Developmental Origins of Health and Diseases in horses
Proof of concept and significance in horse breeding

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1. SETTING THE SCENE : DOHAD, EPIGENETICS AND CRITICAL PERIODS

2. PROOFS OF CONCEPT IN EQUIDAE

3. IMPORTANCE FOR HORSE BREEDING

4. CONCLUDING REMARKS

The developmental origins of health and disease (DOHaD)

Adverse nutrition or metabolic status of the parents

Barker's

DOHaD Society

International Society for Developmental Origins of Health and Disease

Fetal growth adaptation

transcriptome, cells, tissues, organs,

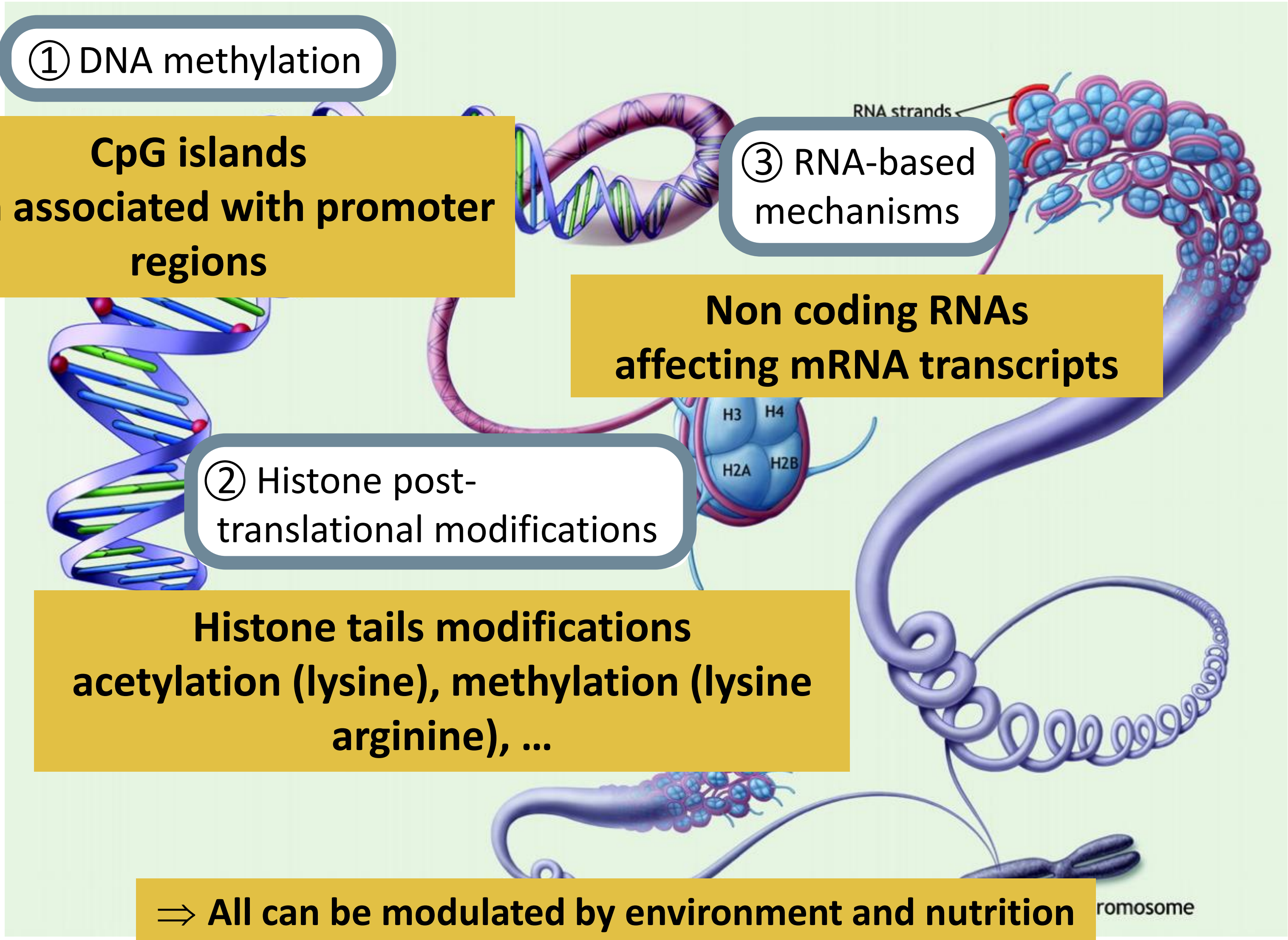
Neonatal Phenotype

Predictive

Metabolic disorders

Type 2 diabetes, osteoporosis, obesity + many other diseases…

Epigenetic marks: the basis of memory



① DNA methylation

CpG islands
often associated with promoter regions

③ RNA-based mechanisms

Non coding RNAs
affecting mRNA transcripts

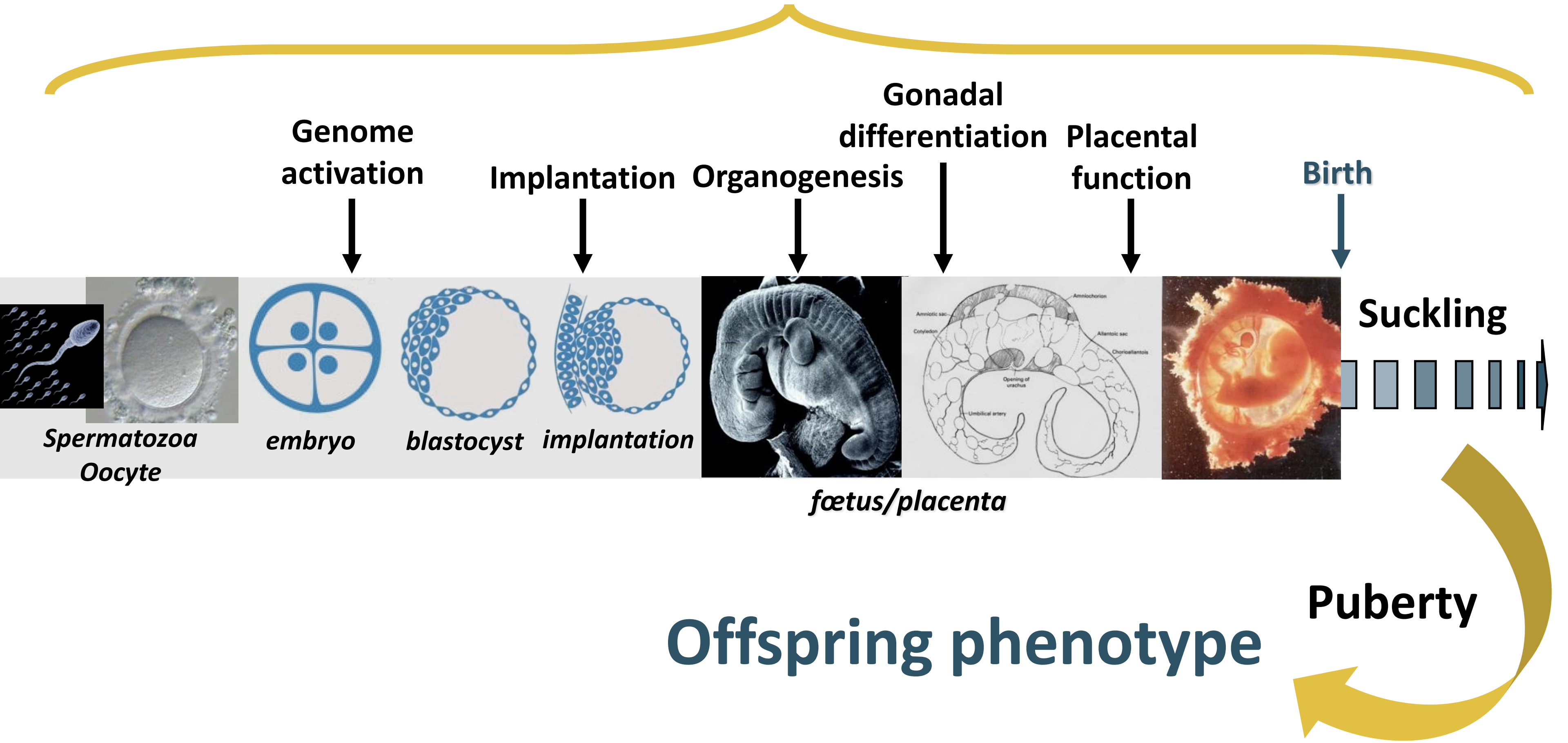
② Histone post-translational modifications

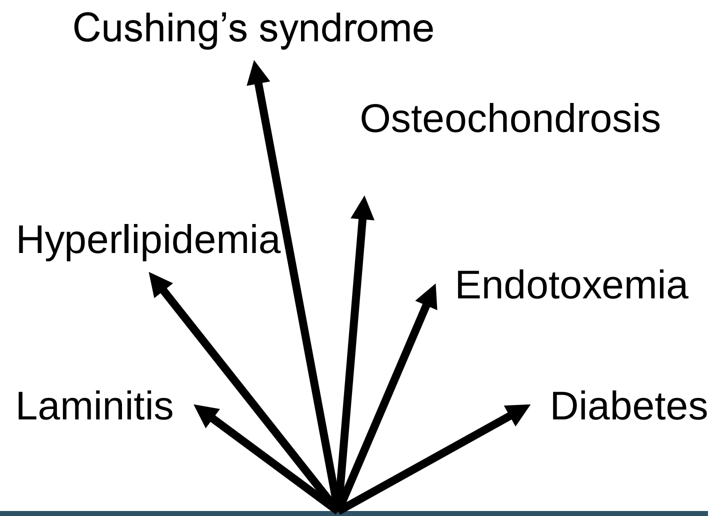
Histone tails modifications
acetylation (lysine), methylation (lysine arginine), …

⇒ All can be modulated by environment and nutrition
See recent review Chavatte-Palmer et al. Animal, 2018

Critical periods of development

Nutrition and environment





Insulin resistance in horses

EMS - Durham et al. JVIM 2019

There may be truth in focusing on stallions and mares

DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE (DOHaD)

Not only will the broodmare supply half the genes, but she will carry the foal for nearly 11 months and nurture it for at least its first 6 months.

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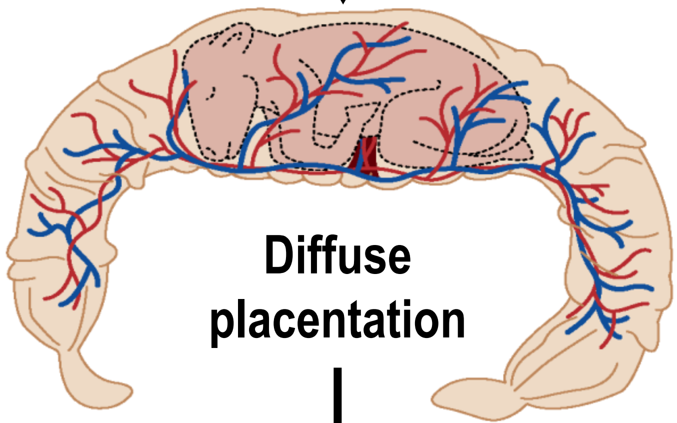
Maternal size

↓

Uterine capacity

↓



↓

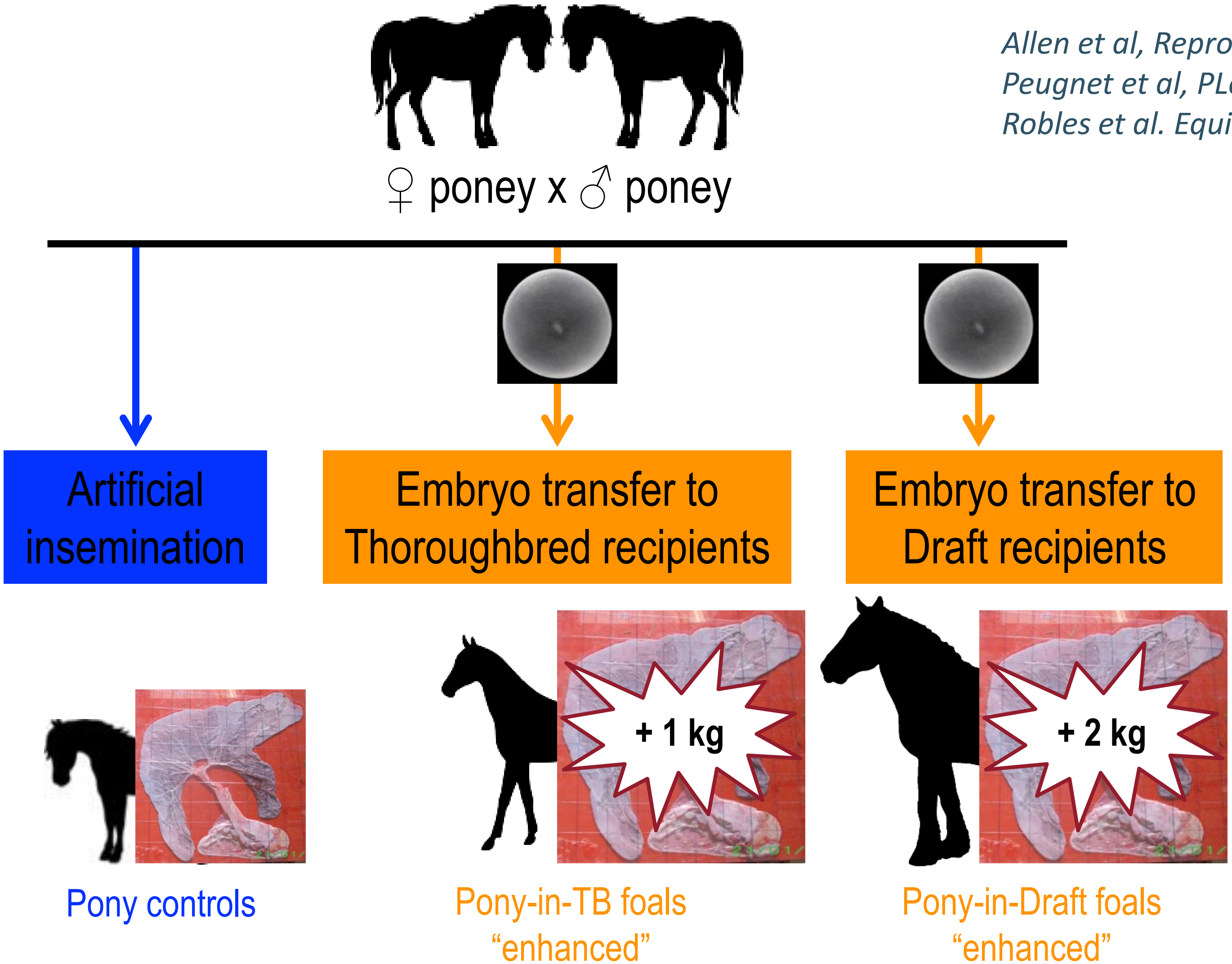
Contact surface placenta / endometrium
Nutritional supply to the fetus

"In equidae, fetal growth can be enhanced above or restricted below the normal genetic potential for the breed by varying maternal size."

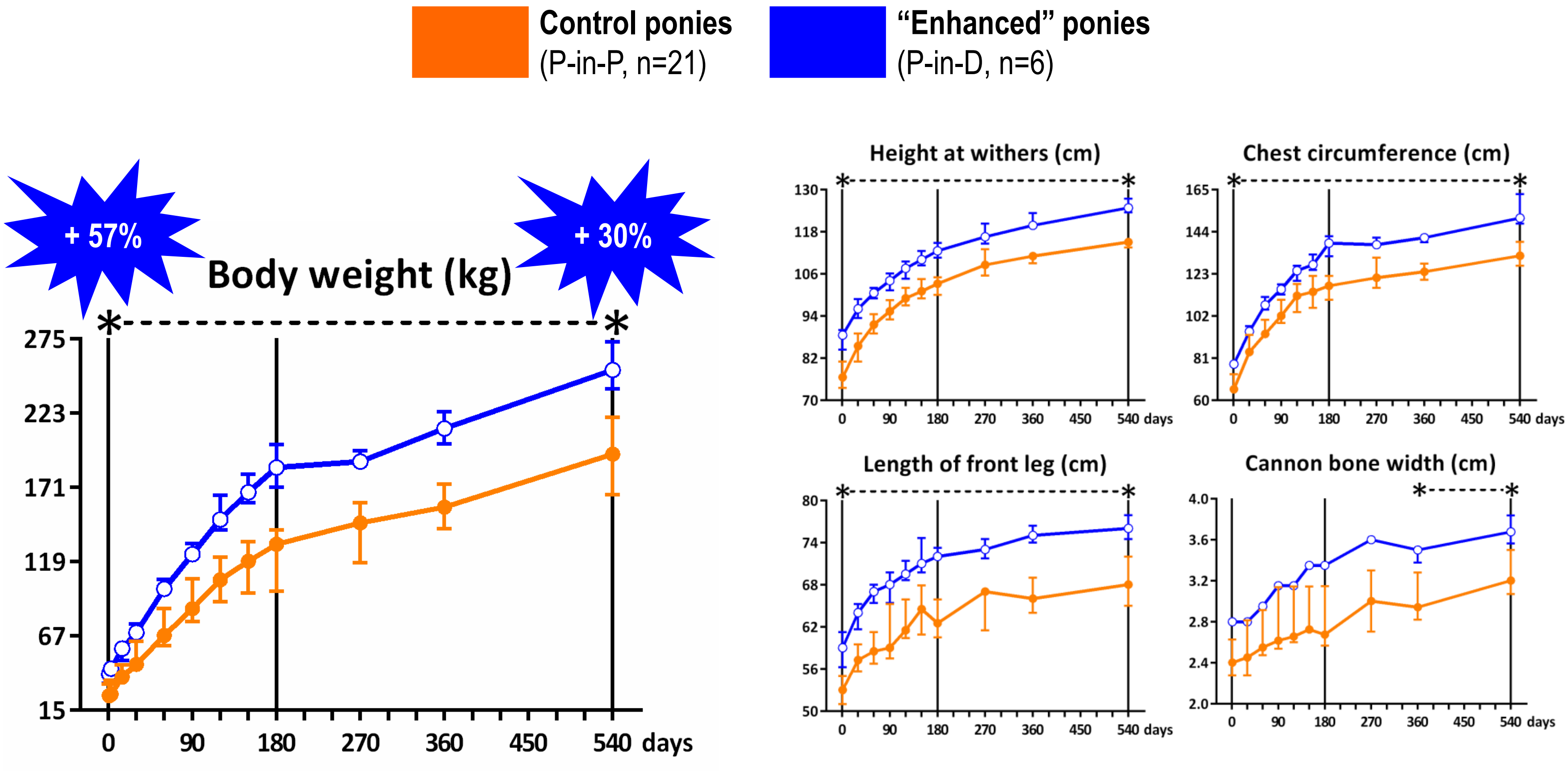
(Walton & Hammond, 1938; Allen et al, 2002a; Forhead et al, 2004)

Models of increased fetal growth
Lessons from between-breed embryo transfers

Allen et al, Reproduction, 2002
Peugnet et al, PLoS ONE, 2014
Robles et al. Equine vet J, 2018



Models of increased fetal growth



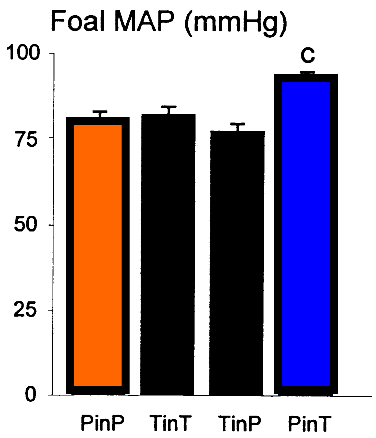
Growth of "enhanced" foals (Pony-in-Draft) is amplified in an harmonious way until age 1 year ½.

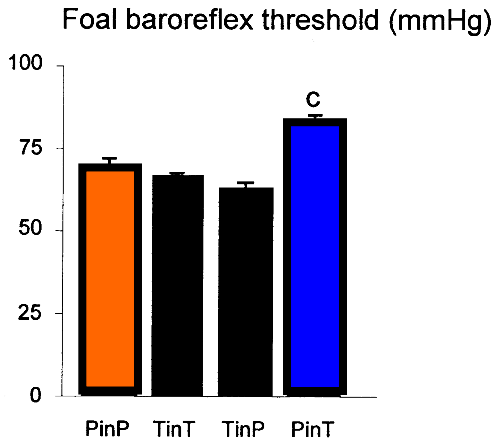
Peugnet et al, J Eq Vet Sci, 2016 - see also Allen et al, Reproduction, 2002; Allen et al, Reproduction, 2004

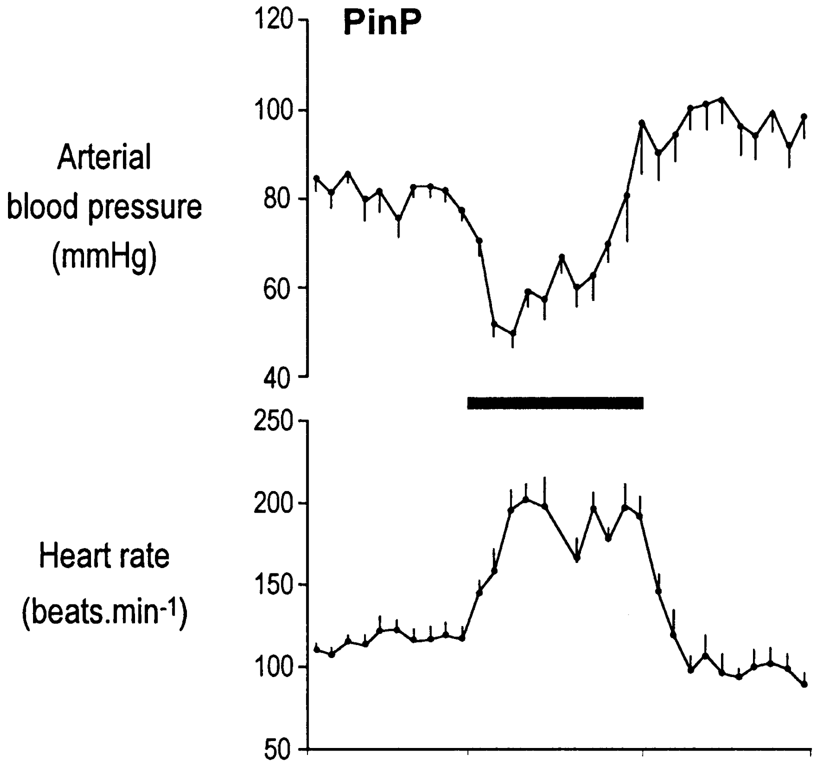
Models of increased fetal growth

Poney in Thoroughbred, 6 days of age

Induction of hypotension using sodium nitroprusside:
- Similar fall in arterial blood pressure in the 4 groups.
- Similar increase in heart rate in the 4 groups.
- Higher secretion of catecholamines in PinT *vs* PinP.







"Enhanced" foals (Pony-in-Thoroughbred) have elevated arterial blood pressure, lower baroreflex sensitivity and lower sympathetic stimulation at 6 days of age.

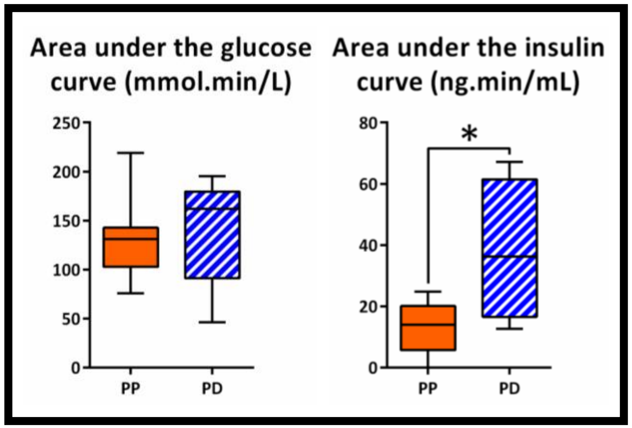
Giussani et al, J Physiol, 2003

Models of increased fetal growth

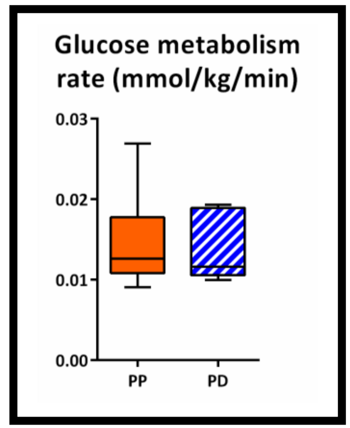
Control ponies (P-in-P, n=21)

"Enhanced" ponies (P-in-D, n=6)

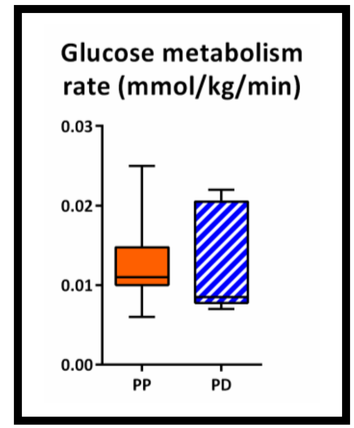
Newborn - age 3 days
IV glucose tolerance test



Weanling - age 200 days
Hyperinsulinemic euglycemic clamp



Yearling - age 540 days
Hyperinsulinemic euglycemic clamp

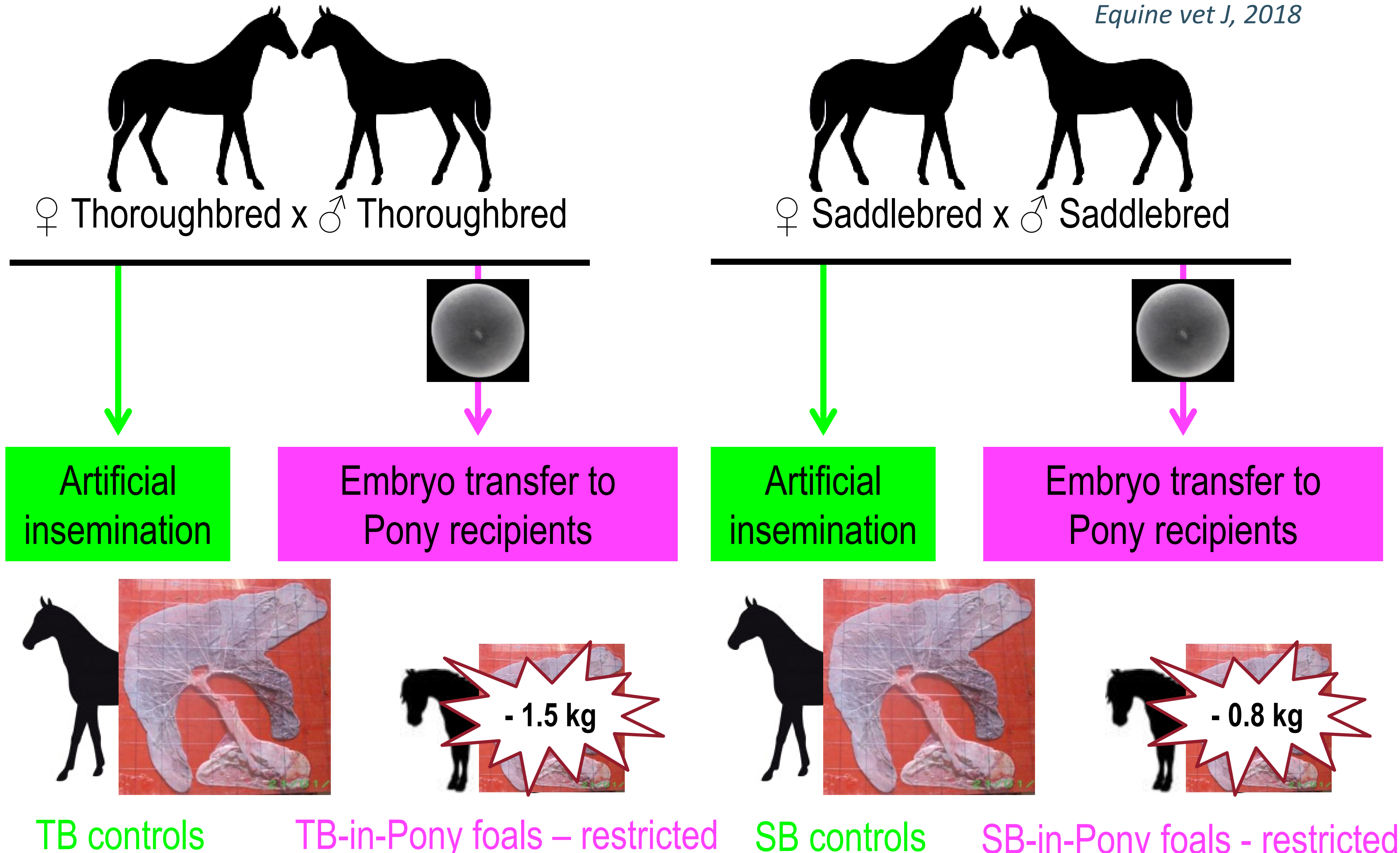


"Enhanced" foals (Pony-in-Draft) show an early resistance to insulin, but no further effect is observed until 1 year ½.

Peugnet et al, PLoS ONE, 2014; Peugnet et al, J Eq Vet Sci,2016; see also Forhead et al, J Endocrinol, 2004

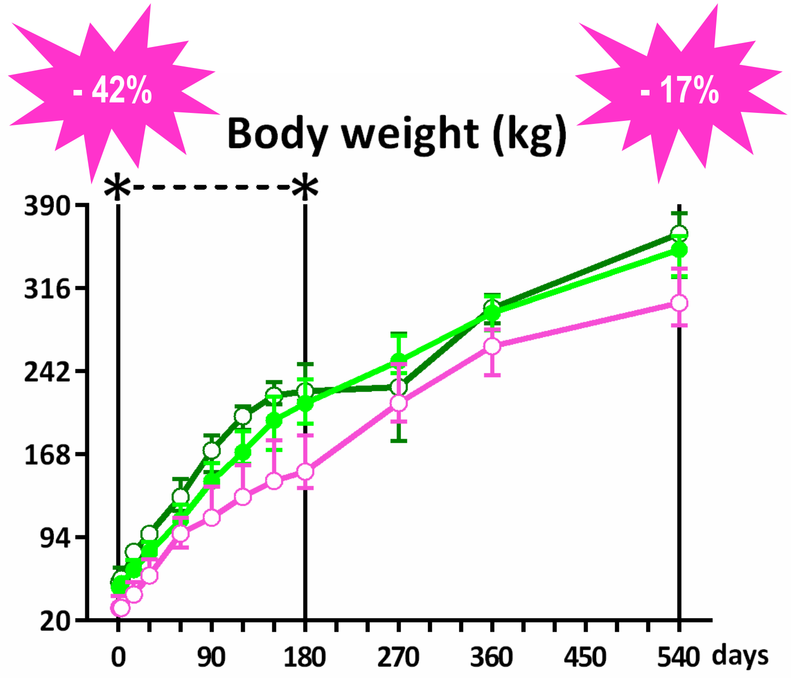
Models of restricted fetal growth
Lessons from between-breed embryo transfers

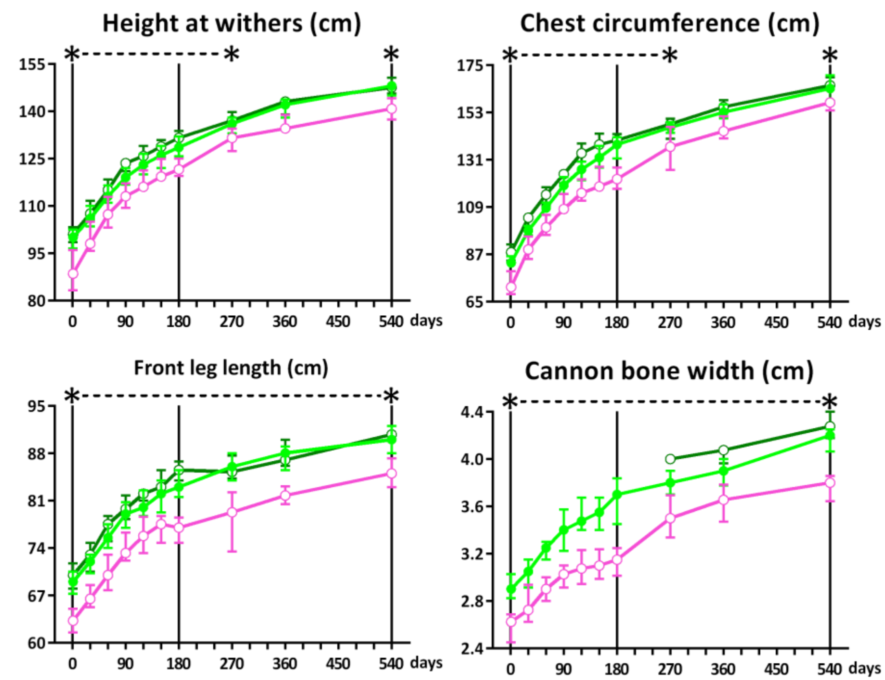
Allen et al, Reproduction, 2002, Peugnet et al, PLoS ONE, 2014, Robles et al. Equine vet J, 2018



Models of restricted fetal growth

"Restricted" saddlebreds (S-in-P, n=6)
Control saddlebreds (S-in-S, n=28)
"Enhanced" saddlebreds (Saddle-in-Draft, n=8)





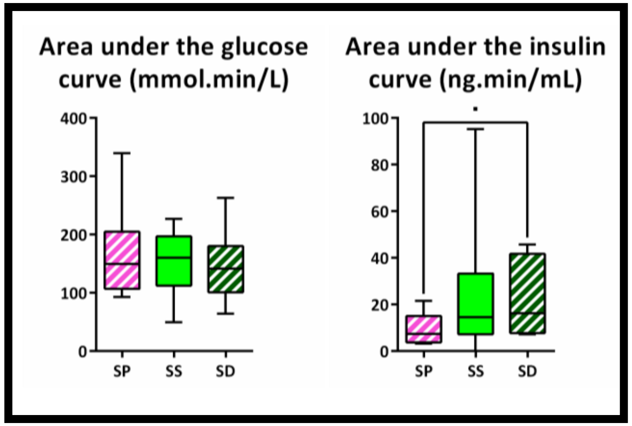
Peugnet et al, J Eq Vet Sci, 2016, see also Allen et al. 2002

Growth of "restricted" foals (Saddlebred-in-Pony) is slowed until weaning, then a disharmonious catch-up growth occurs.

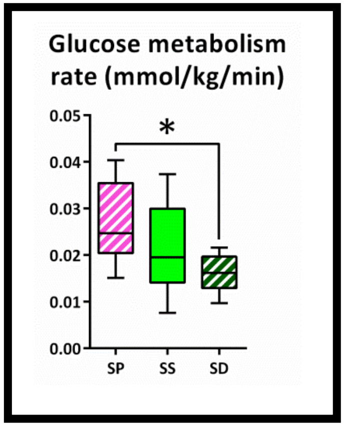
Models of restricted fetal growth

"Restricted" saddlebreds (S-in-P, n=6) | **Control saddlebreds** (S-in-S, n=28) | **"Enhanced" saddlebreds** (S-in-D, n=8)

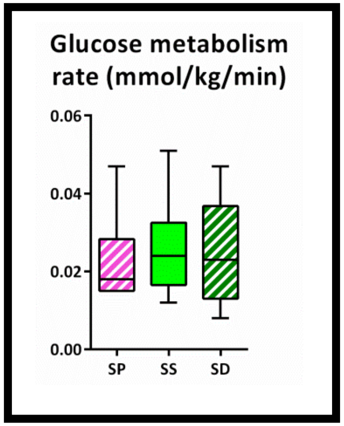
<u>Newborn - age 3 days</u>
IV glucose tolerance test



<u>Weanling - age 200 days</u>
Hyperinsulinemic euglycemic clamp



<u>Yearling - age 540 days</u>
Hyperinsulinemic euglycemic clamp



"Restricted" foals (Saddlebred-in-Pony) are more sensitive to insulin until weaning, but the effect disappears by age 1 year ½.

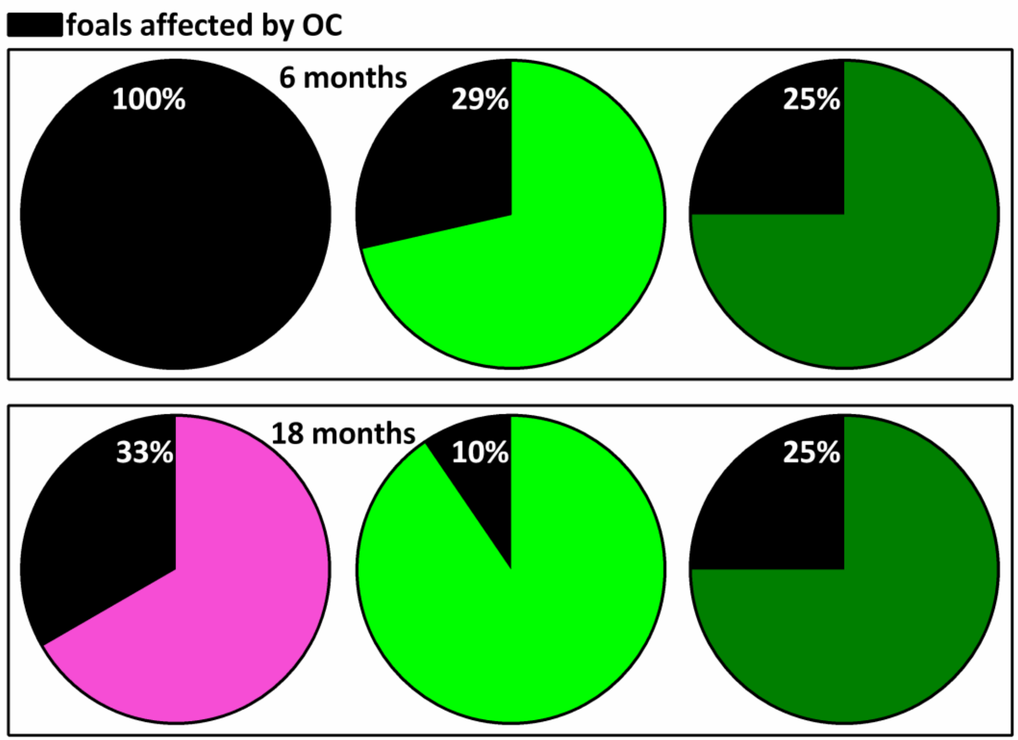
Peugnet et al, PLoS ONE, 2014; Peugnet et al, J Eq Vet Sci, 2016; Forhead et al, J Endocrinol, 2004

Models of restricted fetal growth

"Restricted" saddlebreds (S-in-P, n=6)

Control saddlebreds (S-in-S, n=28)

"Enhanced" saddlebreds (S-in-D, n=8)



Peugnet et al, J Eq Vet Sci, 2016

"Restricted" foals (Saddlebred-in-Pony) have an increased risk of osteochondrosis at age 6 months, but not any more at 18 months of age

In summary

	Enhanced ponies	Restricted horses
Environnement maternel	↗↗uterine capacity 🡐 Maternal glycemia reduced /ponies 🡐 ↗↗ milk production 🡐	🡒 ↘↘ uterine capacity 🡒 Maternal glycemia higher / SB or TB 🡒 ↘↘ milk production
Croissance fœtale	INCREASED 🡐	🡒 RESTRICTED
Croissance placentaire	Large placentas 🡐 No functional adaptation 🡐	🡒 Small placentas 🡒 ↘↘ *IGF-II* et *SNAT2* expression
Croissance postnatale	**AMPLIFIED / HARMONIOUS** 🡐	🡒 **SLOW / DYSHARMONIOUS CATCH-UP**
Homéostasie glucidique	Hypoglycemia 🡐 ↗↗ NEFA concentrations 🡐 Early insulin resistance 🡐 Later on, insulin resitance as for ponies 🡐	🡒 Hyperglycemia 🡒 ↘↘ NEFA concentrations 🡒 Early improved insulin sensitivity 🡒 Later on, insulin sensibility as for SB
Ostéochondrose	No effect on osteochondrosis (but ponies) 🡐	🡒 More osteochondrosis at 6 months of age
Triiodothyronine	↘↘ T3 concentrations 🡐	🡒 ↗↗ T3 concentrations

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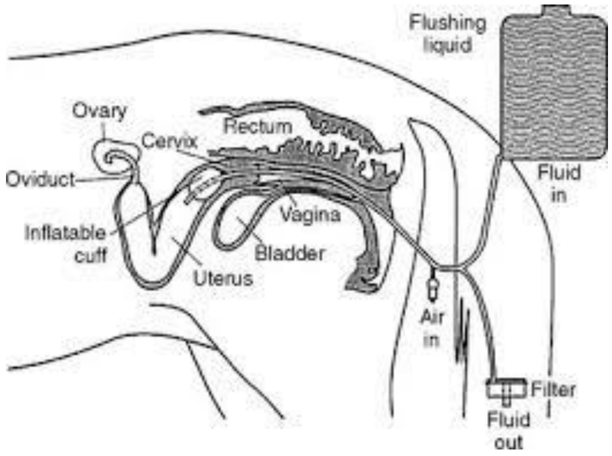
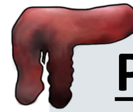
Obvious importance for embryo transfer



The recipient mare should be of the same size / breed as embryo

Effect of maternal undernutrition on health of foals

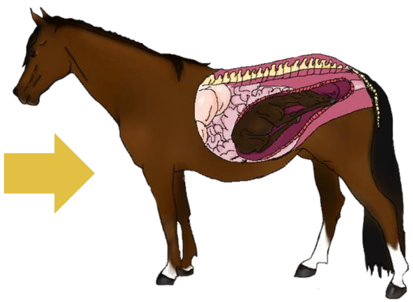


Placental adaptations

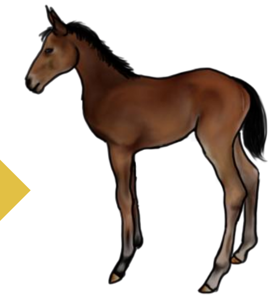
↗ vascularisation

↗ amino acids and vitamins transport and metabolism *Robles et al., 2018*

Moderate undernutrition (80% of energy needs)



No effect ***on in utero*** **and early post-natal growth**



Sutton et al., 1977; Banach and Evans, 1981; Henneke et al., 1984; Hines et al., 1987; Peugnet et al., 2015; Robles et al., 2017

Long-term effects

Delayed testicular maturation at 12 months of age

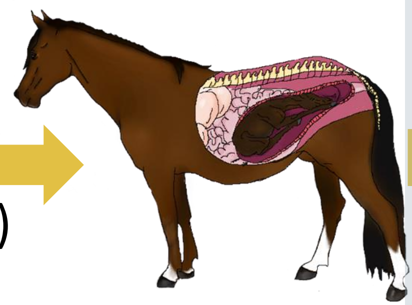
Reduced insulin sensitivity at 19 months of age

Reduced cannon width from 19 to 24 months of age (end of study)

Robles et al., 2017

Effect of maternal undernutrition on health of foals

Severe undernutrition (*Streptococcus equi*)



Placental adaptations

↗ placental gross area
BUT ↘ placental volume
➔ Decreased surface of microcotyledons

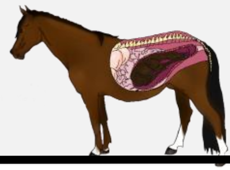
↘ birthweight

Wilsher and Allen, 2006

Long-term effects ?

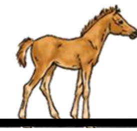
Primiparity: a physiological fetal growth restriction model?

Primiparous mare

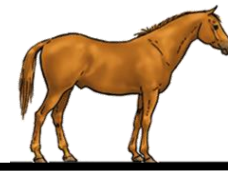


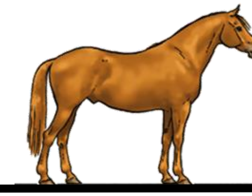
Conception

Birth



6 months
Weaning





Gestation
11 months

Lactation

Puberty
1-2 years

Adult
4-5 years

Impaired maternal metabolic adaptation (late gestation insulin resistance) to pregnancy
Robles et al. 2019
Reduced placental exchange surface
Wilsher & Allen 2003 ; Mereilles et al. 2017 ; Robles et al. 2018

Maternal milk production: inconsistent data
Doreau 1991 ; Wilsher & Allen 2003; Robles et al. 2018

Reduced size and weight
Hintz et al. 1979 ; Pool-Anderson et al 1994 ; Cymbaluk & Laarveld 1996 ; Fernandes et al 2014 ; Klewitz et al 2015 ; Meirelles et al. 2017 ; Robles et al. 2018

Delayed maturation of glucose metabolism
Robles et al. 2018

Delayed testicular maturation
Robles et al. 2018

Smaller and lighter foals
Doreau et al. 1991 ; Lawrence et al 1992 ; Pool-Anderson et al 1994 ; Cymbaluk & Laarveld 1996 ; Wilsher & Allen 2003 ; Elliott et al. 2009 ; Fernandes et al 2014 ; Klewitz et al 2015 ; Affonso et al 2016 ; Meirelles et al. 2017 ; Robles et al. 2018

Primiparity: a physiological fetal growth restriction model?

RACING

Performance and winning prizes positivement correlated to size at 1 year of age

Pagan et al., 2005 ; Smith et al., 2006

SHOW JUMPING and RACING

Performance is slightly reduced when born to primiparous mare

Barron, 1995 ; Palmer et al., 2018, 2019 (abstracts), Robles et al. in preparation

On going work to study effects of age and parity and how to prevent them

Emilie Derisoud, PhD student

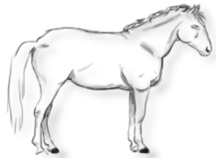
Effect of maternal overnutrition and obesity on health of foals

Overnutrition during pregnancy
(Obesity in late gestation)

Growth

Birth	**2 months of age**	**3 months of age**
		
No effect	↘ weight ↘ thoracic perimeter	No effect
Henneke et al., 1984; Kubiak et al., 1988 Robles et al. 2018	*Kubiak et al., 1991*	*Henneke et al., 1984, Robles et al. 2018*

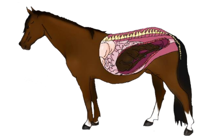
Effect of maternal overnutrition and obesity on health of foals



Obesity from early gestation

Insulin resistance, low-grade inflammation

Robles et al., 2018



Placental adaptations

Early gestation

↘ milk production?

Modification of milk quality (fatty acids)

<u>Endometrium</u>: inflammation and cytokines, Lipid regulation, mitochondrial stress

<u>Embryos</u>: inflammation, lipid regulation, ER, oxidative and mitochondrial stress

Altered uterine environment and embryo development at 8 and 16 days post ovulation

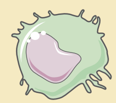
Sessions-Bresnahan et al., 2018

Robles et al. in preparation

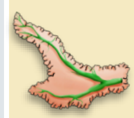
Whole gestation

Robles et al. in preparation

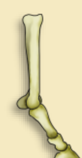
n foal growth



↗ low-grade inflammation until 6 months



↗ insulin resistance at 6 and 18 months

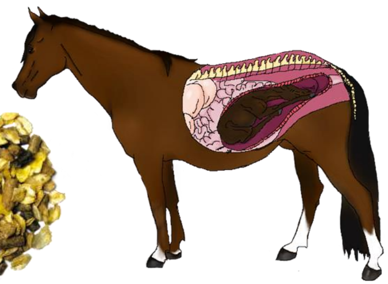


↗ osteochondrosis at 12 months

Robles et al., 2018

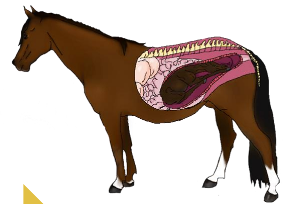
Both under & overnutrition alter long-term offspring health

Quantity of starch in the pregnant mares



Cereal concentrates

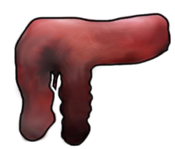
Flattened barley



Maternal hyperglycemia and increased insulin resistance

Peugnet et al. Plos One, 2015

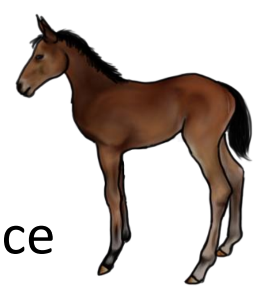
Foal and placental biometry not affected at birth



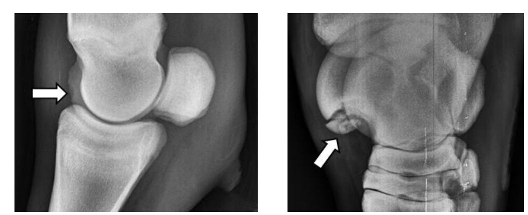
Placental morphological and gene expression modifications similar to observed in the placenta of diabetic women or of mares with laminitis

Robles et al. Placenta, 2018

↘ colostrum quality



↗ insulin resistance



Increased risk of developing osteochondrosis

Caure and Lebreton, 2004; Vander Heyden et al., 2012; Peugnet et al., 2015

Quantity of starch in the pregnant mares

BUT

- Quality of forage,
- Quantity of forage

Are often insufficient to meet nutrients needs in pregnancy

➔ **Distribution of concentrated feeds remains important during gestation**

Is it possible to provide enough energy to broodmares without affecting the health of their future foals?

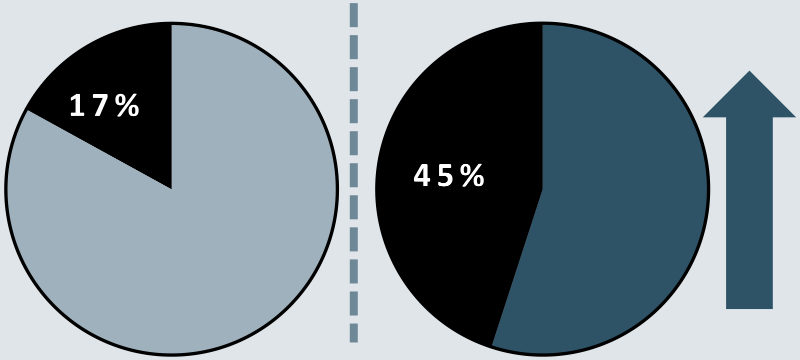
Quantity of starch in the pregnant mare

Flattened barley

Last 4.5 months of gestation

0g starch /100kgBW/meal

167g starch /100kgBW/meal



Increased number of foals with osteochondrosis lesions at 6 months of age

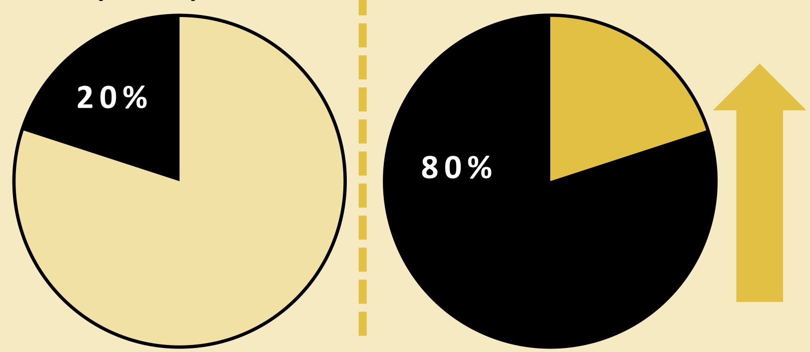
Peugnet et al., 2015

Flattened barley

Last 2 months of gestation

MAX 75g starch /100kg BW/meal (n=5)

MIN 110g starch /100kg BW/meal (n=5)

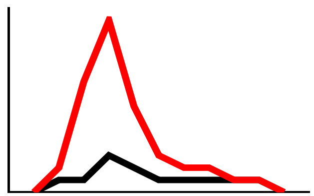


Increased number of foals with osteochondrosis lesions at 12 months of age

Robles et al., unpublished

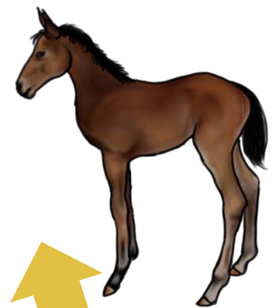
Quantity of starch in the growing foal

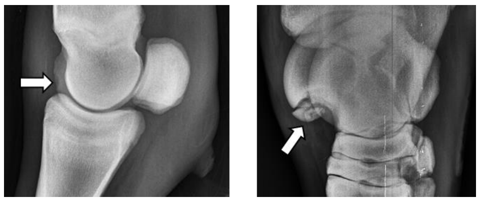
Mendoza et al., 2016



Glycemic and insulinemic response

Ralston, 1996, Pagan et al., 2001 Peugnet et al., 2015, Caure and Lebreton, 2004





Increased risk of developing osteochondrosis

Excessive growth rate
Overnutrition

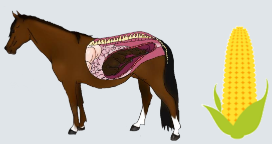
Donabedian et al., 2006

Maximum amount of starch for pregnant mares and growing foals: 100g/100kgBW/meal

Fat and fatty acids

Limits of presented studies: Diets richer in starch are also richer in energy

- Starch amount ?
- Energy amount ?
- Starch x energy ?



Diet rich in starch

Corn, **>100**g starch / 100kgBW / meal

Diet rich in fat

Corn oil 14% fat, **<15**g starch / 100kgBW / meal

- ↗ basal plasma glucose and insulin concentrations until 80 days of age
- ↗ insulin resistance at 160 days of age

George et al., 2009

Fat may be a good source of energy to decrease the amount of starch in diet of pregnant mares

Fat and fatty acids

Supplementing diet of stallions with ω-3

Quality of fat source?

Immunomodulatory properties
Fœtal neuronal development
Fertilit
Infla

Improves sperm quality in stallions

Brinsko et al., 2005, Freitas et al., 2016

Supplementing diet of mares with ω-3

Algae or fish source

Early gestation

↗ hospitable uterine environment

↗ embryo and trophoblast development

Jacobs et al., 2018

Late gestation & lactation

↗ ω-3 (EPA) in milk

↗ DHA transfer at birth

↗ social behaviour, memory and learning ability

Adkin et al., 2013, Adkin et al., 2015, Hodge et al., 2017

No effect: colostrum Ig concentration *(Adkin et al., 2013, Hodge et al., 2017)*, gestation length *(Adkin et al., 2013)*, placental and foal weight at birth *(Feirrera et al., 2012, Adkin et al., 2013)*, interval to first postpartum ovulation *(Adkin et al., 2013)*.

Proteins and amino acids

Growing foals
Excess proteins → No effect on development of osteochondrosis

Savage et al., 1993

In other species … (humans, mice, cows, pigs…)

Inadequate intake of proteins/amino acids (excess or defficiency)

Behaviour

Food intake
Taking risk behaviour

Health

Intrauterine growth restriction
Development of: Skelettal muscle, thymus gland, bone, hypothalamic-pituitary-gonadal axis
Glucose metabolism
Regulation of blood pressure

Lifespan

Depends on growth during early life

Chen et al., 2009; Jahan-Mihan et al., 2015; Herring et al., 2018

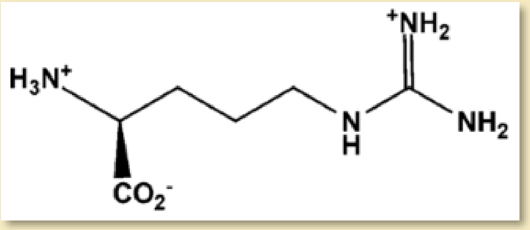
Proteins and amino acids

L-arginine *Wu et al., 2009*

Essential during pregnancy and growth in horses

Abondant in mares' milk compared to other species *Davis et al., 1994*

Protein synthesis ←



→ Creatine **Glucose tolerance**

↙ Nitric oxide **Vascularization**

↘ Urea cycle-Ornithine-Polyamines **Growth factors**

Early gestation

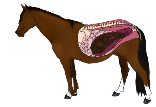
0.0125% of BW (68g) - from 15 to 45 days after ovulation

No effects on embryo size, increased early growth at D45?

Aurich et al., 2019

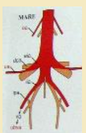
Arginine supplementation

Late gestation



Last 4 months of gestation (100g/d) *Robles et al., 2019*

- ↗ physiological insulin resistance in response to pregnancy in primiparous mares
- ↗ birthweight of foals born to primiparous dams
- ↗ placental gene expression of *GLUT1* and *CD36* in primiparous placentas



21 days before foaling (100g/d) *Mortensen et al., 2011*

- ↗ uterine artery bloodflow (non-pregnant horn)
- ↘ gestation length (-12 days)
- No effect on foal and placental weight at birth

Improves sperm quality in humans

Stanislavov et al., 2014, Kobori et al., 2015

Lactation (50g/d) *Hunka et al., 2016*

- No effect on milk composition
- No effect on growth of foals
- No effect on adiposity of foals

1. SETTING THE SCENE : DOHAD, EPIGENETICS AND CRITICAL PERIODS

2. PROOF OF CONCEPT IN EQUIDAE

3. IMPORTANCE FOR HORSE BREEDING

4. CONCLUDING REMARKS

Conclusion

- The effects of prenatal environment are so far probably underestimated and need to be further evaluated
- The choice and nutrition of the dam (and stallion…) are essential
- Markers of prenatal programming are urgently needed, as well as epigenetic tools in equidae €€€€€

Because…

The origins of the phenotype

$$P = G + E + Ep + G \times E \times Ep + M$$

Phenotype

Genetics

Environment

Epigenetics

Interactions

Metagenomics

Show jumping performance *(Palmer et al. 2018, 2019 abstracts; Robles et al in preparation)*

Random effect	% of the variance explained
Genetic value for performance	24.6 %
Genetic « maternal value » (concerns recipient in case of ET)	**1.4%**
Permanent environment of individual horse *(explains partly repeated successive performance)*	23.4%
Common environment of offspring of the same mare *(concerns recipient in case of ET)*	**1.6%**
Residual	49.6%

UMR Biologie du Developpement et Reproduction

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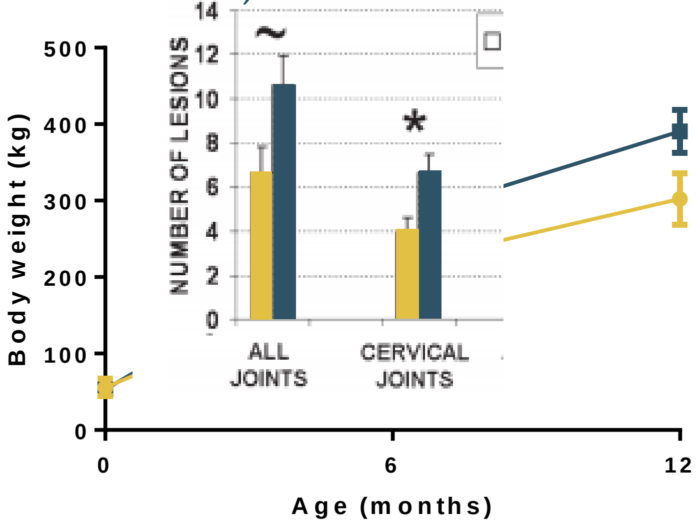
INRA SCIENCE & IMPACT

Eric PALMER
Provider of unlimited moral support and endless scientific questioning

Thank you

Growth rate of foals

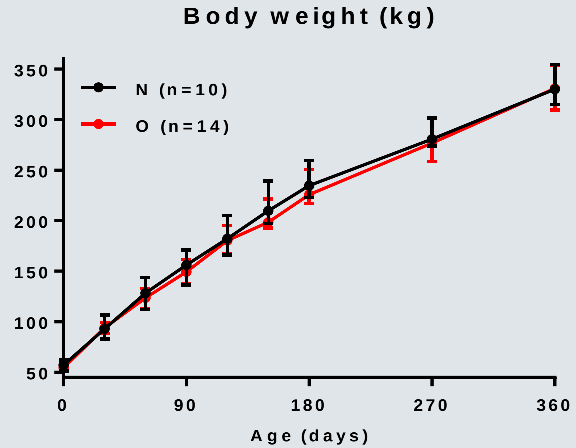
Donabédian et al., 2006

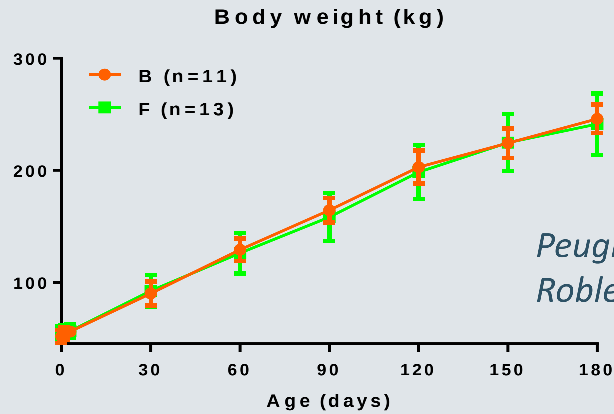


Fast growth rate is linked to osteoarticular diseases

Donabédian et al., 2006; Lepeule et al., 2009 ; Makvandi-Nejad et al., 2012; Teyssedre et al., 2012; Orr et al., 2013; Naccache et al., 2018

Normal growth rate is not a marker of osteoarticular diseases





Peugnet et al., 2015; Robles et al., 2018

↗ OC+ foals at 12 months of age

↗ OC+ foals at 6 months of age

Interests of vitamin supplementation

Improves sperm quality in stallions

Denniston et al., 2000
Deichsel et al., 2008
Gee et al., 2008
Freitas et al., 2016

Natural vitamin E

RRR-α-tocopherol, 1678mg/day
Last 3 months of gestation

Hoffman et al., 1999, Bondo et al., 2011

olostrum, milk and plasma of neonatal foals
d IgM colostrum concentration
n foal plasma concentration at 3 days of age

Oocyte and embryo quality?

Beta-carotene

1000mg/day
From 2 weeks before foaling until 6 weeks after

Kuhl et al., 2012

↗ β-carotene in colostrum and plasma foals at 1 day of age

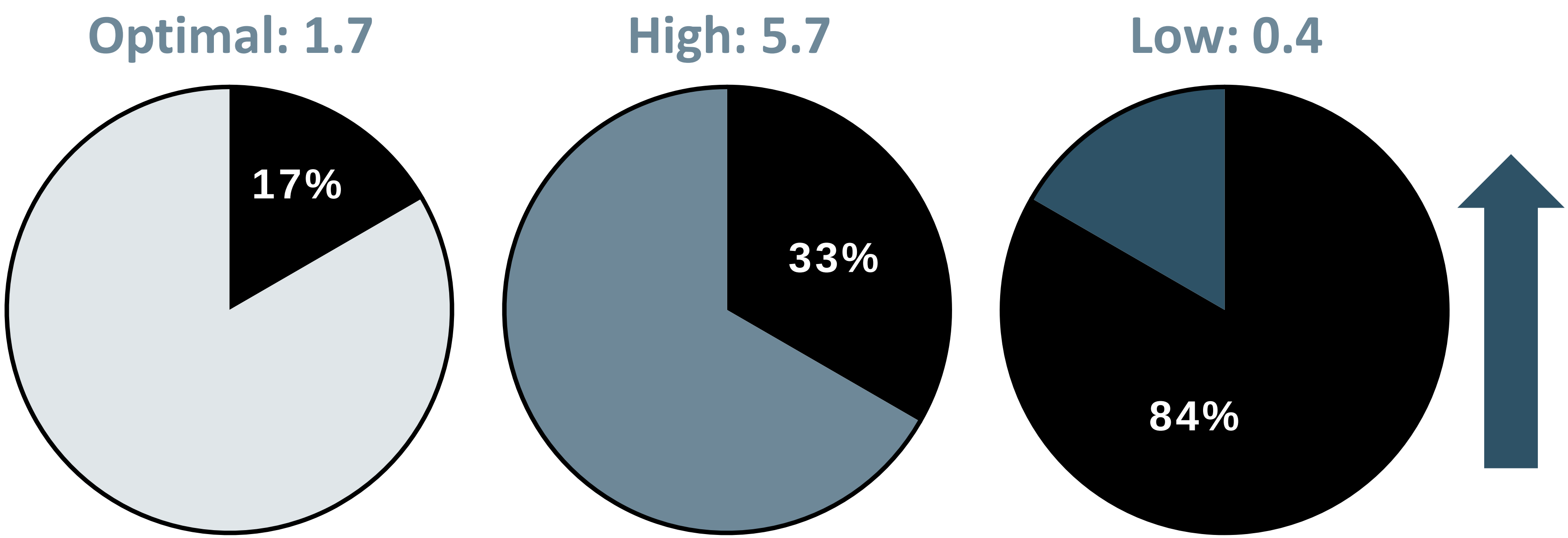
Supplementation of liposoluble vitamins improves the colostrum quality

Role of antioxidants in placental and fetal development ?

Calcium and phosphorus

Phosphocalcic ratio (Ca/P)

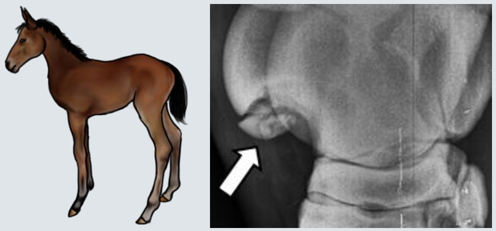
Between 4 and 8 months of age



Savage et al., 1993

Pregnancy ? Lactation ?

Copper and zinc

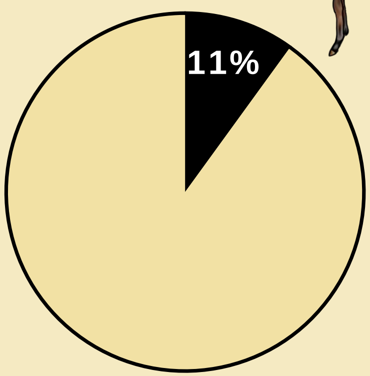


Osteoarticular diseases

- Plasma copper concentration below normal
- Occasionally zinc toxicosis
- Low copper storage in liver

Carbery et al., 1978, Bridges et al., 1984, Van weeren et al., 2003, Coskun et al., 2016

Foals between 3 and 9 months of age

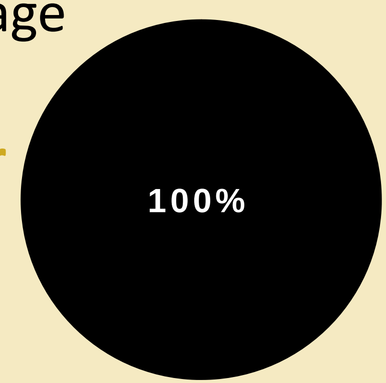


High Copper 25ppm

3 lesions

Low Copper 8ppm

29 lesions



Hurtig et al., 1993

Copper defficiency is associated to development of osteochondrosis lesions in foals

So… Supplementing the mare of the foal ?

21 Thoroughbred mares *Pearce et al., 1998*

REMINDER – NRC RECOMMANDATIONS

9 months → 0.2mg/kgBW

Then 0,25mg/kgBW

Control

0.1 to 0.2 mg/kgBW

Cu+

+0.5 mg/kgBW

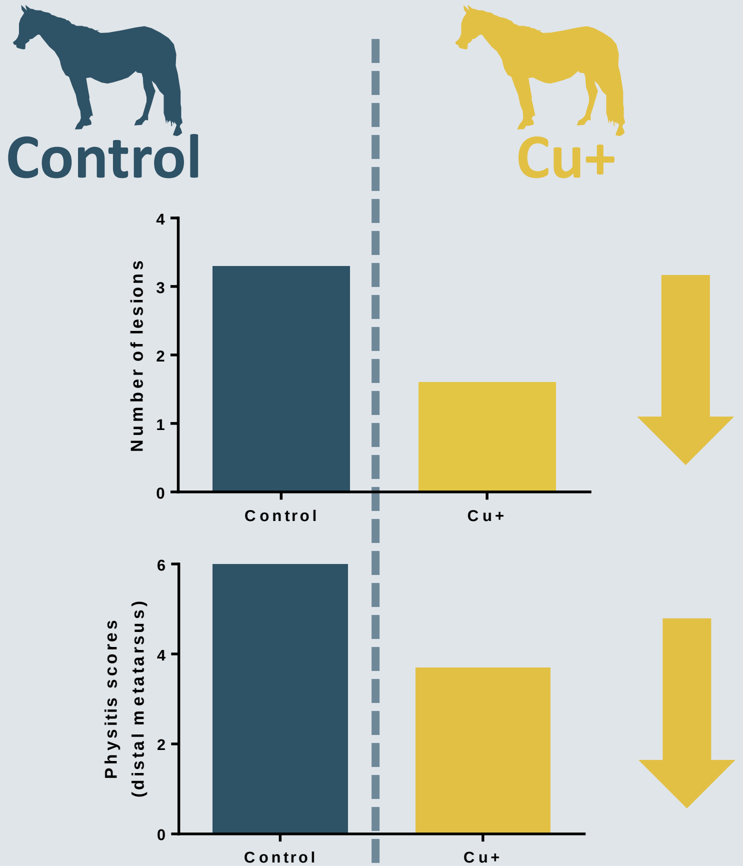
0.6 to 0.7 mg/kgBW

3 to 6 months before foaling

Control | **Cu+** | **Control** | **Cu+**

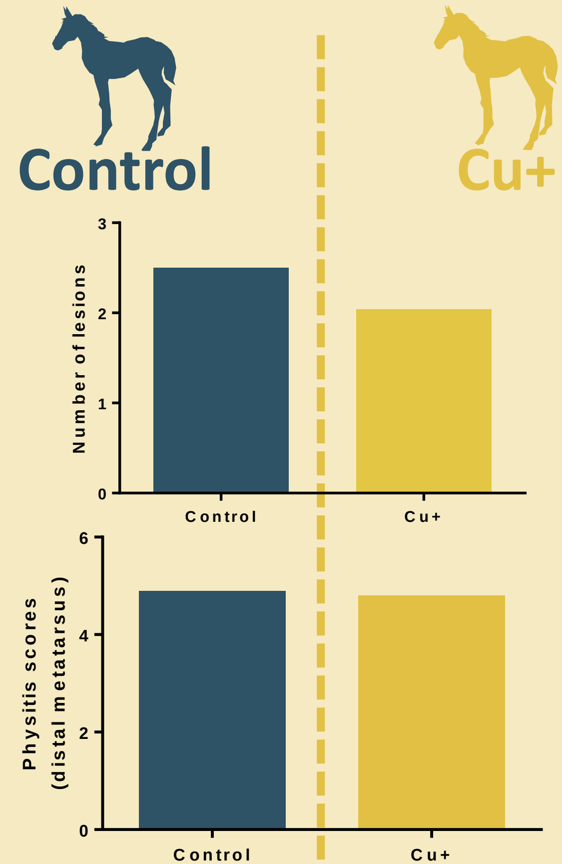
So… Supplementing the mare of the foal ?

Maternal supplementation



Decreased number and severity of lesions in supplemented group

Foal supplementation



No difference

Pearce et al., 1998

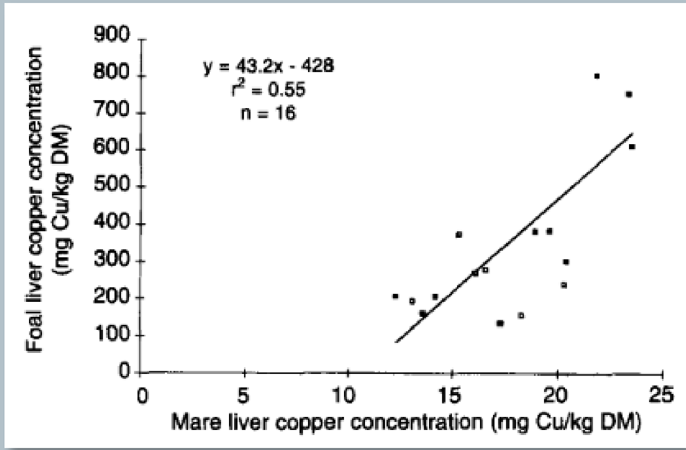
So… Supplementing the mare or the foal ?

BOTH

to avoid copper difficiencies

BUT

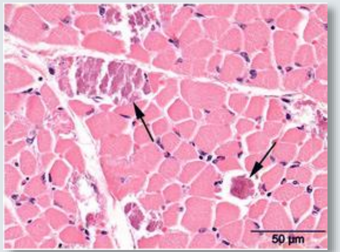
Pregnant mares (especially during the 3 last months of gestation) will benefit from supplementation above the recommandations



With respect to the balance between copper and zinc

Selenium

Delesalle et al., 2017



Selenium defficiency during pregnancy

Lofstedt, 1997

White muscle disease

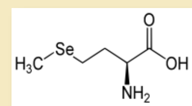
Myodegenerative pathology
Affecting skeletal and cardiac muscles
Lead to death of foal in most cases

Form of selenium

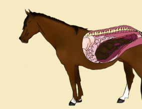
Selenium yeast

0.65ppm *vs* 0.35ppm/day
Last 4m of gestation

↗Se concentration in plasma and muscles *(Karren et al., 2010)*
↘ Leptin plasma concentration 36h after birth *(Cavinder et al., 2012)*
No effect on glutathione peroxidase



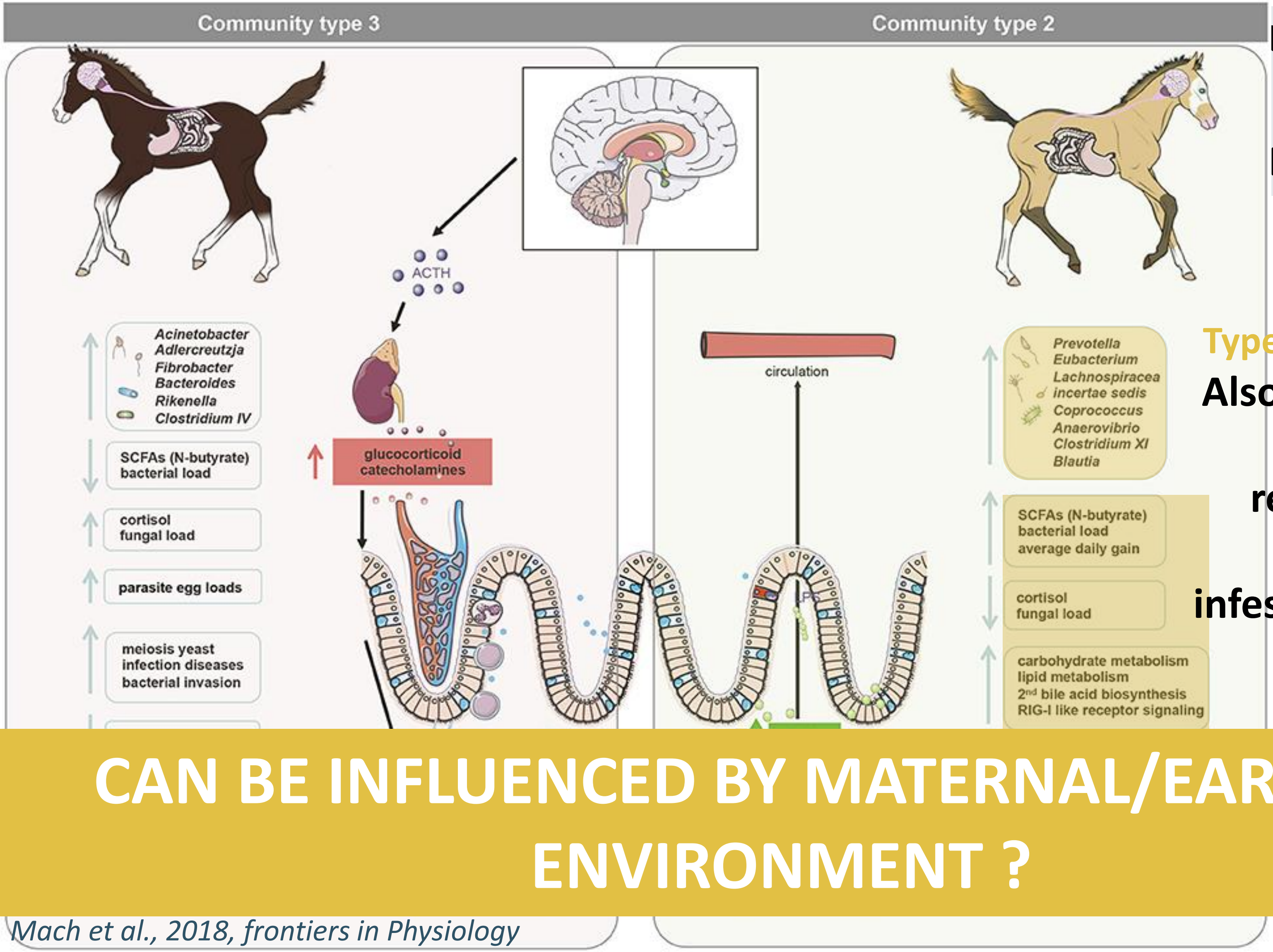
Selenomethionine *vs.* Sodium selenite



2mg/day
Last 3m of gestation and 1stm of lactation

↗Se in milk at 7 and 30 days of lactation
↗ plasma Se and specific alkaline phosphatase concentration at 30 days
↗ blood gluthatione peroxidase activity at 30 days of age *(Leleu et al., 2017)*

Pre-pro-postbiotics supplements and intestinal microbiota



...tabolic health

Growth

Behaviour

Type 2 community

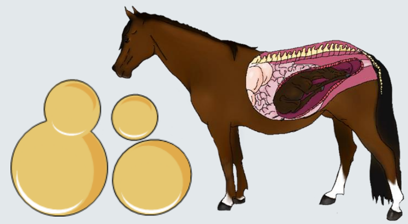
Also associated to increased resistance to strongyle infestation in adult horses

CAN BE INFLUENCED BY MATERNAL/EARLY LIFE ENVIRONMENT ?

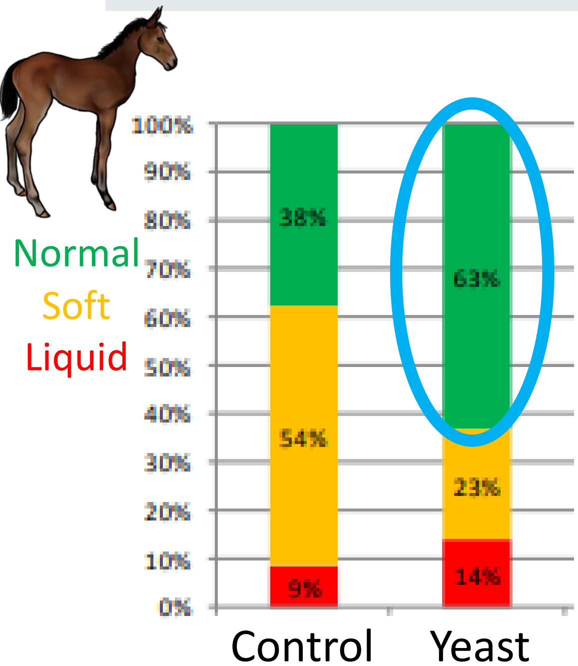
Mach et al., 2018, frontiers in Physiology

Pre-pro-postbiotics supplements and intestinal microbiota

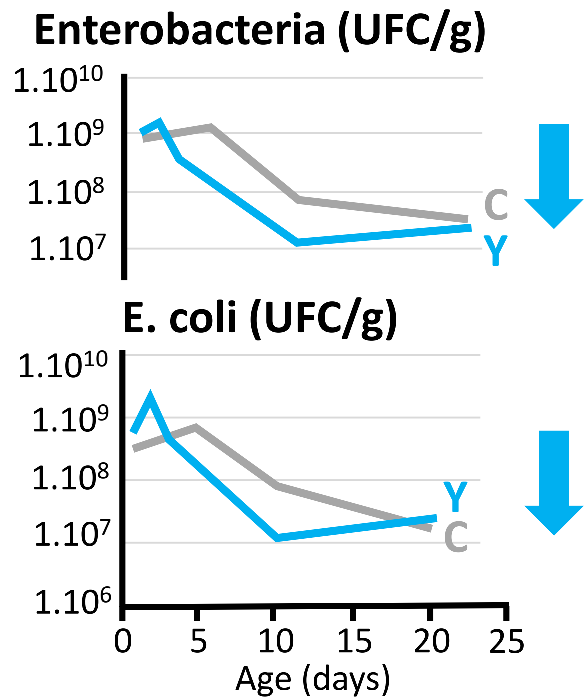
Betsch et al., 2014
Proceedings of the Journée de la Recherche Equine



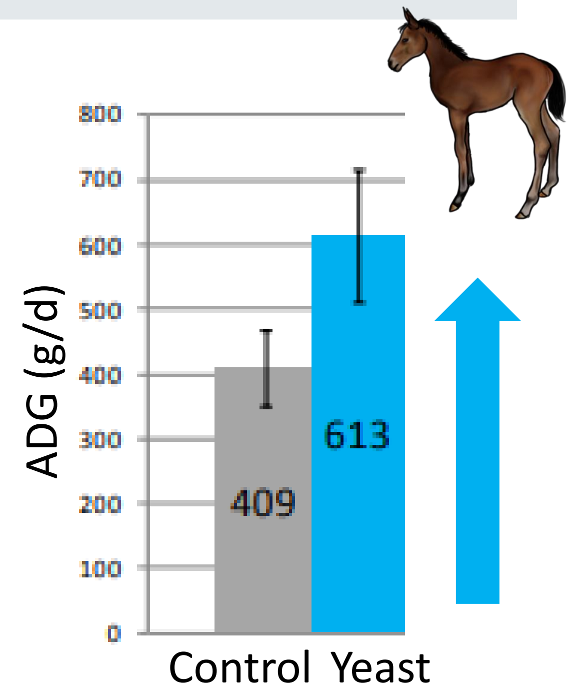
S. cerevisiae **CNCM-I1079**
7.1010CFU/day
8 days before to 4 days after foaling



↗ **proportion of normal-looking faeces**



↘ ***E.coli*** **and enterobacteria in faeces of foals at 10 days of age**



↗ **(tendency) of average daily gain (ADG) between birth and 20 days of age**

General synthesis – Pregnant mares and growing foals

Component	Recommendation	Remarks
Macronutrients		
Starch	100g/100kg BW/meal **MAX**	Quality of starch? → Limits glucose and insulin response

What remains unexplored ?

OTHER PHYSIOLOGICAL STAGES

Lactating mare and stallion

Periconception

DEVELOPMENT OF THE OFFSPRING

Very long term effects? Performance?

Muscular and cardio-vascular development, bone strength and resistance?

Reproductive capacity?

Behaviour?

Microbiota?

Milk?

NUTRITIONAL ADVICES

Other minerals and vitamins (chromium, iron, fluorine…)?

Protein intake and amino acid (methionine?)?

Chondroprotectors? Maca? Antioxidants? Beta-glucans?

Pre-pro-postbiotics?

PART 3 : EARLY MARKERS TO PREDICT THE DEVELOPMENT OF THE FOAL

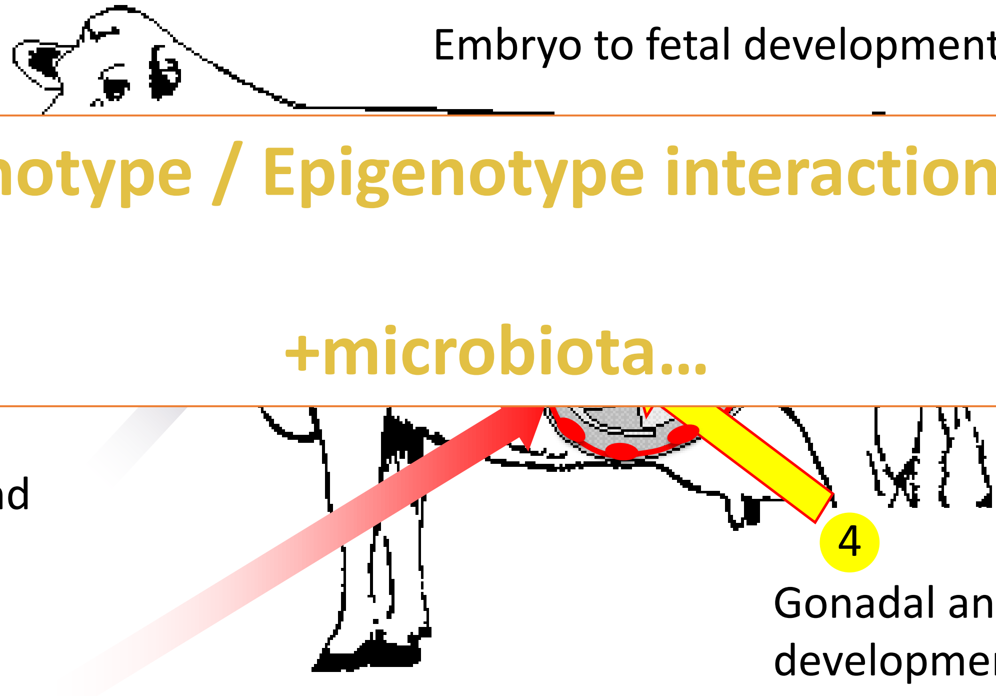
Pascale CHAVATTE-PALMER,
UMR BDR, INRA, ENVA, Université Paris Saclay, 78350, Jouy en Josas, France

Conclusion…

+ Large variations depending on genotype

1 The environment affects the gametes (F0)

Embryo to fetal development



Genotype / Epigenotype interactions….

+microbiota…

2 Embryo development and epigenetic marks are affected

3 The placenta regulates fetal growth and nutrition (F1) and adapts according to the fetal sex. Fetal adaptive mechanisms are conveyed by epigenetic marks

4 Gonadal and gamete development may be affected, thus having an effect on the next generation (F2) (inter-generational effects)

5 Epigenetic modifications in the gametes induce trans-generational effects (F>2)

1. REPRODUCTION: IMPORTANCE OF THE DAM

2. MONITORING THE BODY CONDITION AND METABOLISM OF THE PREGNANT MARE

3. PLACENTA MARKER OF PREGNANCY DISORDERS

1. REPRODUCTION: IMPORTANCE OF THE DAM

2. MONITORING THE BODY CONDITION AND METABOLISM OF THE PREGNANT MARE

3. PLACENTA MARKER OF PREGNANCY DISORDERS

Monitoring the body condition of the pregnant mare

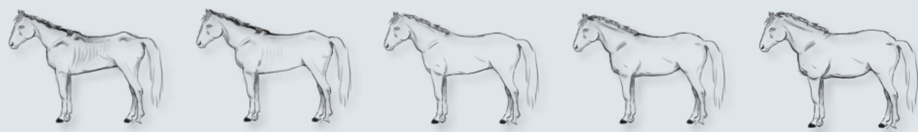
Why?

Because both under and overnutrition affect the long-term health of the foal

How?

Body condition score

Cresty neck score



Conformation changes

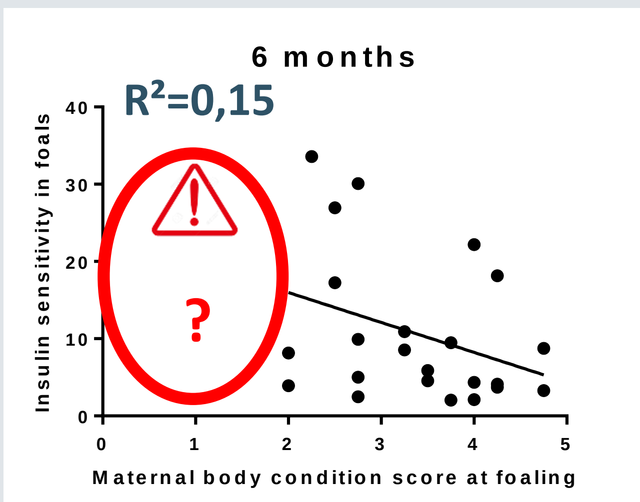
Shoulder and withers

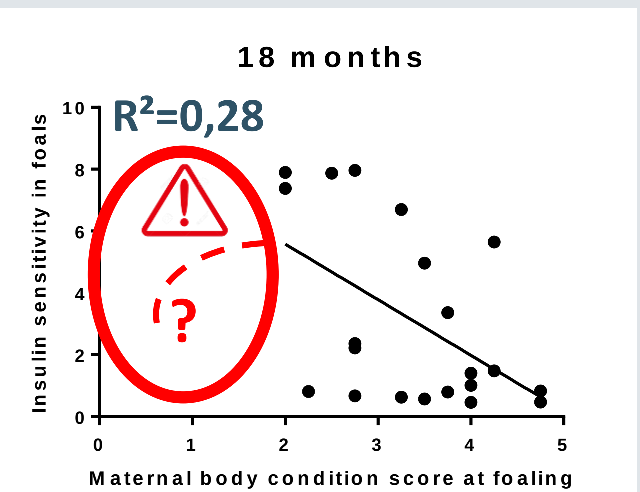
Musculature stretch

Henneke et al., 1984

Prediction of foal metabolism ?

CNS
Prediction of maternal insulin resistance?





Robles et al., 2017

Monitoring the body condition of the pregnant mare

Why?

Because both under and overnutrition affect the long-term health of the foal

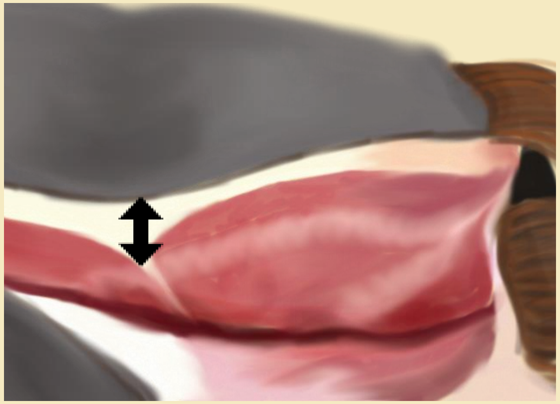
How?

Ultrasonography

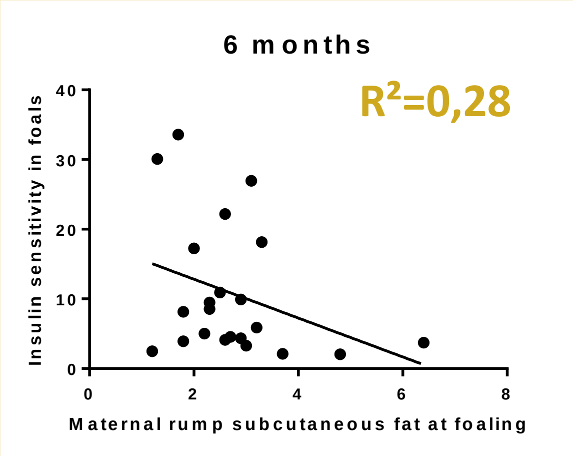
Main measures: rump

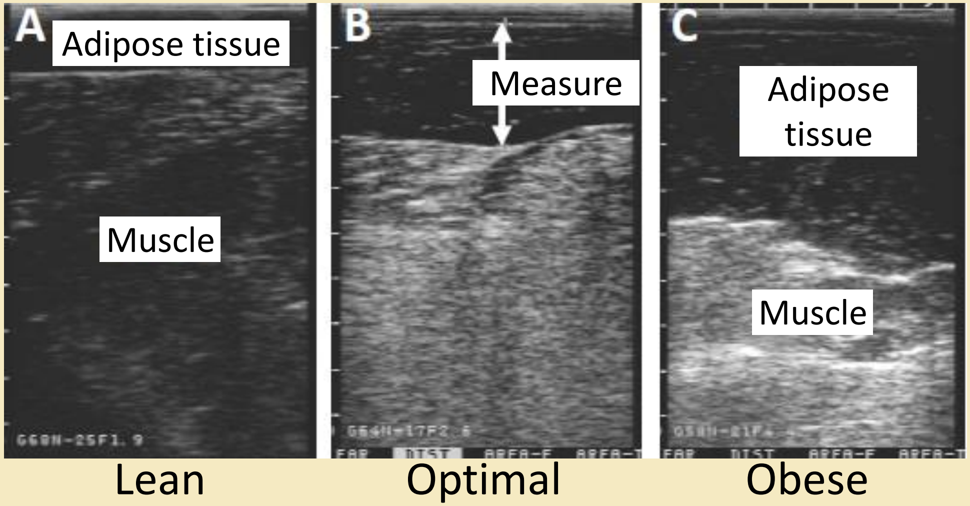
Other sites?

Correlation with metabolism?



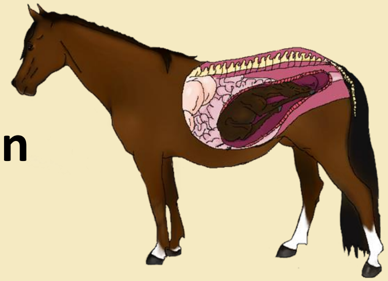
Prediction of foal metabolism ?

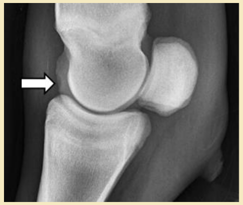


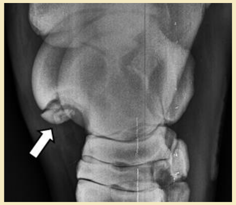


Monitoring the metabolic health of the pregnant mare

Maternal insulin resistance







Increased risk of developing osteochondrosis lesions

Caure and Lebreton, 2004; Peugnet et al., 2015; Robles et al., 2018

Basal/fasting glucose, insulin concentrations

Derived proxies (HOMA, RISQI, QUICKY)

No correlation with foal metabolism and osteochondrosis lesions

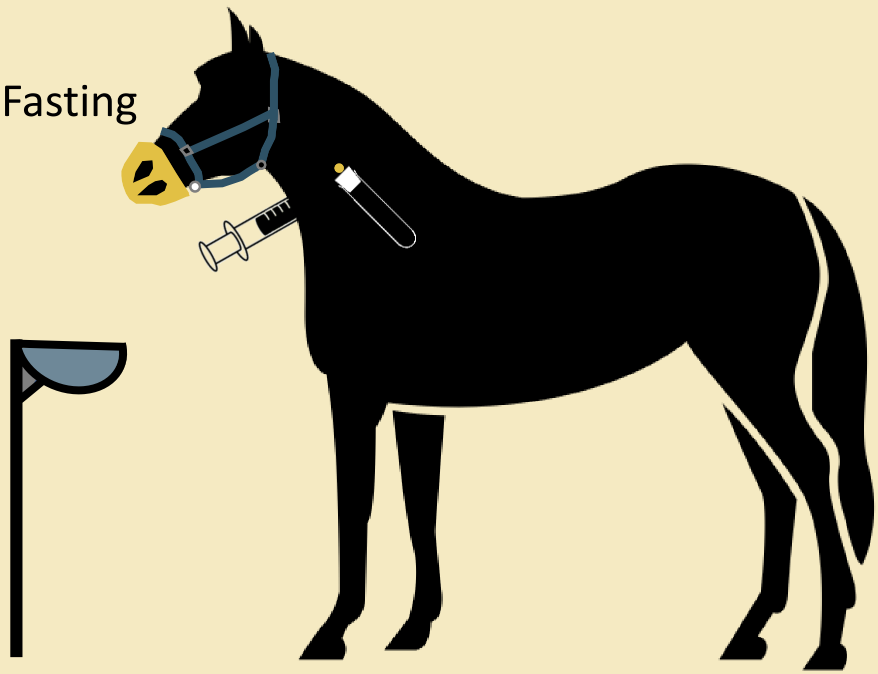
Peugnet et al., 2015; Robles et al., 2017; Robles et al., 2018

More complex and complete methods are needed

Euglycemic hyperinsulinemic clamps **IVGTT** **OGTT** **FSIGT**

IVGTT: Intravenous Glucose Tolerance Test

PROCEDURE



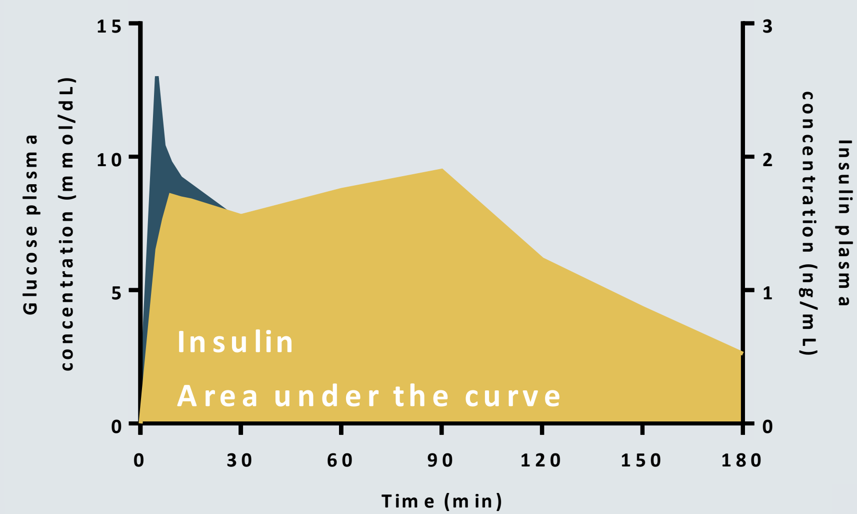
- Fasting sampling
- Intravenous glucose injection (250mg/kg)
- 3 hours sampling

ANALYSES

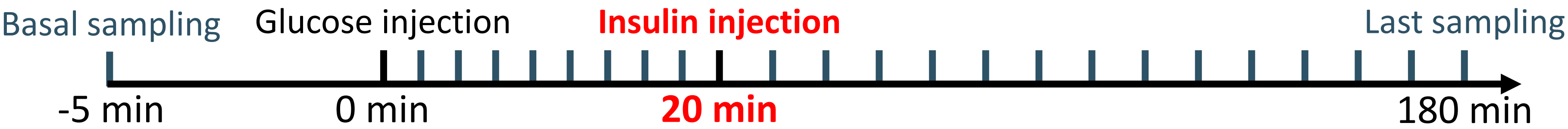
- Normalization
- Area under the curve of glucose and insulin plasma concentrations

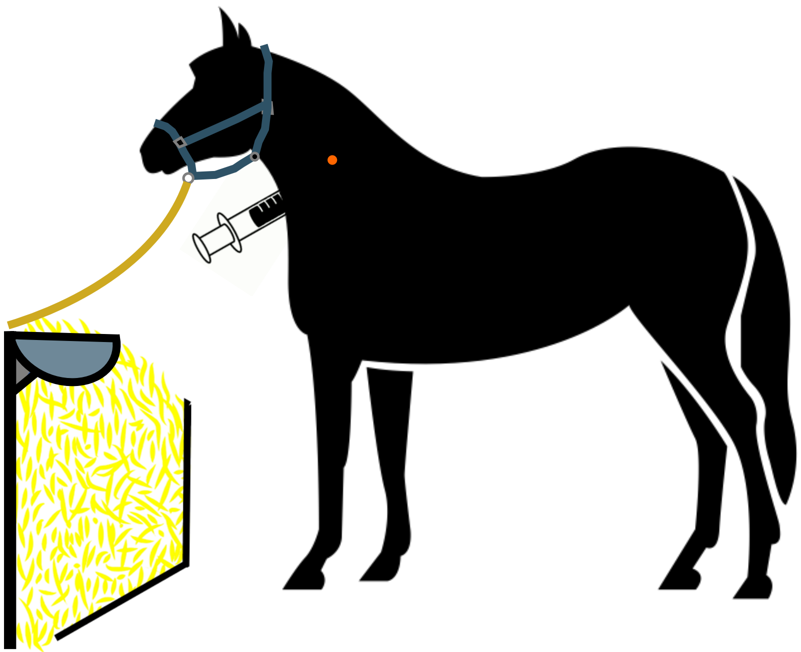
✓ **Glucose clearance**

✓ **Insulin release**



FSIGT: Frequently Sampled Intravenous Glucose Tolerance Test





Simultaneous estimation of insulin sensitivity and glucose tolerance through the calculation of quantitative indexes.

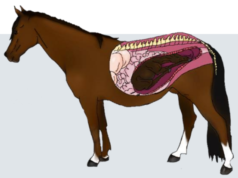
- Insulin sensitivity (SI)
- Short term pancreatic production of insulin (AIRg)
- Glucose mediated glucose transport (Sg)
- Whole body insulin sensitivity (DI=SI*AIRg)

- Basal sampling
- Intravenous glucose injection (100 mg/kg)
- Sampling for 19 min *Toth et al., 2009*
- **20 min, intravenous insulin injection (20 mUI/kg)**
- 3 hours sampling

Black box mathematic model

(Bergman et al., 1989, Boston et al., 2003)

Maternal insulin sensitivity



Pregnancy

↘ SI
↗ Glucose absorption
↗ Pancreas insulin production

Fowden et al., 1984; Hoffman et al., 2003; Peugnet et al., 2015; Beythien et al., 2017

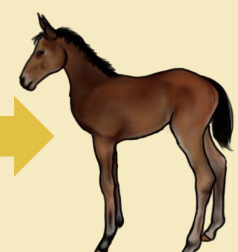
+ differences between breeds and seasons *(Funk et al., 2012; Bamford et al., 2014; Peugnet et al., 2014, Beythien et al., 2017)*

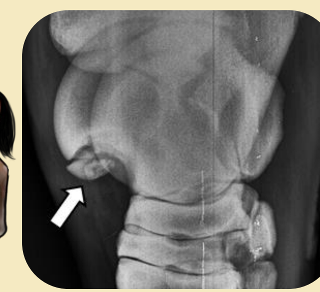
Need to develop reference charts according to stage of gestation, age, parity and breed of the mare

+ interaction with environment of the growing foal (pasture, stable, cereals,…)

French Anglo-Arabian mares

SI<1 at 300 days of gestation





↗ Osteochondrosis lesions

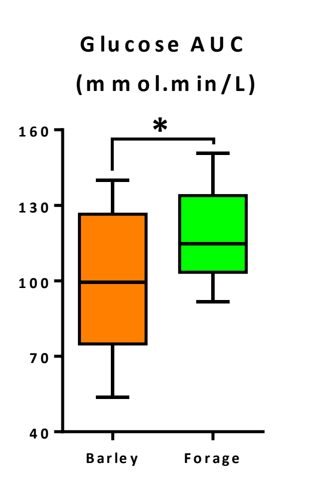
Foal insulin sensitivity

NOT PREDICTIVE

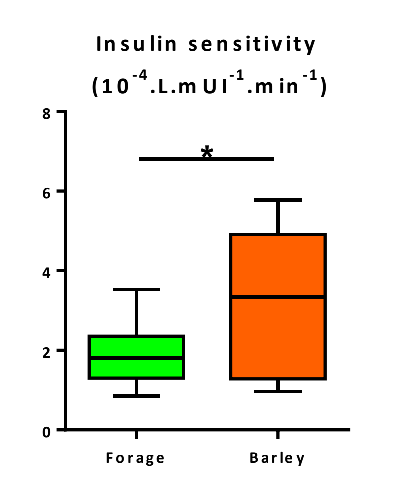
Two examples …

Foals born to mares fed with forage only or forage and barley

↗ glucose tolerance 3 days of age

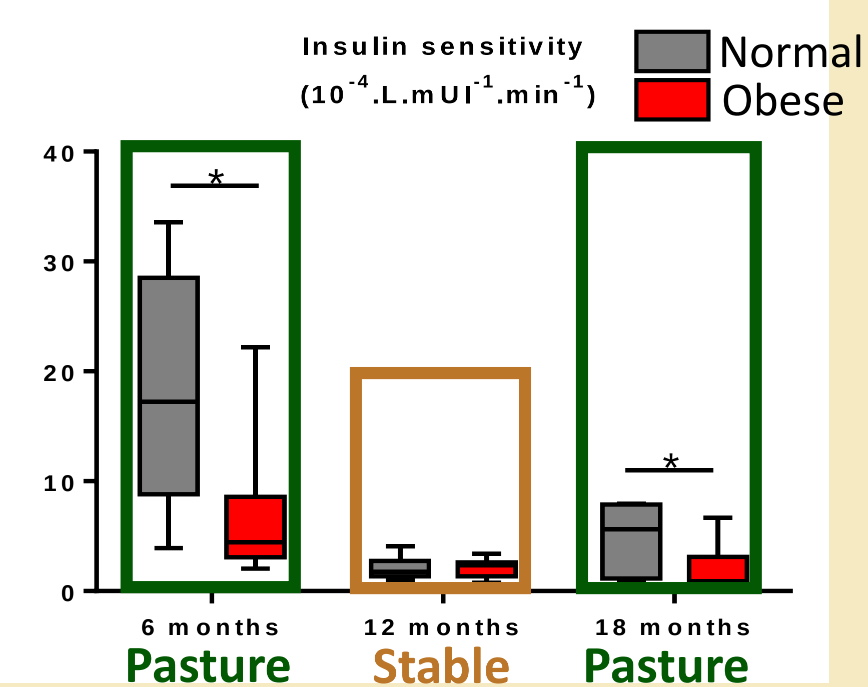


↘ insulin sensitivity 19 months of age



Peugnet et al., 2015; Robles et al., 2017

Foals born to obese or normal mares



Robles et al., 2018

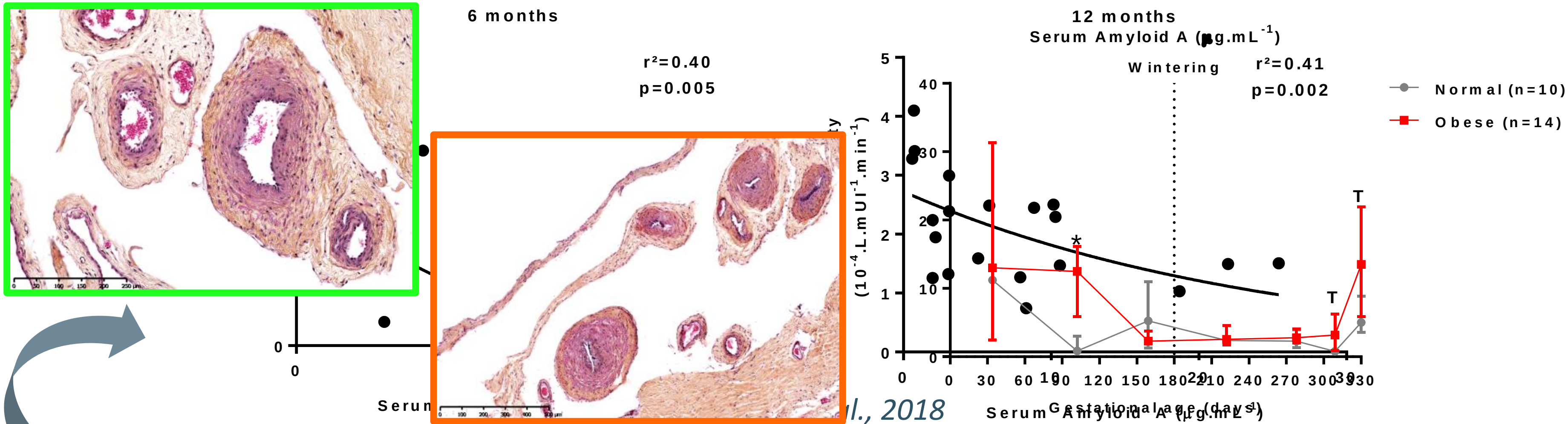
1. REPRODUCTION: IMPORTANCE OF THE DAM

2. MONITORING THE BODY CONDITION AND METABOLISM OF THE PREGNANT MARE

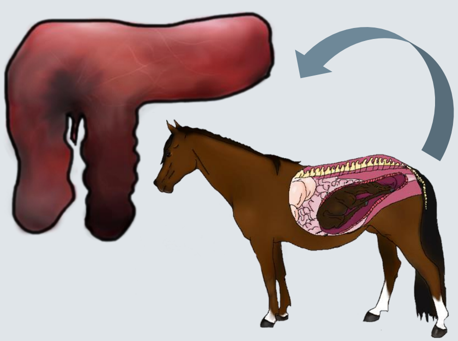
3. PLACENTA MARKER OF PREGNANCY DISORDERS

Maternal systemic inflammation

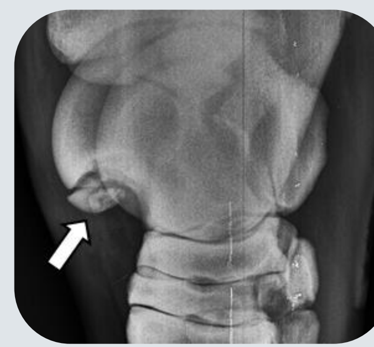
Insulin resistance is correlated to systemic inflammation



, 2018



Maternal and placental inflammation

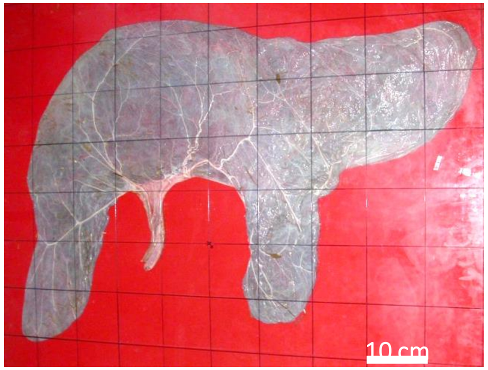


↗ Osteochondrosis lesions

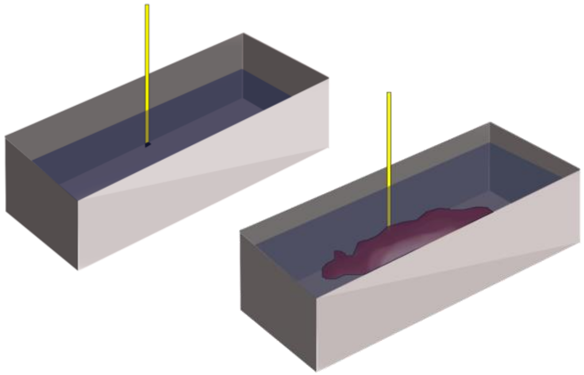
Robles et al., 2018, Placenta; Robles et al., 2018, PLOS ONE

Measurements of placentas and foals at birth

Weight + placental efficiency



Surface



Volume

Peugnet et al., 2014, PLOS ONE; Robles et al., 2018, Placenta
Robles et al., 2018, Equine Veterinary Journal; Robles et al., 2018, Theriogenology

NOT GOOD PREDICTIVE MARKERS

Measurements

- Strongly correlated to maternal wither's height *(Allen et al., 2002, Robles et al., 2018)*
- ↘ in primiprous pregnancies *(Wilsher and Allen, 2003; Elliott et al., 2009; Meirelles et al., 2017; Robles et al., 2018)*
- ↘ in very young and old mares *(Eliott et al., 2009)*

Measurements of placentas and foals at birth

NOT GOOD PREDICTIVE MARKERS

BUT

Examine carefully the placenta: missing parts, white patches, abnormal coloration of the villous side

When measurement strongly differ from expectations

Intra uterine growth retardation

Weight below the 10th percentile

Embryo transfer between breeds

Primiparity

Affects:

- Metabolism
- Osteoarticular development
- Reproductive maturity
- Cardiovascular function

Giussani et al., 2003; Forhead et al., 2004; Peugnet et al., 2014, 2016; Robles et al., 2017

Overgrowth

Weight above the 90th percentile

Embryo transfer between breeds

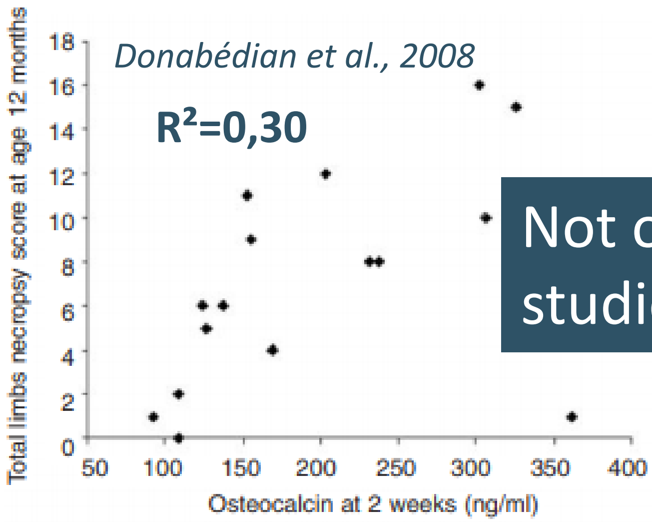
Affects:

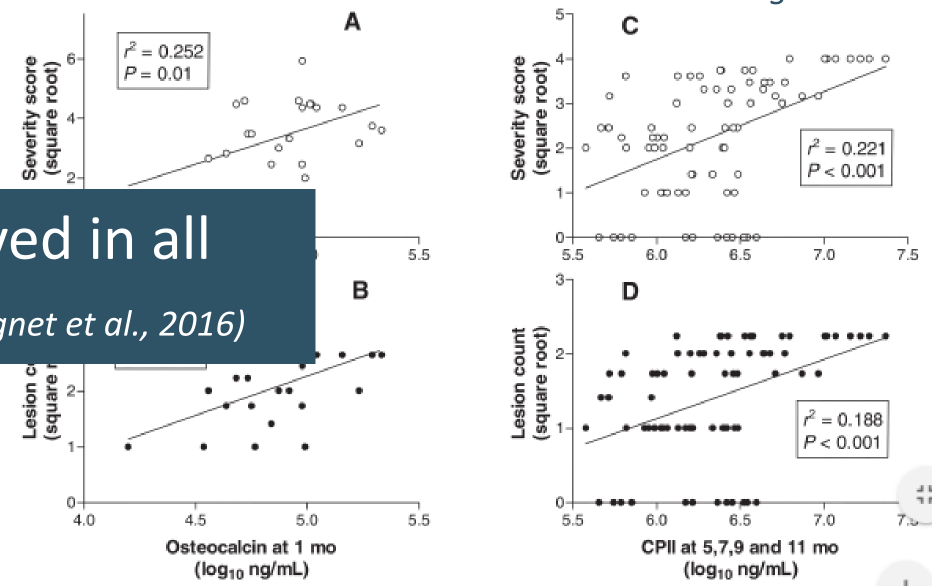
- Metabolism
- Cardiovascular function

Giussani et al., 2003; Forhead et al., 2004; Peugnet et al., 2014, 2016

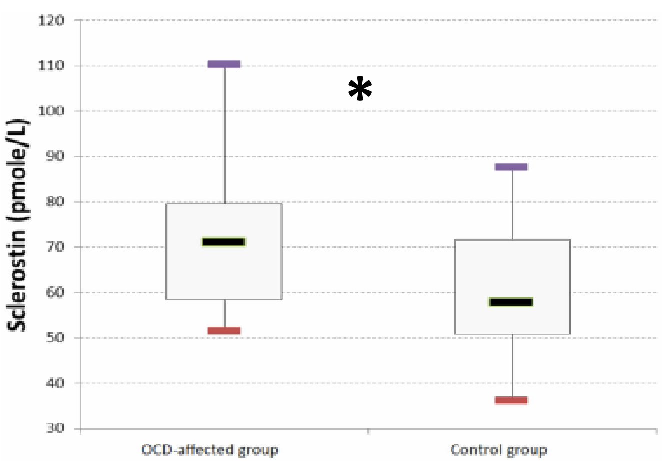
Blood markers of osteochondrosis ?

Clark Billinghurst et al., 2004





Not observed in all studies *(Peugnet et al., 2016)*



Serteyn et al., 2014

No correlation between CTX-II, Bone-specific alkaline phosphatase and hydroxyproline, markers of collagen synthesis and degradation, and osteochondrosis lesions

Nicholson et al., 2010; Peugnet et al., 2016

Role of colostrum quality ?

Composition colostrum vs milk

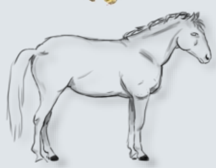
Energy: ↗ fatty acids (expecially long-chain saturated), ↗ proteins (whey proteins, free amino acids),

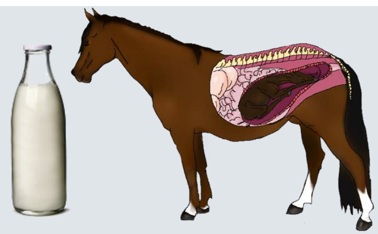
Vitamins and minerals: ↗ zinc and copper, ↗ liposoluble vitamins,

Immune system: Immunoglobulines, native immune cells and cytokines, oligosaccharides

Csapo-Kiss, et al., 1995; Pikul et al., 2007; Burton et al., 2009; Secor et al., 2012; Perkins et al., 2014, Difilippo et al., 2015; Robles et al., unpublished

~~Starch intake~~

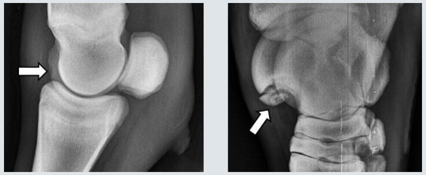




↘ colostrum quality

↘ IgG

↗ concentration in palmitic (C16:0) and linoleic acids (ω6) fatty acids



Caure and Lebreton, 2004; Robles et al., unpublished

Direct or indirect effect ?
Maternal metabolism
Transplacental transfer