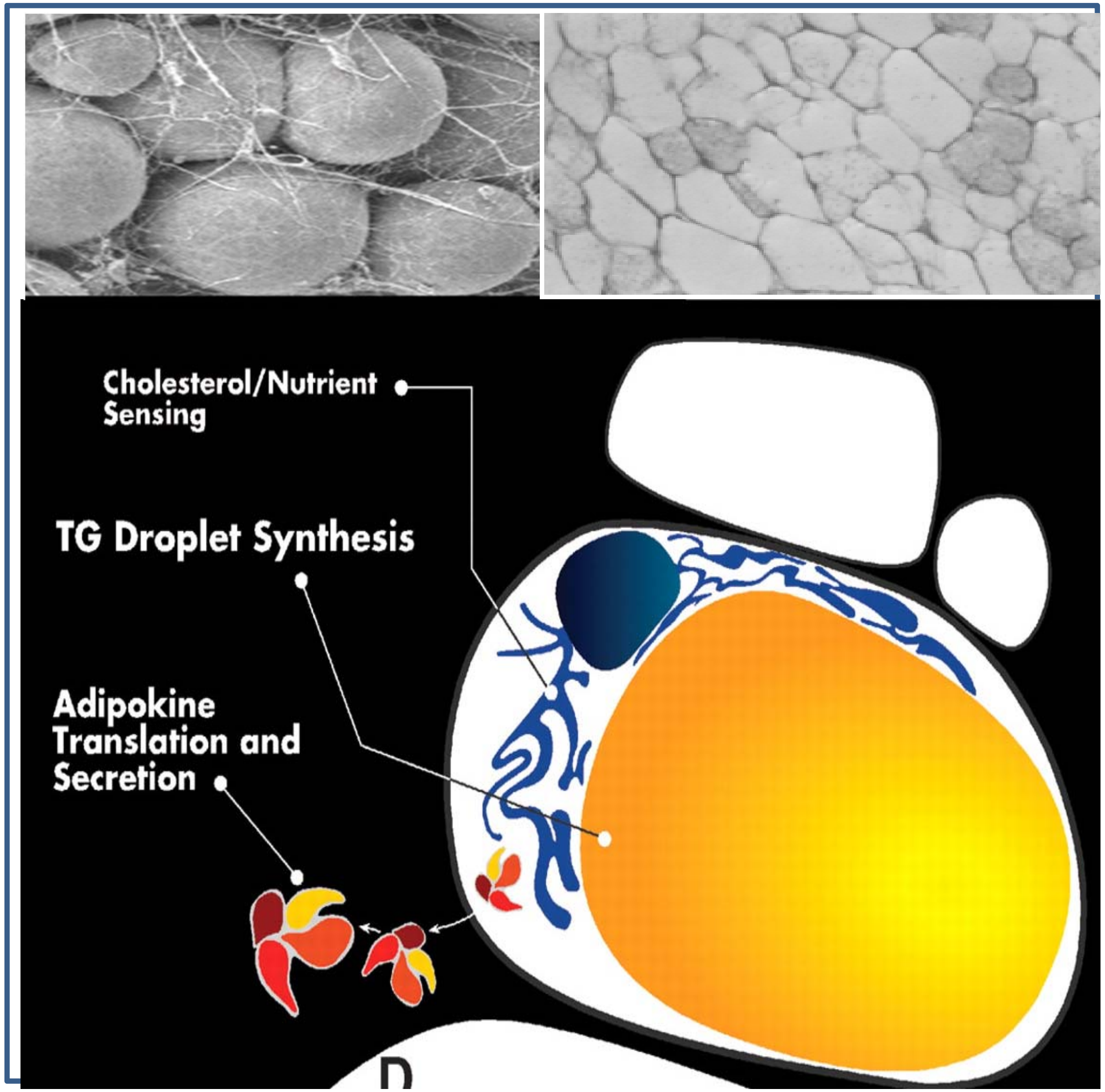
Collagenase digestion Centrifugation



Pellet = stromal-vascular fraction

Adipocytes
→ a floating layer
(triglyceride density = 0.96)

White adipocytes



- Predominating cells in adipose tissue (40-50% of total cells)
- White adipocytes = spherical cells with a wide range of diameters (10-120 µm)
- Cells detected in several tissues

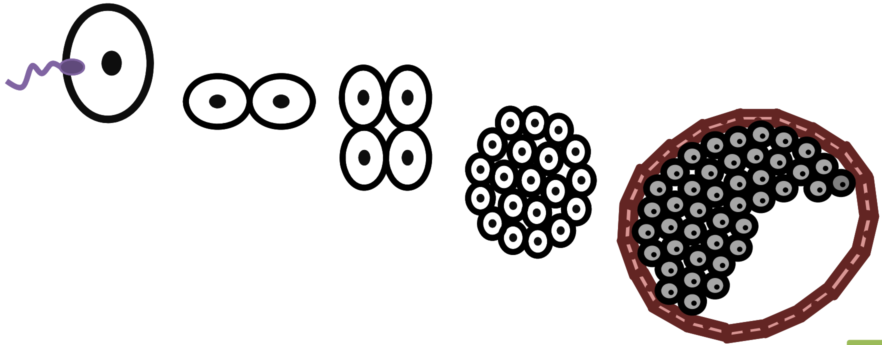
The recent identification of beige adipocytes in white adipose tissue

- **Also identified as "brite" or "brown/white"**
- **First detected in mice (Wu *et al*., 2012) and observed in human (Pisani *et al*., 2011) and sheep (Pope *et al*., 2014)**
- **Energy storage depot with the potential to express the mitochondrial membrane uncoupling protein 1 (UCP1)**
- **Role of beige adipocytes?**

White adipocyte features compared with brown and beige adipocytes

	Brown	Beige/Brite	White
			
Shape of lipid droplets	Multiple, small droplets		Single, large lipid droplet
Mitochondria	+++++		++
UCP1	High expression	Expression after cold exposure	Not detected
Function	Heat production with energy dissipation		Energy storage (triglycerides)

Variety of stem cells

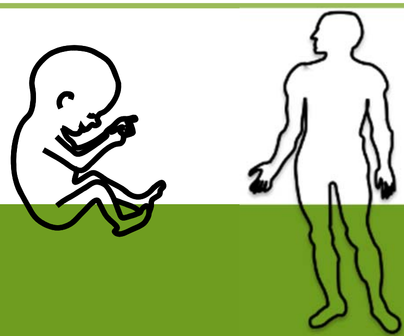


Embryonic stem cells

- Found in blastocyst - a very early embryo
- **Pluri**potent: can make all types of specialized cells in the body

Tissue stem cells = "Adult" stem cells

- Reside in most tissues (from fetus to adult)
- **Multi**potent: can make multiple types of specialized cells, but not all types
- Maintain the integrity of tissue (replacement of cells)



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Features of adipocyte stem cells also called adipocyte derived stromal cells (ADSC)

Several tools are needed to identify those cells.

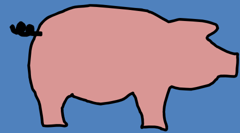
<u>Identification of ADSC based on</u>

- **expression of a subset of cell-associated surface antigens**

- **their differentiation ability *in vitro***

Compared with human and rodents, limited information in domestic animals

ADSC and cell surface markers

Cell surface markers		
CD34; hematopoietic stem cells	+	+
CD90; mesenchymal stem cells	+	
CD56; neurons, muscle cells	+	
CD73; MSCs		+
CD105; endoglin		+
CD31; endothelial cells	-	-
CD45; hematopoietic cells	-	-
CD11b; immune cells	-	-
CD14; immune cells	-	-

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Planat-Benard *et al.*, 2004; Perruchot *et al.*, 2013

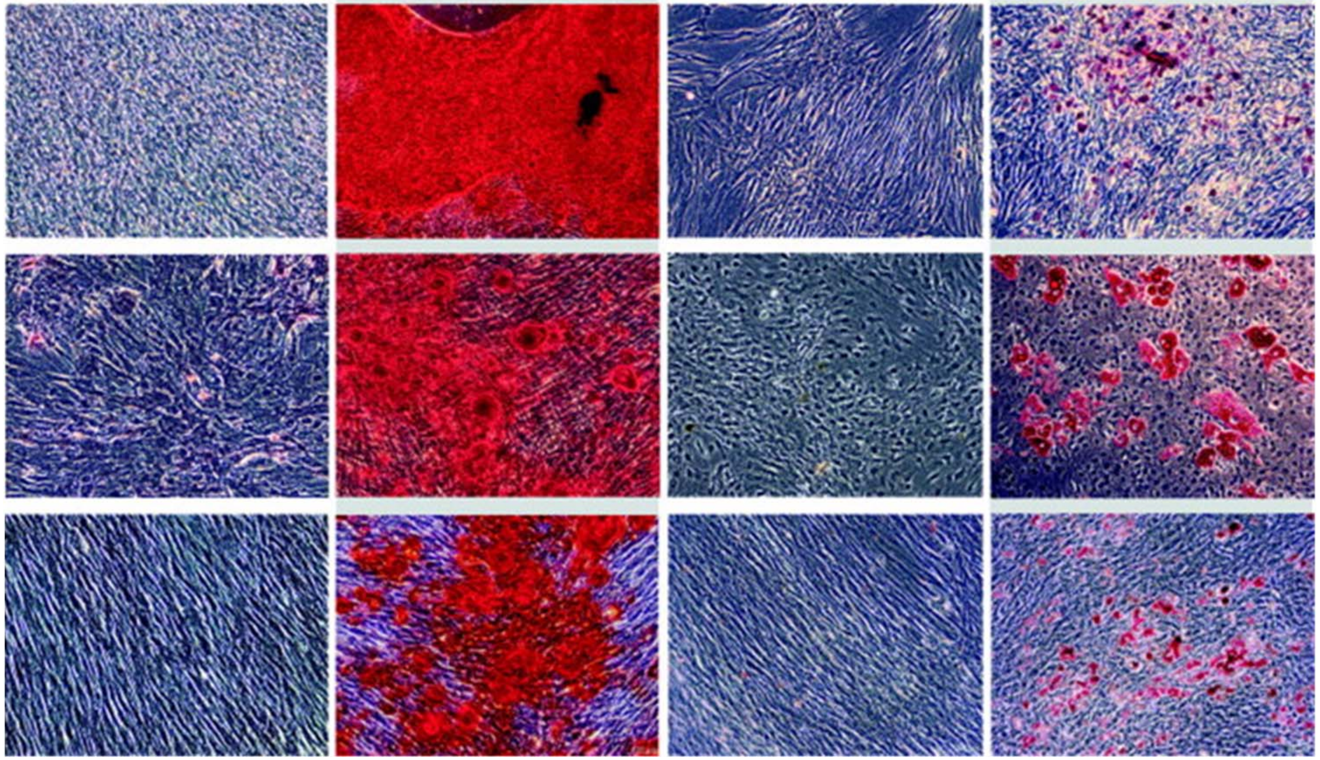
Features of ADSC *in vitro* in different species

Control | Osteogenic diffentiation | Control | Adipogenic diffentiation

Equine ADSC

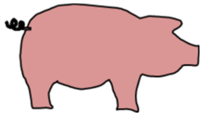
Canine ADSC

Porcine ADSC

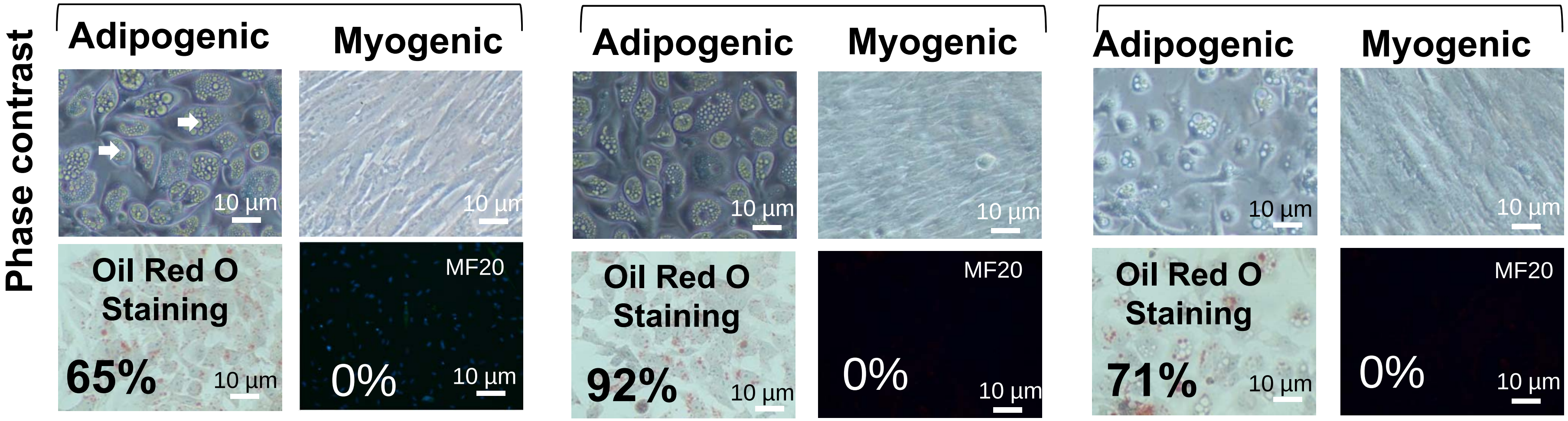


ADSC: adipogenic and osteogenic capacity

ADSC and differentiation ability



Total SV cells	CD90+ cells	CD56+ cells



➡ **a high capacity to differentiate into adipocytes**

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Stem and progenitor cells of adipocytes?

Birth

Puberty

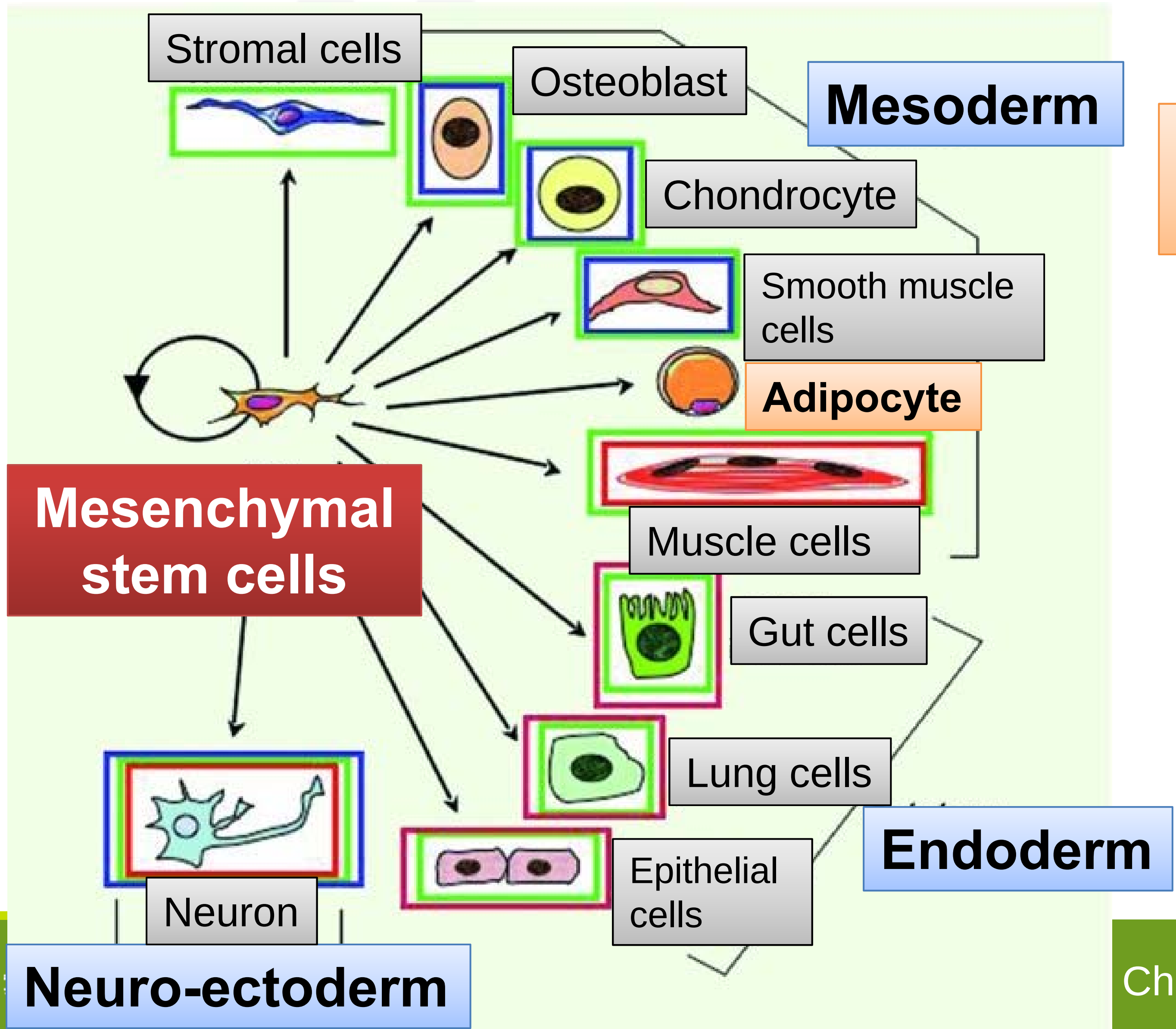
Embryo | Fetus

Limited information especially in farm animals

↑ information on ADSC

In rodents and human, most available studies have been performed in adults

Several differentiated cells with a common origin



Adipose tissue

⇩

Mesoderm + Neural crest (?)

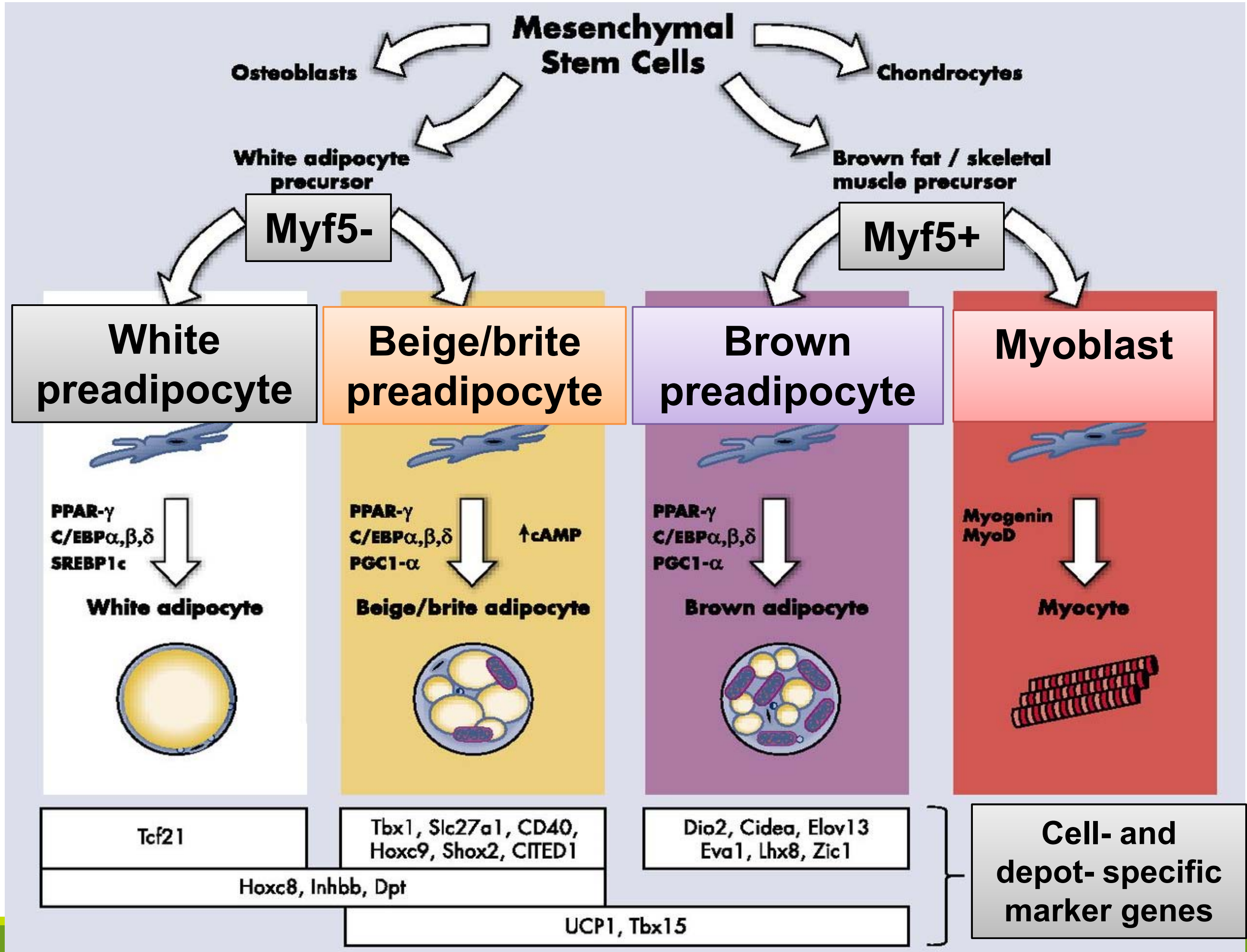
Charbord & Casteilla, 2011

Origin of adipocytes?

- In contrast to other tissues, the embryonic origin of adipose cells remains the subject of debate.

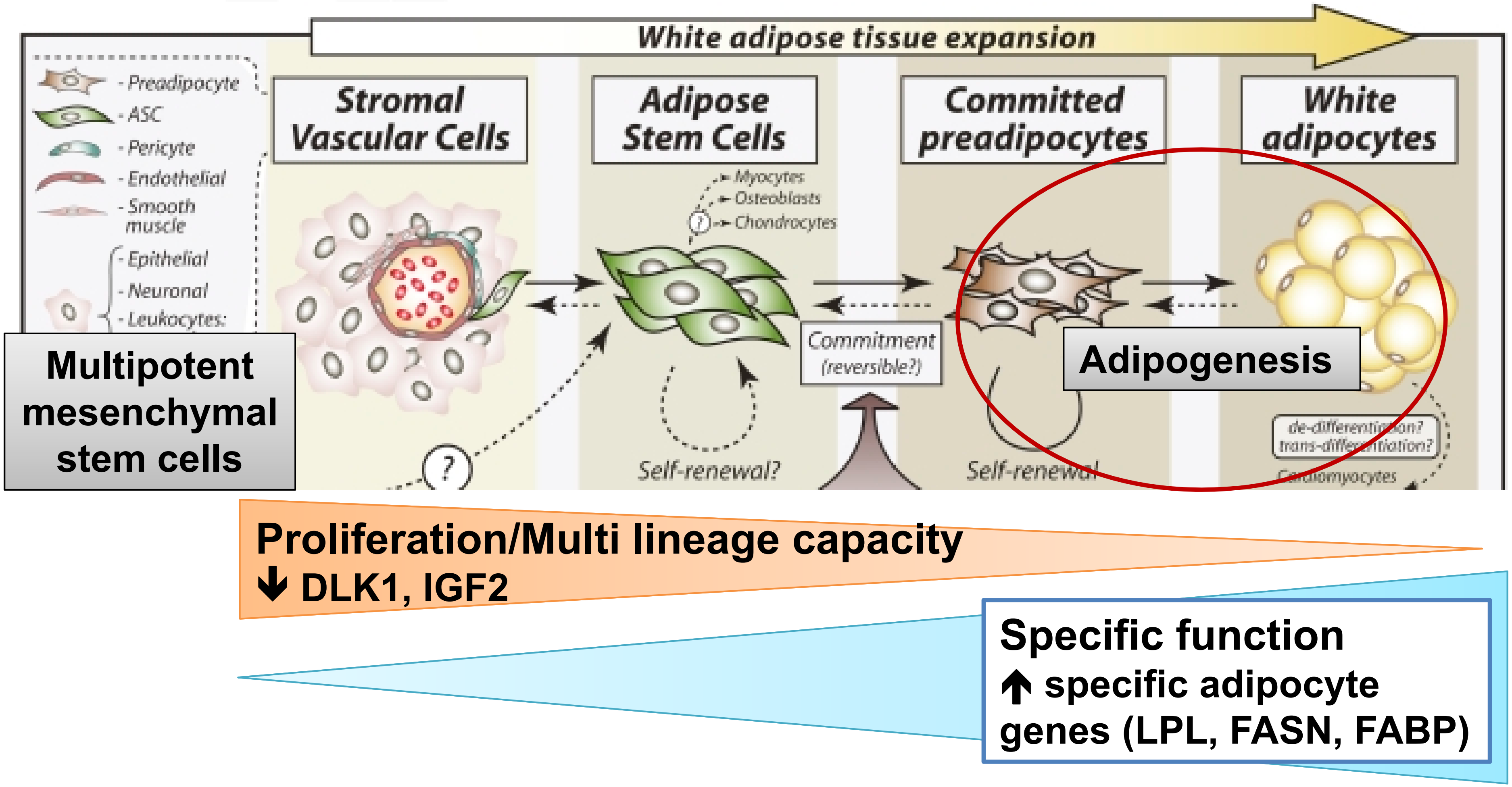
- Available data support the idea that stem and progenitor cells are heterogeneous and may have different embryonic origin.

Origins of white, beige and brown adipocytes

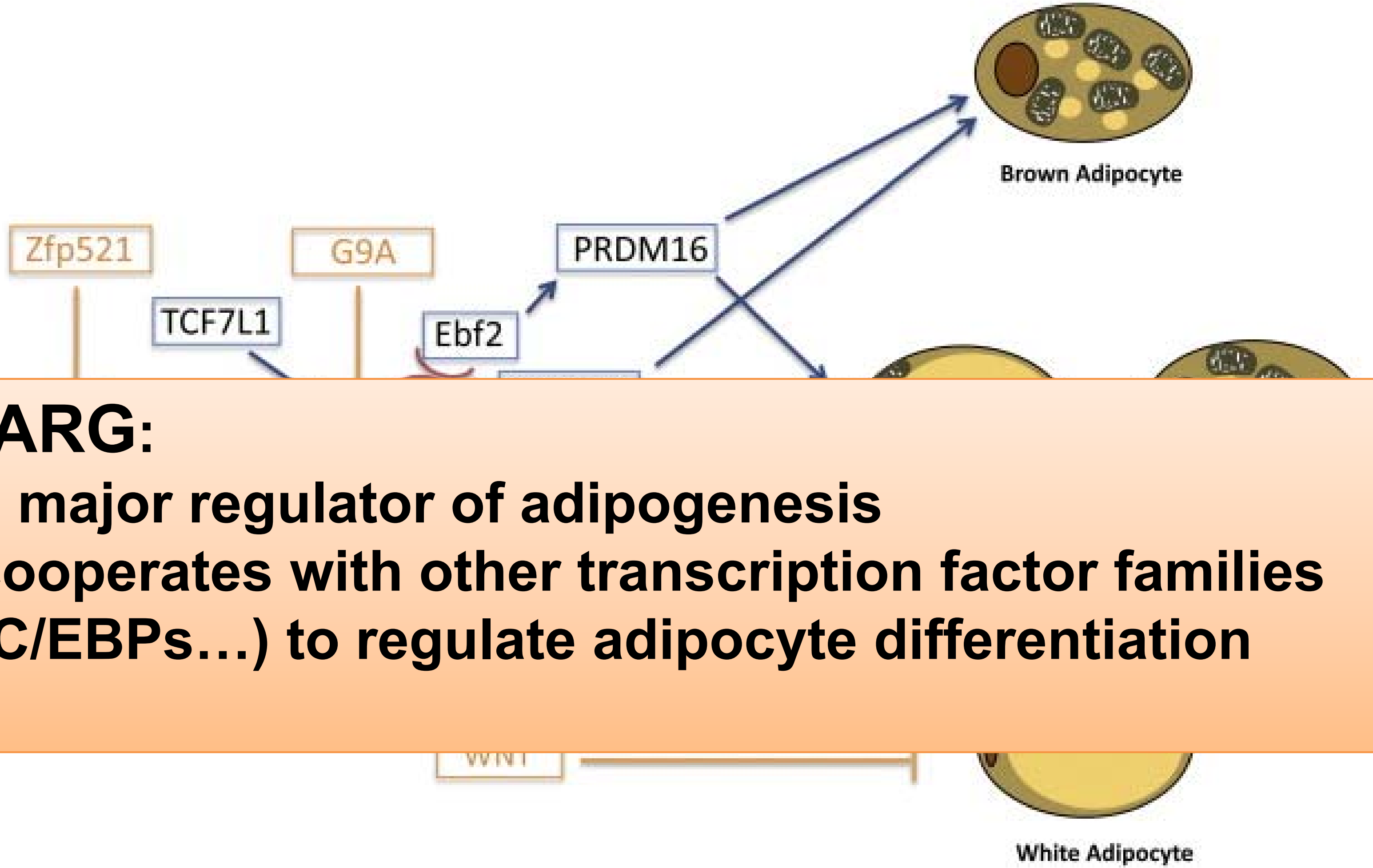


Lee *et al.*, 2013

From stem cells to white adipocytes



Transcriptional regulators that affect the differentiation of white, beige and brown adipocytes



PPARG:

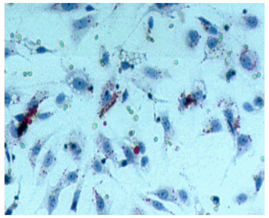
- **a major regulator of adipogenesis**
- **cooperates with other transcription factor families (C/EBPs…) to regulate adipocyte differentiation**

The red curved lines indicate protein–protein interactions.

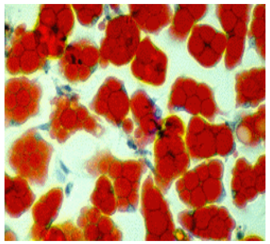
Mueller, 2014

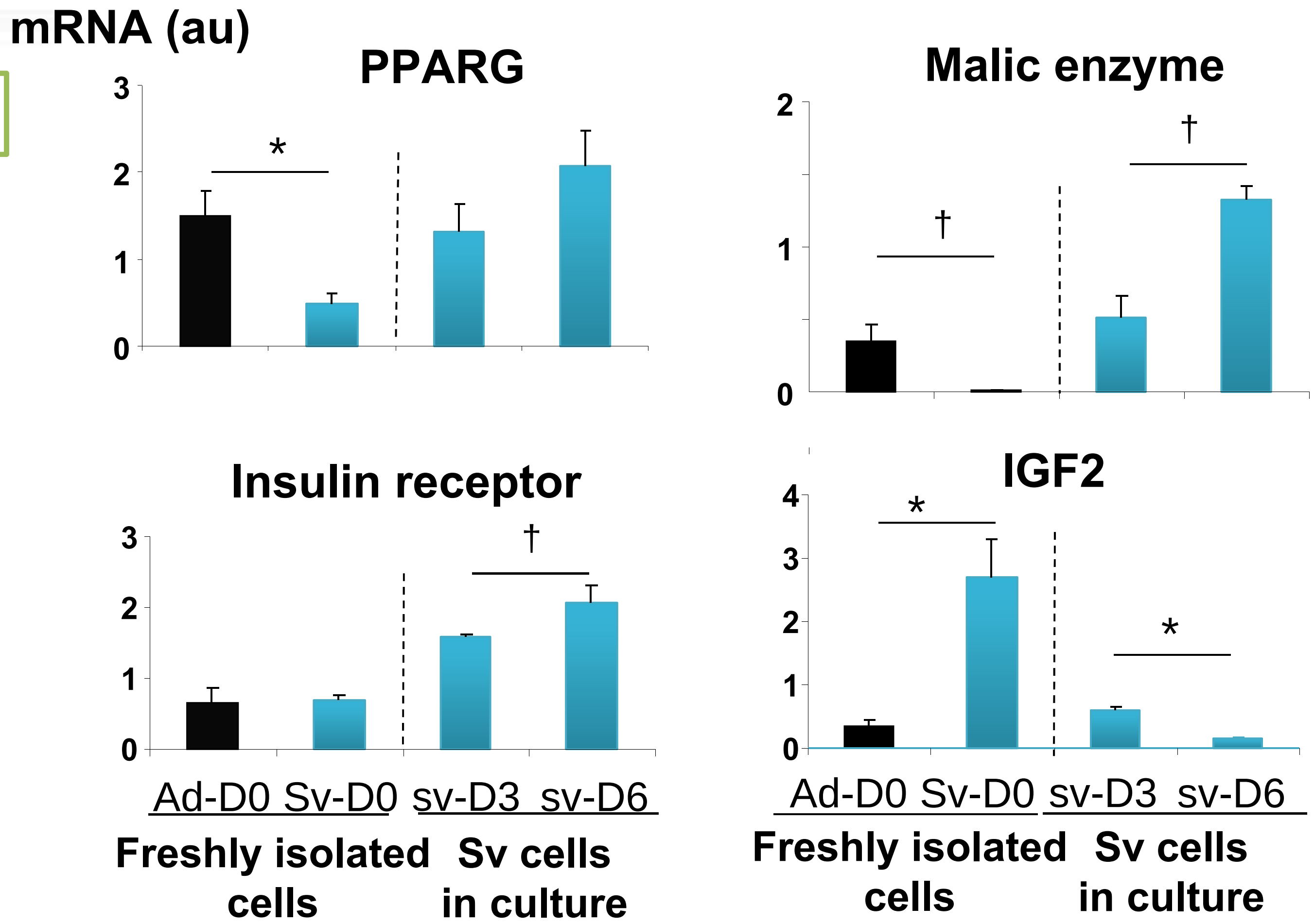
Differentiation of pig sv cells in primary culture

Day 1 of culture



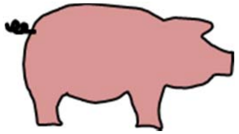
Day 9 of culture





Gerfault *et al.*, 1999 ; Gardan *et al.*, 2008

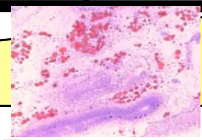
Growth/development of adipose tissue



Birth

Conceptus (days pc)

35 45 70 90 114



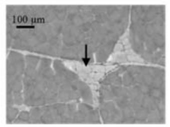
Subcutaneous adipose tissue

First clusters of fat cells

Accelerated fat deposition

Fat cell clusters surrounded by loose connective tissue

Perirenal adipose tissue (PAT)

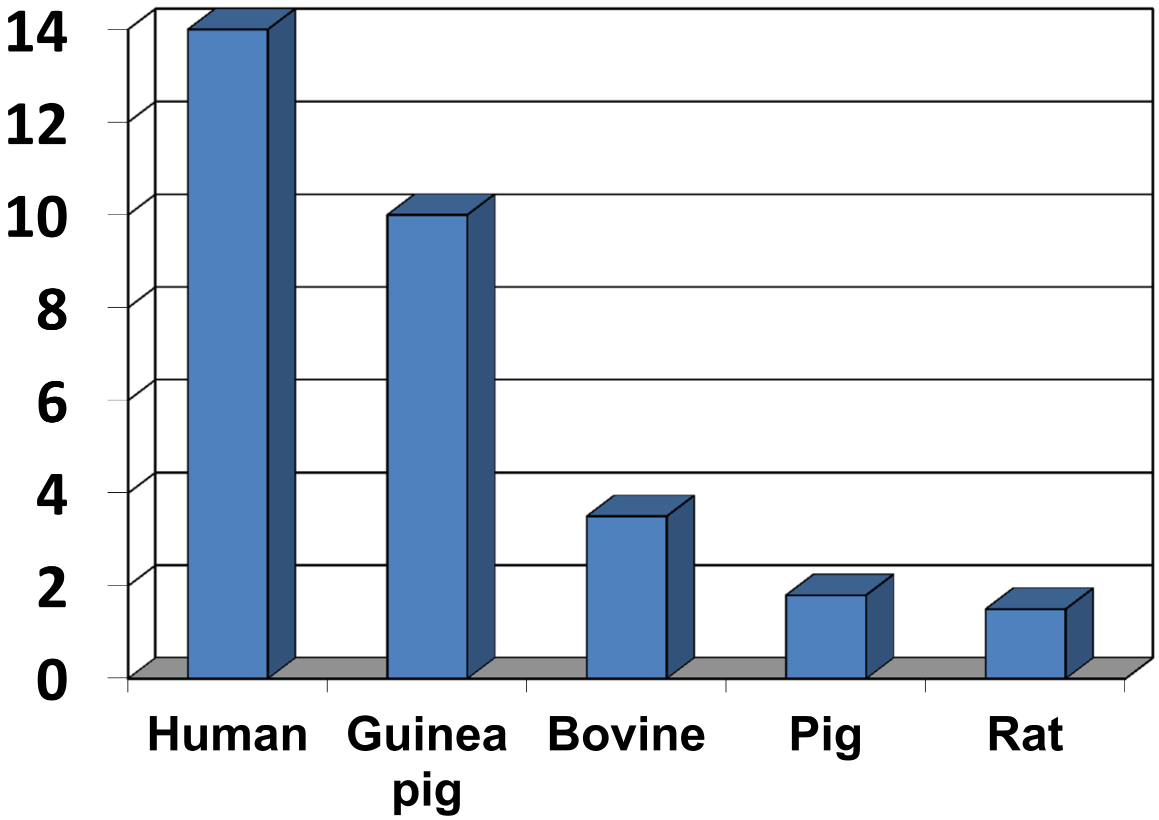


Intramuscular adipose tissue

Adipose tissue development differs according to depot and according to species (PAT: the first detected in cattle)

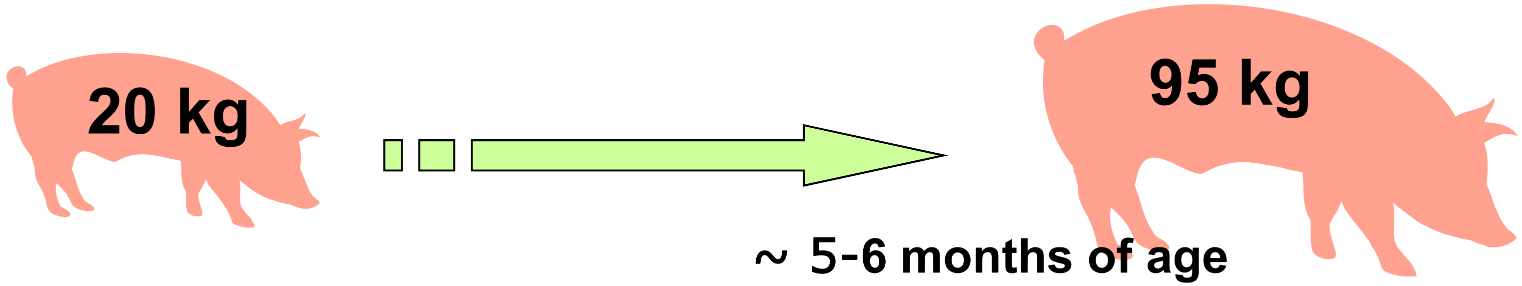
↑ adipose mass throughout life

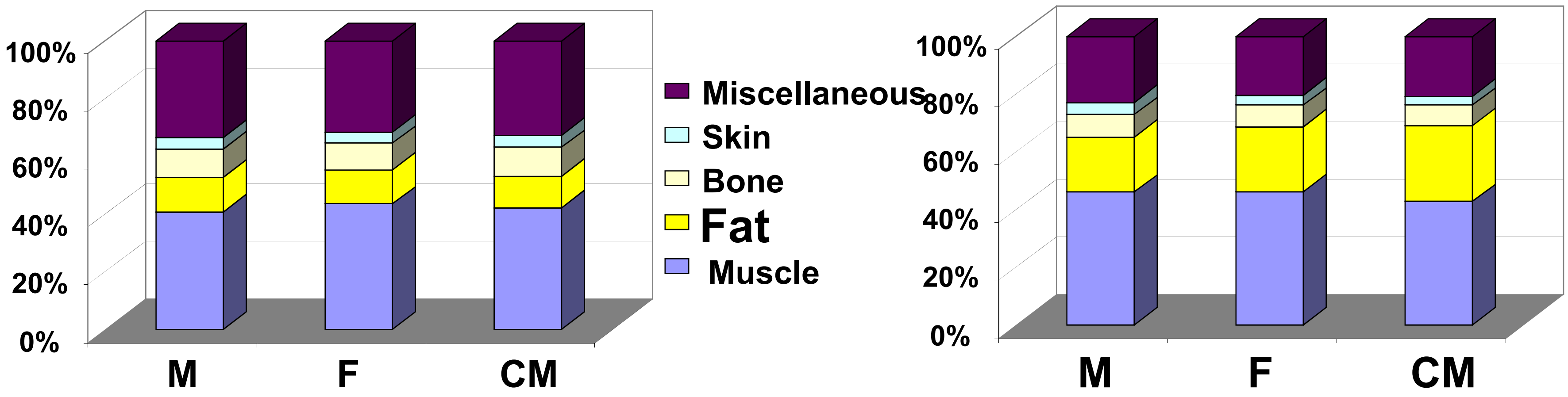
Body fat mass (%) at birth



Large differences between species

Influence of age on body fat mass

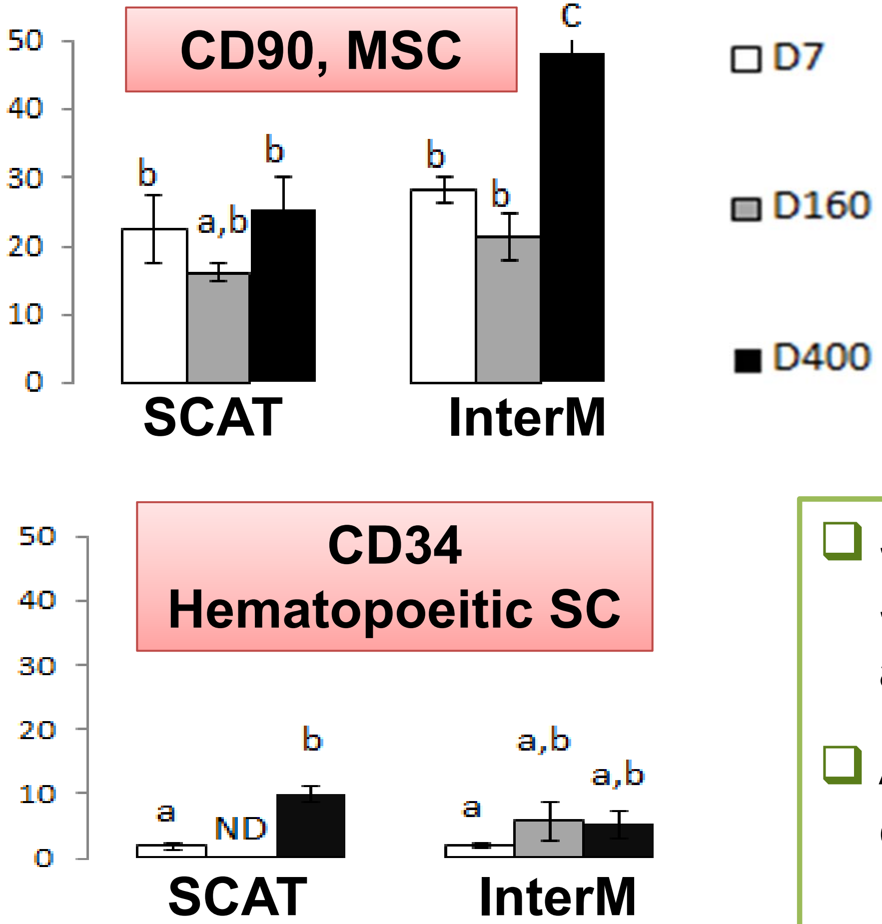


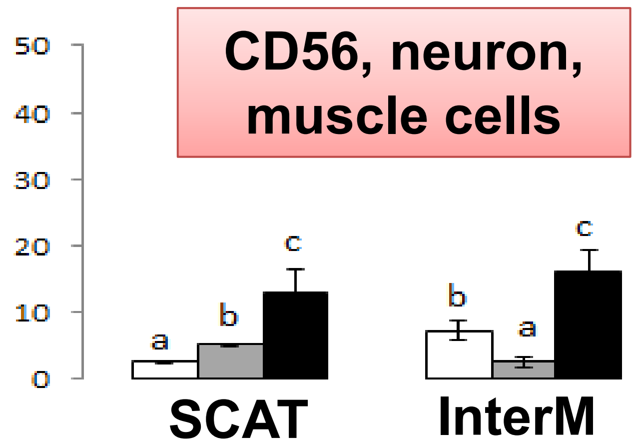


↑ adipose tissue mass with age with CM > F > M

Karege, 1991

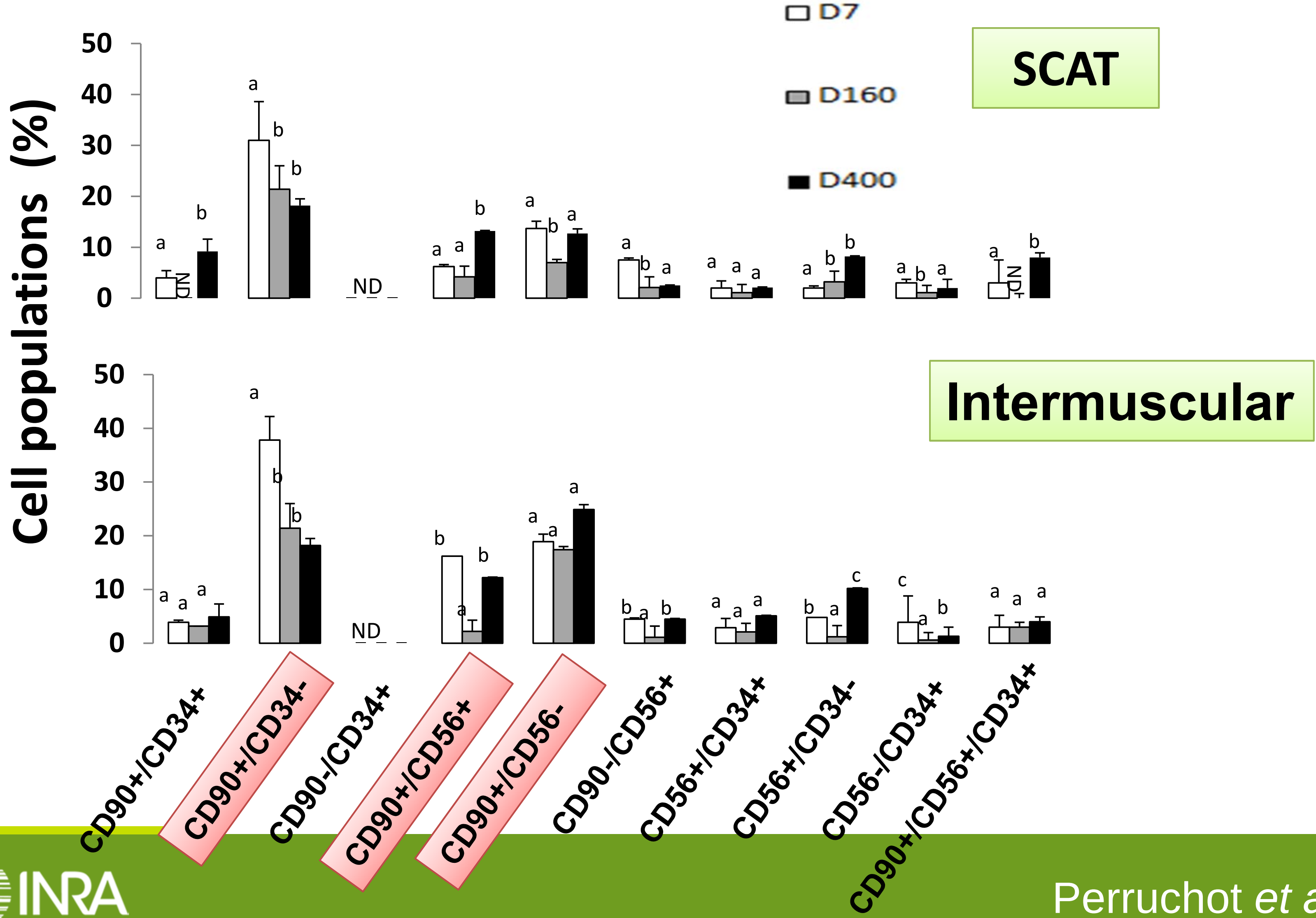
Age-related changes in the features of porcine ADSC from adipose tissue





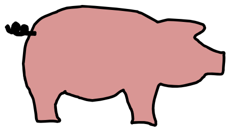
- ❑ Several cell populations in SCAT and intermuscular adipose tissue
- ❑ Age-related differences in some cell populations with differences between adipose depots

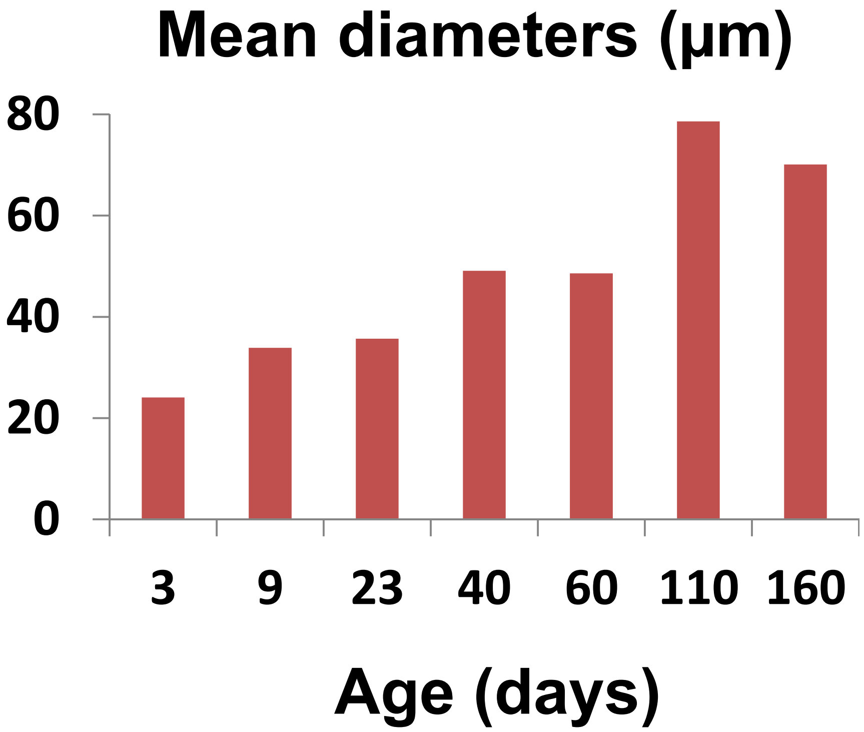
Age-related changes in the features of porcine ADSC isolated from adipose tissue

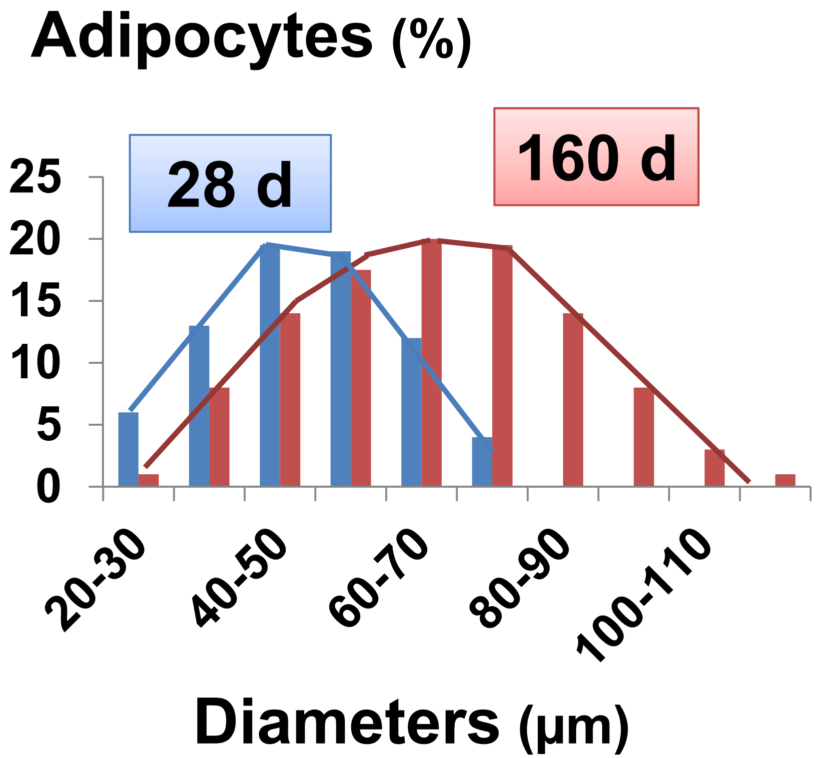


Perruchot *et al.*, 2013

Age-related changes in adipocyte diameters





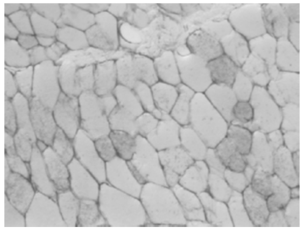


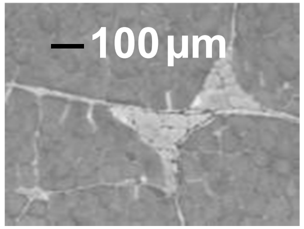
Adapted from Mersmann *et al*., 1975; Sarr *et al.*, 2010

Site-specific development of adipocyte diameters

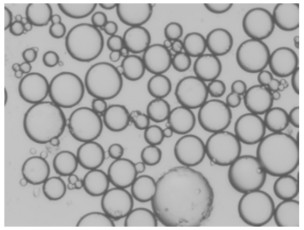
Adipose tissue | **Muscle (*trapezius*)**

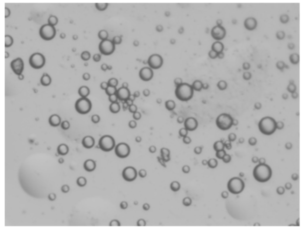
Histological sections





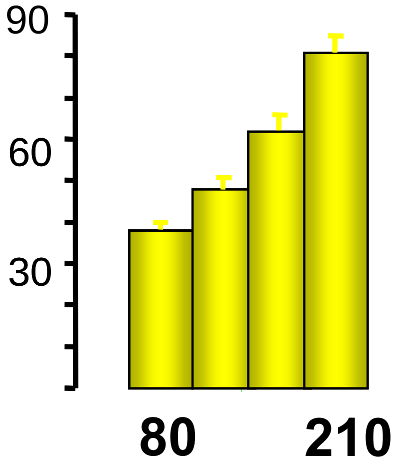
Isolated adipocytes

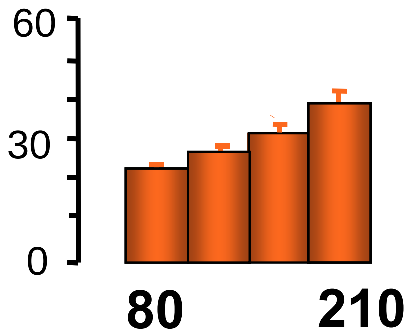




- ↗ adipocyte diameters with age
- Smaller adipocytes in muscle than in adipose tissue for a given age

Average diameters (µm)

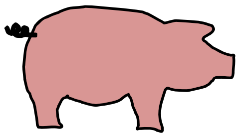




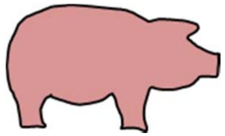
Age (days)

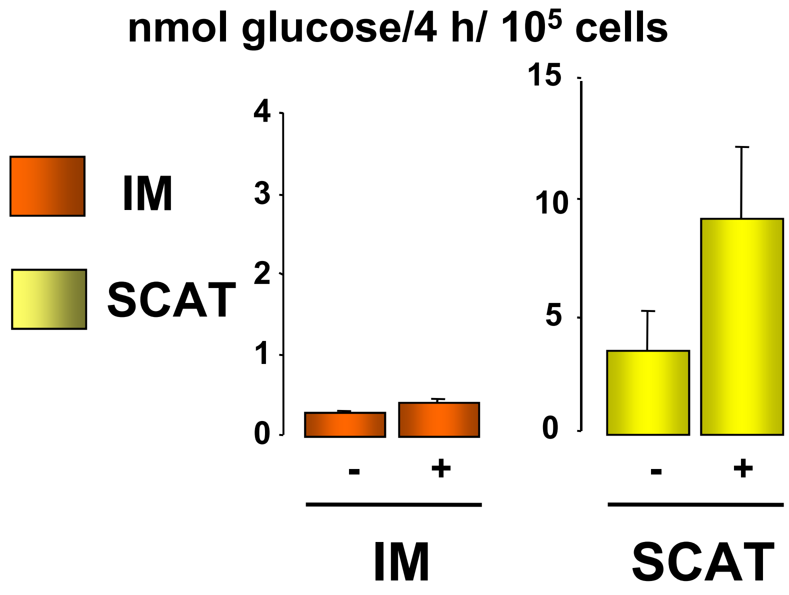
IM

SCAT



Lipid metabolism in adipocytes



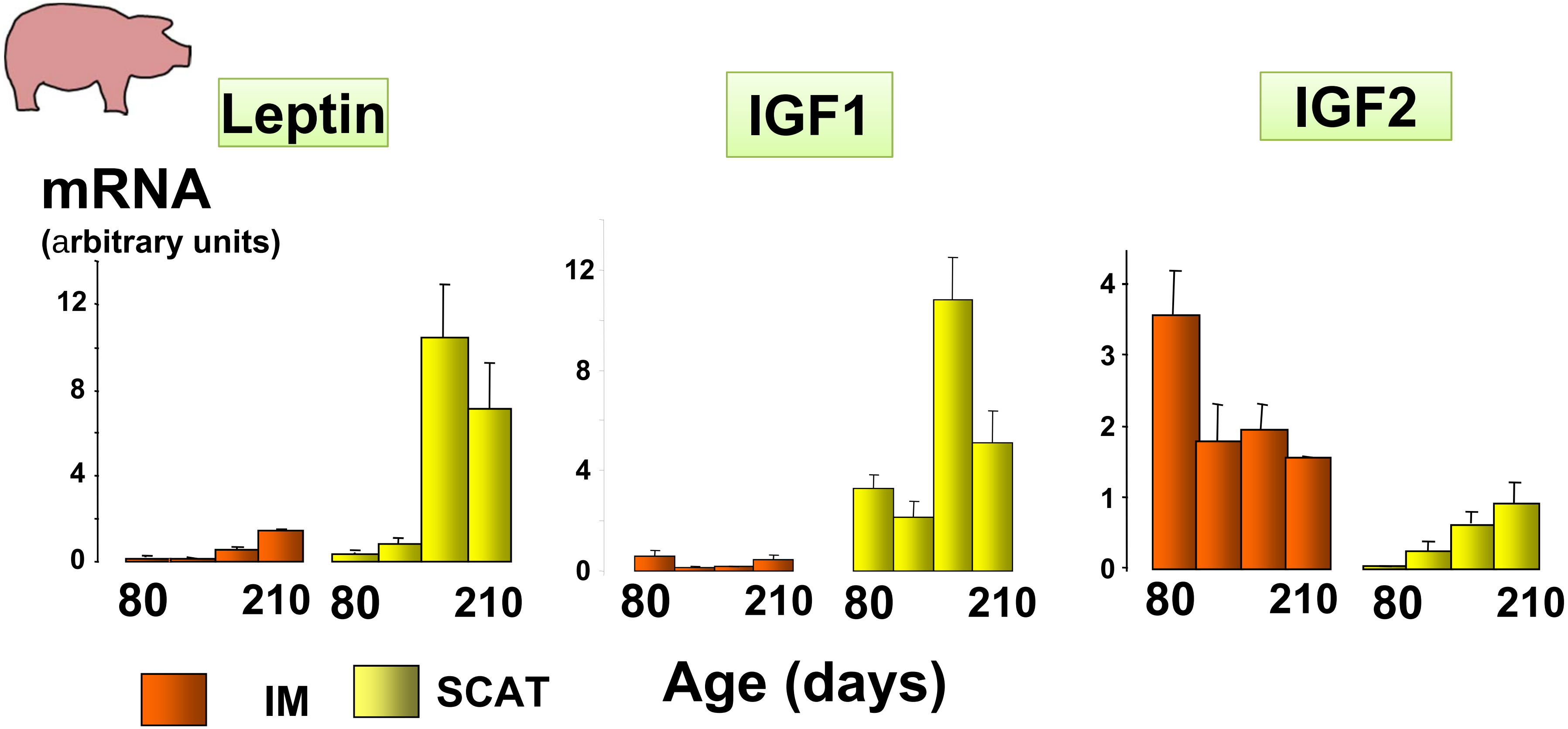


Lipogenesis
(adipocytes from 80-d-old pigs)

- **Basal rate: IM << SC**
- **insulin-stimulated rate: IM << SC**

FAS and malic enzyme activities: higher in SCAT adipocytes than in IM adipocytes between 80 and 210 days of age

Leptin, IGF1, IGF2 mRNA in adipocytes



Leptin and IGF1 expression: SCAT >> IM

IGF2 expression: IM >> SCAT

Development of white adipose tissue in summary

- A quite low body fat mass (<2% in pigs) at birth but a large increase in the fat mass thereafter .

- ↑ fat mass is associated with changes in cell populations of ADSC.

- Age-related ↑ in adipocyte diameters with differences between adipose depots.

- Developmental differences in the physiology of adipocytes according to adipose depots.

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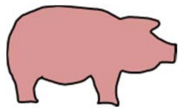
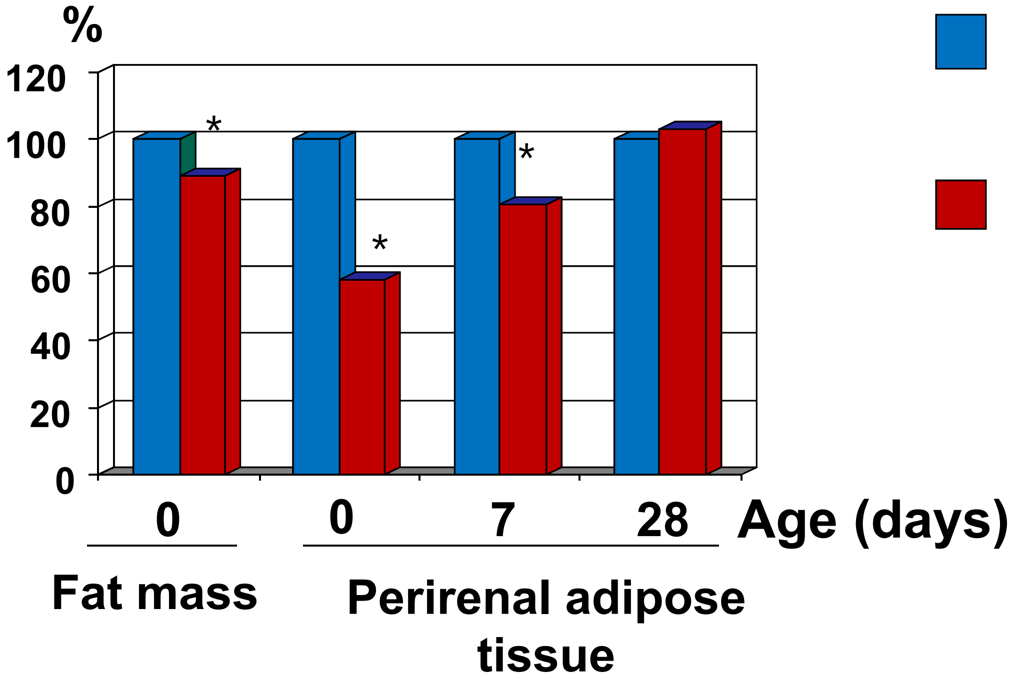
Adipose tissue: a dynamic tissue able to adapt to a variety of environmental and genetic factors

Two examples associated with early development and that may be linked to newborn survival and that may influence the postnatal growth of adipose tissue

- **Fetal adipose tissue development in IUGR and control fetuses**
- **Fetal adipose tissue development in two breeds of pigs differing in maturity and vitality at birth.**

Fetal adipose tissue development and IUGR





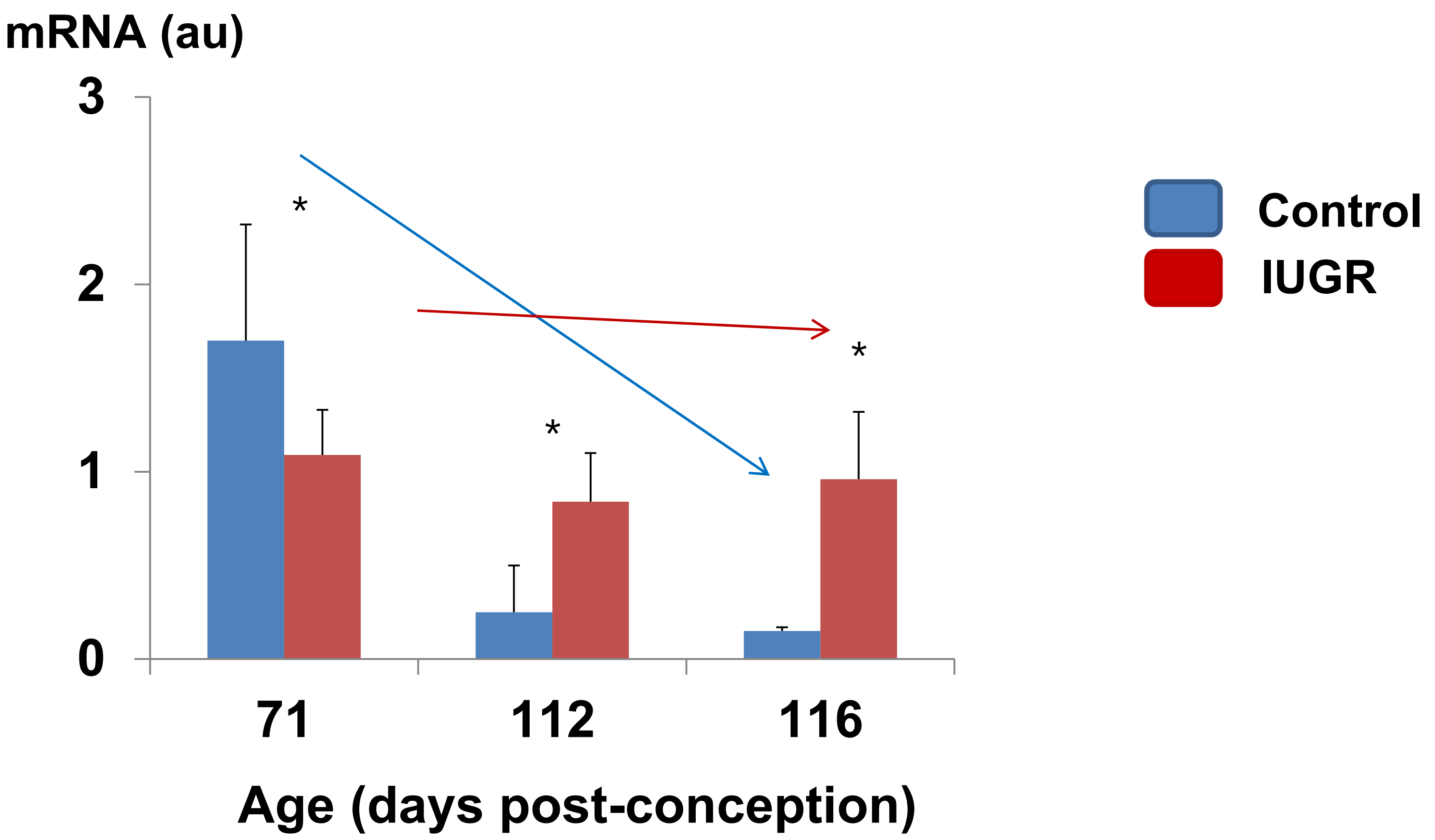
Control
- birth weight: 1.4-1.6 kg

IUGR
- birth weight: 0.8-1.0 kg
- lower fat mass at birth
- adiposity ± modified at 6 months of age

Expression of genes involved in cell cycle arrest, differentiation, and adipocyte physiology during fetal development in adipose tissue ?

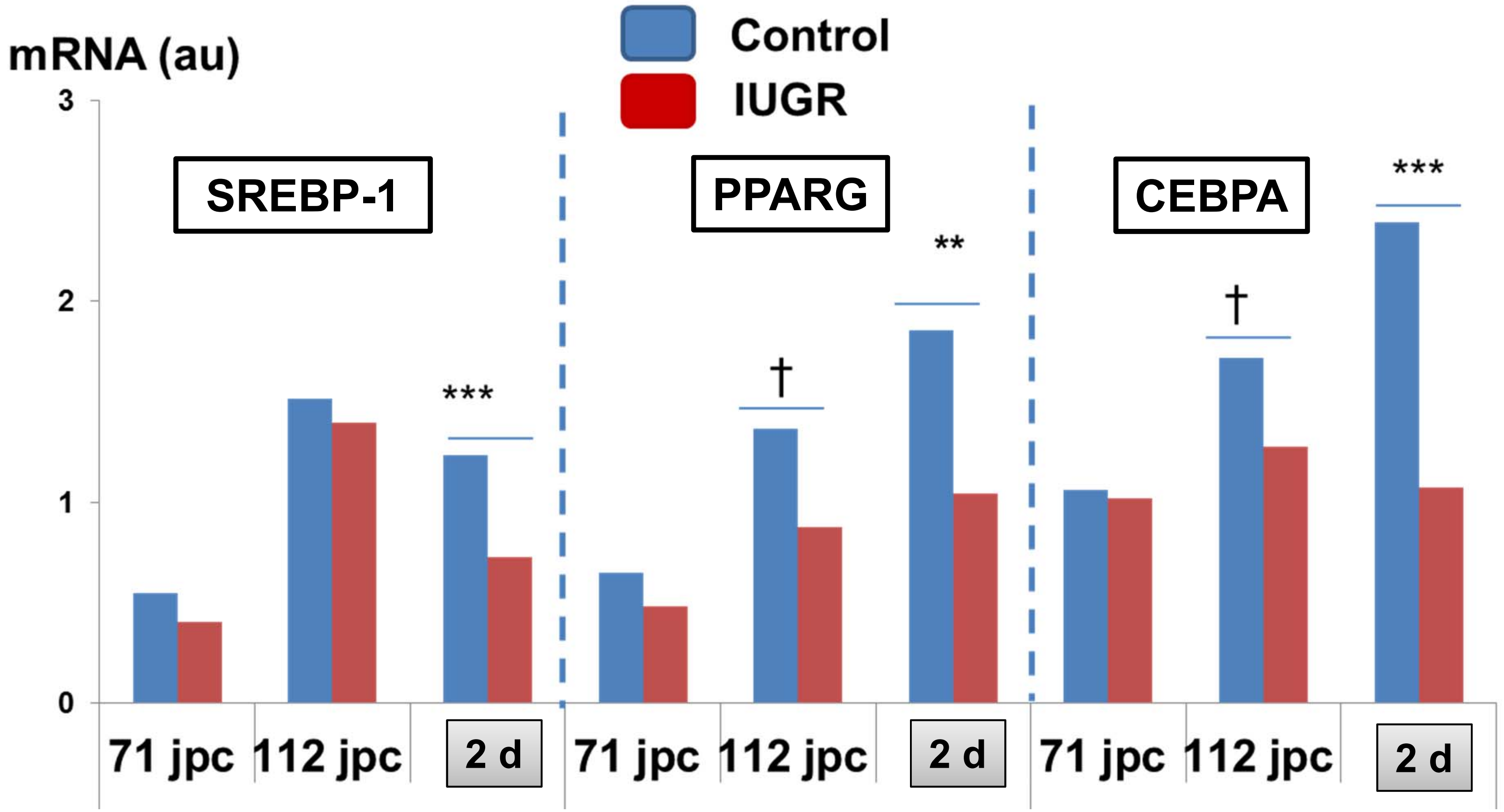
Rehfeldt & Khun, 2006;
Morise *et al.*, 2007

Expression of the gene encoding DLK1/Pref1

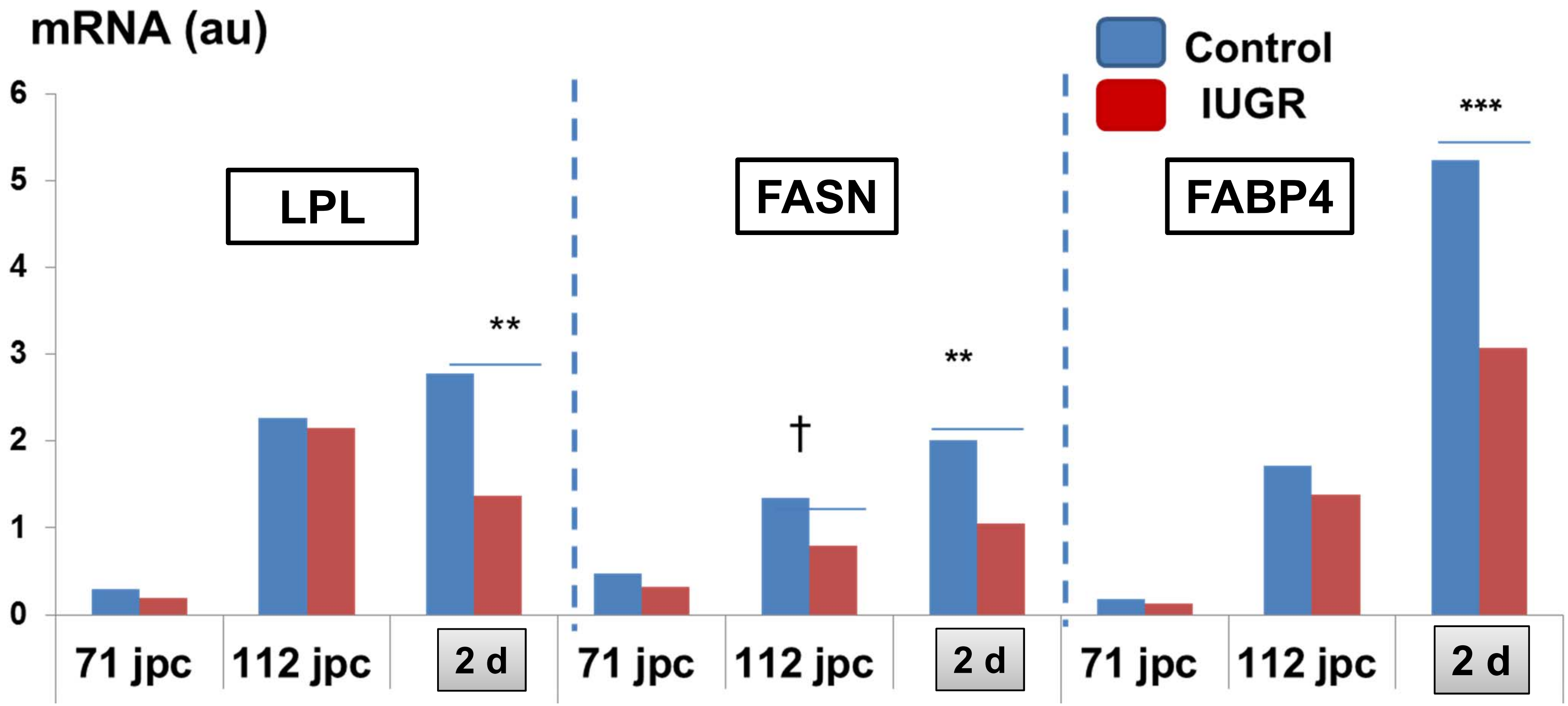


DLK1: a negative regulator of adipocyte differentiation

Expression of genes encoding transcription factors

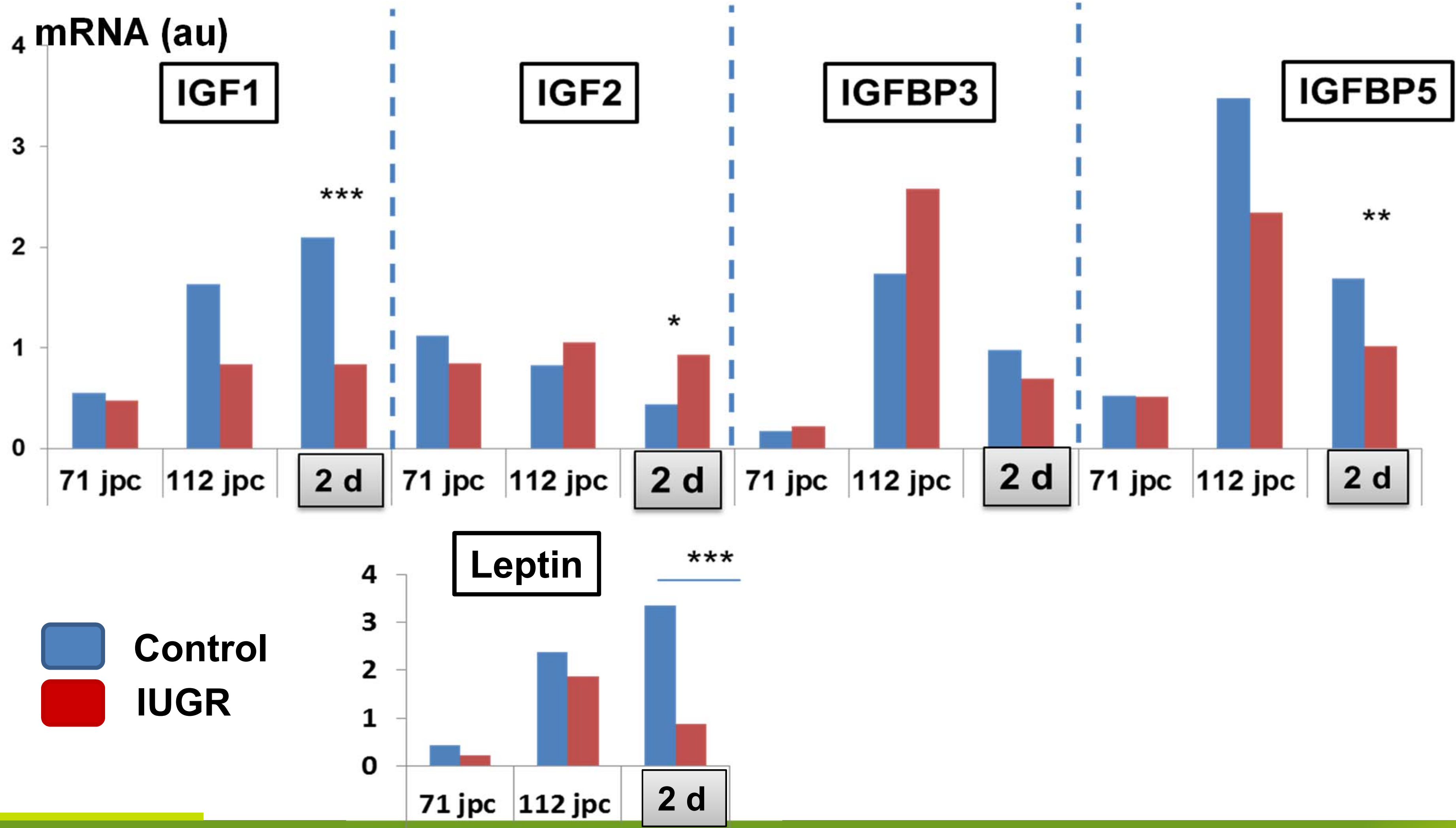


Expression of genes involved in lipid metabolism



Gondret *et al.*, 2013

Expression of genes associated with the secretory function



Expression of genes in adipose tissue of control and IUGR pigs

- The expression levels of genes involved in
  - adipocyte differentiation
  - lipid metabolism
  - secretory function (with the exception of IGF2)

  depressed in IUGR compared with control animals

- The differences between animals was much greater in 2-day-old piglets than in fetuses.

Adipose tissue differentiation process is delayed in IUGR animals. It may influence later development

Fetal adipose tissue development according to breed

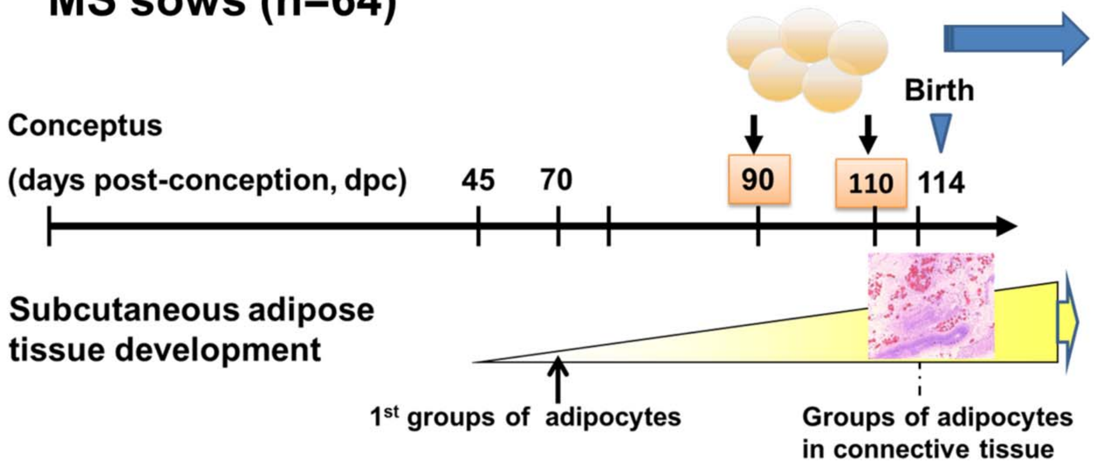
LW

MS

Second parity sows inseminated with a mixed semen (LW+MS)

MS piglets have better survival and vitality after birth than LW piglets

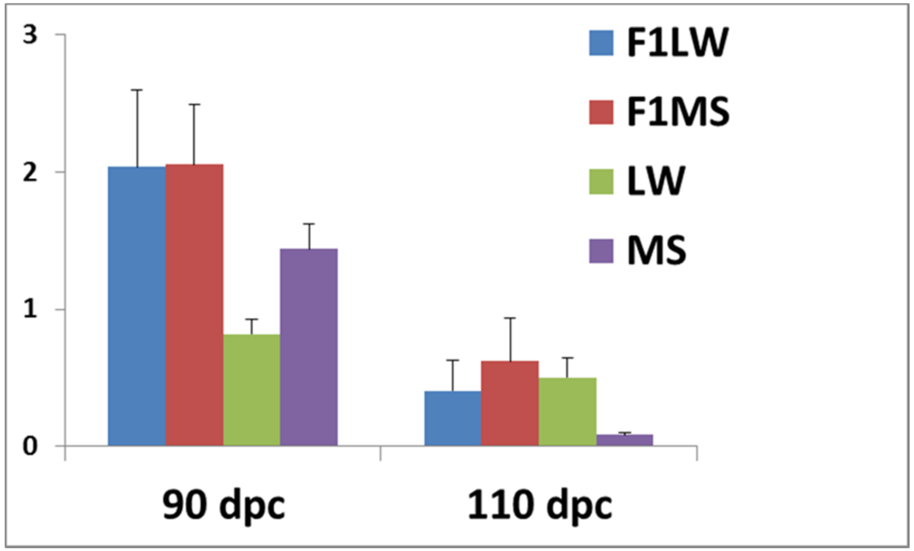
Fetuses: LW and F1 in LW sows; MS and F1 in MS sows (n=64)



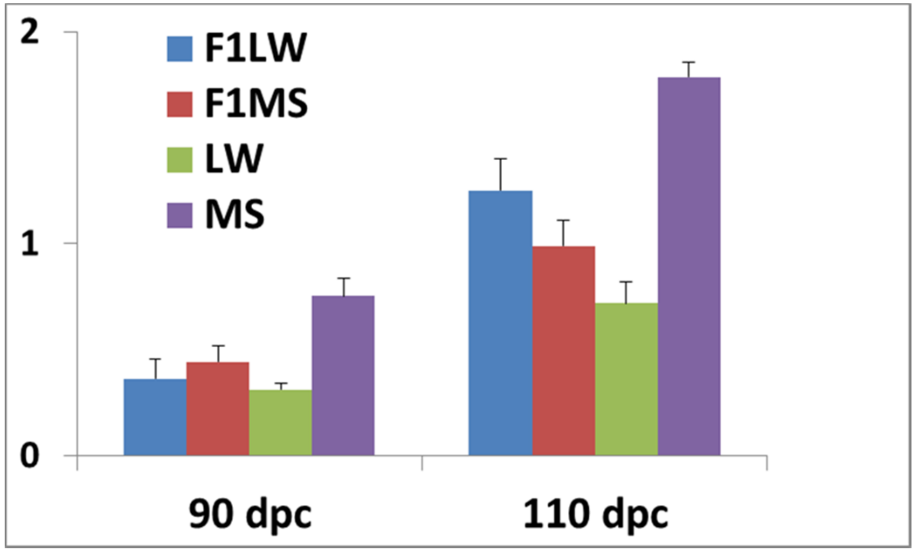
Louveau *et al.*, ICPR 2013

Fetal adipose tissue development according to breed

DLK1, relative mRNA levels



FABP-A, relative mRNA levels



DLK1 mRNA (negative regulator of differentiation) : ↓ with age in all groups.

FABP-A mRNA, involved in fatty acid binding were more expressed in 110 dpc fetuses and in MS fetuses.

Accelerated maturation of adipose tissue in MS fetuses compared with other fetuses: this may contribute to the higher mobility of MS piglets at birth.

Outline

- Introduction
- Diversity of adipose tissue cell types
- Origin and development of adipose tissues
- Adipose tissue: a dynamic tissue able to adapt to a variety of environmental and genetic factors
- **Conclusion & Perspectives**

Conclusion & Perspectives

- White adipose tissue plays a key role in the regulation of energy balance.
- Adipose tissue development differs according to depots and according to species
- A dynamic tissue able to adapt to a variety of genetic and environmental factors including in fetuses:

  ↳ Epigenetics modifications?

Conclusion & Perspectives

- Adipocyte derived stromal cells (ADSC): an emerging field of research for livestock species

  - ADSC are found in pig adipose tissues and their proportion are influenced by age

  - Diversity of cells and their origins: further studies are needed

  - Significance of adult stem cells for the control of adipose tissue mass?

Thank you for your attention

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