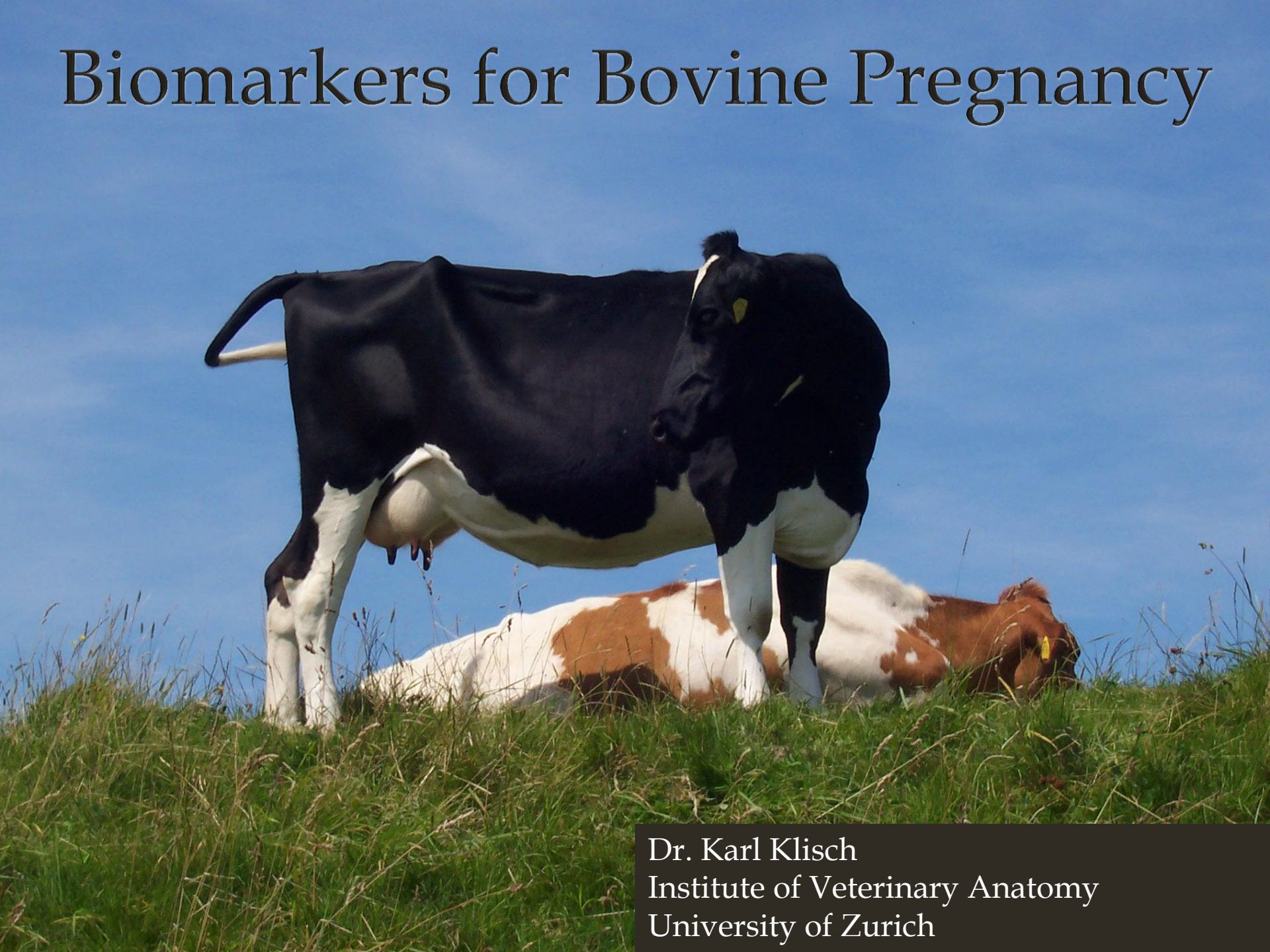
Biomarkers for Bovine Pregnancy



Dr. Karl Klisch
Institute of Veterinary Anatomy
University of Zurich

The ideal bovine pregnancy test:

-early (day 20)
-non invasive
-reliable
-cheap
- ....

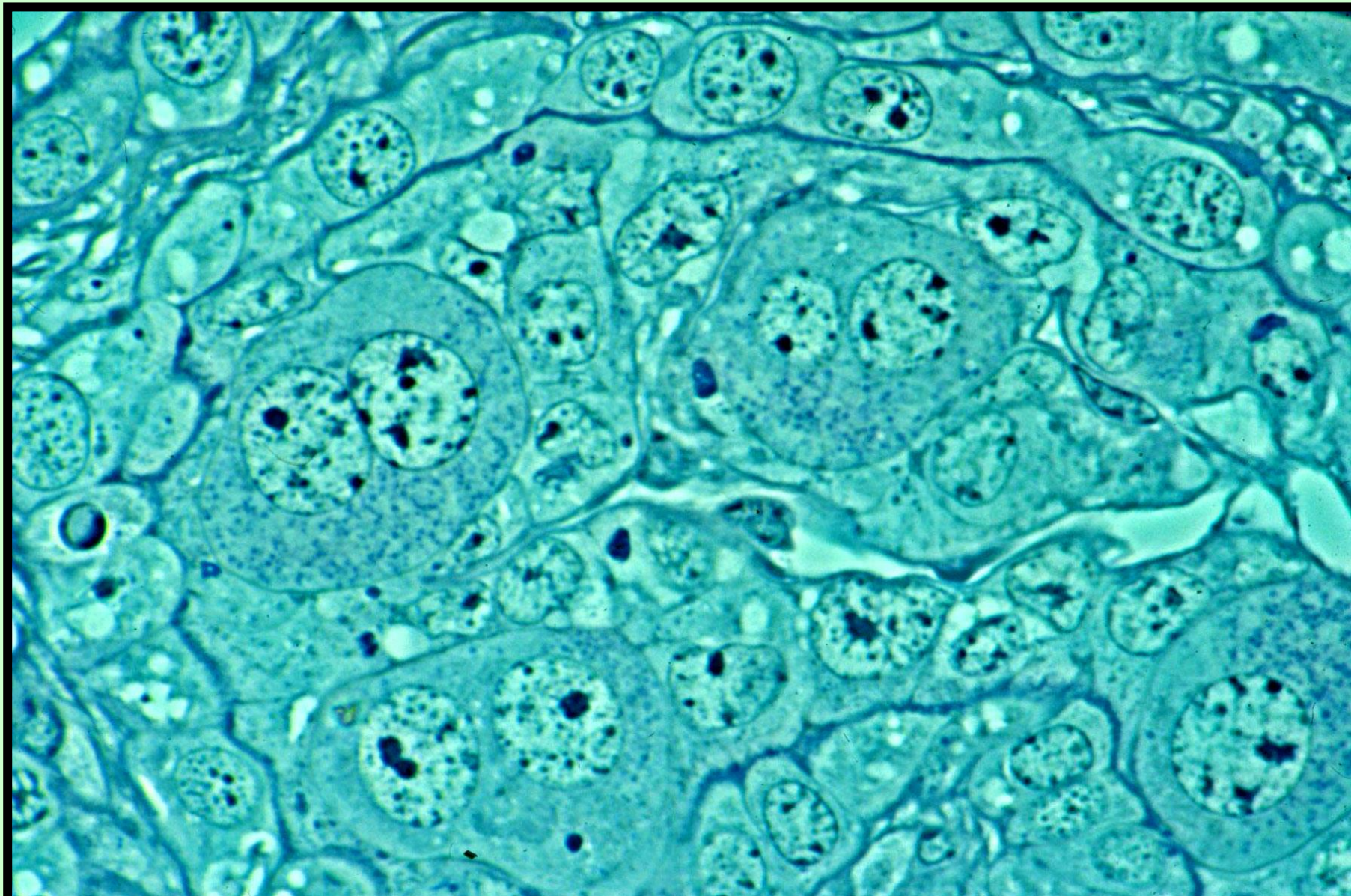
What is available?

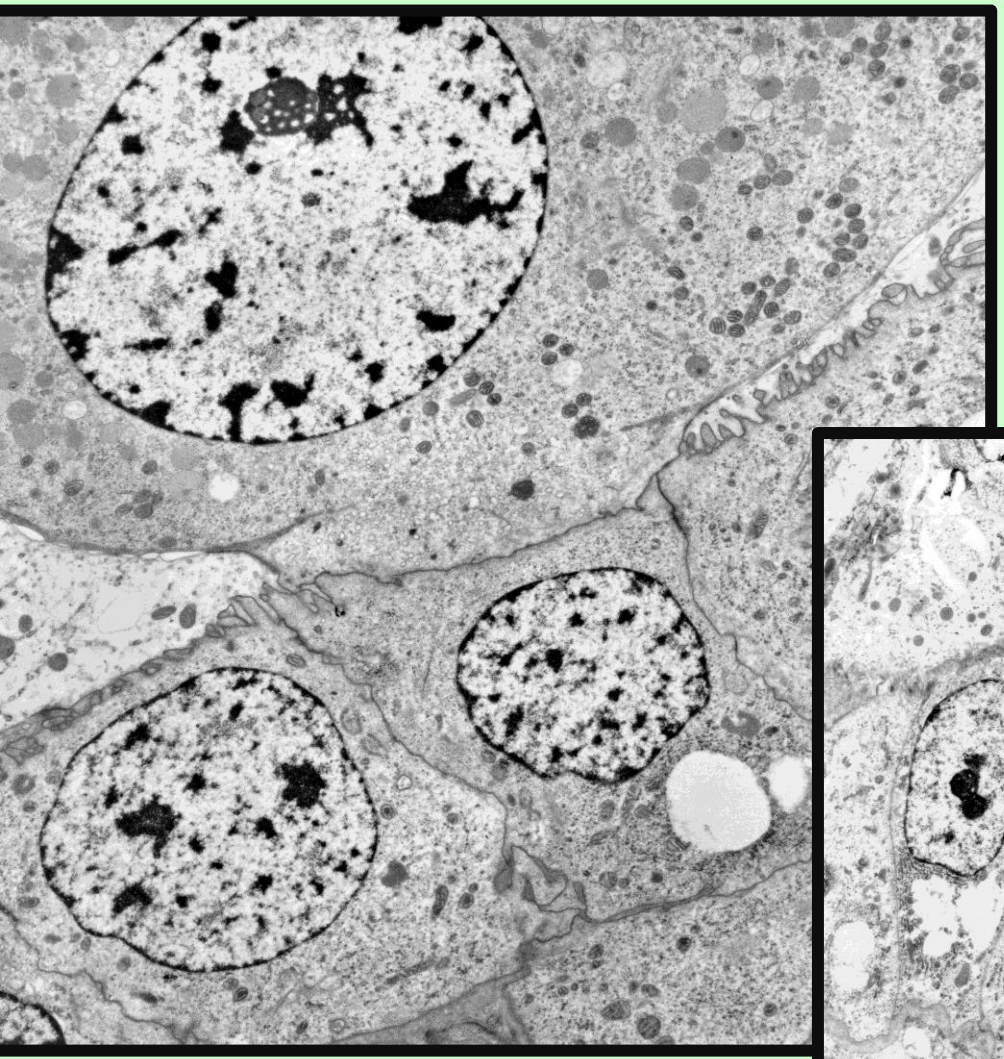
-Rectal Palpation
-Ultrasound
-Progesterone
-Oestrone sulphate

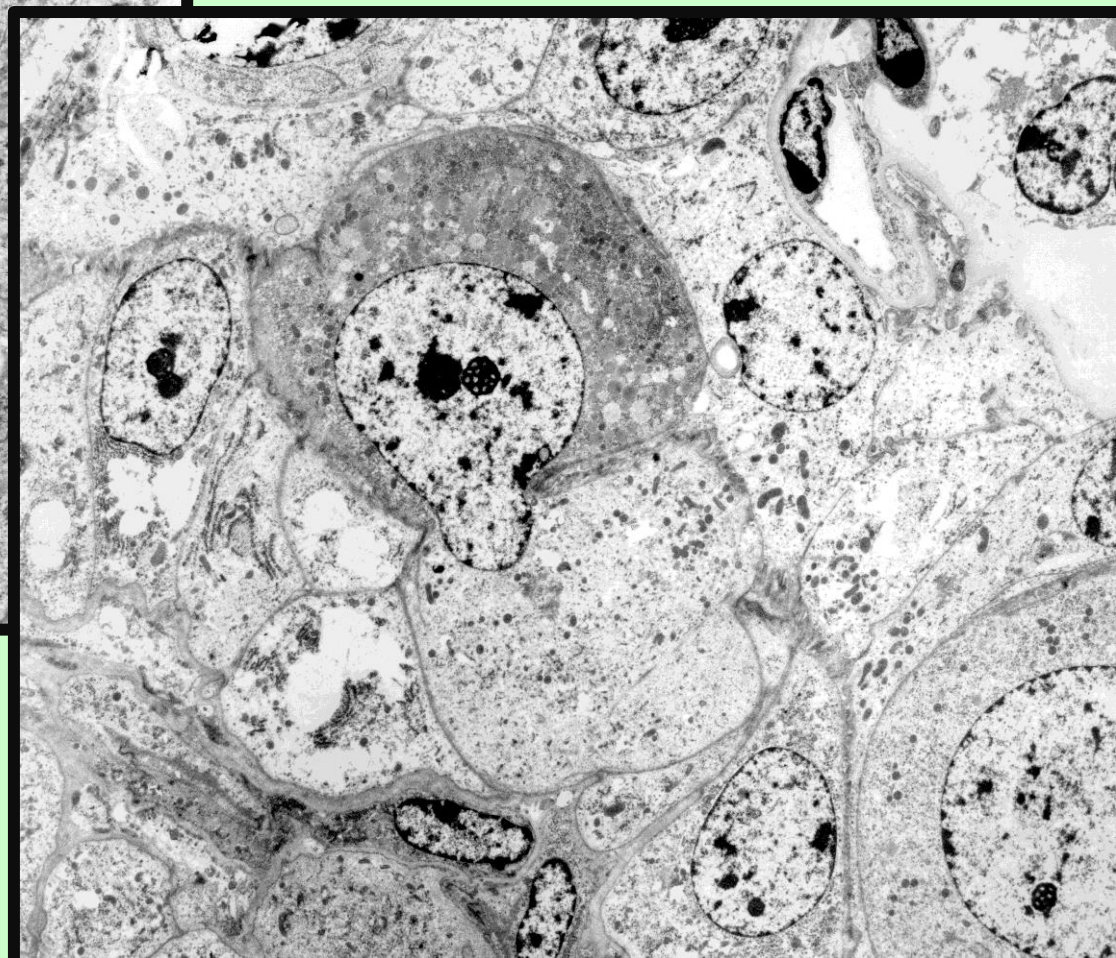
-Pregnancy Associated Glycoproteins (PAGs)
-Interferon-tau stimulated gene expression in PBLs
-Circulating Fetal Nucleic Acids
-Early Pregnancy Factor
-Preimplation factor
-Seed Germination Inhibition

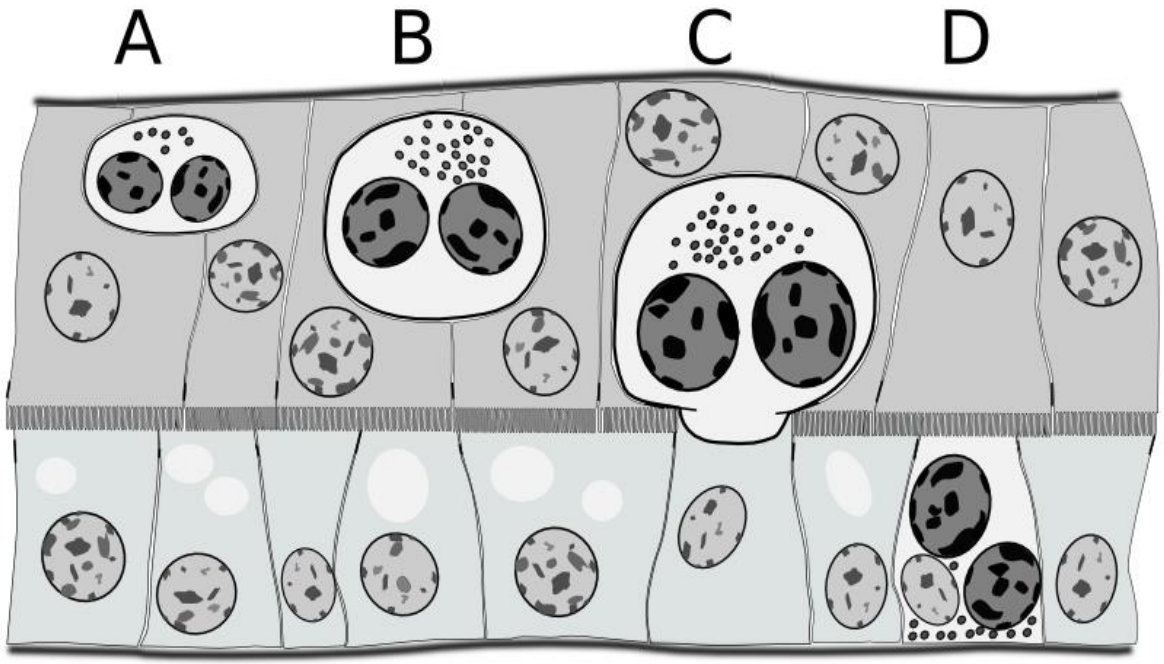
Bovine Placenta

-binucleate trophoblast (giant) cells (BNC)









Granules contain:

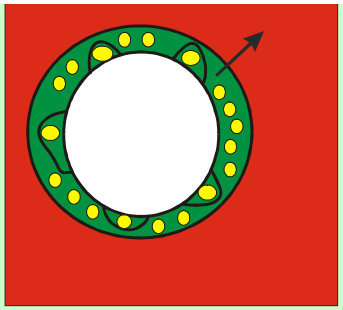
-pregnancy associate glycoproteins (PAG)

-placenta lactogen (PL)

-prolactin related protein-1 (PRP1)

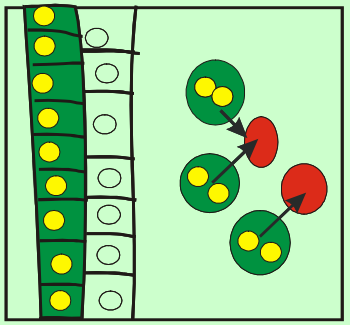
Several ways to deliver trophoblast derived proteins into the mother:

hCG



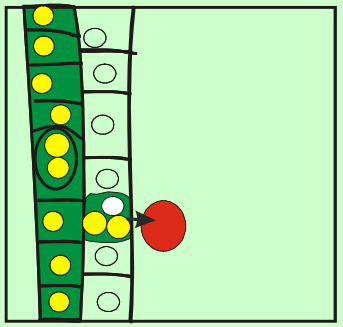
humans

Girdle cells (eCG)



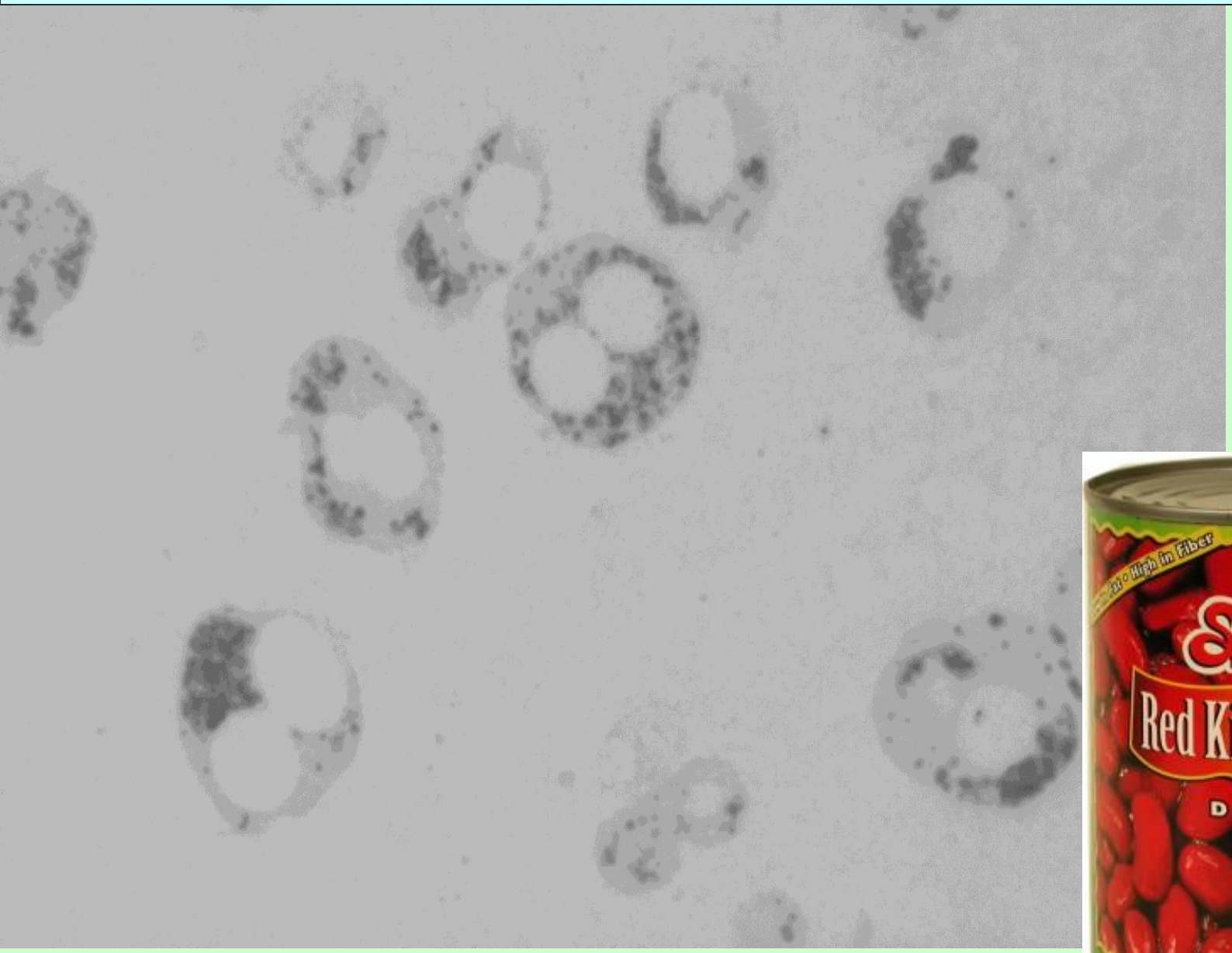
equids

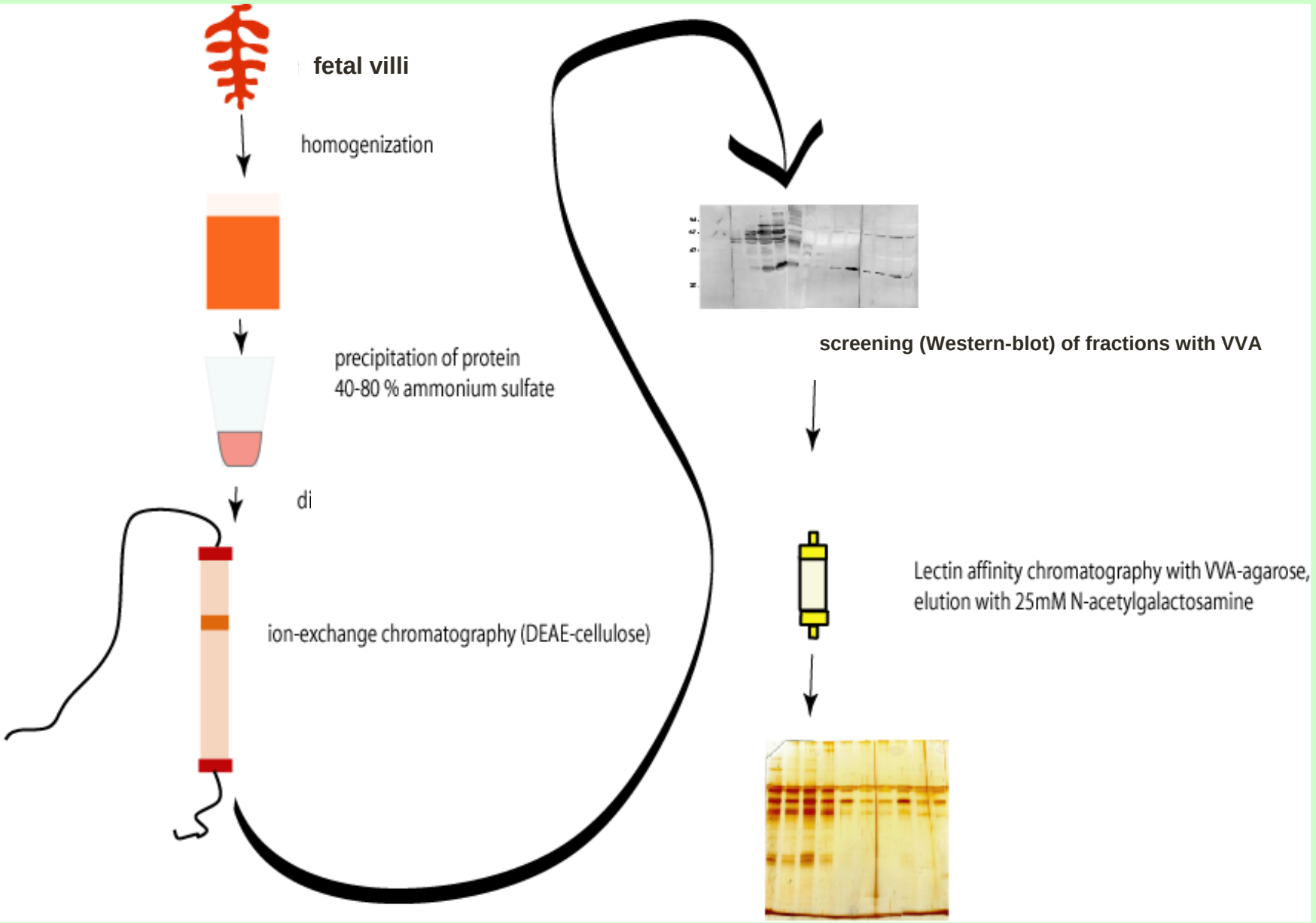
BNC (PAGs, PL)



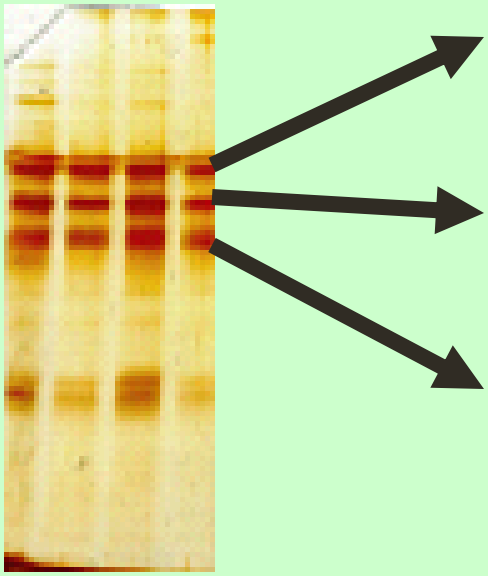
ruminants

Phaseolus vulgaris leucoagglutinin (PHA-l)




fetal villi
homogenization
precipitation of protein
40-80 % ammonium sulfate
di
ion-exchange chromatography (DEAE-cellulose)
screening (Western-blot) of fractions with VVA
Lectin affinity chromatography with VVA-agarose,
elution with 25mM N-acetylgalactosamine

N-terminal protein sequencing



RGSXLTIHPLRNIRD (PAG-7)
RGSXLT HPLRNIRD (PAG-6)

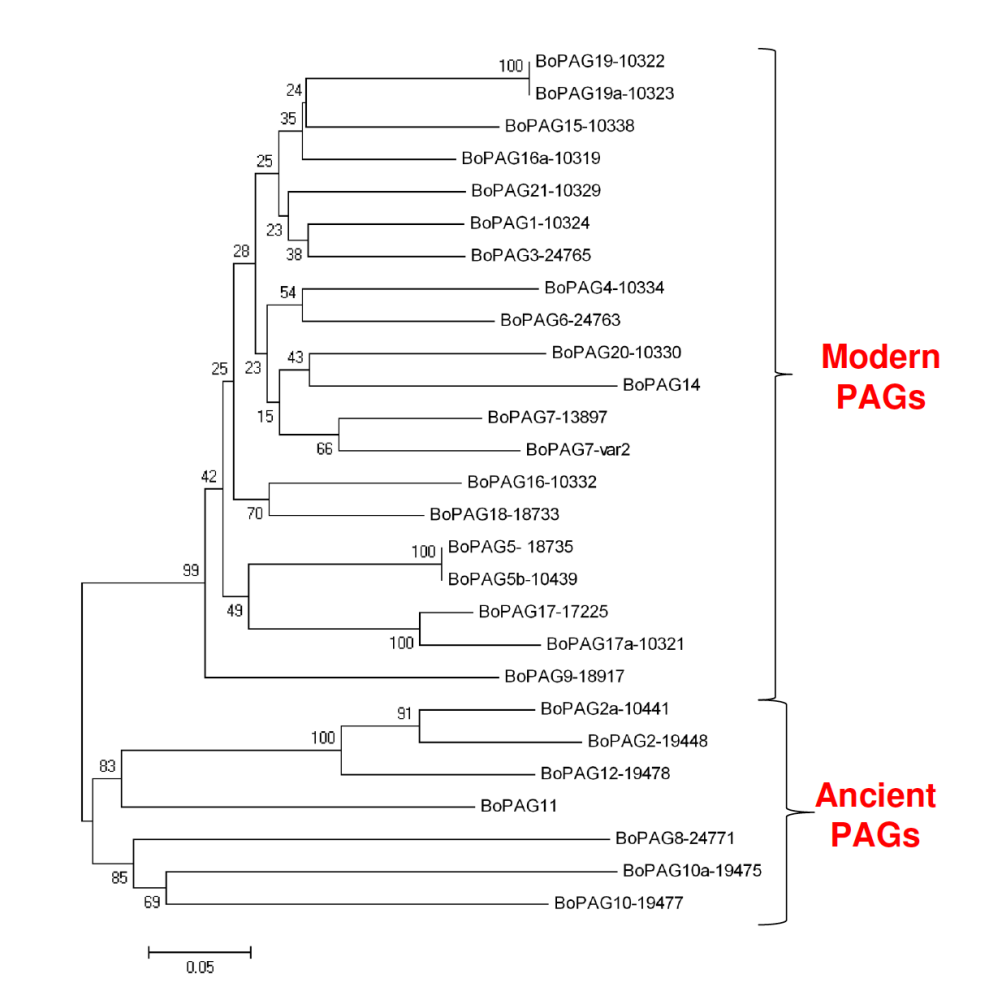
RGSXLTTHPLRNIKD (PAG-1)

SQISSRGSXLTIHP (PAG-17)

=> Pregnancy-associated glycoproteins (PAGs)

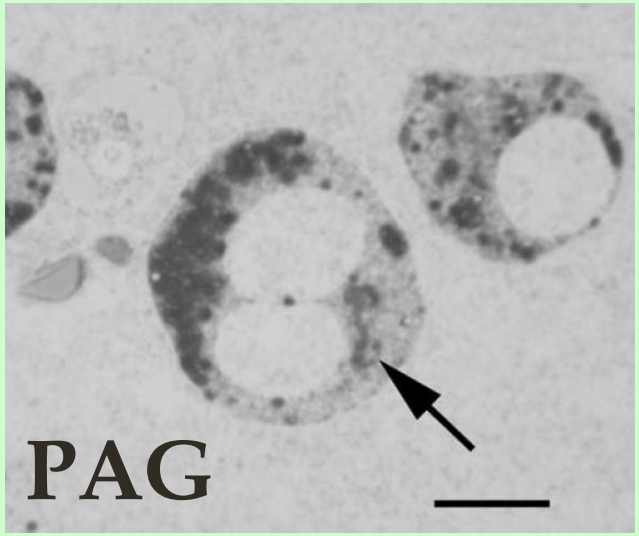
Pregnancy-associated glycoproteins (PAGs)

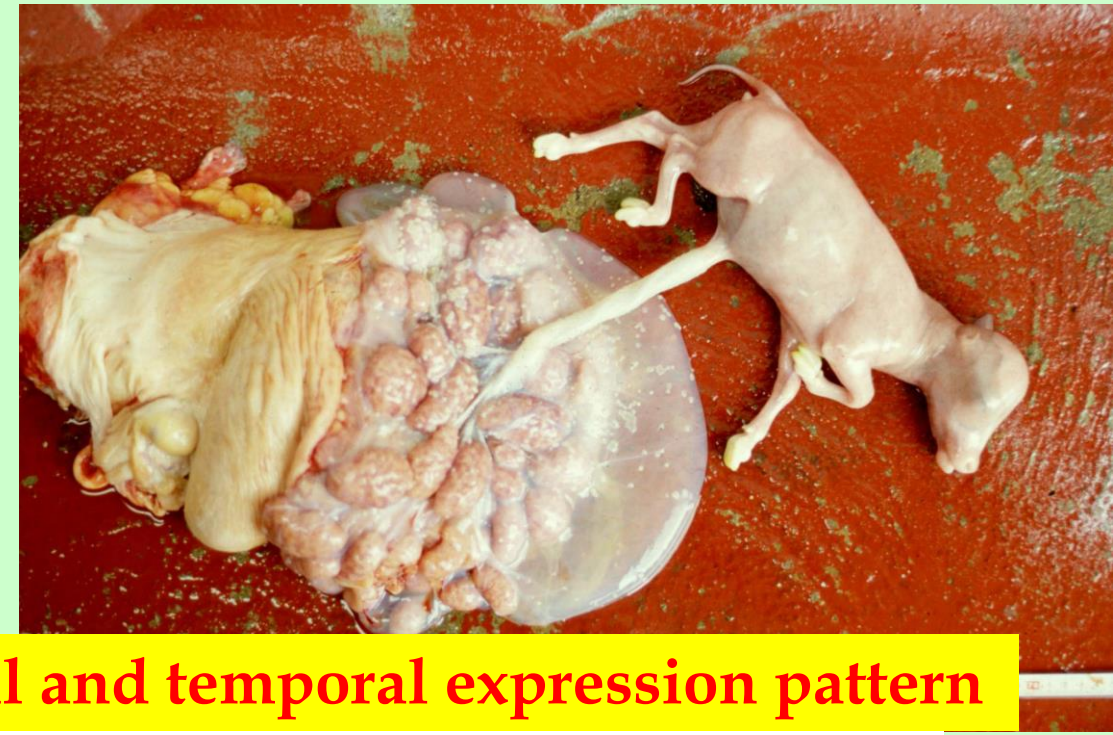
-aspartic proteinases (related to pepsin) => inactive
-expressed in artiodactyl trophoblast cells
-very diverse in ruminants: ancient and modern PAGs



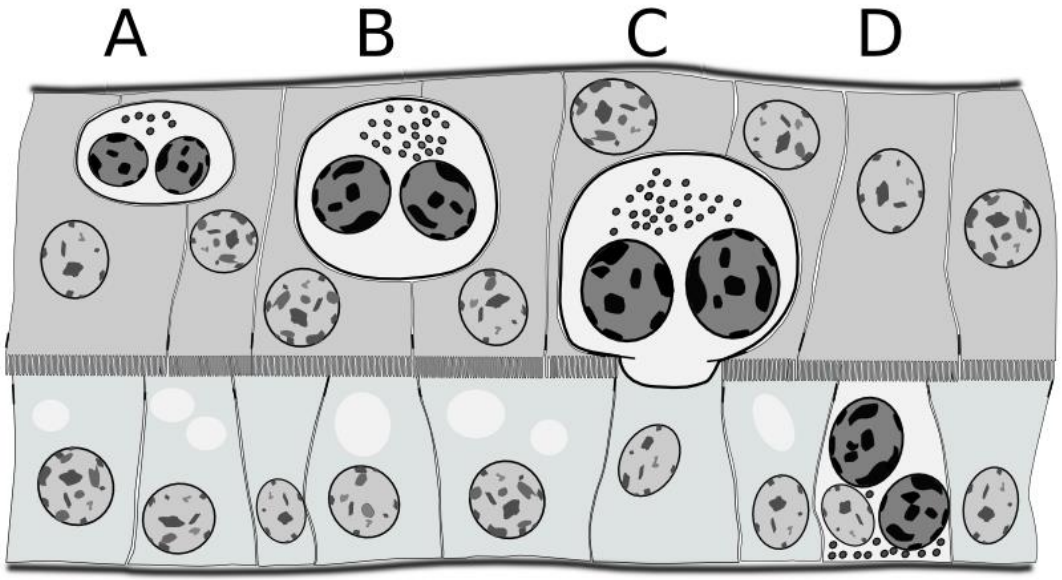
Telugo et al.
BMC Genomics 2009, 10:185

Pregnancy Associated Glycoproteins (PAGs)



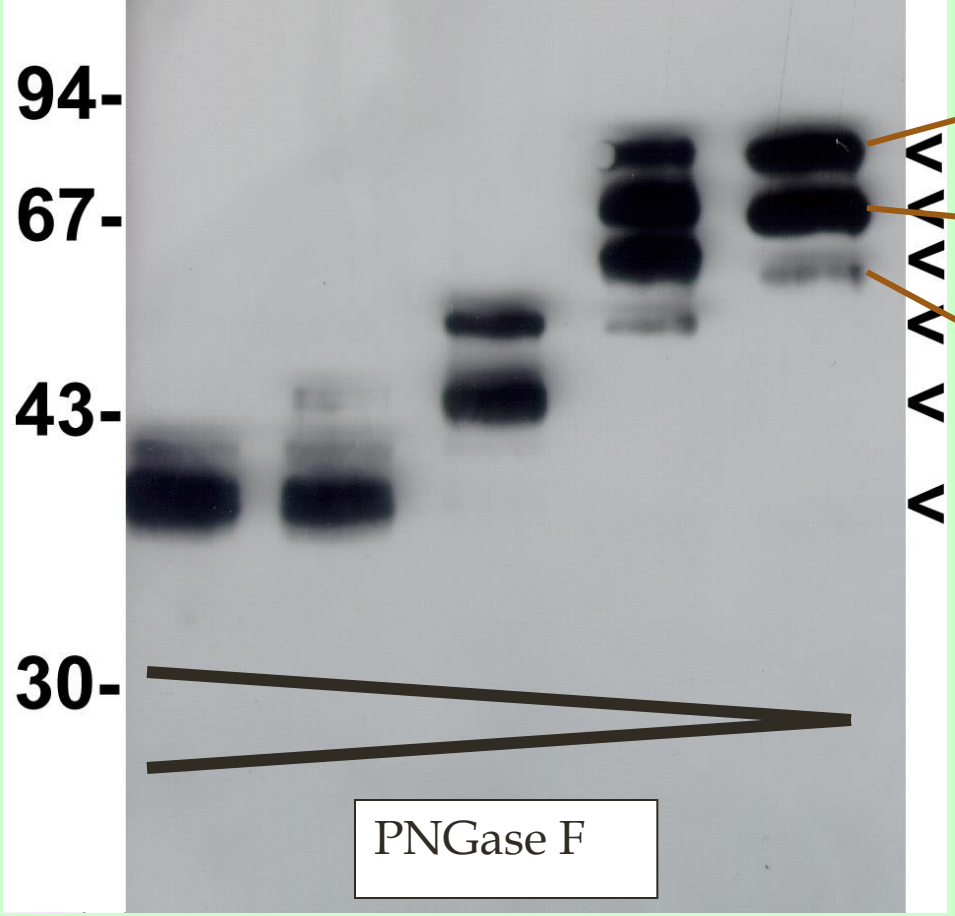


-complex spatial and temporal expression pattern



Trophoblast

Uterine epithelium

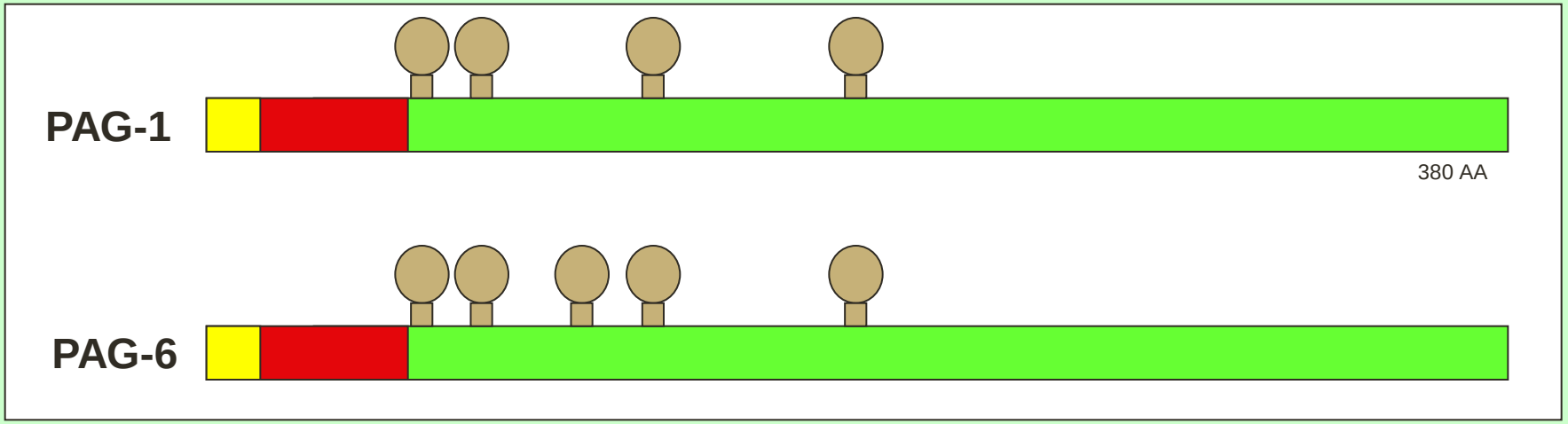


RGSXLTIHPLRNIRD (PAG-7)
RGSXLT HPLRNIRD (PAG-6)

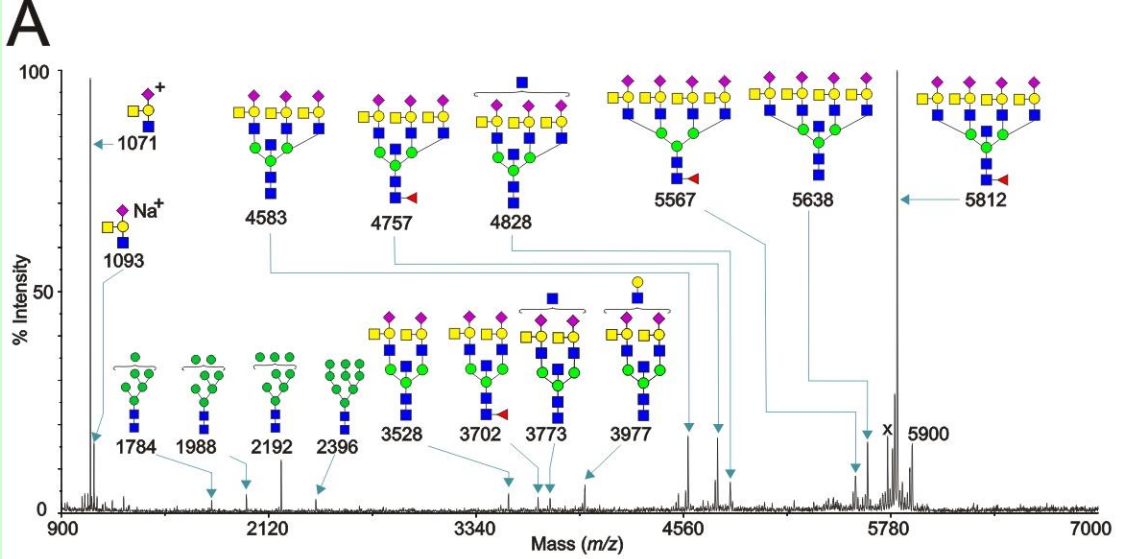
RGSXLTTHPLRNIKD (PAG-1)

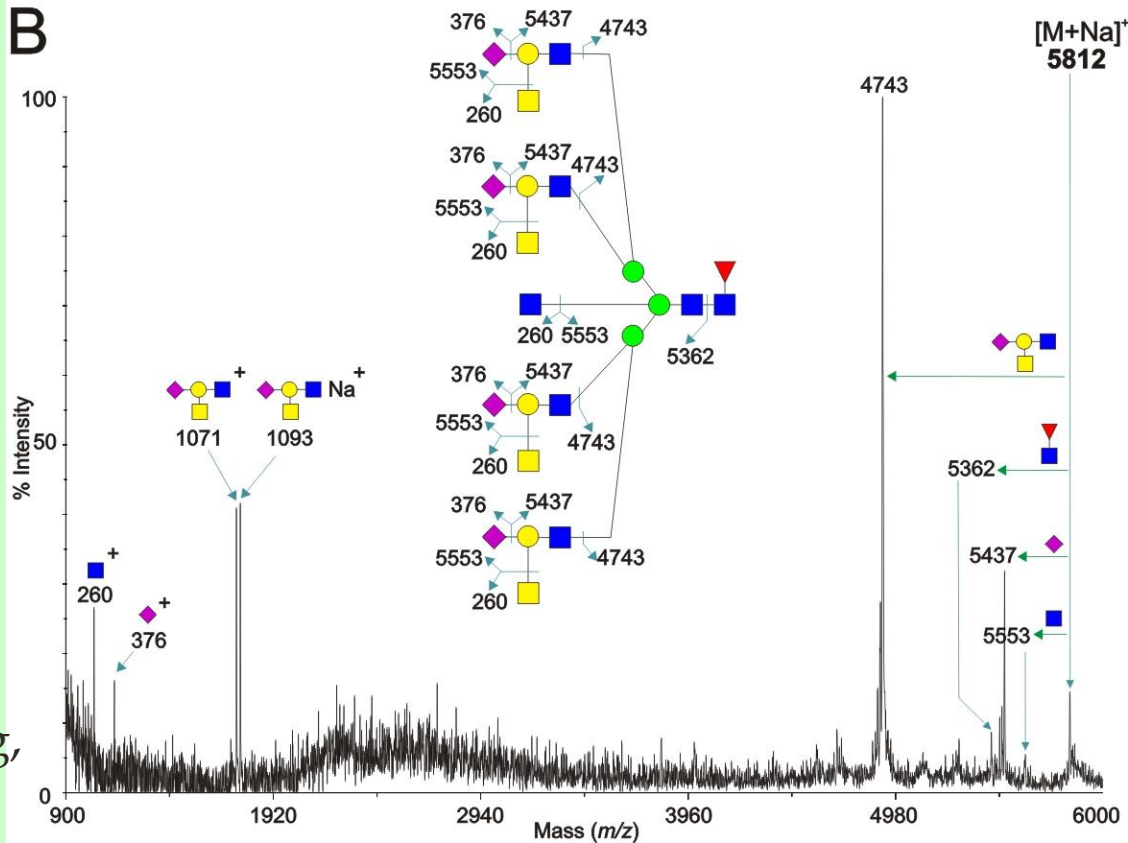
SQISSRGSXLTIHP (PAG-17)

Pregnancy-associated glycoproteins

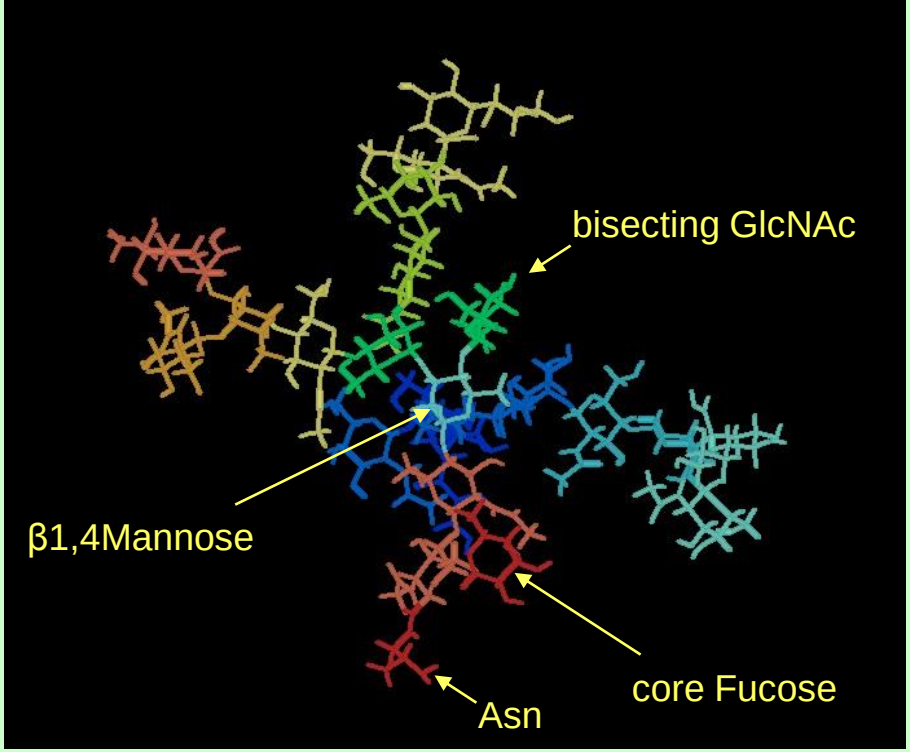


Analysis of PAG-Glycans by MALDI-Mass spectrometry

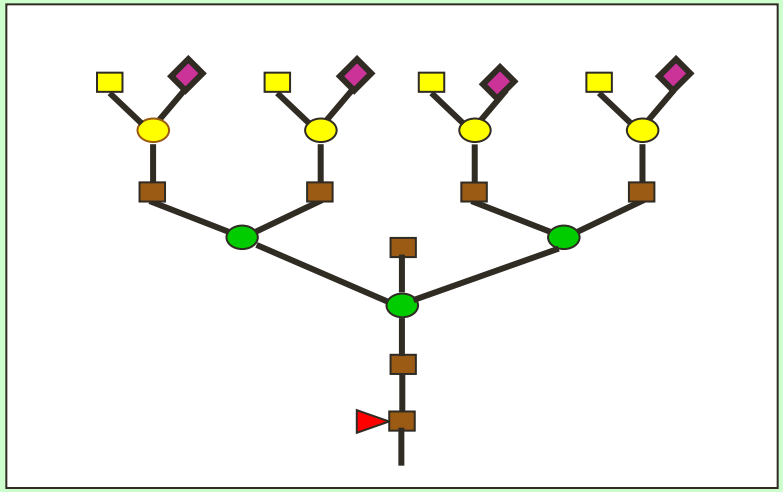


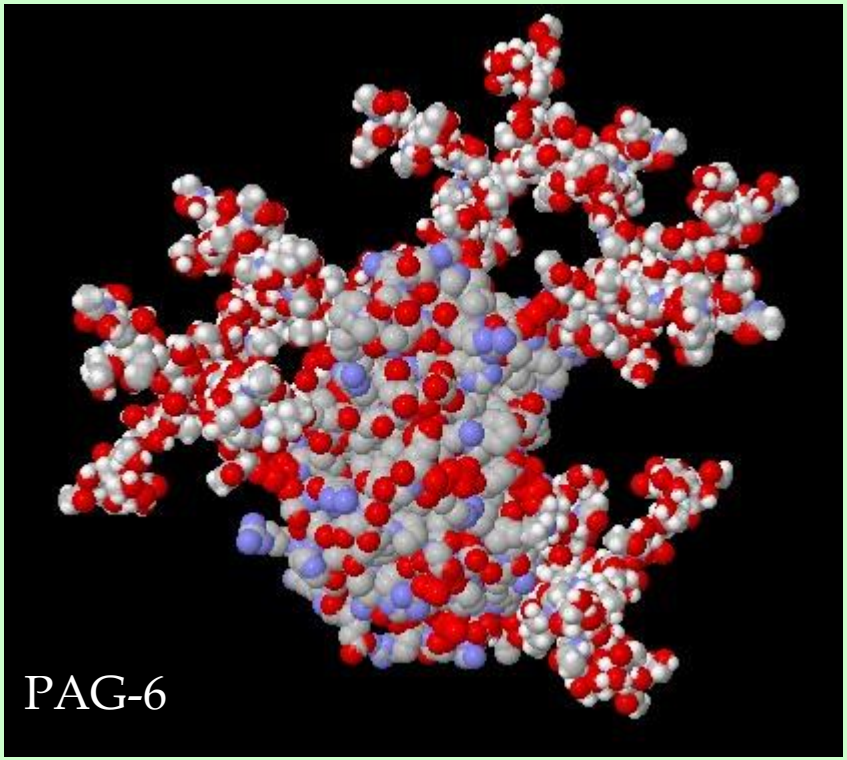


MS by Anne Dell / Poh-Choo Pang, Imperial College London



http://www.glycosciences.de/modeling/sweet2/doc/index.php



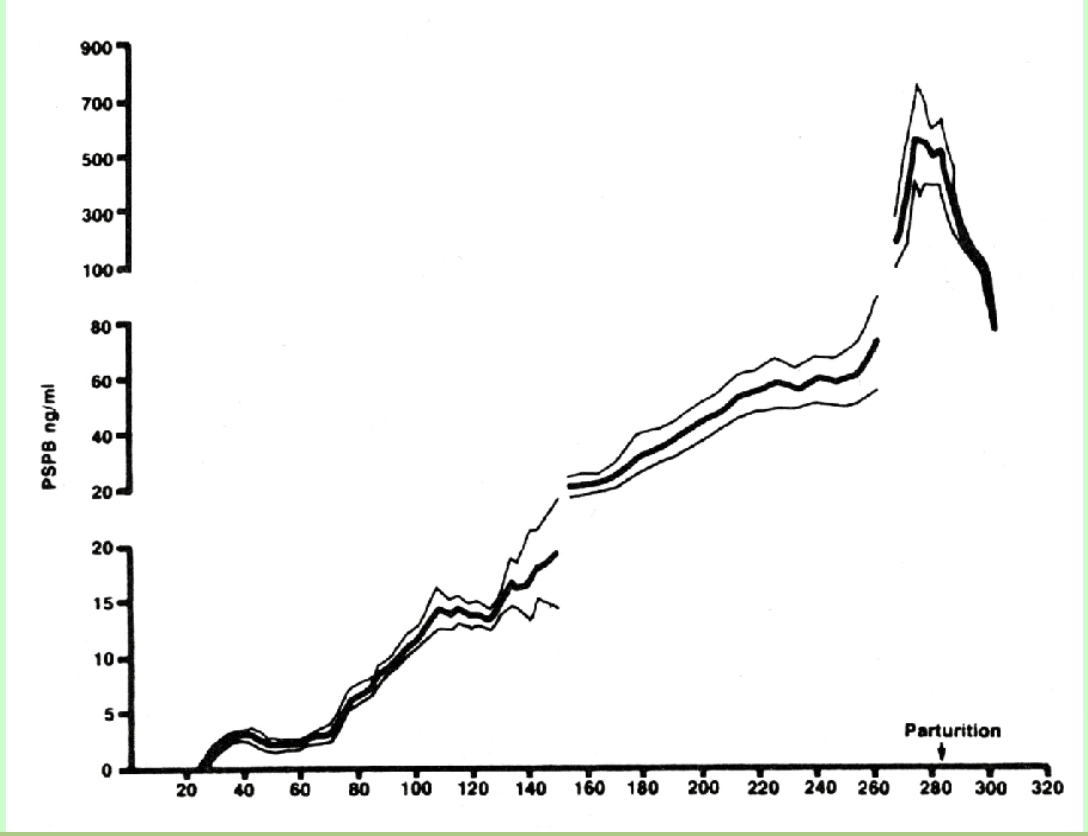


Pregnancy Associated Glycoproteins (PAGs)

in Serum/ Plasma

-rise in late 20s

-very long half life (about 8d) in serum



Sasser et al., 1986,
Biol. Reprod. 35, 932-942

Reprod Dom Anim **45**, 142–146 (2010); doi: 10.1111/j.1439-0531.2008.01287.x
ISSN 0936-6768

Establishment of an ELISA for Measuring Bovine Pregnancy-Associated Glycoprotein in Serum or Milk and Its Application for Early Pregnancy Detection

M Friedrich and W Holtz

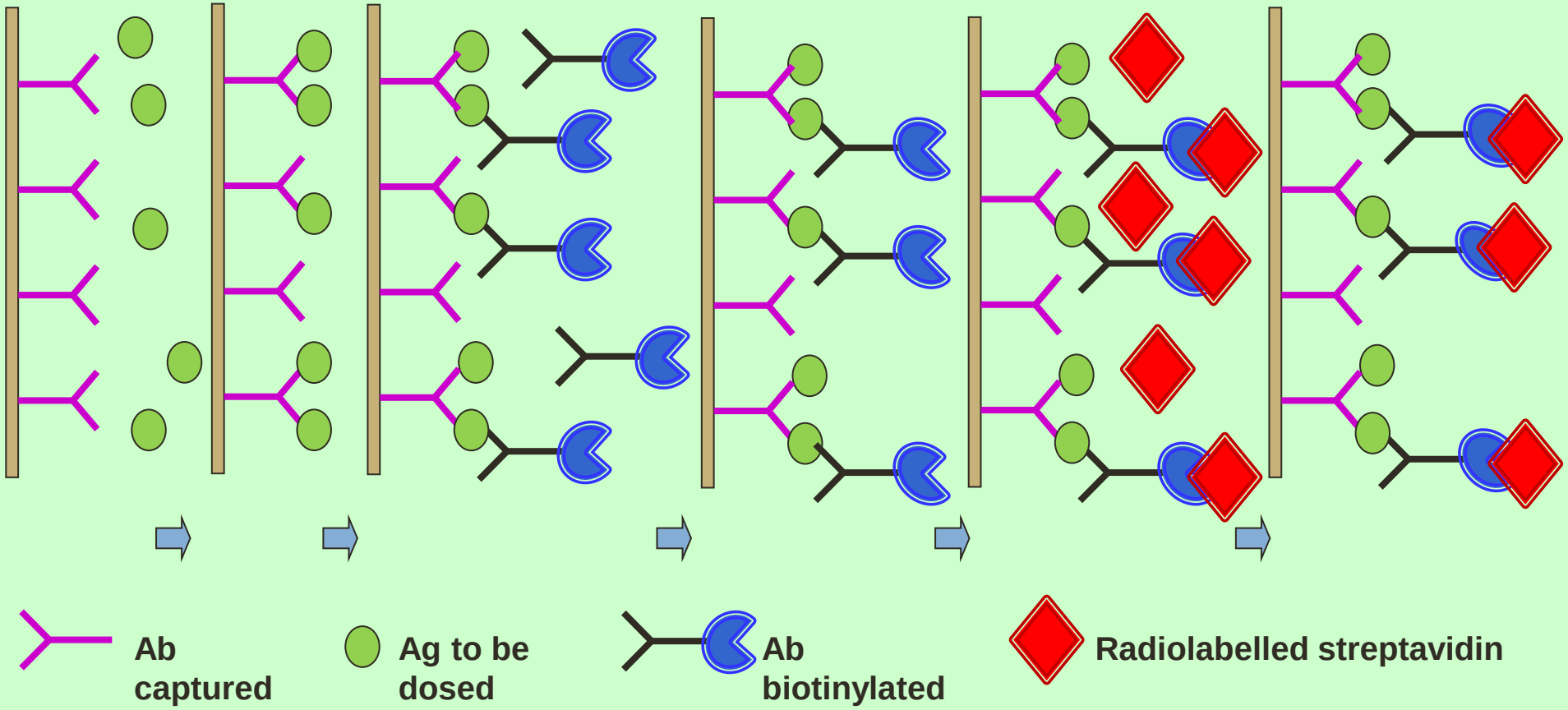
Institute of Animal Breeding and Genetics, Göttingen, Germany

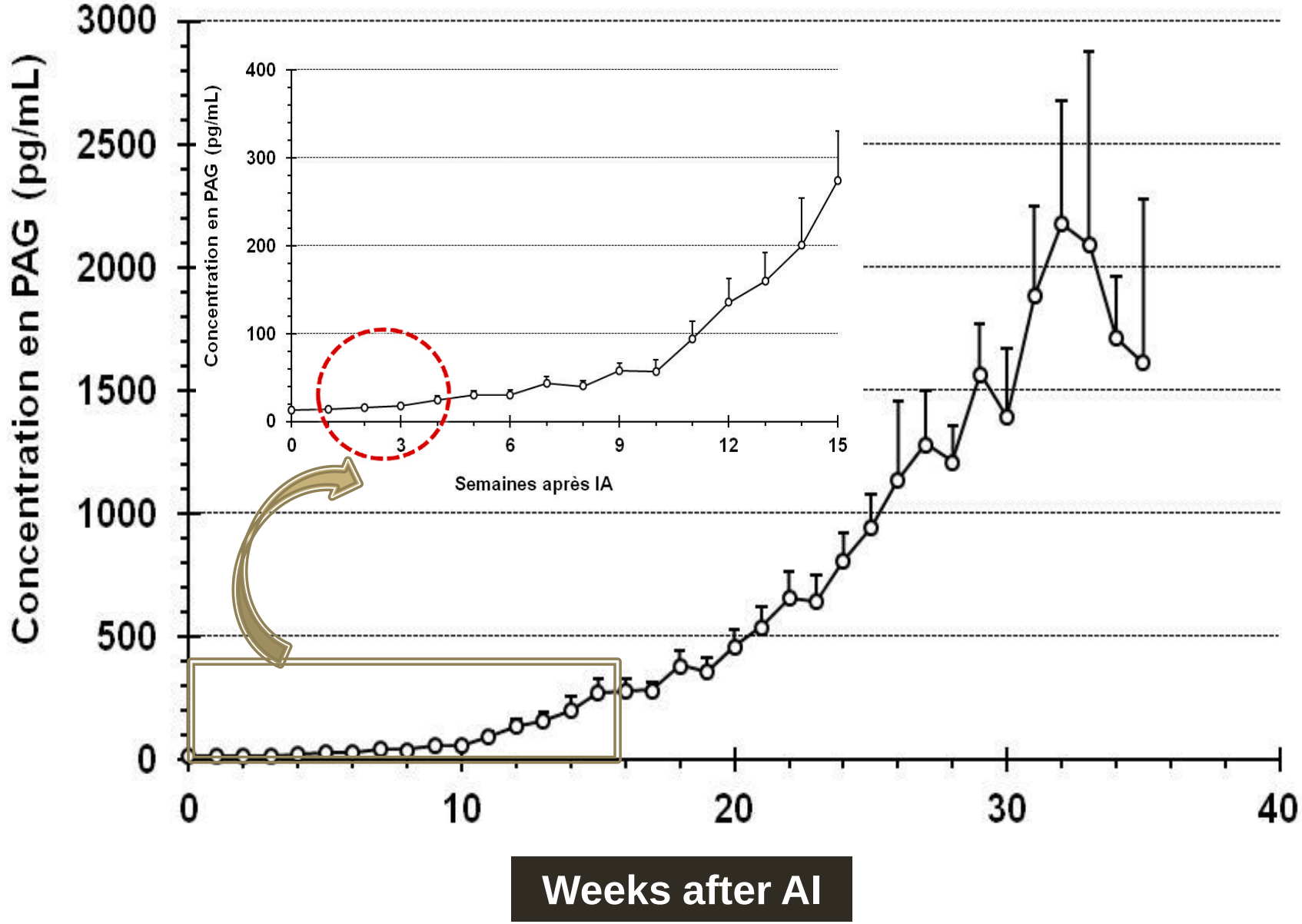
=>

The outcome was that, pregnancy could reliably be diagnosed from day 28 onwards in serum and from **day 150** onwards in milk.

Sousa N.M. & Beckers J.F.
University of Liege, Belgium

...development of an quantitave Immuno-Radio-Metric-Assay (IRMA)





Mean PAG concentrations (+ SEM) measured in milk during pregnancy and before dry-off period.

Pregnancy Associated Glycoproteins (PAGs)

-BioPRYN (BioTracking) test for PSPB (PAG) in blood

http://www.biotracking.com/dairy

Confirms pregnancy

- -28 days post breeding
- -at least 73 (90) days post-calving

-IDEXX Milk Pregnancy Test

https://www.idexx.com/livestock-poultry/ruminant/lpd-bovine-pregnancy-test.html

Confirms pregnancy

- -28 days post breeding
- -from 60 days post calving

Pregnancy Associated Glycoproteins (PAGs)

- possible to determine pregnency from d28 onwards in milk
- unlikely that it will be possible much earlier

Interferon-tau stimulated gene expression in PBLs

-**Interferon-tau =>** molecule of maternal regognition of pregnancy

-produced in uninucleated trophoblast cells

-released at very low concentrations into maternal blood

-induces alterations in gene expression in PBLs

Interferon-tau stimulated gene expression in PBLs

-aims at pd at day 17 => PGF2a at day 18
-RNA from leucocytes
-worked better in heifers

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Measurement of interferon-tau (IFN-τ) stimulated gene expression in blood leukocytes for pregnancy diagnosis within 18–20 d after insemination in dairy cattle

J.C. Green [a], C.S. Okamura [a], S.E. Poock [b], M.C. Lucy [a,*]

[a] Division of Animal Sciences, University of Missouri, 158 Animal Science Research Center, 920 East Campus Drive, Columbia, MO 65211, United States
[b] College of Veterinary Medicine, University of Missouri, Columbia, MO 65211, United States

ARTICLE INFO

Article history:
Received 10 February 2010
Received in revised form 28 April 2010
Accepted 12 May 2010
Available online 20 May 2010

Keywords:
Interferon-stimulated genes
Pregnancy detection

ABSTRACT

The objective was to diagnose pregnancy within 18 d after insemination by measuring interferon-tau (IFN-τ)-stimulated genes (ISG) expression in circulatory leukocytes. Based on microarray results, three genes were selected [2′-5′ oligoadenylate synthetase 1(Oas1), myxovirus resistance gene 2 (Mx2), and interferon-stimulated gene 15 kDa protein (Isg15)] because they were known to be interferon-stimulated genes (ISG) and were also differentially expressed in the samples. The respective genes were assayed by using real time reverse transcriptase polymerase chain reaction (RTPCR). In the first experiment, RNA was isolated from leukocytes of pregnant ($n = 5$) and non-pregnant ($n = 15$) dairy cows on each

Interferon-tau stimulated gene expression in PBLs

-aims at pd at day 17 => PGF2a at day 18
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Interferon-tau stimulated gene expression in PBLs

Kizaki *et al. Reproductive Biology and Endocrinology* 2013, **11**:6
http://www.rbej.com/content/11/1/6

REPRODUCTIVE BIOLOGY AND ENDOCRINOLOGY

RESEARCH Open Access

Differential neutrophil gene expression in early bovine pregnancy

Keiichiro Kizaki[1], Ayumi Shichijo-Kizaki[1], Tadashi Furusawa[2], Toru Takahashi[2], Misa Hosoe[2] and Kazuyoshi Hashizume[1*]

Abstract

Background: In food production animals, especially cattle, the diagnosis of gestation is important because the timing of gestation directly affects the running of farms. Various methods have been used to detect gestation, but none of them are ideal because of problems with the timing of detection or the accuracy, simplicity, or cost of the method. A new method for detecting gestation, which involves assessing interferon-tau (IFNT)-stimulated gene expression in peripheral blood leukocytes (PBL), was recently proposed. PBL fractionation methods were used to examine whether the expression profiles of various PBL populations could be used as reliable diagnostic markers of bovine gestation.

Methods: PBL were collected on days 0 (just before artificial insemination), 7, 14, 17, 21, and 28 of gestation. The gene expression levels of the PBL were assessed with microarray analysis and/or quantitative real-time reverse transcription (q) PCR. PBL fractions were collected by flow cytometry or density gradient cell separation using Histopaque 1083 or Ficoll-Conray solutions. The expression levels of four IFNT-stimulated genes, interferon-stimulated protein 15 kDa (*ISG15*), myxovirus-resistance (*MX*) *1* and *2*, and 2′-5′-oligoadenylate synthetase (*OAS1*), were then analyzed in each fraction through day 28 of gestation using qPCR.

Results: Microarray analysis detected 72 and 28 genes in whole PBL that were significantly higher on days 14 and 21 of gestation, respectively, than on day 0. The upregulated genes included IFNT-stimulated genes. The expression levels of these genes increased with the progression of gestation until day 21. In flow cytometry experiments, on day 14 the expression levels of all of the genes were significantly higher in the granulocyte fraction than in the other fractions. Their expression gradually decreased through day 28 of gestation. Strong correlations were observed between the expression levels of the four genes in the granulocyte fractions obtained with flow cytometry and with density gradient separation.

Conclusions: The expression profiles of *ISG15*, *MX1*, *MX2*, and *OAS1* could be a useful diagnostic biomarker of bovine gestation. Assessing the expression levels of these genes in a granulocyte fraction obtained with density gradient separation is a practical way of detecting gestation in cows within three weeks of insemination.

Interferon-tau stimulated gene expression in PBLs

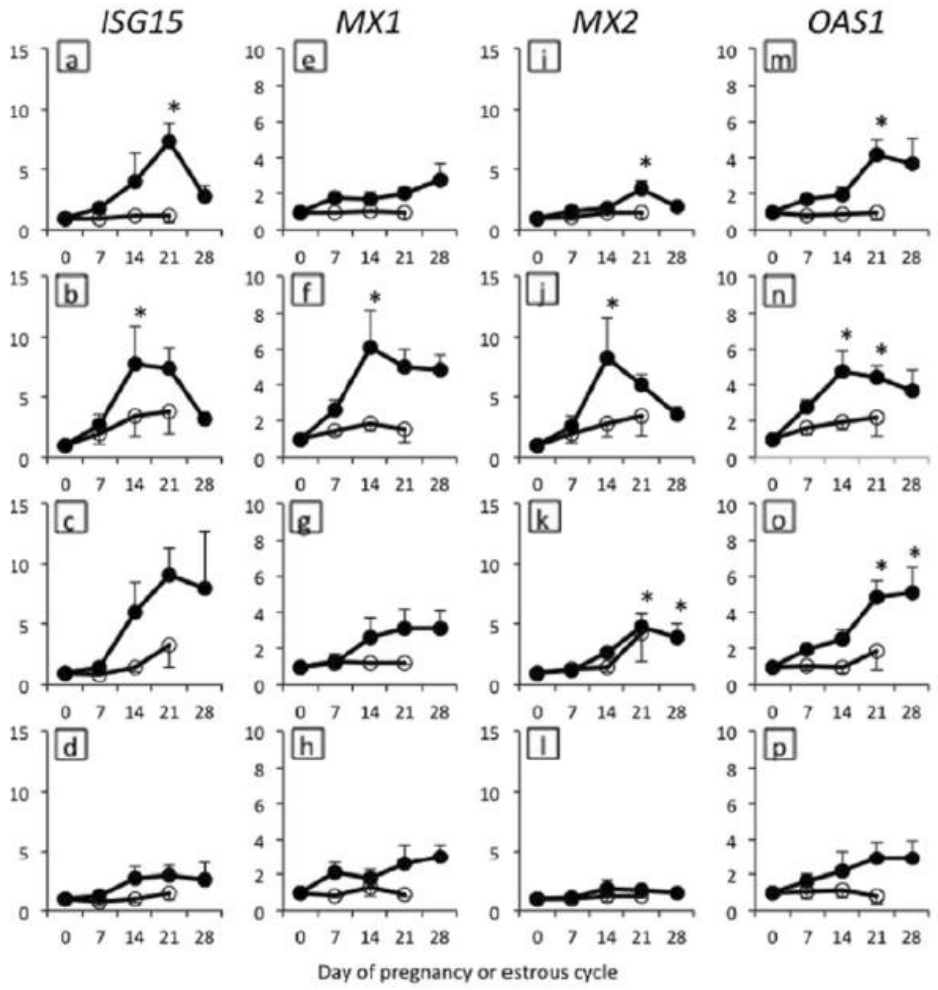
-interferon-stimulated protein 15 kDa (ISG15)
-myxovirus-resistance (MX) 1
-myxovirus-resistance (MX) 2
-20 -50 -oligoadenylate synthetase

all leucocytes

granulocytes (G)

monocytes (M)

lymphocytes (L)



from Kizaki et al., 2013

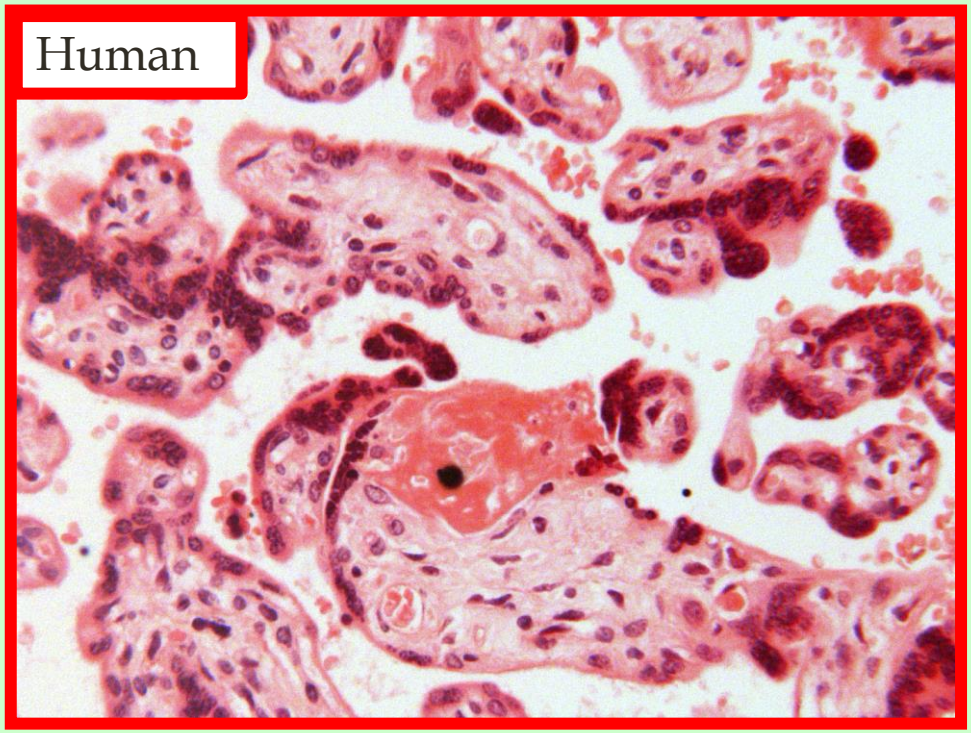
Questions:

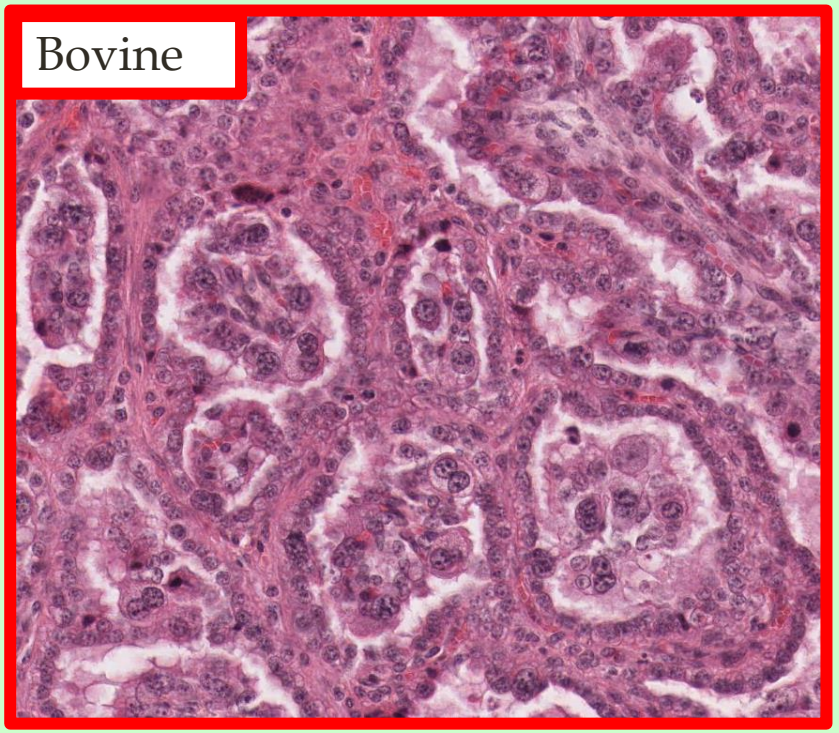
-what causes the very early changes in gene expression?
-is it affected by infections?
-would it be possible to meassure it in cells in milk?

Circulating fetal nucleic acids

Transfer of fetal nucleis acids into the maternal circulation

-used for prenatal diagnostics in humans
-different placental barriere in cattle





Circulating fetal nucleic acids

Placenta 32 (2011) 912–913

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Short communication

Bovine fetal DNA in the maternal circulation: Applications and implications

D.C. Lemos [a], P.L. Takeuchi [a], Á.F.L. Rios [a,b], A. Araújo [a], H.C. Lemos [a], E.S. Ramos [a,c,*]

[a] Departamento de Genética, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil
[b] Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, RJ, Brazil
[c] Departamento de Ginecologia e Obstetrícia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil

ARTICLE INFO

Article history:
Accepted 27 July 2011

Keywords:
Bovines
Placenta
TSPY gene
Embryos
Sexing

ABSTRACT

Objective: The main aim of the present study was to detect bovine fetal DNA in the maternal circulation, a relatively unexplored subject in the literature.
Study design: DNA was extracted from blood of 84 primipara cows (*Bos indicus*) at different gestational ages (30–270 days) and from 100 adult animals (50 males and 50 non-pregnant cows). The samples were analyzed using PCR with primers for *TSPY* gene.
Results: Molecular results matched the fetal phenotypic gender in all 47 male and 37 female fetuses, including early pregnancy, and in control animals.
Conclusions: These results evidence a bovine transplacental fetal DNA passage.
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Table 1
Distribution of the phenotypic fetal gender according to the time after *in vitro* fertilization and embryo transference. In all cases the samples were positives for the presence of a *β-actin* gene sequence. The males were *TSPY*-positives and the females were *TSPY*-negatives (sensitivity and specificity = 100%).

Gestational Age (days)	Fetal Gender		
	Female (n)	Male (n)	Males + Females (n)
30	7	5	12
60	4	8	12
90	5	5	10
120	5	5	10
180	5	5	10
210	5	5	10
240	2	8	10
270	4	6	10
Total	37	47	84

amplification control, we used a bovine *β-actin* gene sequence [10]. The amplification products were visualized in 2% agarose gels stained with ethidium bromide. Each sample results (at least three repetitions) was compared with the phenotypic data.

DNA from lysed blood
-PCR for TSPY-gene
=> Identification of male fetusses

Circulating fetal nucleic acids

Theriogenology 79 (2013) 173–179

Contents lists available at SciVerse ScienceDirect

Theriogenology

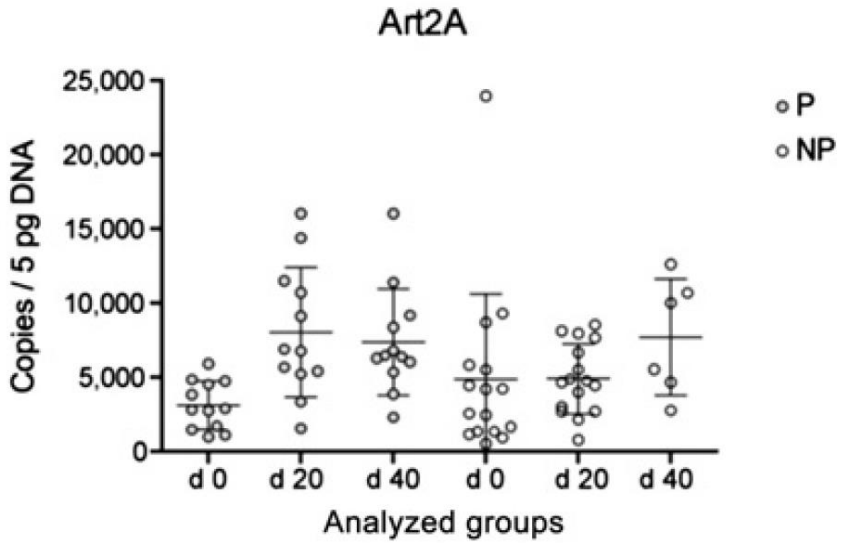
journal homepage: www.theriojournal.com

Early pregnancy diagnosis in dairy cows using circulating nucleic acids

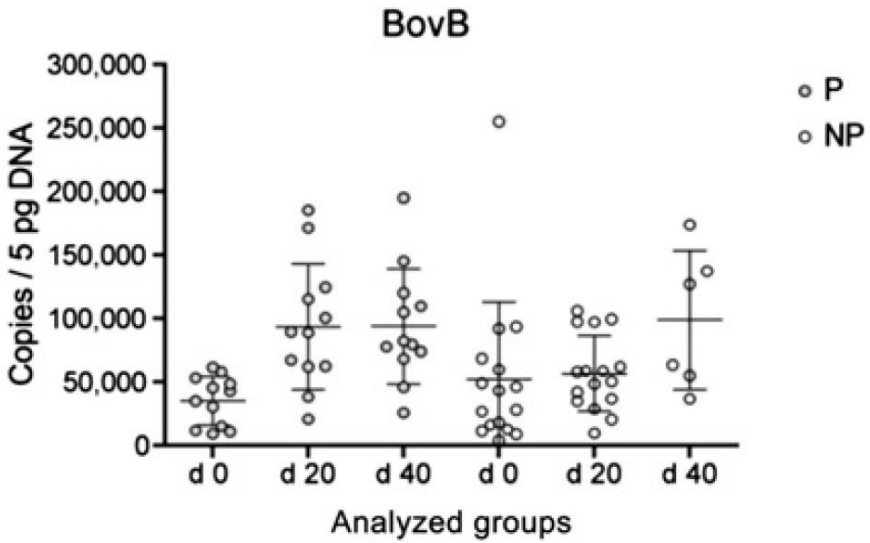
Jennifer Mayer [a], Jan T. Soller [a], Julia Beck [b], Vanessa Purwins [a], Wilhelm Wemheuer [a], Ekkehard Schütz [a,b], Bertram Brenig [a,*]

[a] *Institute of Veterinary Medicine, University of Göttingen, Göttingen, Germany*
[b] *Chronix Biomedical GmbH, Göttingen, Germany*

-days 0, 20, and 40 of pregnancy
-DNA isolated from serum
-identification of sequence tags which are increased at d20
-quantification of 2 repetitive sequences



pregnant non-pregnant



pregnant non-pregnant

Table 3
Summary of ROC statistics for Art2A and BovB.

Test	Cutoff	Number of P	Number of NP	J	AUC	P	Sensitivity	Specificity
Art2A	>5064	10	6	0.46	0.73	0.04	0.83	0.63
	≤5064	2	10					
BovB	>62,122	10	4	0.58	0.78	0.02	0.83	0.75
	≤62,122	2	12					

Abbreviations: AUC, area under the receiver operator characteristics curve; NP, nonpregnant; P, pregnant; ROC, receiver operator characteristics.

Early Pregnancy Factor (EPF)

Enigmatic Pregnancy Factor

(F.M. Clarke, Journal of Assisted Reproduction and Genetics, Vol. 14, No. 9, 1997)

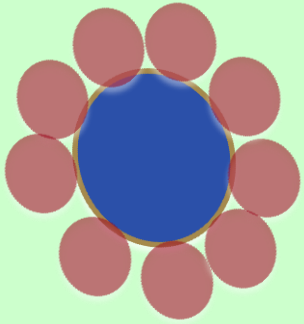
-discovered in the 1970s in rat and human
-very early (6-24 h after fertilisation)
-detected in a bioassay ***–rosette inhibition test-***

-later EPF was identified as:

- -heat shock protein: extracellular chaperonin 10
- -200 kDa glycoprotein (ECF)
- -67 kDa protein
- -21 kDa protein
- -12 kDa protein thioredoxin (plus PAF)
- -.....

Early Pregnancy Factor

Rosette inhibition test (original):

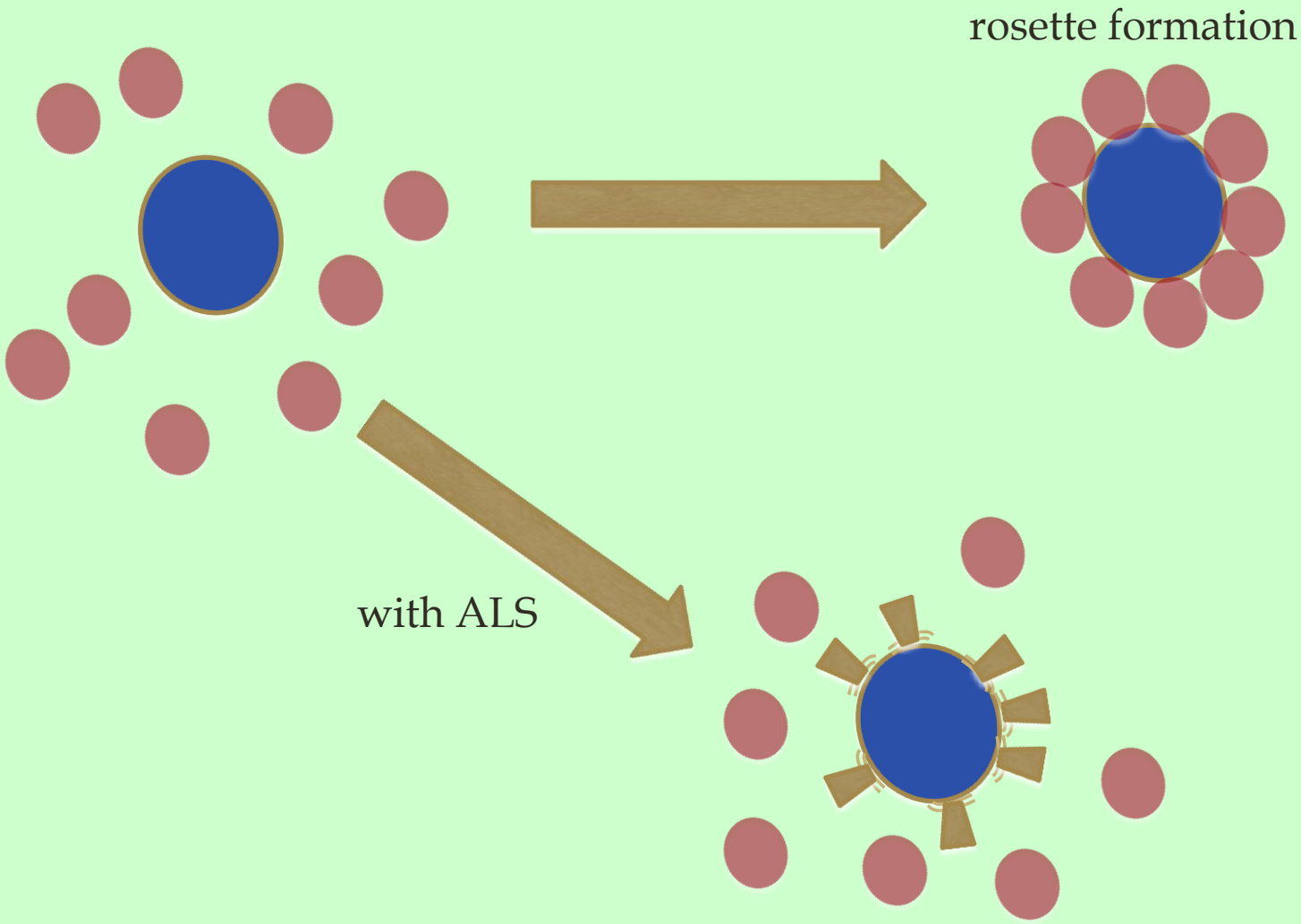


Materials:
-lymphocytes (e.g. mouse)
-human erythrocytes
-(human) serum
-serum sample to be tested
-anti lymphocyte serum (ALS) in dilutions

Procedure:
-Lymphocytes are incubated with serum samples
-incubation with dilutions of ALS (plus human serum)
-ovine erythrocytes are added => rosette formation counted in haemocytometer

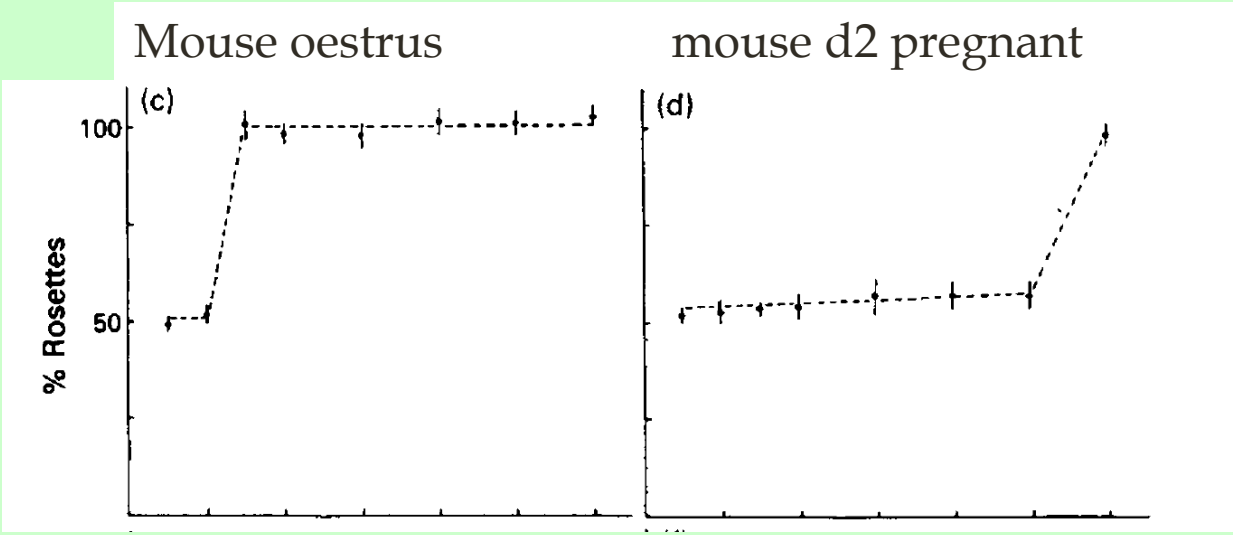
-rosette inhibition titer (RIT) = highest dilution which inhibits 25% of rosettes, compared to controls

Early Pregnancy Factor



CD2 (T-cells, NK cells) => erythrocyte receptor

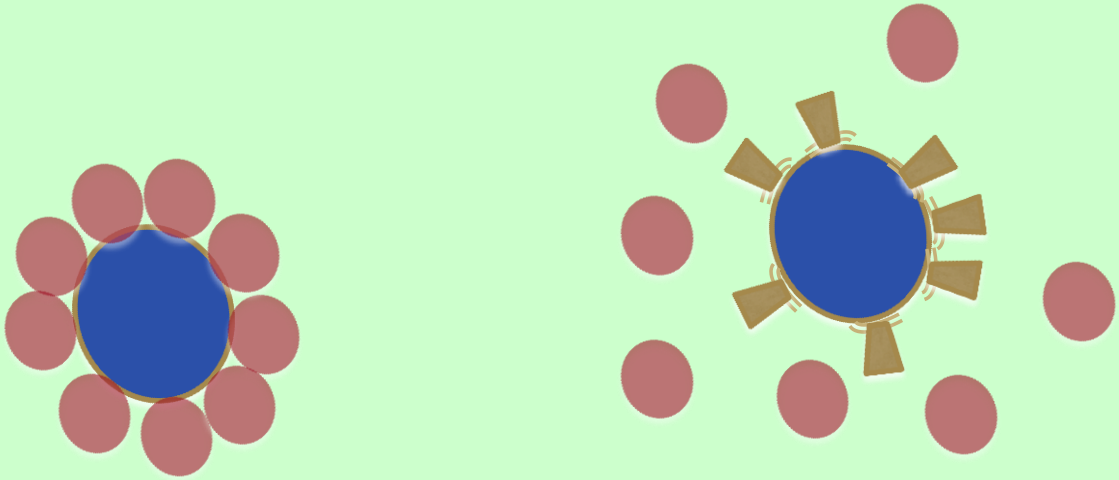
Early Pregnancy Factor



ALS dilution

Inhibitory effect of ALS is enhanced by EPF

Early Pregnancy Factor



–rosette inhibition test-

-difficult to do
-time consuming

ELISA for Early conception factor
(200kDa glycoprotein, Concepto Diagnostics, Knoxville,)

J Dairy Sci. 2001 Aug;84(8):1884-9.

Assessment of a commercially available early conception factor (ECF) test for determining pregnancy status of dairy cattle.

Cordoba MC[1], Sartori R, Fricke PM.

Table 1. Early Conception Factor (ECF) test results for blood sera collected from pregnant and nonpregnant dairy cattle on d 6 after estrus.[1]

			Pregnancy status[2]	
Test	Reader	ECF test result	Pregnant	Nonpregnant
1	1	Pregnant (+)	16	16
		Nonpregnant (–)	2	1
	2	Pregnant (+)	14	17
		Nonpregnant (–)	4	0
2	1	Pregnant (+)	17	17
		Nonpregnant (–)	1	0
	2	Pregnant (+)	15	15
		Nonpregnant (–)	3	2

We conclude that the ECF test is an unreliable method for determining pregnancy status of dairy cattle on day 6 after estrus.

Theriogenology. 2001 Sep 1;56(4):637-47.

Evaluation of the early conception factor (ECF) test for the detection of nonpregnancy in dairy cattle.

Gandy B[1], Tucker W, Ryan P, Williams A, Tucker A, Moore A, Godfrey R, Willard S.

Collectively, these data indicate that the current ECF test cannot accurately identify the nonpregnancy cow with the precision needed by the dairy producer.

Early Pregnancy Factor

ScienceDirect

Theriogenology

ELSEVIER

Theriogenology 70 (2008) 105–107

www.theriojournal.com

Measurement of EPF for detection of cow pregnancy using rosette inhibition test

V. Ghaffari Laleh [a,*], R. Ghaffari Laleh [a], N. Pirany [b], M. Moghadaszadeh Ahrabi [c]

[a] *Young Researchers Club of Tabriz, Islamic Azad University of Tabriz, Iran*
[b] *Department of Animal Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran*
[c] *Department of Immunology, Constituent of Young Researchers Club of Tabriz, Faculty of Veterinary Medicine, Islamic Azad University of Tabriz, Iran*

Received 9 February 2007; received in revised form 12 February 2008; accepted 13 February 2008

Rosette inhibition test:
-bovine lymphcytes (from non-pregnant animal)
-ovine erythrocytes
-serum sample to be tested (day 1-3 and day 3-5)

Lymphocytes are incubated with **dilutions of serum samples**

Table 1
No. of cows showing different rosette inhibition test (RIT) values

Period	Total	Status	RIT titer score[a]											Mean ± SE
			1	2	3	4	5	6	7	8	9	10	11	12	
1–3	11	AI								6	4	1			8.27 ± 0.34b
1–3	7	Control			4	2	1								4.00 ± 0.42c
5–7	12	AI								1	7	4			9.25 ± 0.32a
5–7	11	Control		1	5	4	1								3.45 ± 0.34c

(a–c) $P < 0.05$.
[a] Based on negative base two logarithm.

-AI = pregnant?

Early Pregnancy Factor

- interesting time period
- rosette inhibition test seems to work
- milk?

Preimplanation factor

Ramu *et al. Reproductive Biology and Endocrinology* 2013, **11**:105
http://www.rbej.com/content/11/1/105

RB&E REPRODUCTIVE BIOLOGY AND ENDOCRINOLOGY

RESEARCH Open Access

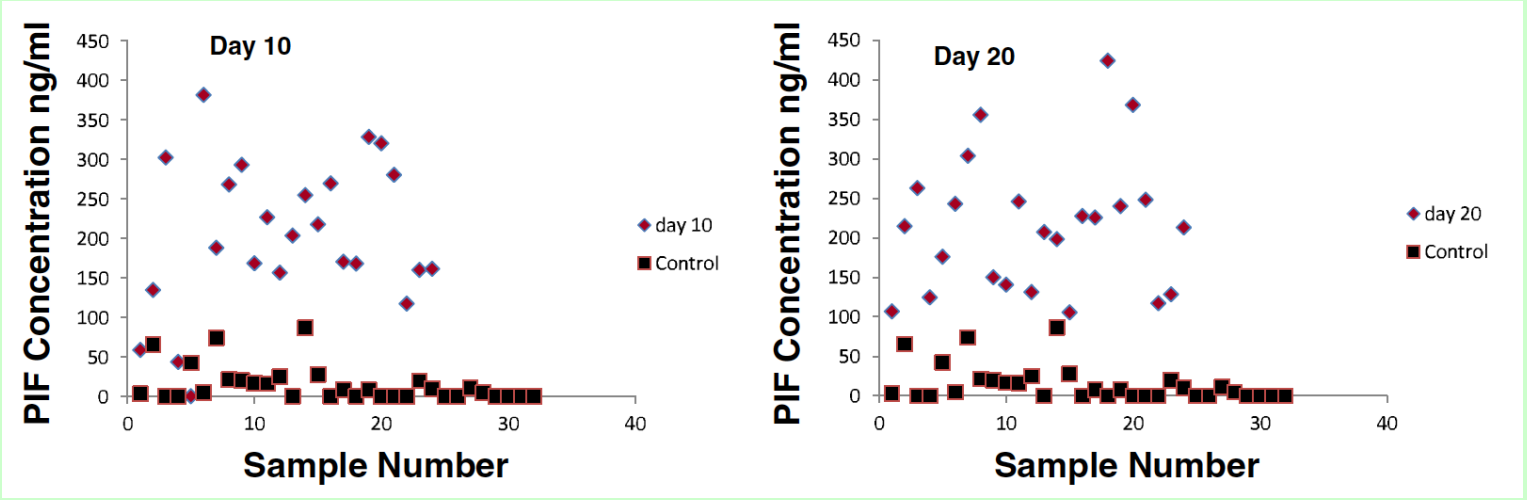
PreImplantation factor (PIF) detection in maternal circulation in early pregnancy correlates with live birth (bovine model)

Sivakumar Ramu[1], Christopher Stamatkin[1], Leo Timms[2], Marshall Ruble[2], Roumen G Roussev[1,3] and Eytan R Barnea[4,5*]

Preimplanation factor

Samples: serum from 21– 23 multiparous dairy Angus beef cows following AI (day 10, 15, 20)
Controls: serum from 30 known non-pregnant heifers and 2 steers

ELISA with a monoclonal antibody against the PIF peptide



day-10, 22/23 (95.6%)
day 15, 18/21 (85%)
day 20, 23/23 (100%)

Preimplanation factor

What is PIF?

PreImplantation Factor (PIF) (MVRIKPGSANKPSDD)

circumsporozoite protein, partial [Plasmodium falciparum]

Sequence ID: gb|AEI28994.1| **Length:** 64 **Number of Matches:** 1

Range 1: 40 to 53 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Positives	Gaps
39.2 bits(85)	0.021	12/14(86%)	13/14(92%)	0/14(0%)

```
Query  2   VRIKPGSANKPSDD  15
           VRIKPGSANKP D+
Sbjct  40  VRIKPGSANKPKDE  53
```

Is the placenta the source and the only source?

What happens in later pregnancy?

Preimplanation factor

Assays for preimplantation factor and preimplantation factor peptides

US 7273708 B2

Publication number	US7273708 B2
Publication type	Grant
Application number	US 10/482,244
PCT number	PCT/US2002/020599
Publication date	Sep 25, 2007
Filing date	Jun 28, 2002
Priority date	Jul 2, 2001
Fee status	Paid
Also published as	CA2490538A1, 29 More »
Inventors	Eytan R. Barnea, Reuben Renee Gonzales Perez, Paul C. Leavis
Original Assignee	Bioincept, Llc
Export Citation	BiBTeX, EndNote, RefMan

Patent Citations (3), Non-Patent Citations (47), Referenced by (8), Classifications (16), Legal Events (2)

External Links: USPTO, USPTO Assignment, Espacenet

ABSTRACT

The present invention relates to assay methods used for detecting the presence of PIF, and to PIF peptides identified using this assay. In particular, the present invention relates to flow cytomery assays for detecting PIF. It is based, at least in part, on the observation that flow cytometry using fluorescently labeled antilymphocyte and anti-platelet antibodies demonstrated an increase in rosette formation in the presence of PIF. It is further based on the observation that flow cytometry demonstrated that monoclonal antibody binding to CD2 decreased in the presence of PIF. The present invention further relates to PIF peptides which, when added to Jurkat cell cultures, have been observed to either (I) decrease binding of anti-CD2 antibody to Jurkat cells; (ii) increase expression of CD2 in Jurkat cells; or (iii) decrease Jurkat cell viability. In additional embodiments, the present invention provides for ELISA assays which detect PIF by determining the effect of a test sample on the binding of anti-CD2 antibody to a CD2 substrate.

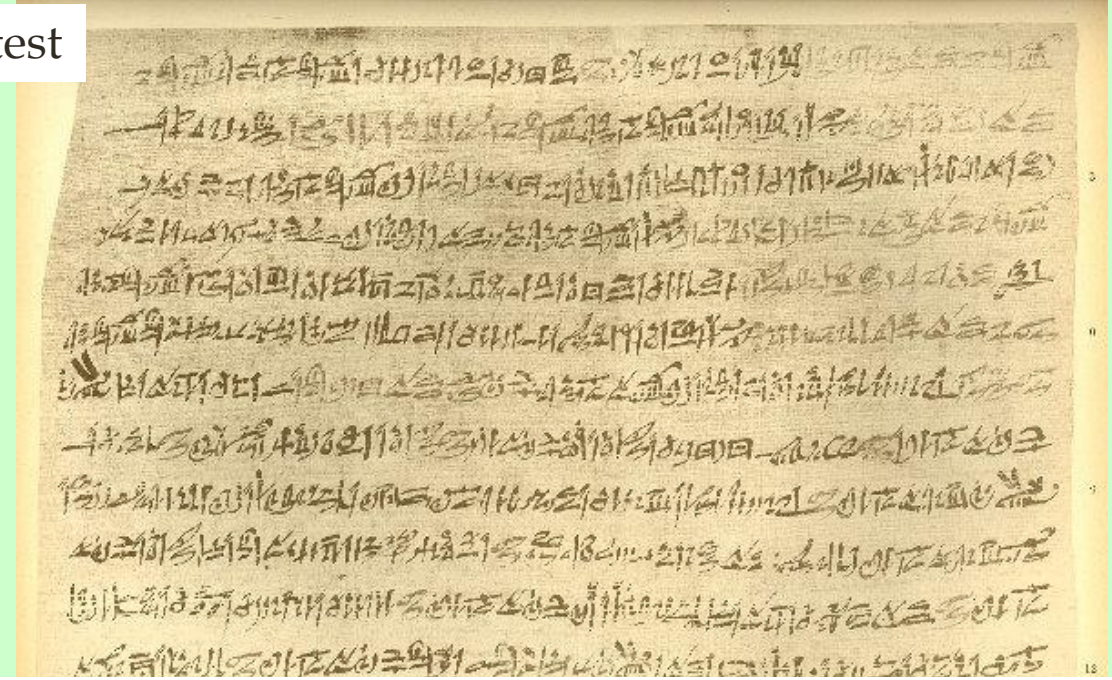
Preimplanation factor

Preimplantation Factor ("PIF") can be detected by mixing
-lymphocytes
-platelets
-heat inactivated serum from a pregnant subject
-guinea pig complement
-T11 (anti-CD2) monoclonal antibody

=>rosette formation between platelets and lymphocytes is increased by PIF in pregnant subjects

(from patent **US 7670850 B2**)

Seed inhibition test



Barley [and] wheat, let the woman water [them] with her urine every day with dates [and] the sand, in two bags. If they [both] grow, she will bear. If the barley grows, it means a male child. If the wheat grows, it means a female child. If both do not grow, she will not bear at all

from the Brugsch Papyrus, cited from Braunstein, Clinical Chemistry 60:1 18–21 (2014)

Seed inhibition test

Dtsch med Wochenschr 1934; 60(22): 822-824

Experiments on Pregnancy Diagnosis in Urin

FORSCHUNGSERGEBNISSE

Aus dem Botanischen Institut und der Frauenklinik der Universität Heidelberg

Versuche zur Schwangerschaftsdiagnose aus dem Harn

Von WALTHER HOFFMANN, Botanisches Institut

In der Arbeit von ASCHHEIM und ZONDEK (1) „Die Schwangerschaftsdiagnose aus dem Harn durch den Nachweis des Hypophysenvorderlappenhormons" findet sich ein kurzer Hinweis auf eine Stelle aus dem großen medizinischen Papyrus des Berliner Museums, nach dem die Ägypter vor 3—4000 Jahren bereits Schwangerschaftsdiagnose aus dem Harn getrieben hätten. Es heißt da: Daß eine Frau, die wissen will, ob sie gebären würde, Spelt und Gerste in ein Gefäß mit Erde bringen soll und dieses täglich mit ihrem Urin begießen. Wachsen sie, so wird sie gebären. Wachsen sie nicht, so wird sie nicht gebären. Ja selbst Geschlechtsbestimmung wurde mit dieser Methode schon getrieben.

Ein Unterschied in der Keimung bei Zusatz von Harn schwangerer und nichtschwangerer Frauen konnte nicht festgestellt werden. Seine Methode ist also zur Schwangerschaftsdiagnose unbrauchbar. Dagegen gibt er an, daß aus der Wirkung von Schwangerschaftsurin auf Gersten- und Weizenkörner auf das Geschlecht des zuerwartenden Kindes geschlossen werden kann. In 77 von 100 untersuchten Urinen zeigten sich Wachstumsunterschiede zwischen Gerste und Weizen. Wuchs die Gerste schneller, so wurde ein Mädchen geboren, wuchs der Weizen besser, ein Knabe. In 23 Fällen war die Diagnose unsicher, es zeigte sich aber, daß mit Ausnahme eines Falles alles Knabengeburten waren. Wenn man also die unsicheren Fälle als Knabendiagnose zählt, so wurden 80% der Fälle richtig diagnostiziert. 19% der Diagnosen waren falsch.

Name	12 Korn von jeder Sorte am 22. VI. ausgelegt		
	gegossen mit	gekeimt	
		Datum	Anzahl
Sommerweizen Triticum dicoccum	a) Wasser	27. VI.	12
	b) Schwangerenharn dialysiert	30. VI.	12
	c) Nichtschwangerenharn dialysiert	4. VII.	0
Winterweizen[1] Hohenwettersbacher unbegrannter Dickkopf	a) Wasser	30. VI.	9
	b) Schwangerenharn dyalisiert	4. VII.	8
	c) Nichtschwangerenharn dialysiert	4. VII.	0
Sommergerste Braugerste Hado Original Dippe	a) Wasser	27. VI.	12
	b) Schwangerenharn dyalisiert	30. VI.	12
	c) Nichtschwangerenharn dyalisiert	4. VII.	0
Wintergerste Nordland	a) Wasser	27. VI.	12
	b) Schwangerenharn dialysiert	30. VI.	12
	c) Nichtschwangerenharn dialysiert	4. VII.	0

-Initial experiments with filter paper and Petri dishes did not show any differences
-experiments on soil showed a strong inhibitory influence of urine from non-pregnant women
-influence of fetal gender was not tested

https://www.thieme-connect.de/products/ejournals/pdf/10.1055/s-0028-1129965.pdf

Seed inhibition test

Dtsch med Wochenschr 1934; 60(22): 822-824

Barley seeds irrigated with urine from:

pregnant women non-pregnant women water

Abb. 2. Wintergerste am 22. VI. 1933.
Linker Topf gegossen mit Schwangerenharn, mittlerer Topf gegossen mit Nichtschwangerenharn, rechter Topf gegossen mit Wasser

https://www.thieme-connect.de/products/ejournals/pdf/10.1055/s-0028-1129965.pdf

Seed inhibition test

Indian J. Anim. Res., **43** (1) : 37-40, 2009

AGRICULTURAL RESEARCH COMMUNICATION CENTRE
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EVALUATION OF SEED GERMINATION TEST FOR EARLY DETECTION OF PREGNANCY IN COWS*

S.V. Rao Krishna[1] and T. Veena

Department of Veterinary Physiology, Veterinary College,
KVAFSU, Bangalore–560 024, India

ABSTRACT

The urine samples collected from 40 crossbred cows/heifers during natural micturition early in the morning on day 14, 21, 28, 35 and 45 after artificial insemination (AI) were subjected to seed germination test using wheat seeds and green gram. A significant inhibition of seed germination after 48 hours and shoot growth after four days in both types of seeds, discoloration of either seeds or the germination fluid or both after 48 hours and deepening thereafter when compared to control groups were taken as criteria for declaring positive for true pregnancy. The results indicated that such observations could be noticed for the first time in 68 per cent of the test pregnant group, especially 28 days post AI. But the efficacies for true pregnancy detection on days 35 and 45 were 100 per cent, each. This test may serve as a simple, non invasive, dependable, user friendly, economical and door step technique for early pregnancy detection in cows, to the field veterinarians and farmers equally.

Seed inhibition test

Evaluation of Seed Germination Inhibition Test for Early Detection of Pregnancy in Cross Breed Dairy Cattle in Bangladesh

*[1]Rine M. Z., [2]Hossain M. G., [3]Akter S., [1]Tarif A. M. M., [1]Bari F. Y., and [1]Alam M. G. S.

[1] Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.
[2] Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.
[3] Department of Physiology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

Abstract

This piece of study was aimed to evaluate the seed germination inhibition test to diagnose pregnancy in cross breed dairy cattle in Bangladesh Agricultural University Dairy Farm, Mymensingh. The urine samples collected from fifty crossbred cows and heifers early in the morning on day 14, 21, 28, 35 and 45 after artificial insemination (AI) were subjected to seed germination test using wheat seeds. In each sterile petridish, fifteen (15) wheat seeds were taken on the filter paper and 15 ml of diluted urine was added (Ratio of urine and distilled water is1:4). The seed germination inhibition percentages were observed after three days of sampling whereas shoot length growth inhibition was observed after five days. Germination inhibition percentage of AI cows, significantly higher compare to non AI cows and water control groups at day 28 (p <0.01), 35 (p <0.001), and 45 (p <0.001) respectively. On the other hand shoot length growth of AI cows, significantly higher compare to non AI cows and water control groups at day 28 (p <0.001), 35 (p <0.001 and 45 (p <0.001) respectively. So, the increased seed germination inhibition percentage and reduced shoot length in post AI cross bred cattle was indicative of pregnancy state and from this study it is concluded that the seed germination inhibition techniques useful to detect pregnancy in cross bred cattle as a simple and economical method and it may be applied in the field level in Bangladesh.

Keywords: Seed germination inhibition, artificial insemination, pregnancy and cross breed.

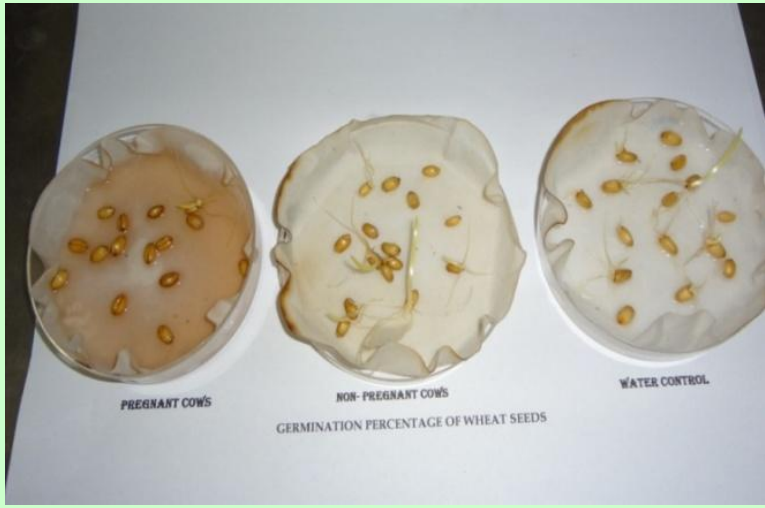
*Corresponding author: Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.
Received on: 29 Oct 2013
Revised on: 15 Dec 2013
Accepted on: 13 Jan 2014
Online Published on: 29 Jan 2014

Seed inhibition test

-25 cows AI (14, 21, 28, 35 and 45 day post insemination)

-25 cows (no AI) => controls

-25 water controls



-15 ml diluted (1:4) urine per Petri dish
-15 wheat seeds / Petri dish

-recording germination at day 3 and 5
-recording shoot length at day 5

Seed inhibition test

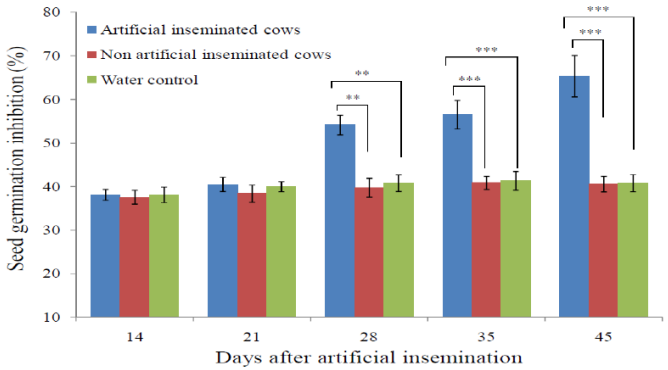


Fig. 5: Graphical presentation of germination inhibition percentages of wheat seeds in different groups on different days after AI. The graph shows the mean ± SE values of germination inhibition percentages of wheat seeds (n=25). SE = Standard error, $^{**}P < 0.01$ and $^{***}P < 0.001$ by one-way ANOVA.

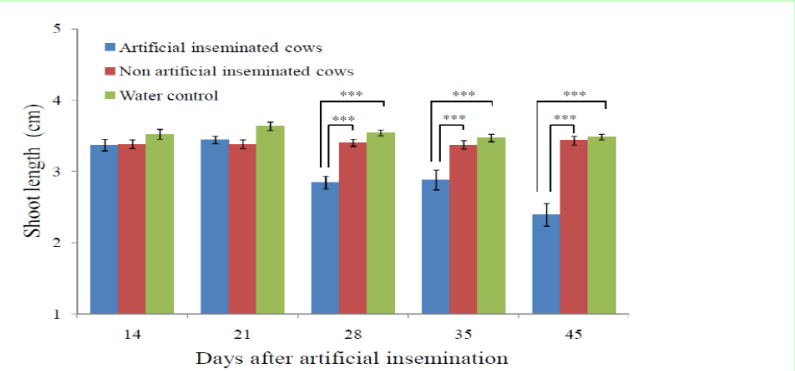


Fig. 6: Graphical presentation of shoot lengths (cm) of wheat seeds in different groups on different days after AI. The graph shows the mean ± SE values of shoot lengths (cm) of wheat seeds (n=25). SE = Standard error, $^{***}P < 0.001$ by one-way ANOVA.

=> Inhibition of germination and shoot growth from day 28 onwards

Seed inhibition test

Tropentag, September 17-19, 2014, Prague, Czech Republic

"Bridging the gap between increasing knowledge and decreasing resources"

Seed Germination Test for Pregnancy Diagnosis from Urine in Alpacas (*Vicugna pacos*)

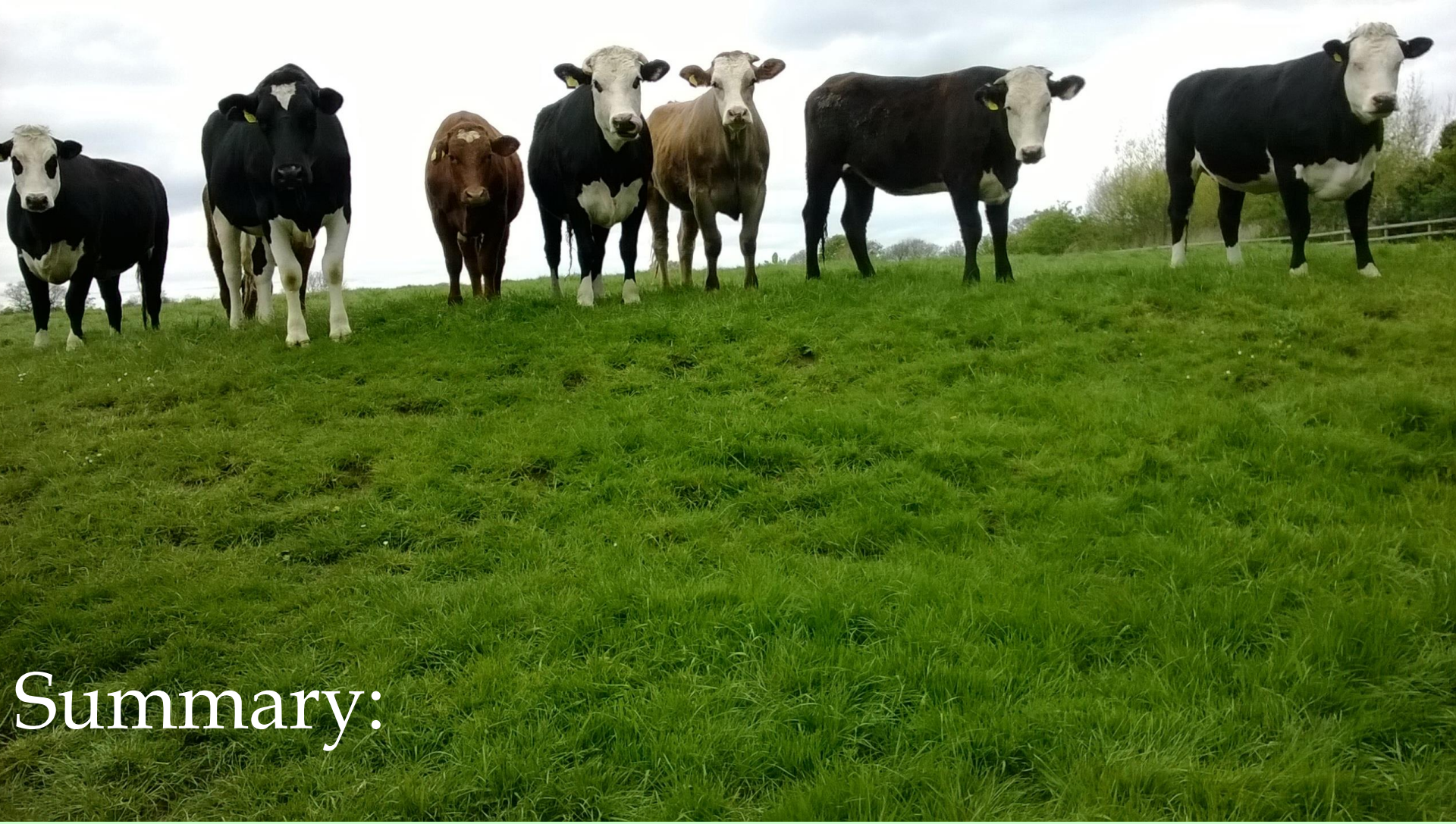
ANNA KUBÁTOVÁ, IVA SKÁLOVÁ, TAMARA FEDOROVA

Czech University of Life Sciences Prague, Fac. of Tropical AgriSciences, Dept. of Animal Science and Food Processing, Czech Republic

-general inhibition of germination by urine
-inhibition by urine from non-pregnant alpacas was stronger

Seed inhibition test

-variation between species
-in bovine so far only shown in India and Bangladesh
-other factors (season, ...) might influence the test
-works after 28 days or later
-milk?



Summary:

- test for PAG in milk is now available (day 28)

- there are several lines of research, which might lead to a test that can detect pregenancy earlier

Thank you very much for your attention