

Novas tecnologias de diagnóstico precoce de gestação: além da ultrassonografia Doppler

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Tópicos

**Métodos de
Diagnóstico de
Gestação
Convencionais**

**Ferramentas para
Antecipar a
Detecção do
Status Gestacional**

**Marcadores
Gestacionais e
Potenciais
Métodos**

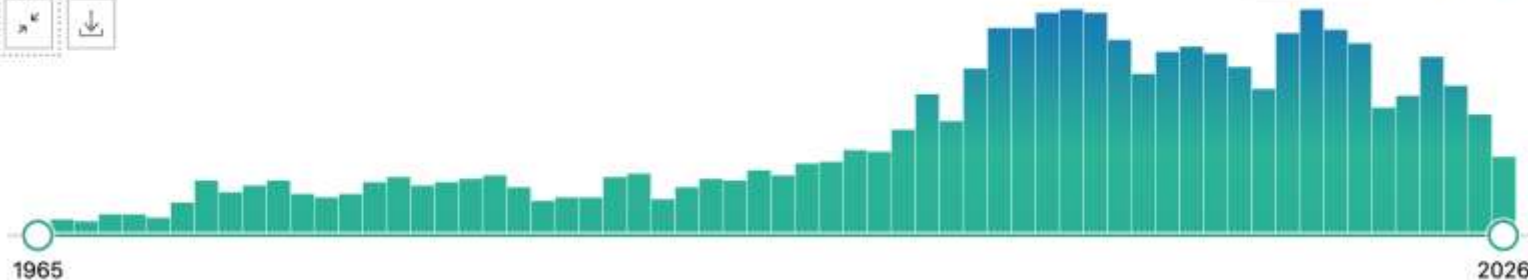
**Considerações
Finais**

Importância nas duas últimas décadas

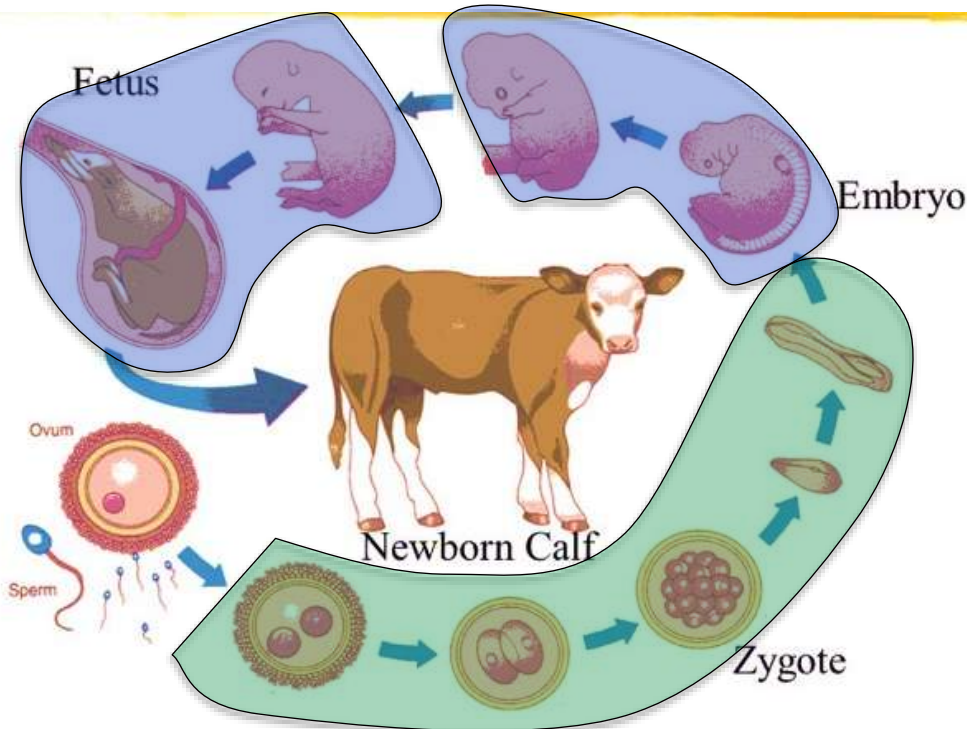
RESULTS BY YEAR

3,021 results

Page 1 of 303



Qual a importância do diagnóstico gestacional?



Embionário Inicial:
Maior perda gestacional

Identificar as fêmeas não-gestantes → Nova chance
Redução dos dias em aberto
Aumento da taxa de serviço

O principal desafio reprodutivo atual não é saber qual vaca está prenhe, mas sim **identificar precocemente qual fêmea está vazia.**



IATF1

30 dias

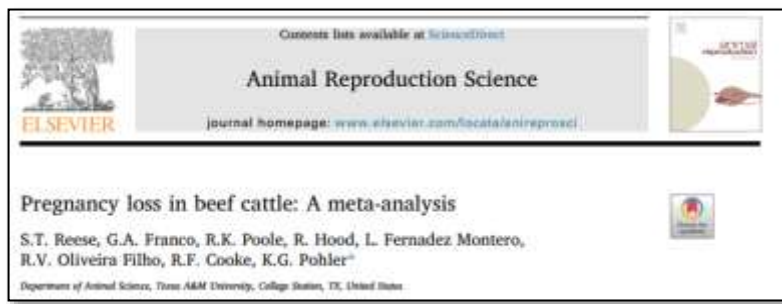
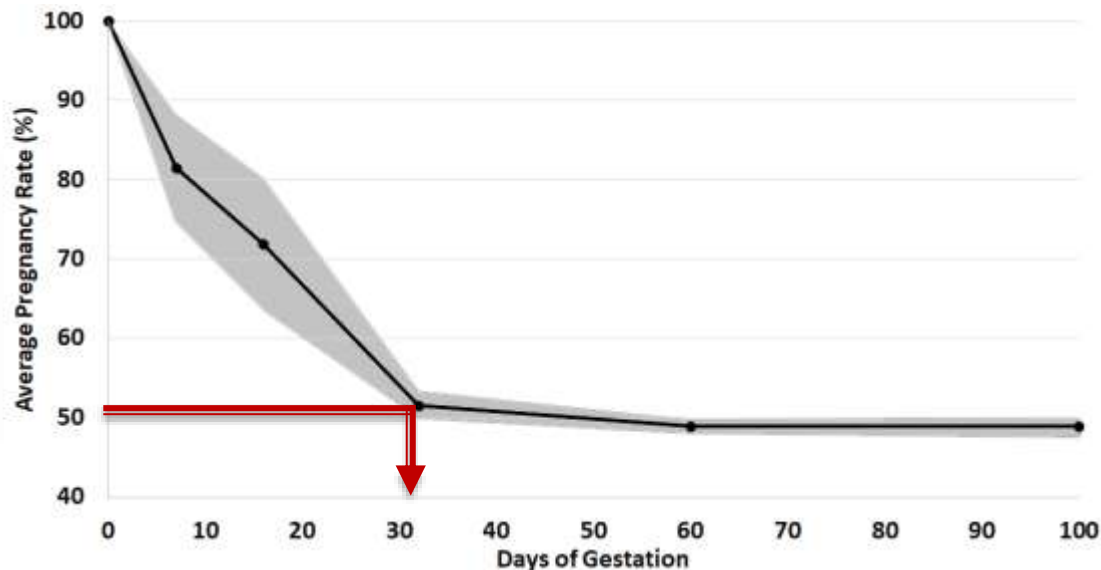


Diagnóstico de gestação +
Ressincronização

10 dias



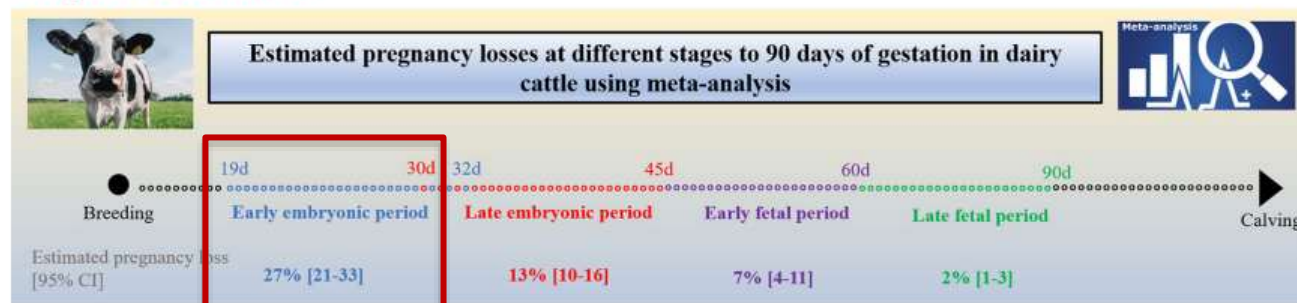
IATF2



Meta-analysis of the incidence of pregnancy losses in dairy cows at different stages to 90 days of gestation

A. Albaaj,^{1*} J. Durocher,² S. J. LeBlanc,³ and S. Dufour¹

Graphical Abstract



Estabelecimento da gestação

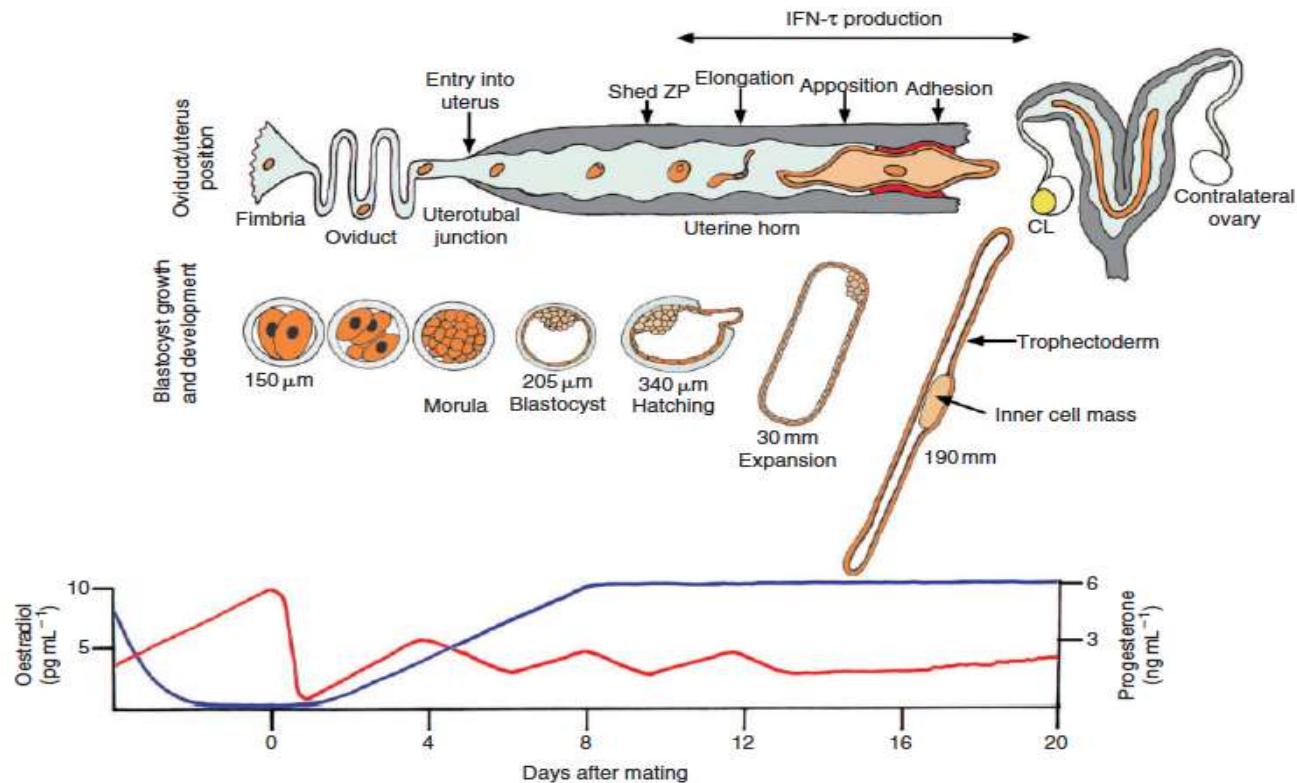
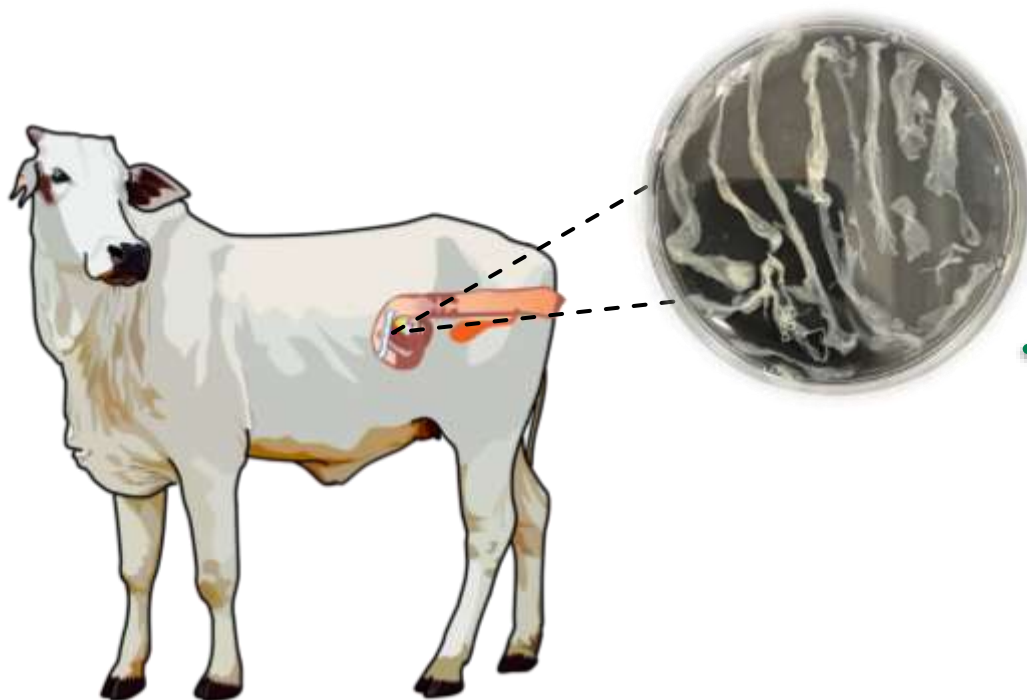


Fig. 1. Early pregnancy events in sheep. This schematic summarises changes in embryo/blastocyst development after fertilisation in relation to

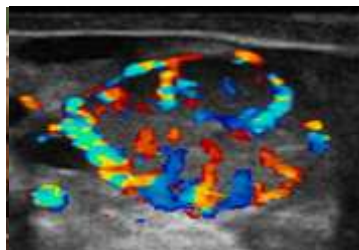
Período Crítico – 14° e 19° dia pós-estro



- ✓ **Reconhecimento Materno da sinalização embrionária**
 - Produção adequada de moléculas sinalizadoras (IFN-tau)
- ✓ **Bloqueio da Luteólise**
 - Manutenção da produção de P4
- ✓ **Modulação do sistema imune materno**
- ✓ **Genes estimulados por Interferon-tau (ISGs)?**

Abordagens para DG à campo

Ultrassonografia
Retrometral
Doppler



Ultrassonografia
Transretal



Palpação
Transretal

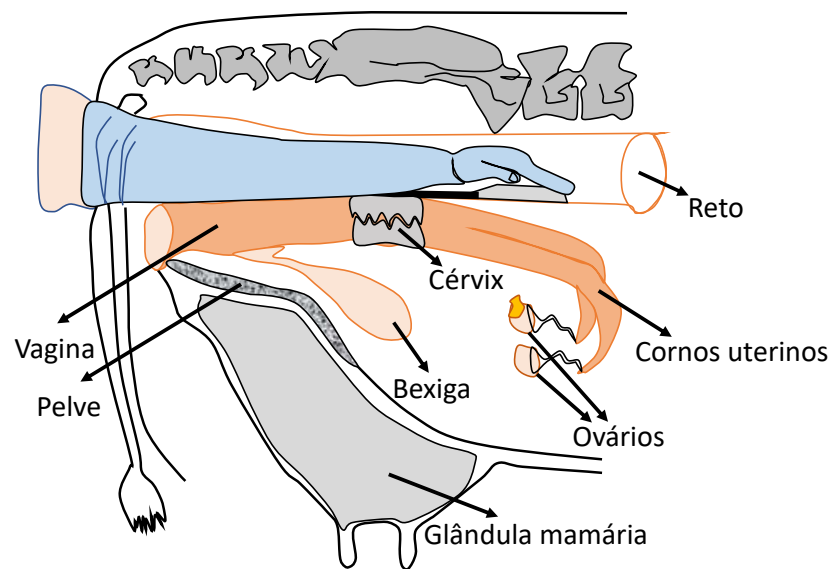


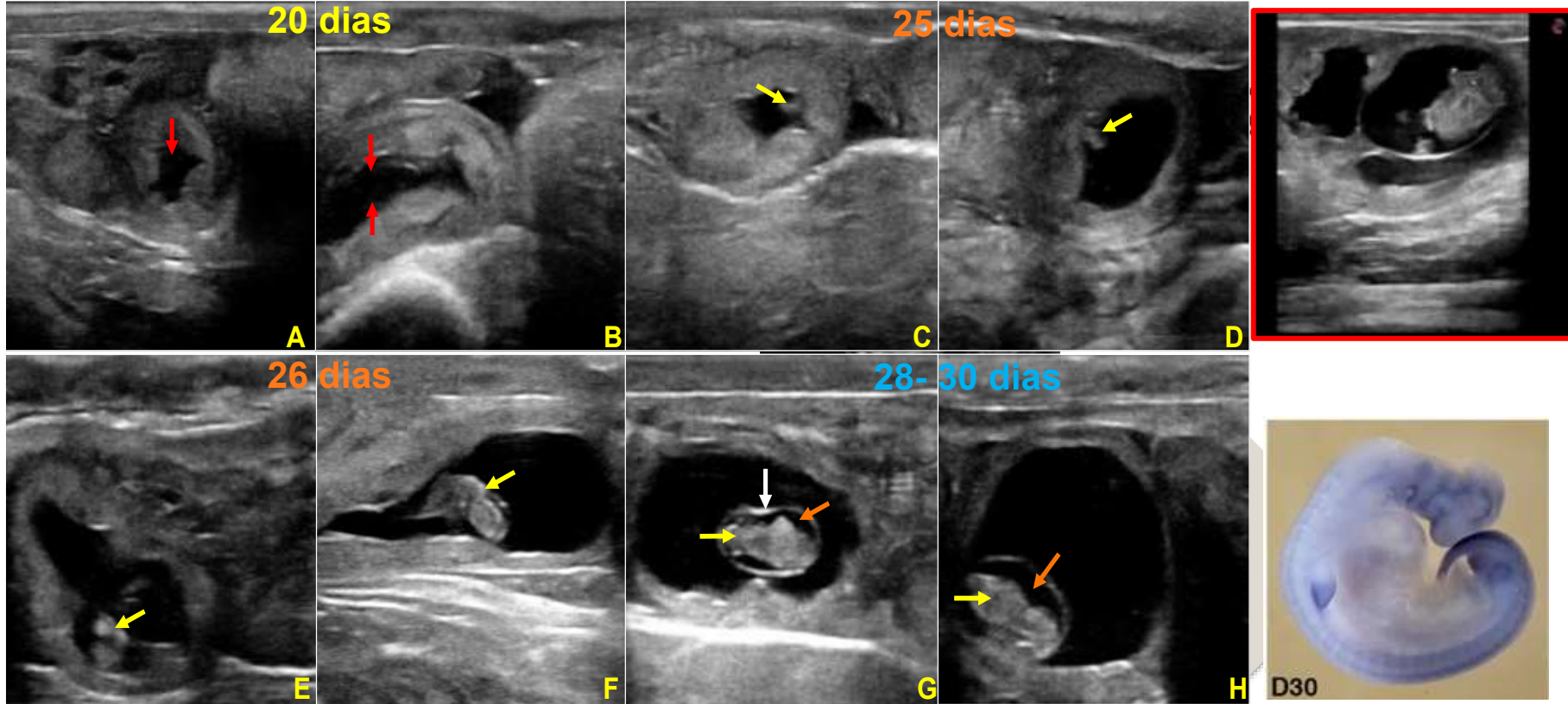
IA

29-24 dias

28 dias

40 dias





Imagens ultrassonográficas em modo B de corno uterino. As setas vermelhas indicam o acúmulo de fluido no lúmen uterino (imagem anecogênica) de fêmeas bovinas aos **20 dias de gestação (Painéis A e B)**. As setas amarelas indicam embriões bovinos (estrutura hiperecogênica) aos **25 dias (Painéis C e D)** aos **26 (Painéis E e F)**, **28 (Painel G)** e **30 (Painel H)** dias de gestação. As setas laranjas indicam o líquido amniótico (estrutura anecogênica) e a seta branca indica a membrana amniótica (estrutura hiperecogênica).

Tópicos

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Gestacionais e
Potenciais
Métodos**

**Considerações
Finais**

Como detectar mais cedo o status gestacional?

☐ Inovações no diagnóstico precoce da gestação em bovinos

Processo: 15/10606-9

Linha de fomento: Auxílio à Pesquisa - Jovens Pesquisadores

Vigência: 01 de dezembro de 2016 - 30 de novembro de 2021

Área do conhecimento: Ciências Agrárias - Medicina Veterinária - Reprodução Animal

Pesquisador responsável: Guilherme Pugliesi   

Beneficiário: Guilherme Pugliesi   

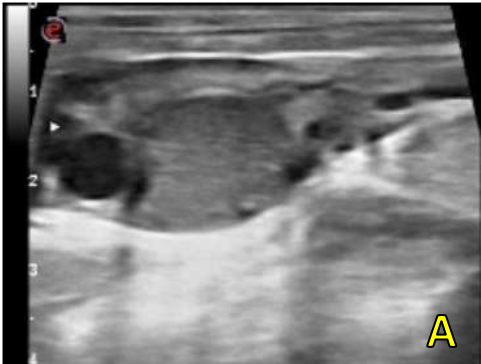
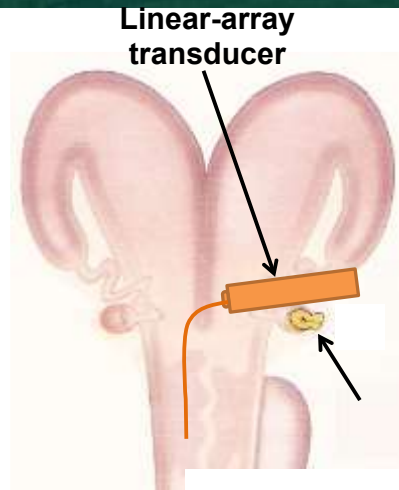
Instituição-sede: Faculdade de Medicina Veterinária e Zootecnia (FMVZ). Universidade de São Paulo (USP).
São Paulo, SP, Brasil

Pesquisadores associados: Adroaldo Jose Zanella ; Augusto Hauber Gameiro ; Claudia Maria Bertan Membrive ;
Ed Hoffmann Madureira ; Fernando Silveira Mesquita ; Luciano Andrade Silva ; Luiz
Lehmann Coutinho ; Mario Binelli ; Sônia Cristina da Silva Andrade

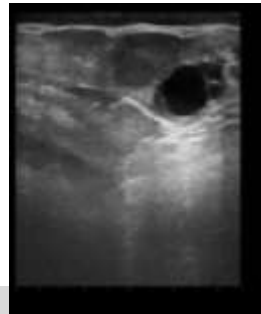
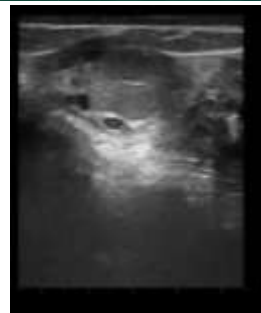
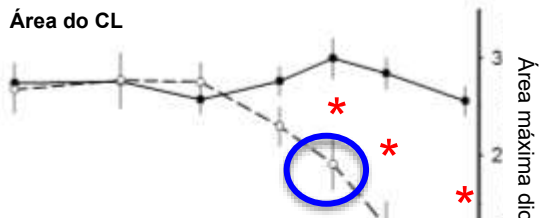
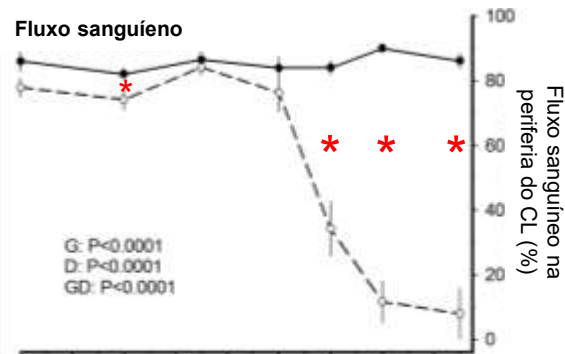
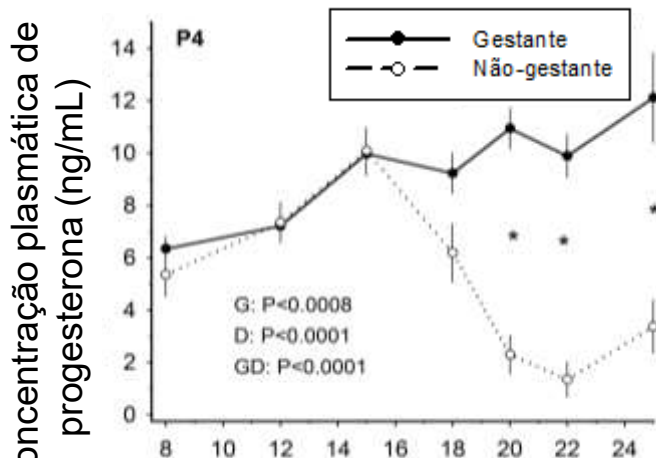


Dia 16-20

Avaliação do CL com o Doppler



Avaliação do corpo lúteo gestacional



Uso para diagnóstico precoce das fêmeas não-gestantes



J. Dairy Sci. TBC:1–12
<http://dx.doi.org/10.3168/jds.2013-6814>
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Color Doppler flow imaging for the early detection of nonpregnant cattle at 20 days after timed artificial insemination

L. G. B. Siqueira,* V. S. Areas,† A. M. Ghatti,‡ J. F. Fonseca,* M. P. Palhao,§ C. A. C. Fernandes,§
and J. H. M. Viana*¹



Acurácia da Ultrassonografia Doppler no D.G. precoce

BIOLOGY OF REPRODUCTION (2014) 91(4):95, 1–12
Published online before print 10 September 2014,
DOI 10.1095/biolreprod.114.121525

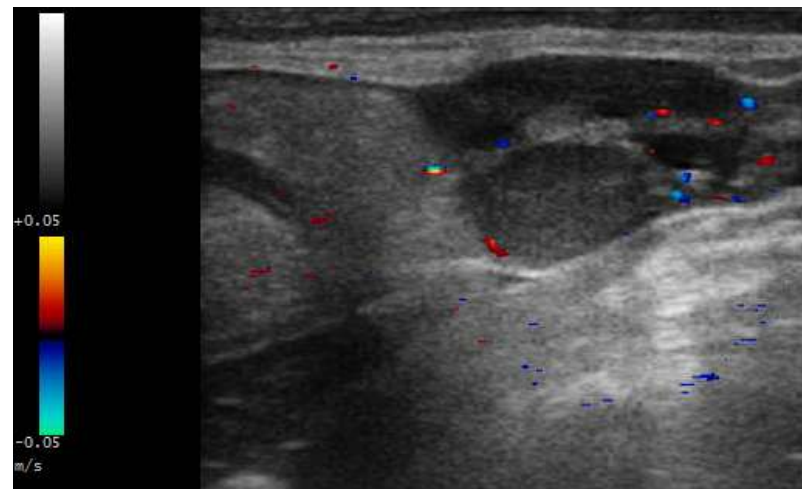
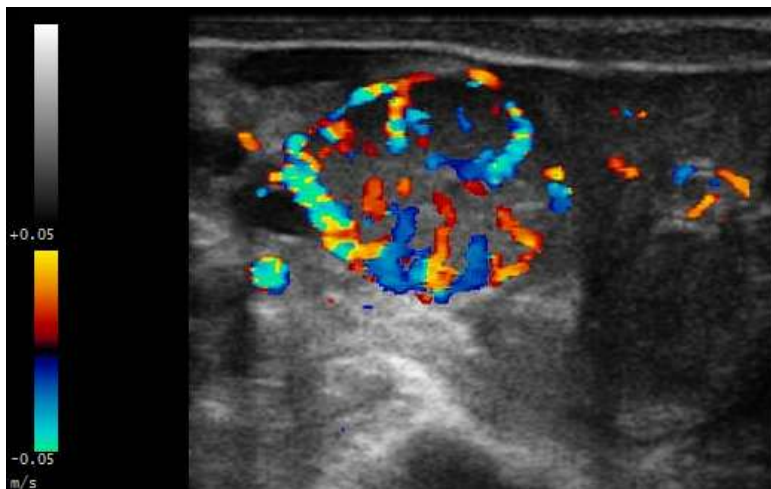


Conceptus-Induced Changes in the Gene Expression of Blood Immune Cells and the Ultrasound-Accessed Luteal Function in Beef Cattle: How Early Can We Detect Pregnancy?¹

Guilherme Pugliesi,^{2,3,4} Bruna T. Miagawa,³ Yasmin N. Paiva,³ Moana R. França,³ Luciano A. Silva,⁴
and Mario Binelli³

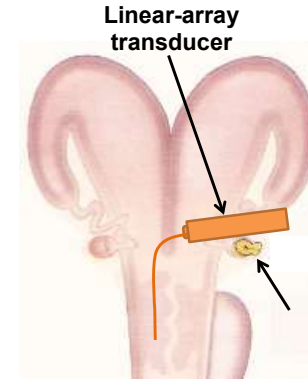
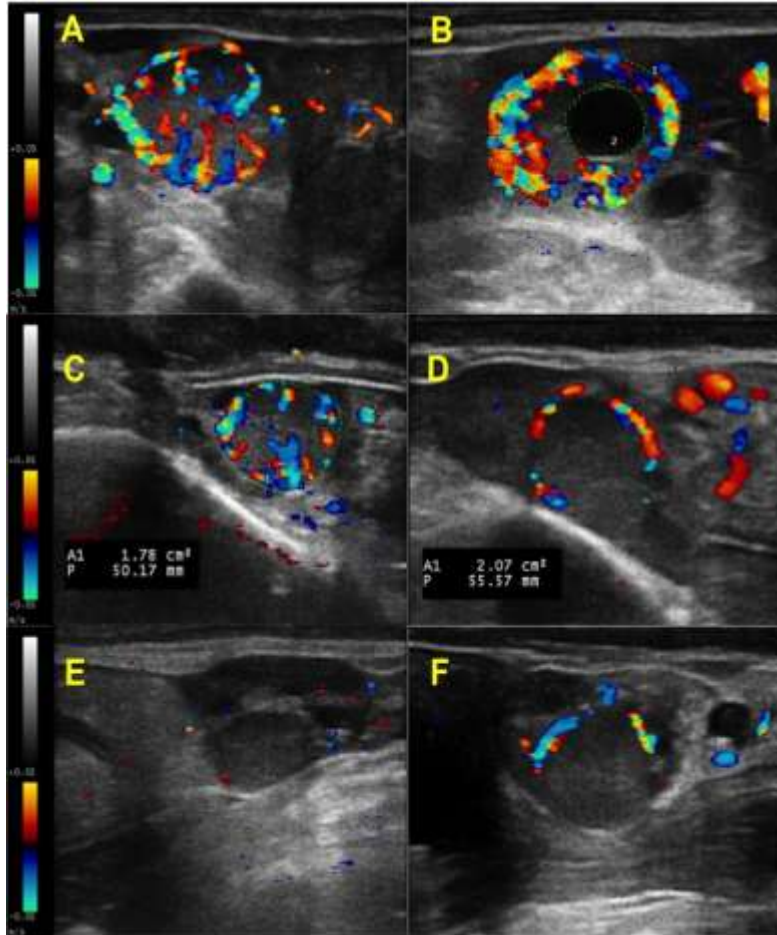
Gestante ou Não-gestante ???

É possível detectar com alta acurácia a
gestação aos 20 dias pela ultrassonografia
Doppler em bovinos???



Dia 20 pós-IATF

Doppler-US method – day 20 after TAI



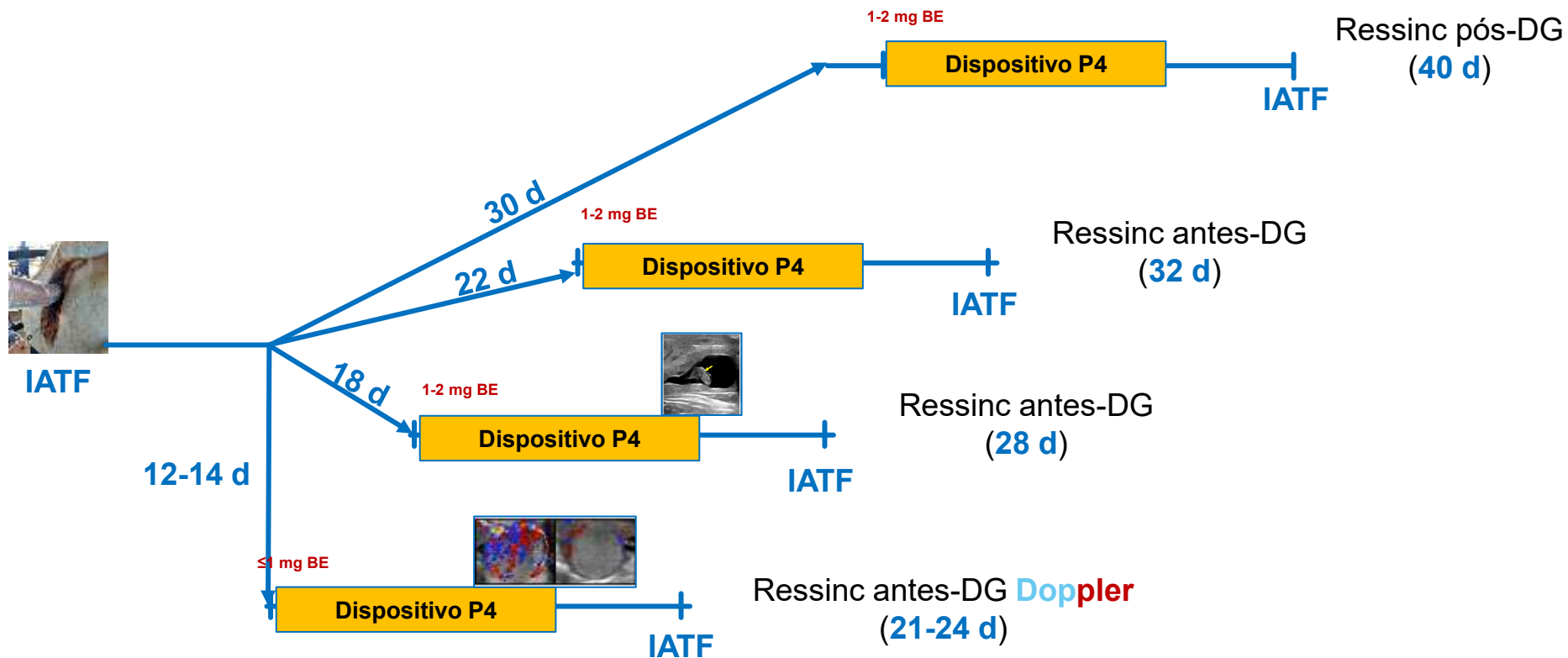
Detection of luteolysis
(Non-pregnant females)

Criteria:
Blood perfusion $\leq 25\%$.

Authors	Type/breed	Parity order	Type of service	Moment of gestation, d	Animals, n	Conception rate, %	Accuracy, %	False positive rate, %	False negative rate, %	PPV, %	NPV, %
Utt et al., 2009	Beef (Crossbreed)	Cows	TET	17 19 21	50	46	60 68.8 71.4	30.6 24.6 21	9.4 6.6 7.6	54.4 61.4 64.8	73.3 82.9 81.5

Siqueira et al., 2013	(Siqueira et al., 2013)  Accuracy: 75% False-negative: 0.5% False-positive: 24.5% 	4.8	97.7
Pugliesi et al., 2014		7.2	98
Guimarães et al., 2015		0.8	100
Scully et al., 2015 ¹		8.9	100
Pugliesi et al., 2018		6.6	70.3
Ataide Jr. Et al., 2019	(Pugliesi et al., 2014)  Accuracy: 91% False-negative: 0% False-positive: 9%	9.5	88
Andrade et al., 2019		0.8	87.8
Dalmaso Melo et al., 2020		8.8	97.5
Wellert et al., 2020		0.4	100
Dubuc et al., 2020		0.5	100
Holton et al., 2022a		8.8	100
Holton et al., 2022b		7.3	100
Madoz et al., 2022		89	100
Ferraz et al., 2022		1.8	100
		9.4	100
	75	100	
	52	98.1	
	80	100	
	85	100	
	86	100	
	90	100	
	60	98.2	
	38	98	
	48	100	

Antecipação da Ressincronização



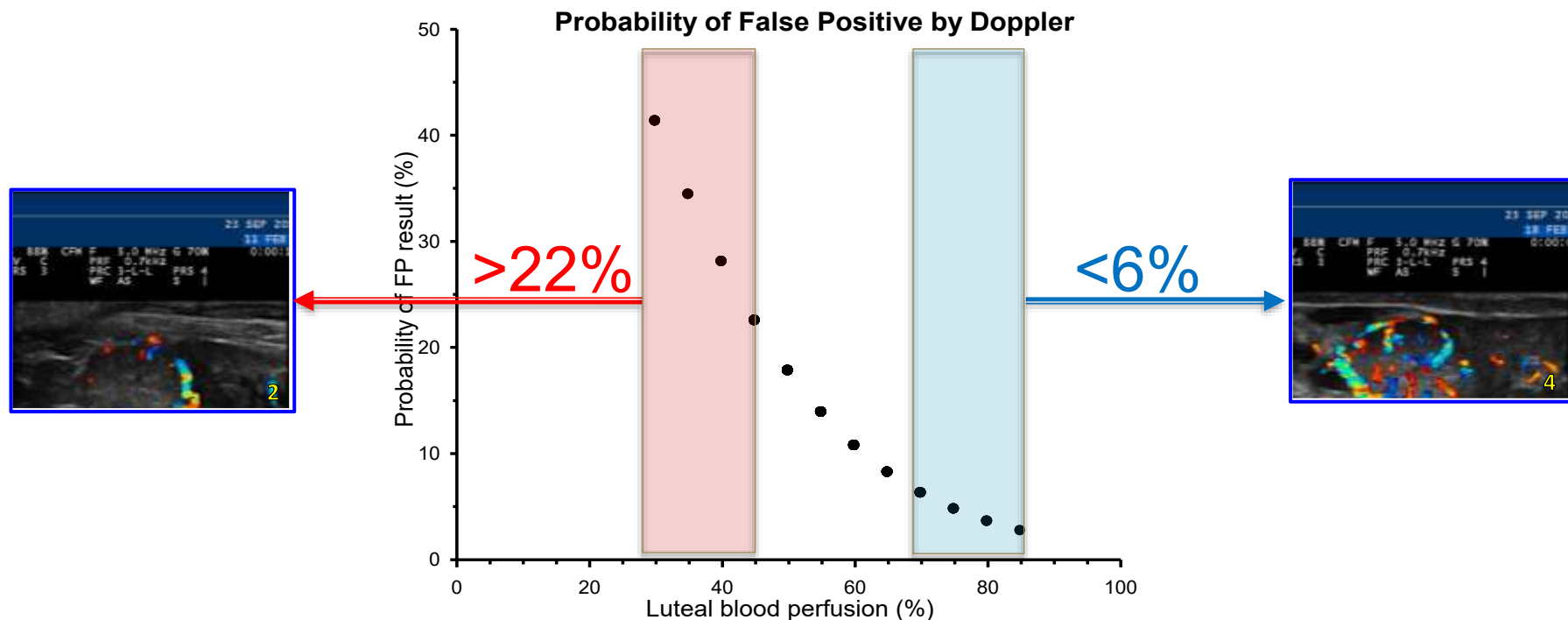
Impactos da Ressinc com Doppler

- Aumento do número de nascimentos no início da estação de nascimento
- Melhora no peso final a desmama
- Maior proporção de novilhas púberes
- Menor custo/kg de bezerro desmamado

(Odeja-Rojas, 2021; 2022)



Relation of CL blood perfusion and FP results



Probability of false positive (FP) result (females identified with a functional CL on day 20 post-TAI by Doppler ultrasonography, but non-pregnant on day 30) according to the luteal blood perfusion at the of Doppler evaluation in beef cattle (n=159). Probability of FP = $\exp(-0.0591x + 1.4201) / 1 + \exp(-0.0591x + 1.4201)$; $P=0.008$.

CL blood perfusion and Pregnancy Loss

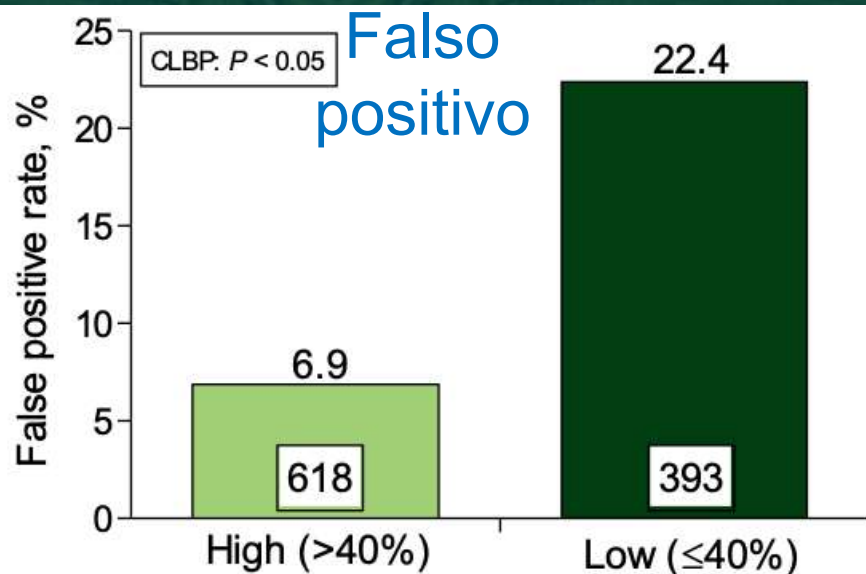


Figure 3. False positive rate (%) in beef females with high (>40%) or low (≤40%) corpus luteum blood perfusion (CLBP) on D22.

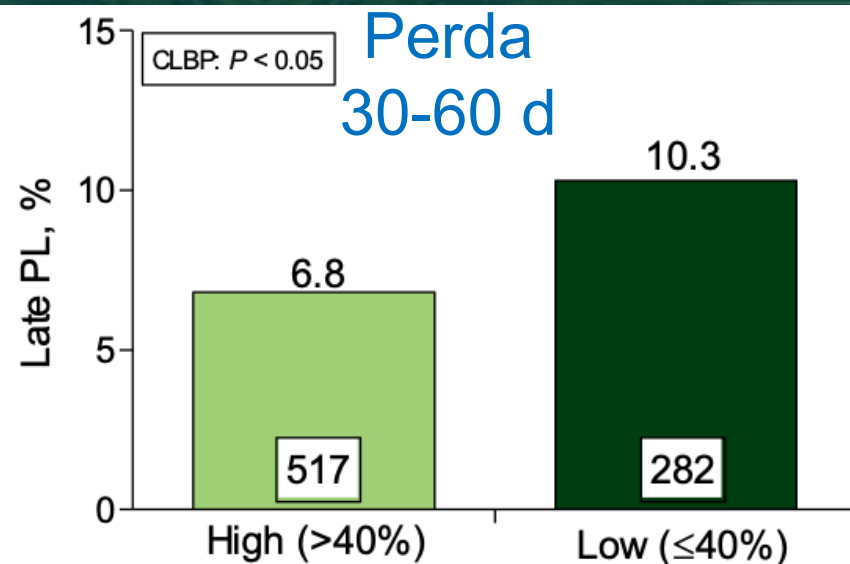
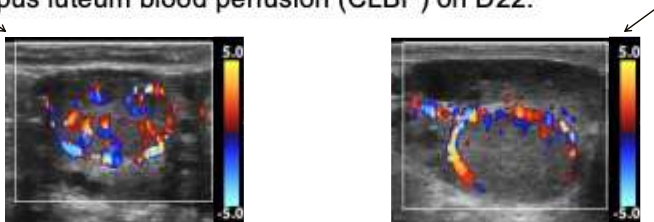
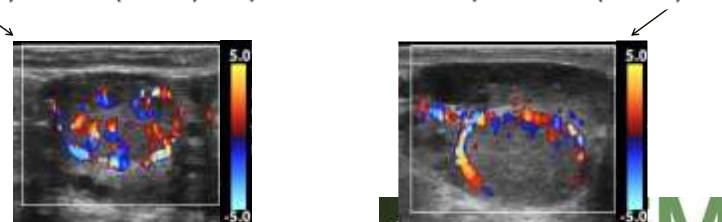


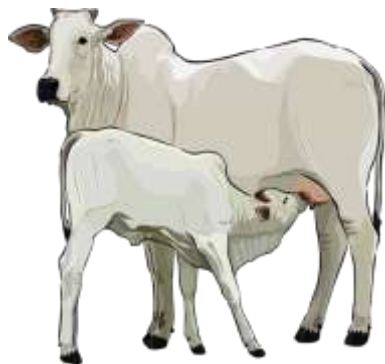
Figure 4. Late pregnancy loss (PD2 to PD3) in beef females with high (>40%) or low (≤40%) corpus luteum blood perfusion (CLBP) on D22.



CL blood perfusion and Pregnancy Loss



Heifers



Primiparous cows

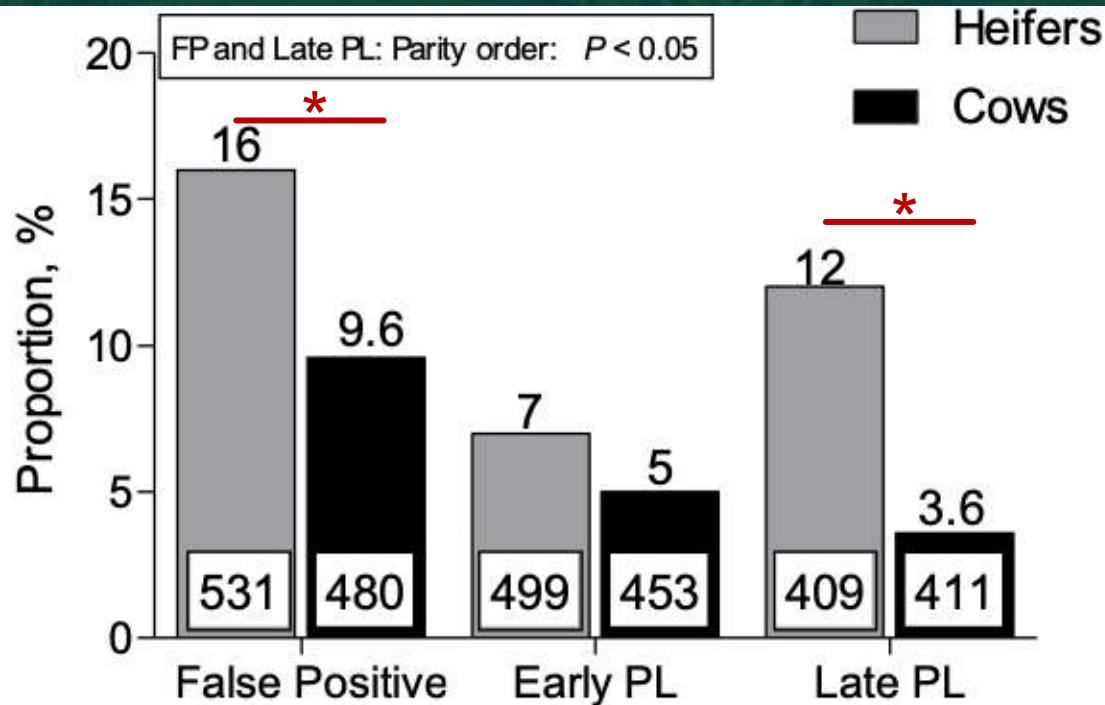


Figure 5. False positive rate (%), early (%; PD1 to PD2) and late pregnancy loss (%; PD2 to PD3) between heifers and cows.

Quais fatores estão relacionados com o Falso +?

- Perda gestacional
- Avulsão tardia no protocolo
- Luteólise tardia (leite vs. corte; novilhas vs. vacas)

Qual a proporção de cada fator na taxa de falso +?

Monitores de atividade (acelerômetros)



J. Dairy Sci. 98:8723–8731
<http://dx.doi.org/10.3168/jds.2015-9645>
 © American Dairy Science Association[®], 2015.

Behavioral and physiological changes around estrus events identified using multiple automated monitoring technologies

K. A. Dolecheck,^{*} W. J. Silvia,^{*†} G. Heersche Jr.,^{*} Y. M. Chang,[†] D. L. Ray,^{*} A. E. Stone,^{*} B. A. Wadsworth,^{*} and J. M. Bewley[‡]

^{*}Department of Animal and Food Sciences, University of Kentucky, Lexington 40546

[†]Research Support Office, Royal Veterinary College, University of London, London, NW1 1TU, United Kingdom



Acurácia em detectar o CIO (vazia)

Table 3. Estrus detection capability¹ and 95% CI of different automated monitoring technologies² and machine-learning techniques (random forest, linear discriminant analysis, and neural network)³

Technique	Technology	Sensitivity	Specificity	Accuracy
Random forest	CowManager SensOor	100.00 (47.82–100.00)	98.57 (84.93–99.83)	98.62 (95.11–99.83)
	HR Tag	60.00 (14.66–94.73)	99.29 (96.08–99.98)	97.93 (94.07–99.57)
	IceQube	80.00 (28.36–99.49)	99.29 (96.08–99.98)	98.62 (95.11–99.83)
	Track a Cow	100.00 (47.82–100.00)	97.14 (92.85–99.22)	97.24 (93.09–99.24)
Linear discriminant analysis	CowManager SensOor	100.00 (47.82–100.00)	100.00 (97.40–100.00)	100.00 (47.82–100.00)
	HR Tag	100.00 (47.82–100.00)	97.86 (93.87–99.56)	97.93 (94.07–99.57)
	IceQube	100.00 (47.82–100.00)	97.86 (93.87–99.56)	97.93 (94.07–99.57)
	Track a Cow	100.00 (47.82–100.00)	96.43 (91.86–98.83)	96.55 (92.14–98.87)
Neural network	CowManager SensOor	100.00 (47.82–100.00)	98.57 (94.93–99.83)	98.62 (95.11–99.83)
	HR Tag	100.00 (47.82–100.00)	96.43 (91.86–98.83)	96.55 (92.14–98.87)
	IceQube	100.00 (47.82–100.00)	100.00 (97.40–100.00)	100.00 (97.49–100.00)
	Track a Cow	100.00 (47.82–100.00)	90.71 (84.64–94.96)	91.03 (85.16–95.14)

¹Sensitivity = TP/(TP + FN); specificity = TN/(TN + FP); accuracy = (TP + TN)/(TP + TN + FP + FN); TP = true positive, TN = true negative, FP = false positive, and FN = false negative.

²CowManager SensOor (Agis Automatisering, Harmelen, the Netherlands); HR Tag (SCR Engineers Ltd., Netanya, Israel); IceQube (IceRobotics Ltd., Edinburgh, Scotland); and Track a Cow (Animart Inc., Beaver Dam, WI).

Monitores de atividade (acelerômetros)



Original Research Article

Relationships between activity monitoring device data and ovarian, uterine, hormonal, and pregnancy variables in beef cows

Cecilia Constantino Rocha ^{a,b}, Ana Beatriz Montevicchio ^a, Alexandra Bennett ^a, Abdul Waheed ^a, Mackenzie Mazziotto ^a, Tatiane S. Maia ^a, McKenzie Lane-Jackson Haimon ^a, Quino A. Hoorn ^a, Masroor Sagheer ^a, Camila J. Cuellar ^a, Oscar Alejandro Ojeda-Rojas ^c, Rebecca Lynn Krisher ^d, Marcello Rubessa ^{e,f}, Ky G. Pohler ^g, Peter J. Hansen ^h, Philippe Moriel ^h, Ricardo C. Chebel ^a, Mario Binelli ^{h,i}

Table 1

Number of true positives, true negatives, false positives, false negatives diagnosis, and sensitivity, specificity and accuracy for determining cows that showed true estrus by accelerometer or ESTROTECT.

Variable	Accelerometer	ESTROTECT
Number of replicates	400	400
True positive	259	289
True negative	103	104
False positive	8	7
False negative	30	0
Sensitivity ^a	90 %	100 %
Specificity ^b	93 %	93 %
Accuracy ^c	91 %	98 %

Alta Acurácia em detectar o CIO (vazia)

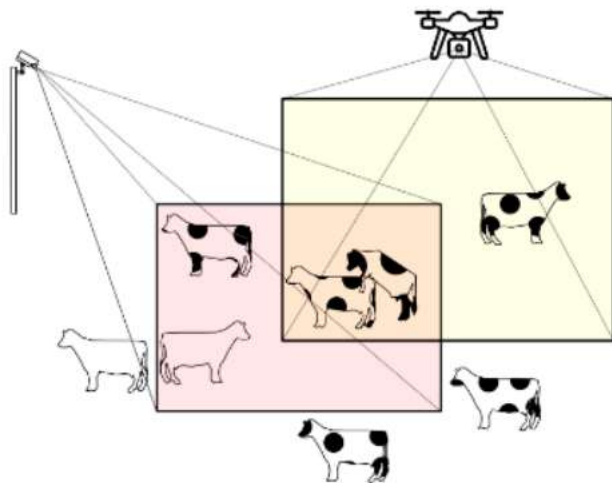


Monitores de atividade associados a IA

Article

Estrus Detection and Dairy Cow Identification with Cascade Deep Learning for Augmented Reality-Ready Livestock Farming

İbrahim Ankan ¹, Tolga Ayav ¹, Ahmet Çağdaş Seçkin ² and Fatih Soygazi ^{2,*}



Monitores de atividade associados a IA

Article Estrus Detection and Dairy Cow Identification with Cascade Deep Learning for Augmented Reality-Ready Livestock Farming

İbrahim Arıkan ¹, Tolga Ayav ¹, Ahmet Çağdaş Seçkin ² and Fatih Soygazi ^{2,*}

Table 5. Comparison of machine learning-based methods in estrus detection and cow identification.

Reference	Sensor	Software	Estrus Detection Accuracy (%)	Cow Identification Accuracy (%)
[23]	IMU	Fuzzy logic model	98	NA
[27]	IMU	Deep learning	97	NA
[29]	IMU	NA	80	NA
[28]	Camera	Deep learning	94	NA
[45]	Camera	Detection of cow images with Deep learning (YOLOv5)	94.3	NA
[49]	Camera	Computer vision	90.9	NA
[50]	Camera	YOLOv3	82.1	NA
[51]	Camera and ear tag	CNN	NA	84
[52]	Camera	YOLO and faster R-CNN	NA	84.4
Proposed CNN model	Camera	Deep learning	98	NA
Proposed VGG-19 model	Camera	Deep learning	99	NA
Proposed YOLO model	Camera	Deep learning	98	95

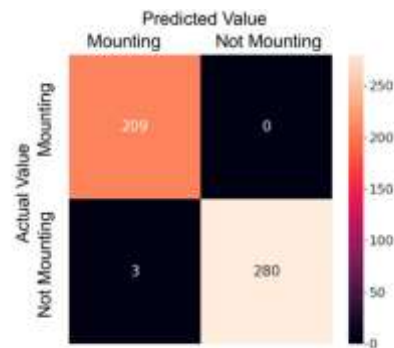


Figure 11. VGG-19 model's confusion matrix of the test dataset.

Limitantes:

- Vacas Anovulares
- Ovulação Silenciosa
- Luteólise tardia
- Perda embrionária

Tópicos



D

C

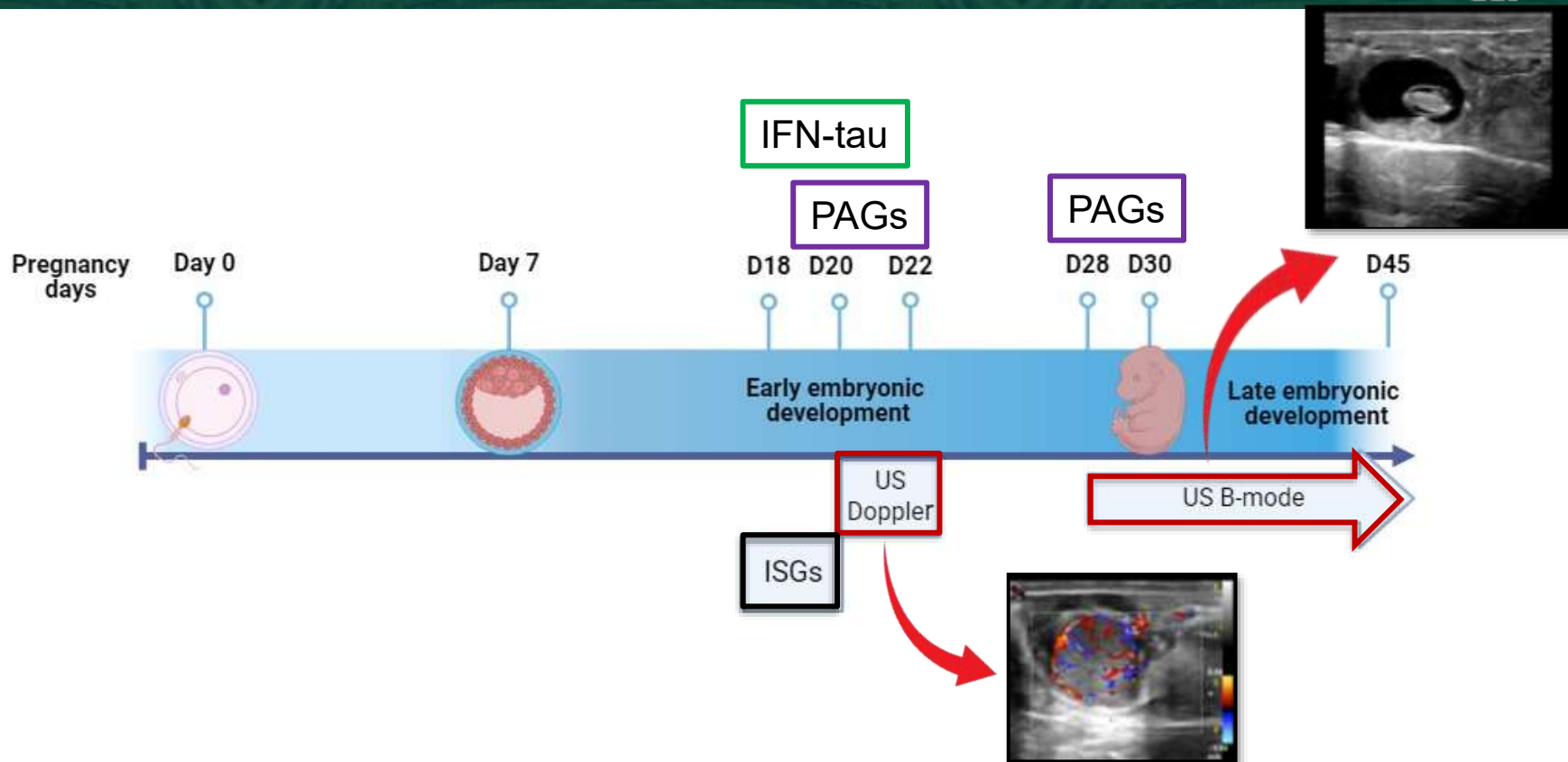
Marcador ou marcadores

- Único marcador
 - Uso de candidatos conhecidos
 - Descoberta de novos candidatos

- Painel de marcadores ou assinaturas
 - Análises ômicas
 - Transcriptoma
 - Metabolômica
 - Lipidômica
 - Proteômica
 - Etc.

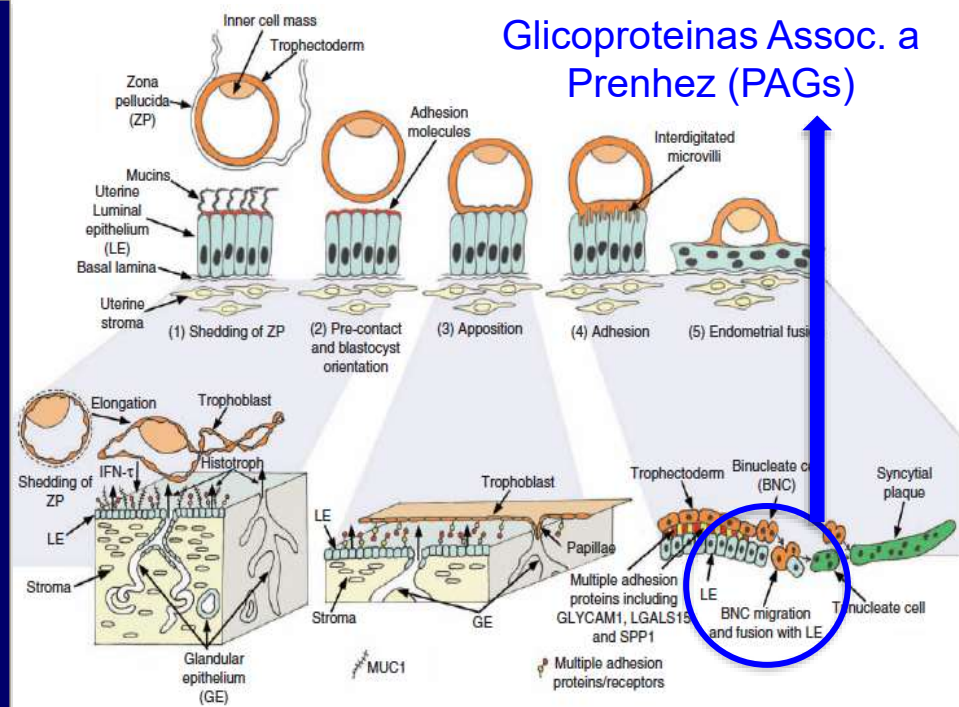


Métodos de Diagnóstico de Gestação



Métodos Laboratoriais (Moleculares)

Glicoproteínas Assoc. a
Prenhez (PAGs)



PAGs (Glicoproteínas associadas com a gestação)

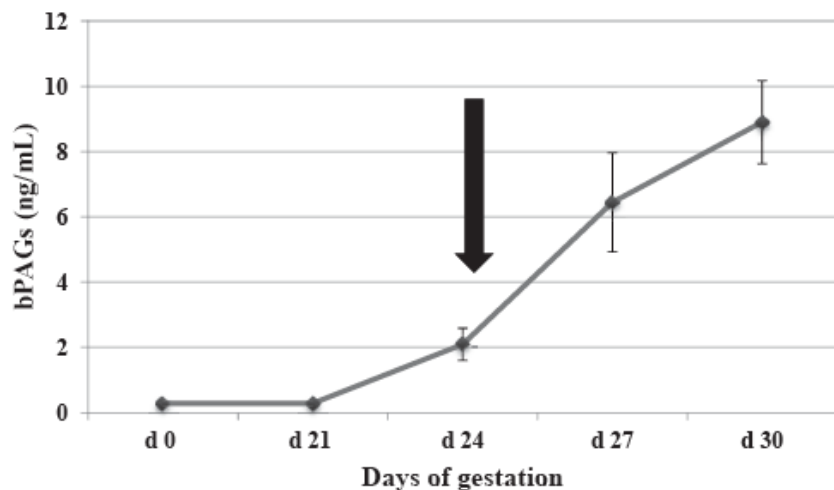


Fig. 1. Serum concentrations of bovine pregnancy-associated glycoproteins (bPAGs) in pregnant Nelore cows (mean $\pm$ SEM; $n = 22$) from Days 0 to 30 of gestation in experiment 1. First significant increase ($P < 0.0001$) in circulating bPAG concentrations occurred on Day 24 of gestation. Arrow head represents the first significant increase in circulating bPAG concentrations.

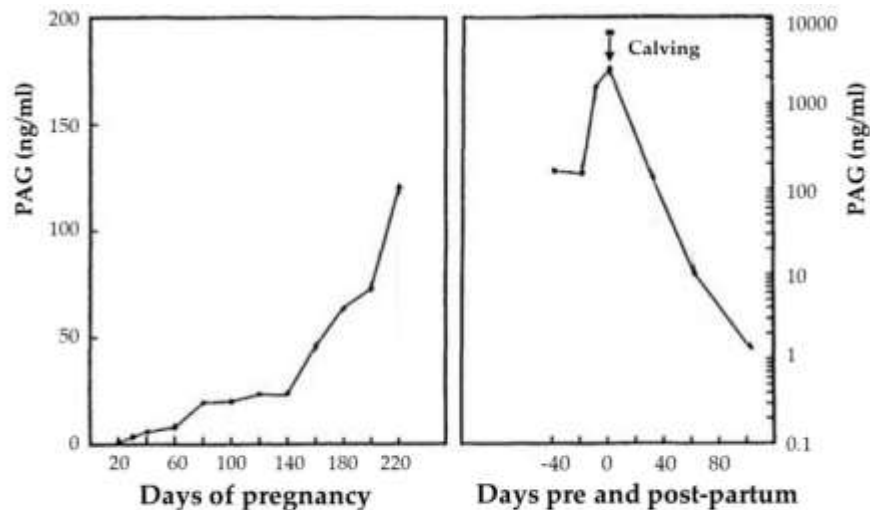


Figure 1. Pregnancy-associated glycoprotein (PAG) plasma profile of a cow during pregnancy and the post-partum period. Adapted from Zoli et al. [16].

PAGs (Glicoproteínas associadas com a gestação)

- Bovinos: 22 genes de PAGs identificados
- Mensuração das concentrações
 - RIA e EIA (“Sandwich”)
 - Envio para laboratórios especializados (USA e Canadá)
 - Kits comerciais para Laboratórios
 - Kits para Campo

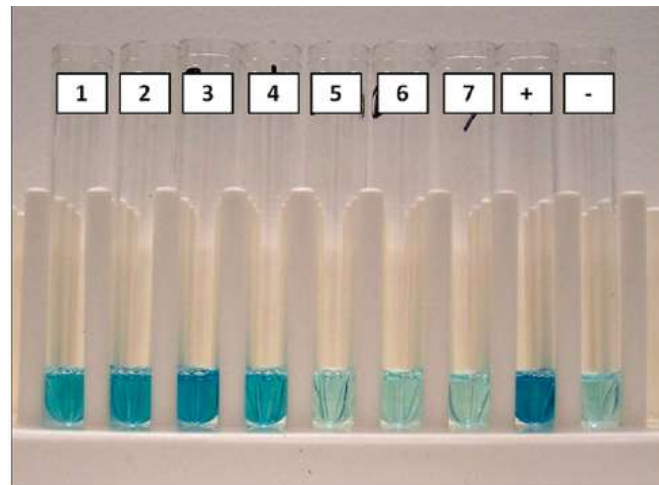


Fig. 2. Representative outcome of the rapid PAG ELISA on Day 25

AntelBio
Bovine PAG ELISA...
to identify open-cows

bioPRYN

CONCEPTION
Animal Reproduction Technologies

IDEXX
LABORATORIES

LFEM
REPRODUÇÃO ANIMAL - FMVZ - USP



10 kits de teste de gravidez de vaca de alta precisão

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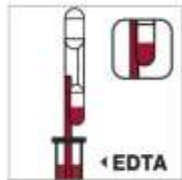
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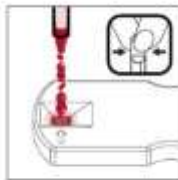
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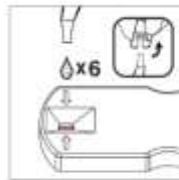
Step 1



Step 2



Step 3



Step 4



Step 5



10% Increase in PSPB between 17 and 24 days

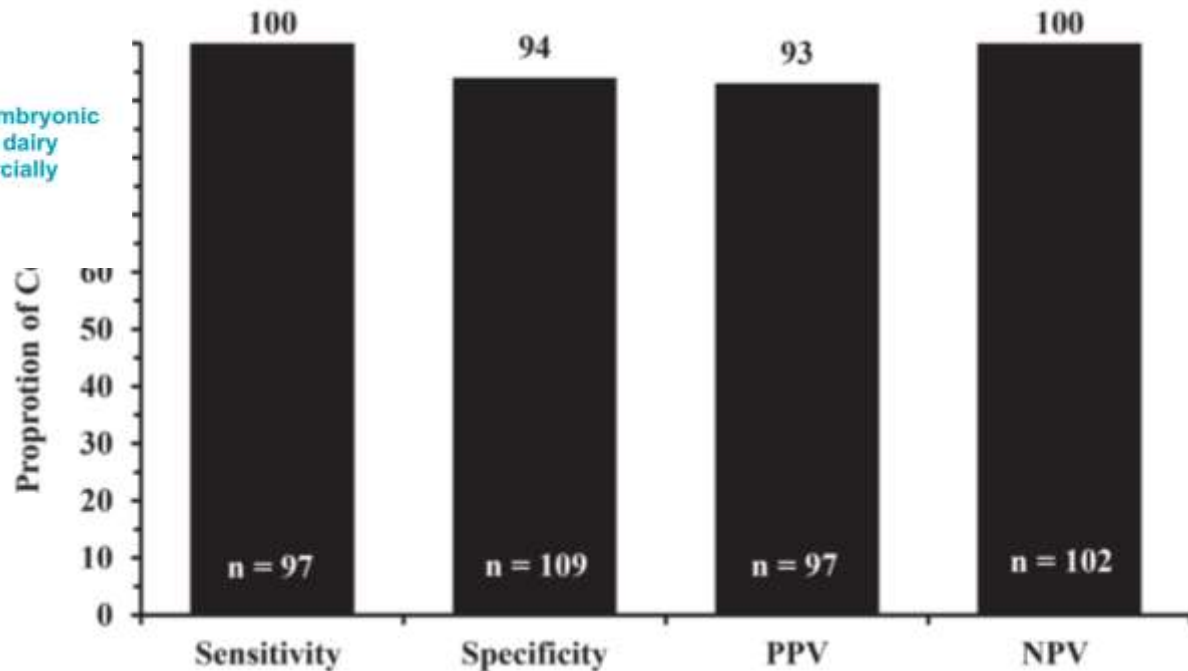


J. Dairy Sci. 102:7570–7575
<https://doi.org/10.3168/jds.2018-15961>
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Short communication: Blood samples before and after embryonic attachment accurately determine non-pregnant lactating dairy cows at 24 d post-artificial insemination using a commercially available assay for pregnancy-specific protein B

E. L. Middleton and J. R. Pursley*
Department of Animal Science, Michigan State University, East Lansing 48824

Validating the 10% increase in PSPB – samples collected on D17 and 24 post-AI



Determining time to increase in PSPB in heifers and multiparous dairy cows

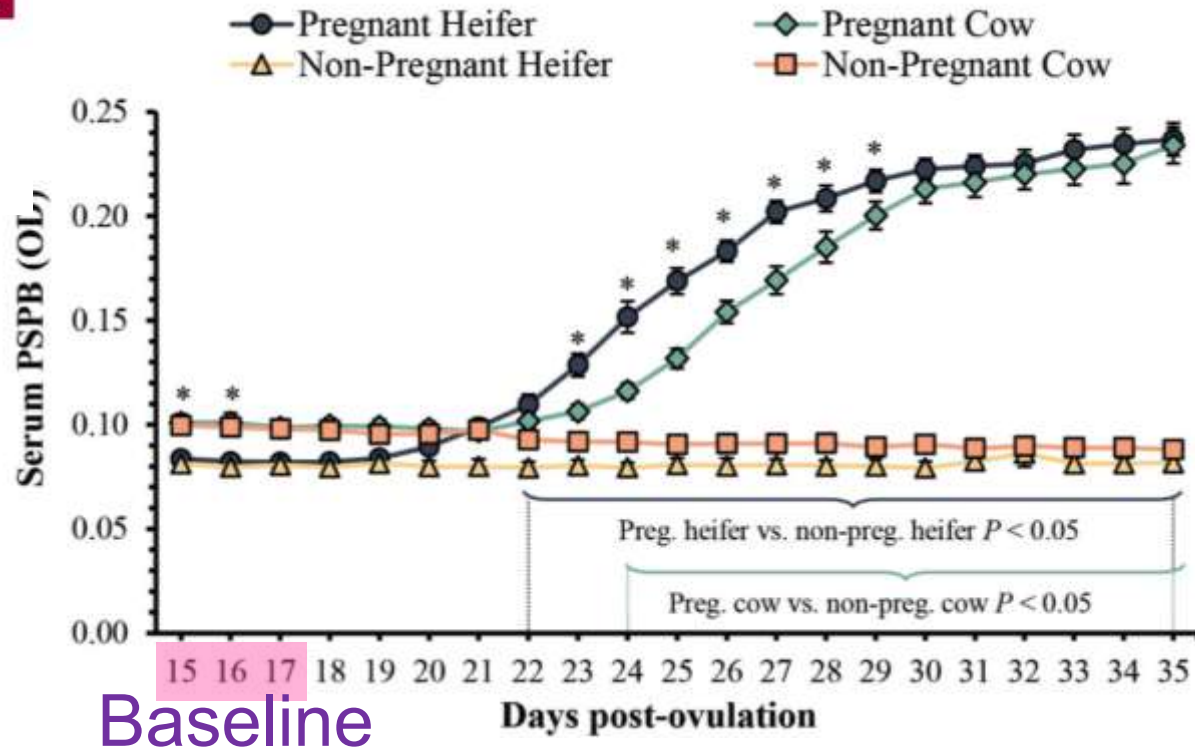
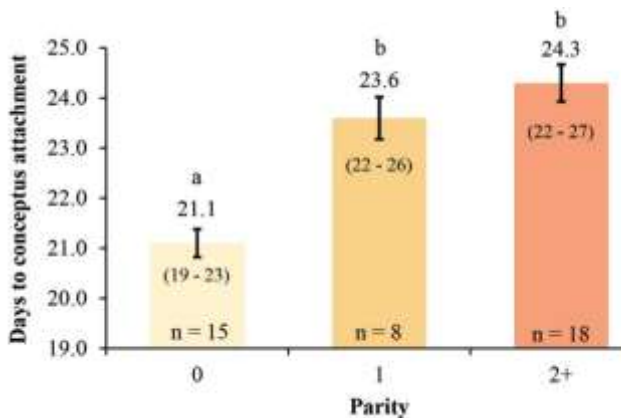


JDS
Communications®
2022; 3:291-295

<https://doi.org/10.3168/jds.2021-0190>
Short Communication
Physiology

Dairy heifers have an earlier increase in serum pregnancy-specific protein B compared with lactating dairy cows. Is this an indicator of earlier conceptus attachment?

E. L. Middleton, T. Mineia, M. Ahearn, H. Arnold, A. Santos, and J. R. Pursley*



Determining the relationship between time of PSPB increase and subsequent pregnancy loss



J. Dairy Sci. 106:3734–3747

<https://doi.org/10.3168/jds.2022-22553>

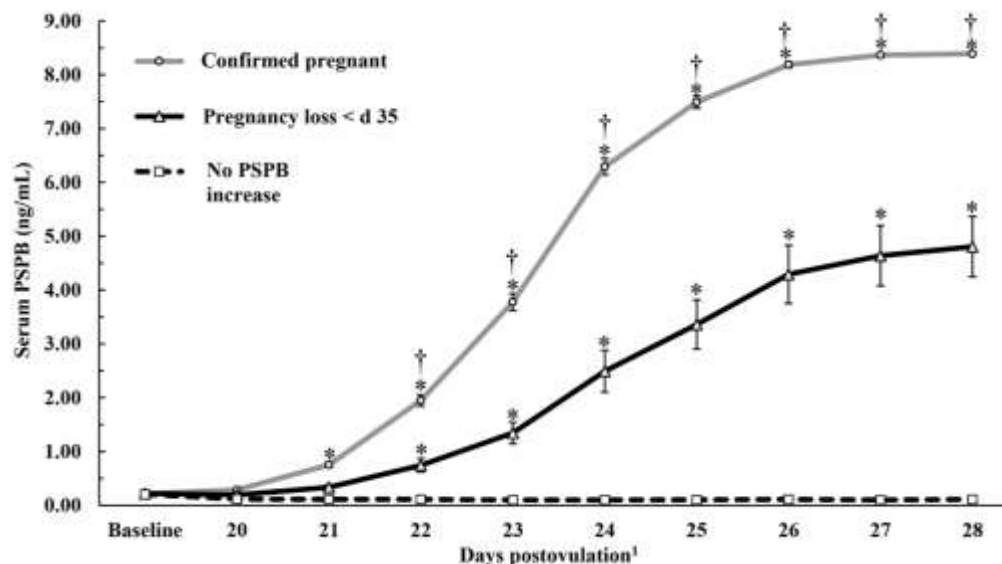
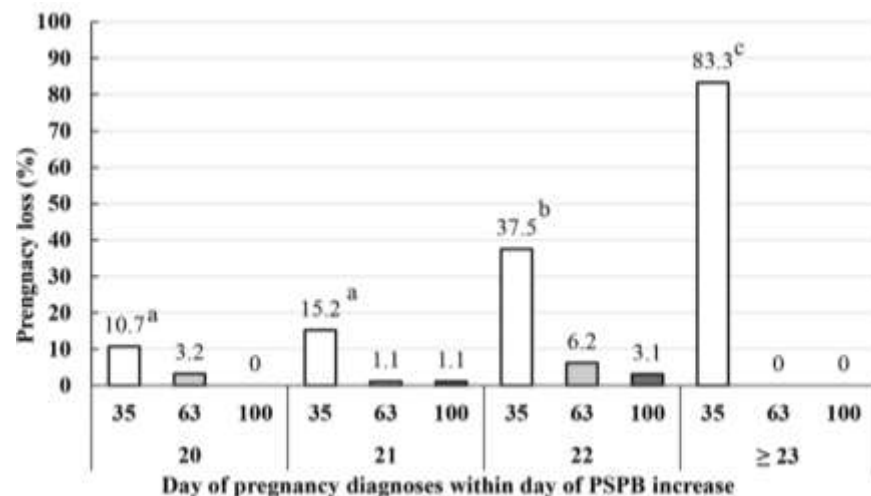
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Time to increase in pregnancy-specific protein B following artificial insemination is a direct determinant of subsequent pregnancy loss in lactating dairy cows

A. Santos,¹ T. Minela,¹ J. Branen,² and J. R. Pursley^{1*}

¹Department of Animal Science, Michigan State University, East Lansing 48824

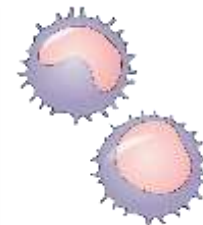
²BioTracking Inc., Moscow, ID 83843



BIOLOGY OF REPRODUCTION (2014) 91(4):95, 1–12
Published online before print 10 September 2014.
DOI 10.1095/biolreprod.114.121525

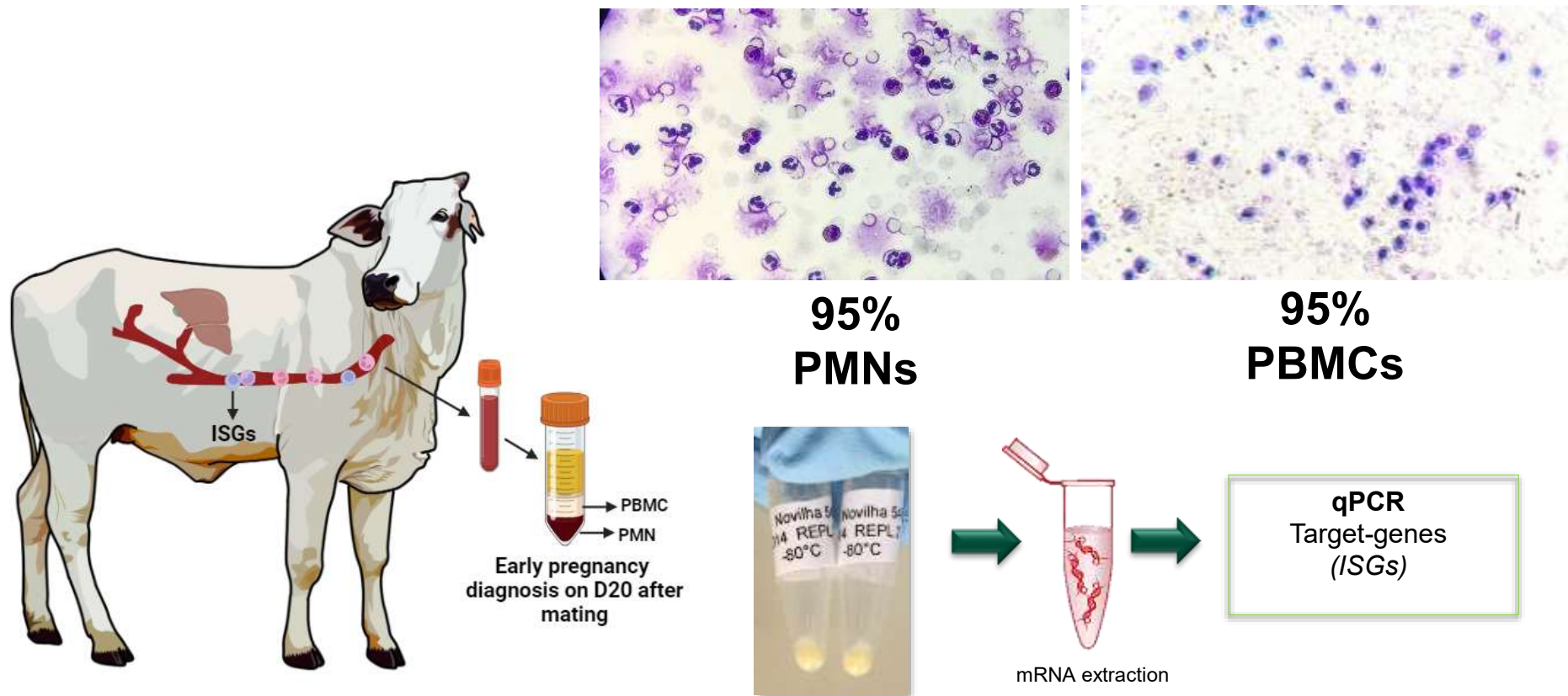
Conceptus-Induced Changes in the Gene Expression of Blood Immune Cells and the Ultrasound-Accessed Luteal Function in Beef Cattle: How Early Can We Detect Pregnancy?¹

Guilherme Pugliesi,^{2,3,4} Bruna T. Miagawa,³ Yasmin N. Paiva,³ Moana R. França,³ Luciano A. Silva,⁴ and Mario Binelli³

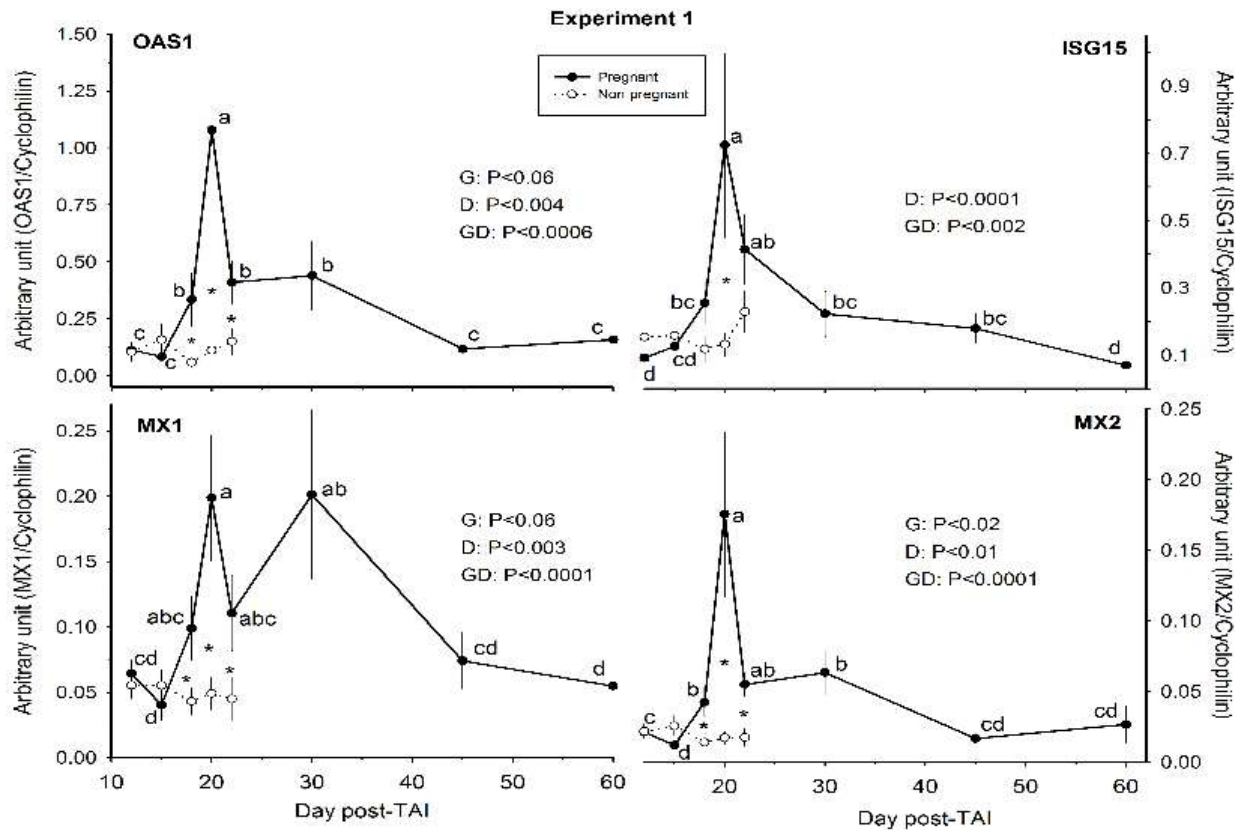


- Multiparous, non-lactating Nelore cows (n=27);
- PBMC isolation on days 12, 15, 18, 20, 22, 30, 45 and 60 post –TAI;
- Classic ISGs (*ISG15*, *OAS1*, *MX1* and *MX2*).

Material and methods



Perfil de ISGs em células imunes circulantes (PBMCs)





ISGs em PBMCs

TABLE 3. Experiment 2: number of TP, TN, FP, and FN diagnoses and sensitivity, specificity, PPV, NPV, and accuracy for determining pregnancy status on Day 20 post-TAI by Doppler-US or ISG-PBMC method.

Endpoint	Doppler US ^a			ISG-PBMC ^b			Doppler US + ISG-PBMC ^c
	Only luteal area	Only luteal blood flow	Both	OAS-1	MX-2	Both	
No. of cows	111	111	111	90	90	90	90
TP	41	41	42	24	26	22	22
TN	61	59	59	44	30	50	54
FP	8	10	10	13	27	7	3
FN	1	1	0	9	7	11	11
Sensitivity ^d	97.6%	97.6%	100%	72.7%	78.8%	66.7%	66.7%
Specificity ^e	88.4%	85.5%	85.5%	77.2%	52.6%	87.7%	94.7%
PPV ^f	83.7%	80.4%	80.8%	64.9%	49%	75.8%	88%
NPV ^g	98.4%	98.4%	100%	83%	81.1%	81.9%	83.1%
Accuracy ^h	92%	90.1%	91%	75.6%	62.2%	80%	84.4%

Genes estimulados por IFN-tau



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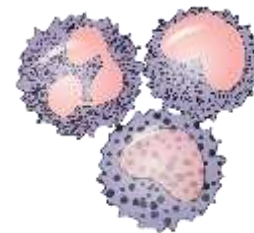


Applied use of interferon-tau stimulated genes expression in polymorphonuclear cells to detect pregnancy compared to other early predictors in beef cattle

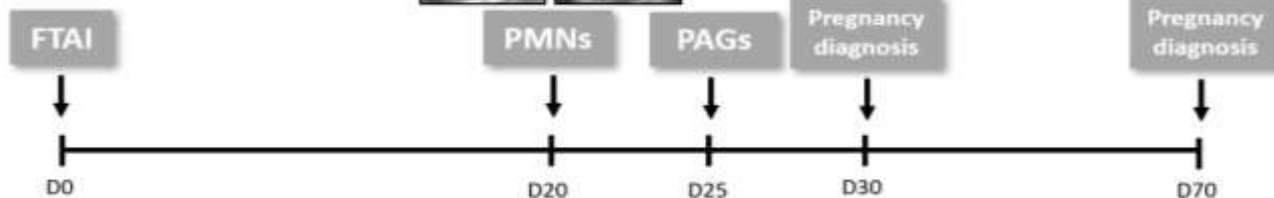
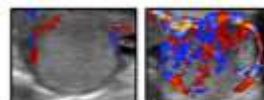


Gabriela Dalmaso de Melo ^a, Barbara Piffero Mello
Carlos Alberto Souto Godoy Filho ^a, Cecilia Constan
Amanda Guimarães Silva ^a, Sydney Taylor Reese ^b, Ed Hommann Madureira
Ky Garrett Pohler ^b, Guilherme Pugliesi ^{a,*}

PMNs



Heifers: N=103
Cows: N=144



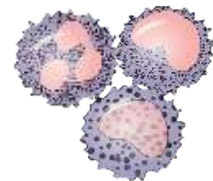
Applied use of interferon-tau stimulated genes expression in polymorphonuclear cells to detect pregnancy compared to other early predictors in beef cattle

Gabriela Dalmasio de Melo¹, Barbara Piffero Mello², Cátia Aparecida Ferreira³, Carlos Alberto Souto Godoy Piffer⁴, Cécilia Constantino Rocha⁵, Amanda Guimarães Silva⁶, Sydney Taylor Reese⁷, Ed Hoffmann Mafureira⁸, Rg Carreni Pabber⁹, Guilherme Pugliesi¹⁰

Número de resultados verdadeiro-positivo (TP), verdadeiro-negativo (TN), falso-positivo (FP) e falso-negativo (FN) e sensibilidade (SENS), especificidade (SPEC), Valor Preditivo Negativo (NPV), Valor Preditivo Positivo (PPV) e acurácia (ACCU) para determinação do status gestacional no dia 20 pós-IATF pelo Doppler-US, ISG15, OAS1 e P4 e no dia 25 pós-IATF pelas PAGs em novilhas e vacas.



Endpoint	Novilhas					Vacas				
	Doppler-US	ISG15	OAS1	P4	PAGs	Doppler-US	ISG15	OAS1	P4	PAGs
N	103	103	103	99	103	144	144	144	136	144
TP (N)	51	42	51	55	53	84	62	63	83	83
TN (N)	37	40	32	35	42	50	42	45	45	52
FP (N)	12	9	17	9	7	10	18	15	8	8
FN (N)	0	12	3	0	1	0	22	21	0	1
SENS (%) ^a	100	78	94	100	98	100	74	75	100	99
SPEC (%) ^b	75	82	65	80	86	83	70	75	85	87
PPV (%) ^c	81	82	75	86	88	89	78	81	91	91
NPV (%) ^d	100	77	91	100	98	100	66	68	100	98
ACCU (%) ^e	85	80	81	91	92	93	72	75	94	94



Genes estimulados por IFN-tau



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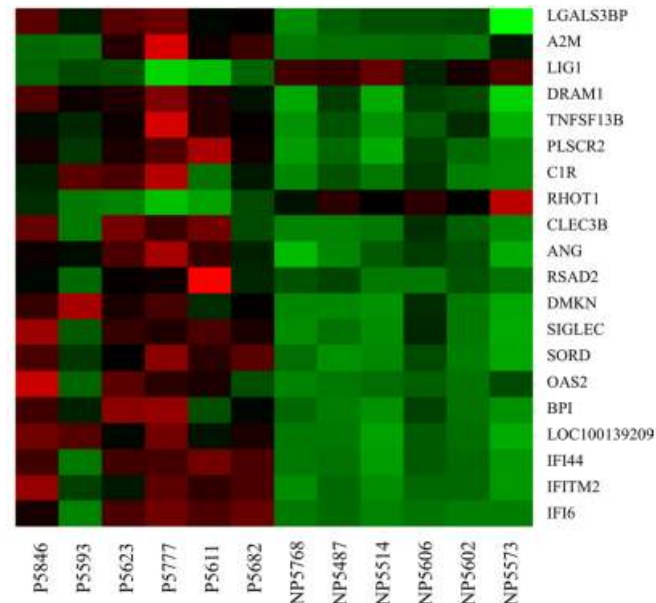


OPEN Early pregnancy-induced transcripts in peripheral blood immune cells in *Bos indicus* heifers

Cecilia Constantino Rocha¹, Sônia Cristina da Silva Andrade², Gabriela Dalmazo de Melo¹, Igor Garcia Motta¹, Luiz Lehmann Coutinho³, Angela Maria Gonella-Díaz⁴, Mario Binelli² & Guilherme Pugliesi^{1,2}

9 potencial biomarkers in PBMC (*IFI6*, *RSAD2*, *IFI44*, *IFITM2*, *TNFSF13B*, *LGALS3BP*, *CLEC3B*, *OAS2*, *LOC100139209*)

5 potencial biomarkers in PMN (*IFI6*, *RSAD2*, *IFI44*, *OAS2*, *LGALS3BP*).



OPEN Conceptus signaling markers in immune cells enhance pregnancy prediction in beef cattle

Isabella Ili Fofre^{1,2}, Gabriela Oliveira Melo³, Pedro Paulo Freitas², Karine Galvão Mendel⁴, Maria Bion⁵, Cláudia Maria Bertan Mendes^{1,2} & Guilherme Pagnini^{2,1}

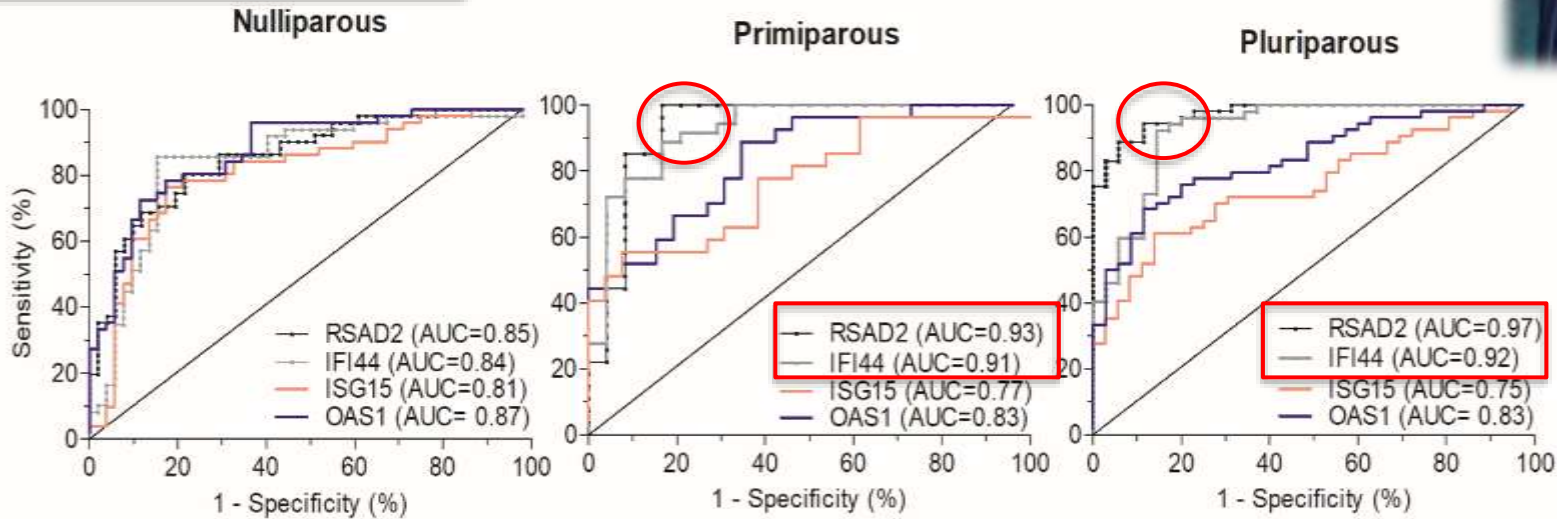


Figure 6. ROC (Receiver Operating Characteristic) curves of the classic (*ISG15* and *OAS1*) and non-classic (*RSAD2* and *IFI44*) ISGs on D20 post-TAI in nulliparous (N=103), primiparous (N=53) and pluriparous (N=91) bovine females. The horizontal and vertical axes represent false positive rate (1 - specificity) and sensitivity, respectively. Non-classic (*RSAD2* and *IFI44*) ISGs provided the most adequate prediction of pregnancy when compared to classic ISGs (*ISG15* and *OAS1*) in primiparous and pluriparous bovine females.

Aumento da sensibilidade usando 4 ISGs em PMNs

Table 3. Number of True-Positive (TP), True-Negative (TN), False-Positive (FP), False-Negative (FN), Sensitivity (SENS), Specificity (SPEC), Positive Predictive Value (PPV), Negative Predictive Value (NPV) and Accuracy (ACCU) for determining pregnancy status on D20 post-FTAI by *RSAD2*, *IFI44*, *ISG15* and *OAS1* in bovine females with a functional CL.

Endpoint	Nulliparous				Primiparous				Pluriparous			
	<i>RSAD2</i>	<i>IFI44</i>	<i>RSAD2/IFI44</i>	<i>RSAD2/IFI44/ISG15/OAS1</i>	<i>RSAD2</i>	<i>IFI44</i>	<i>RSAD2/IFI44</i>	<i>RSAD2/IFI44/ISG15/OAS1</i>	<i>RSAD2</i>	<i>IFI44</i>	<i>RSAD2/IFI44</i>	<i>RSAD2/IFI44/ISG15/OAS1</i>
<i>n</i>	100	100	100	100	50	50	50	50	83	83	83	83
VP (<i>n</i>)	39	42	44	48	27	23	27	27	45	46	48	48
VN (<i>n</i>)	47	47	46	43	22	22	22	21	32	30	30	28
FP (<i>n</i>)	4	4	5	8	1	1	1	2	1	3	3	5
FN (<i>n</i>)	10	7	5	1	0	4	0	0	5	4	2	1
SENS (%)	79.6	85.7	89.8	98.0	100.0	85.2	100.0	100.0	90.0	92.0	96.0	98.0
SPEC (%)	92.2	92.2	90.2	84.3	95.6	95.6	95.7	91.3	97.0	90.9	90.9	84.8
PPV (%)	90.7	91.3	89.8	85.7	96.4	95.8	96.4	93.1	97.8	93.9	94.1	90.7
NPV (%)	82.5	87.0	90.2	97.7	100.0	84.6	100.0	100.0	86.5	88.2	93.7	96.5
ACCU (%)	86.0	89.0	90.0	91.0	98.0	90.0	98.0	96.0	92.8	91.6	94.0	92.8

↑ Marcadores: ↓ Falso Negativo

Aumento da sensibilidade usando 4 ISGs em PMNs na fêmeas com CL ativo

Table 4. Number of True-Positive (TP), True-Negative (TN), False-Positive (FP), False-Negative (FN), Sensitivity (SENS), Specificity (SPEC), Positive Predictive Value (PPV), Negative Predictive Value (NPV) and Accuracy (ACCU) for determining pregnancy status on D20 post-FTAI by *RSAD2*, *IFI44*, *ISG15* and *OAS1* in bovine females with a functional CL.

<i>Genes</i>	<i>ISG15</i>			<i>OAS1</i>		<i>RSAD2</i>	<i>RSAD2/IFI44/</i>
	<i>OAS1</i>	<i>RSAD2</i>	<i>IFI44</i>	<i>RSAD2</i>	<i>IFI44</i>	<i>IFI44</i>	<i>ISG15/OAS1</i>
<i>n</i>	233	233	233	233	233	233	233
TP (<i>n</i>)	108	115	118	122	123	120	124
TN (<i>n</i>)	92	95	95	95	95	98	92
FP (<i>n</i>)	15	12	12	12	12	9	15
FN (<i>n</i>)	18	11	8	4	3	6	2
SENS (%)	85.7	91.3	93.6	96.8	97.6	95.2	98.4
SPEC (%)	86.0	88.8	88.8	88.8	88.8	91.6	86.0
PPV (%)	87.8	90.5	90.8	91.0	91.1	93.0	89.2
NPV (%)	83.6	89.6	92.2	96.0	96.9	93.2	97.9
ACCU (%)	85.8	90.1	91.4	93.1	93.6	93.6	92.7

Acurácia dos ISGs usando células sanguíneas para DG Precoce

Feltrin et al., 2025. (PAINEL de ISG)

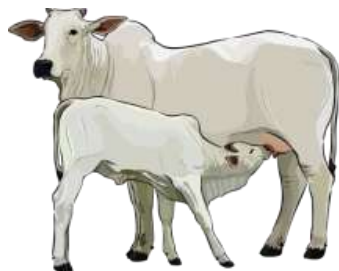


Nulliparous

Accuracy: 91%

FP: 14%

FN: 2%



Primiparous:

Accuracy: 98%

FP: 3%

FN: 0%



Cows:

Accuracy 93%

FP: 9%

FN: 3%

Pugliesi et al., 2014; Dalmaso de Melo et al., 2020.

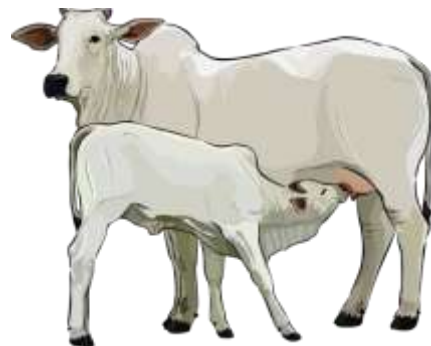


Heifers:

Accuracy: 90%

FP: 12%

FN: 6%



Cows:

Accuracy: 83-85 %

FP: 9-12%

FN: 16-25%



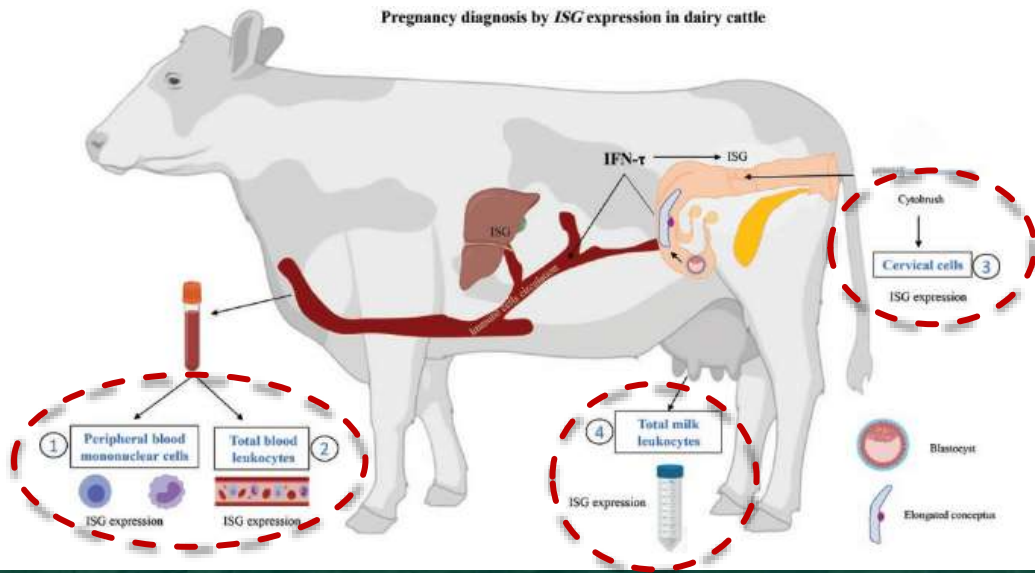
**JDS
Communications**
2021; 2:153-158

<https://doi.org/10.3168/jdsc.2020-0062>
Short Communication
Physiology

Feasibility and accuracy of using different methods to detect pregnancy by conceptus-stimulated genes in dairy cattle

Priscila Assis Ferraz,¹ Carlos Alberto Souto Godoy Filho,² Cecilia Constantino Rocha,² Adomar Laurindo Neto,¹ Gabriela de Andrade Bruni,¹ Thaís Sayuri Imura Oshiro,¹ Pietro Sampaio Baruselli,¹ Fabio Soares Lima,³ and Guilherme Pugliesi^{1*}

Pregnancy diagnosis by ISG expression in dairy cattle



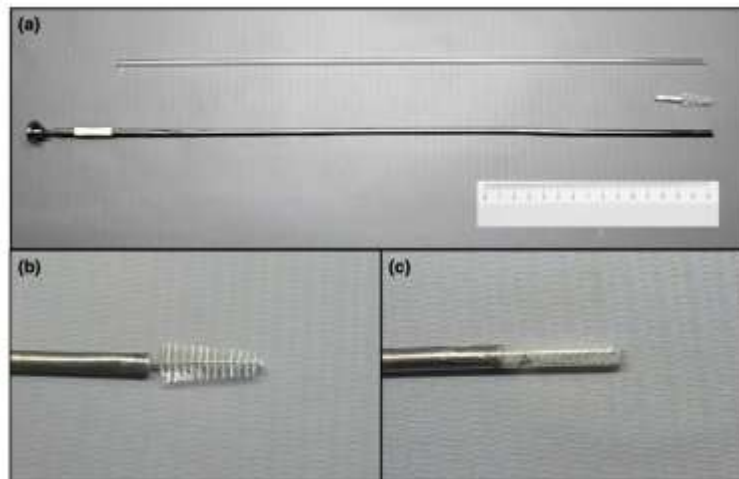
DOI: 10.1111/jvms.13007

SHORT COMMUNICATION

WILEY *Journal of Veterinary Medicine*

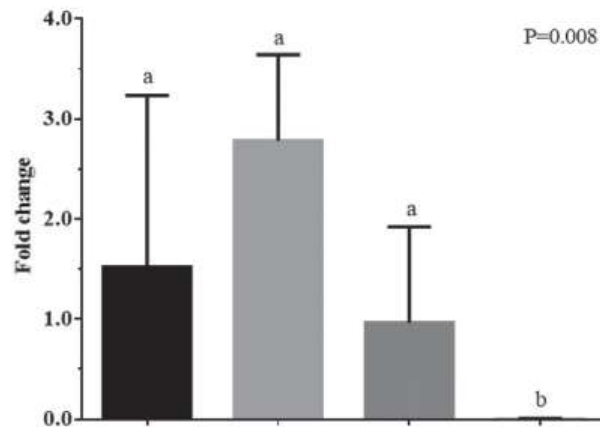
Cytobrush: A tool for sequential evaluation of gene expression in bovine endometrium

B Cardoso | ML Oliveira | G Pugliesi | EOS Batista | M Binelli

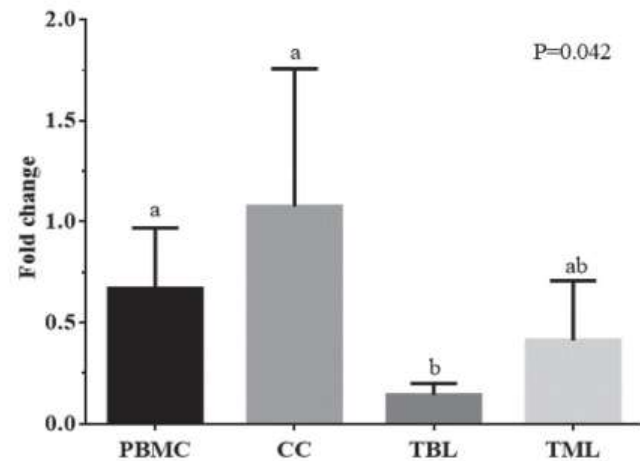


- Vacas (N=12) e novilhas (N=6) Holandesas lactantes;
- Amostras coletadas no **D20 pós-IATF**.

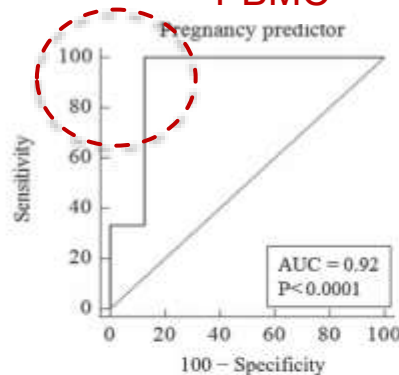
A

ISG15

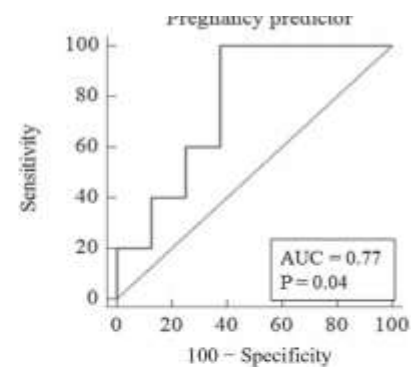
B

LGALS3BP

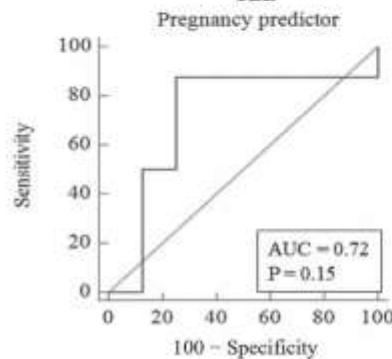
PBMC



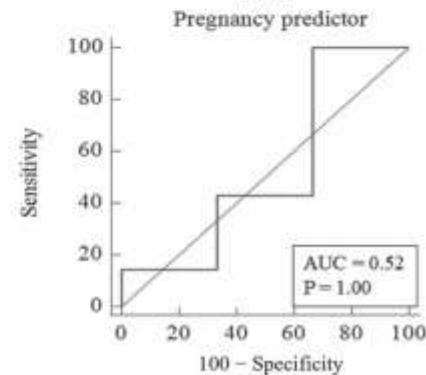
Células da Cérvix



TBL



TML



Leucócitos totais do leite

Leucócitos totais do sangue

Increased expression of interferon-stimulated gene 15 (*ISG15*) in cervical cells on day 14 of pregnancy in Holstein heifers

Graciana R. Mendina,^{1*} Victoria de Brun,² Maria de Lourdes Adrien,¹ Victoria Pons,¹ Rodrigo Vivian Paradizo,³ Jorge Gil,¹ Cecilia C. Rocha,⁴ Mario Binelli,⁴ and Ana Meikle²

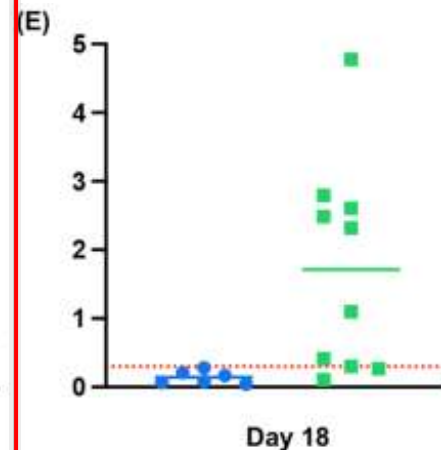
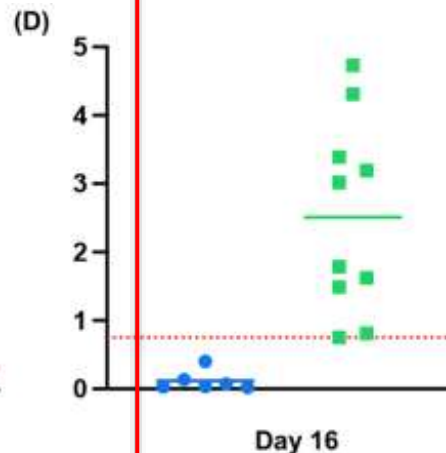
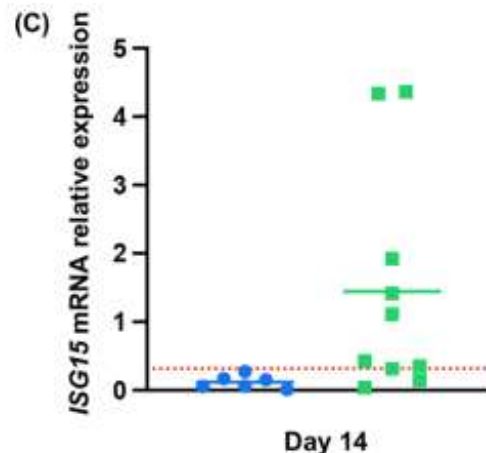
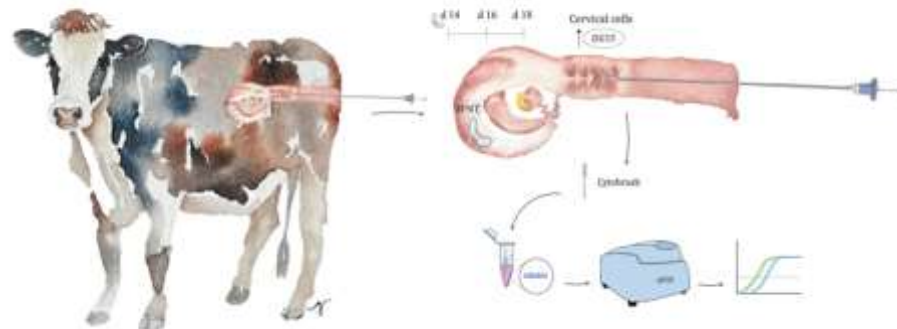


Table 1. Number of animals, true positive (TP), true negative (TN), false positive (FP), and false negative (FN) diagnoses, and sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, and area under the curve (AUC) for determining pregnancy status on d 14, 16, and 18 post-AI by *ISG15* and on d 18 by *PGR* mRNA relative expression

Item	<i>ISG15</i>			<i>PGR</i>
	d 14	d 16	d 18	d 18
No. of animals	16	16	16	16
TP	8	10	8	10
TN	6	6	6	3
FP	0	0	0	3
FN	2	0	2	0
Sensitivity ¹ (%)	80.0	100	80.0	100
Specificity ² (%)	100	100	100	50.0
PPV ³ (%)	100	100	100	76.9
NPV ⁴ (%)	75.0	100	75.0	100
Accuracy ⁵ (%)	87.5	100	87.5	81.3
AUC (%)	86.0	100	93.3	73.3
P-value	0.0027	<0.0001	0.0009	0.0417

Associação do uso de ISGs e PAGs para detector perda gestacional

Biology of Reproduction, 2024, 110(3), 558–568
<https://doi.org/10.1093/biolre/lead164>
Advance access publication date 11 December 2023
Research Article

OXFORD

Profiles of interferon-stimulated genes in multiple tissues and circulating pregnancy-associated glycoproteins and their association with pregnancy loss in dairy cows†

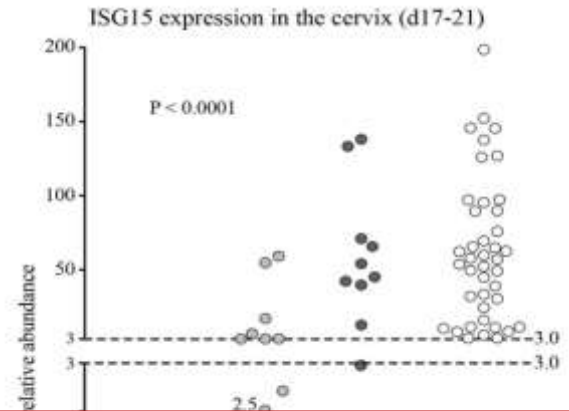
Rafael R. Domingues^{1,2}, Joao Paulo N. Andrade¹, Thiago O. Cunha¹, Guilherme Madureira¹, August S. Hoppman¹, Natalia N. Teixeira¹, Pedro L.J. Monteiro¹, Victor H. Gomez-Leon^{1,3}, Joao Paulo N. Martins⁴ and Milo C. Wiltbank^{1,2,*}

Pregnancy maintenance:
High ISG d17-21
Placenta attachment on d21
Luteal maintenance

ISG15 was determined in blood leukocyte on d19 and in the vagina and cervix on d17, d19, and d21

Embryonic attachment as determined by

↓ ISG ↑ ISG PAG Embrião
↓ PAG



Uso de ISGs em células sanguíneas ainda com considerável ocorrência de Falso negativo

Normal ISG15 on d17
Normal time of luteolysis d22
Lower ISG d21

Pregnancy loss d22-33 (24.6%):

High ISG d17-21
Delayed placenta attachment
Luteolysis or embryo death after d22

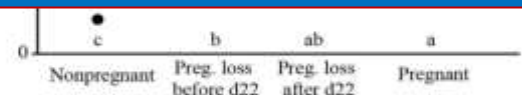


Figure 1. Mean expression of ISG15 in the cervix averaged for d17, d19, and d21 in cows that were not pregnant, pregnancy loss before d22, pregnancy loss after d22, and pregnant.

Biology of Reproduction, 2025, **112**(5), 981–995
<https://doi.org/10.1093/biolre/boaf066>
Advance access publication date 28 March 2025
Research Article

OXFORD

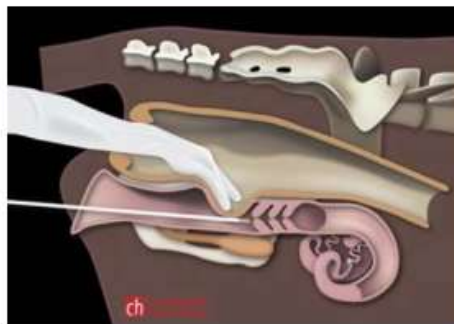
Early identification of bovine pregnancy status and embryonic mortality[†]

Jeanette V. Bishop^{1,†}, Aydin Guzeloglu^{1,†}, Tom Scheller², Joshua J. Docheff³,
Carolina L. Gonzalez-Berrios¹, Hana Van Campen¹, Terry M. Nett¹, Abigail L. Zezeski⁴,
Thomas W. Geary⁴, William W. Thatcher⁵ and Thomas R. Hansen^{1,*}

Equine Swab



Transrectal-Guided Swab

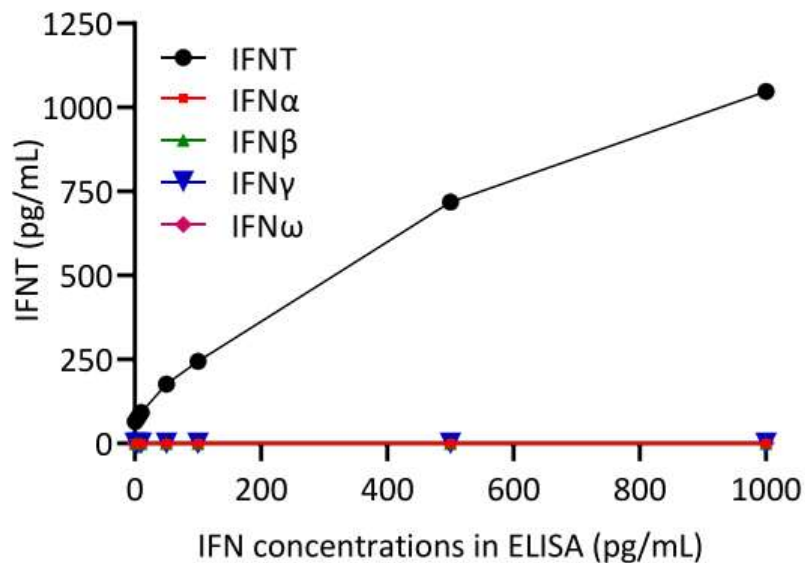


Bovine Swab

S I O A

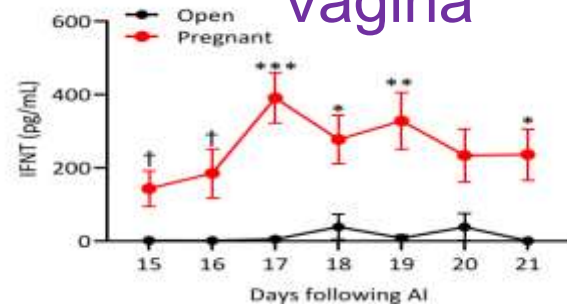


A)



C)

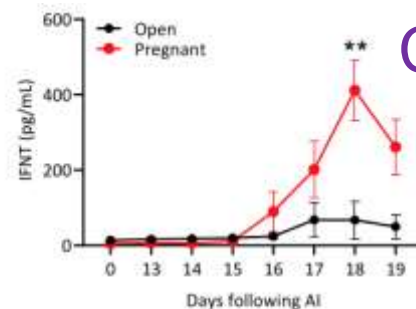
Vagina



	D15	D16	D17	D18	D19	D20	D21
Se	50	50	83	72	72	53	56
Sp	100	100	95	100	100	95	100
FP	0	0	5	0	0	5	0
FN	50	50	17	28	28	47	44

B)

Cervix



	D0	D13	D14	D15	D16	D17	D18	D19
Se	0	0	0	5	18	36	77	55
Sp	100	100	100	100	93	86	93	93
FP	0	0	0	0	7	14	7	7
FN	100	100	100	95	82	64	23	45

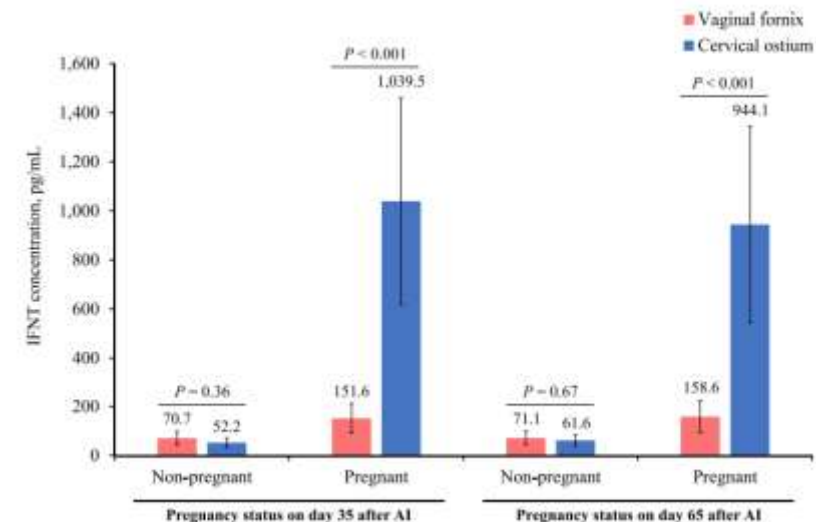
Table 2. Calculated parameters for open cow test (OCT) results based on pregnancy diagnosis by ultrasound in beef heifers and cows after insemination or embryo transfer in five different studies (USDA-ARS, MT, USA).

Endpoints	Definition	Heifers (ES, AI) (OCT Days 18–20) (<i>n</i> = 102) Value	Cows (ES, AI) (OCT Day 18) (<i>n</i> = 135) Value	Cows (ES, ET) (OCT Day 18) (<i>n</i> = 126) Value	Cows (BS, AI) (OCT Day 16) (<i>n</i> = 70) Value	Cows (BS, AI) (OCT Day 18) (<i>n</i> = 66) Value
TP (<i>n</i>)	P by OCT and US	23	75	33	28	23
TN (<i>n</i>)	NP by OCT and US	66	47	80	36	39
FP (<i>n</i>)	P by OCT and NP by US	3	5	11	5	4
FP (%)	(FP/FP + TN)	4.4	9.7	12.1	12	9.3
FN (<i>n</i>)	NP by OCT and P by US	10	8	2	1	0
FN (%)	(FN/FN + TP)	30.3	9.7	5.7	3.4	0
ACC (%)	(TP + TN/ <i>n</i>)	87.3	90.3	89.6	91.4	94
Se (%)	(TP/TP + FN)	69.6	90.3	94.3	96.6	100
Sp (%)	(TN/TN + FP)	95.6	90.3	87.9	87.8	90.7
PPV (%)	(TP/TP + FP)	88.4	93.7	75	84.8	85.2
NPV (%)	(TN/TN + FN)	86.8	85.4	97.5	97.3	100
EEM (%)	(FP/TP + FP + FN)	8	6	24.4	14.7	14.8

P, pregnant; NP, non-pregnant; TP, true positive; TN, true negative; FP, false positive; FN, false negative; ACC, accuracy; Sp, specificity; Se, sensitivity; PPV, positive predictive value; NPV, negative predictive value; EEM, estimated embryonic mortality; AI, artificial insemination; ES, equine swab device; BS, bovine swab device; US, ultrasound. The bold text and numbers represent the most important accuracy estimates for the open cow test.

Interferon-tau concentration at the vaginal fornix and cervical ostium predicted early pregnancy outcomes in lactating dairy cows[†]

Madison Lauryl Newman¹, Victória Alice Bastos Primo¹, Maria Belen Ugarte Marin¹, Phillip Martins Gondim Peixoto¹, Tomás Delfin Gonzalez², Alexandra Bennett³, Cecilia Constantino Rocha⁴, Mário Binelli⁵, Jeanette Victoria Bishop⁶, Aydin Guzeloglu⁸, Thomas Ross Hansen⁶, William Watters Thatcher⁵ and Rafael Sisoneto Bisinotto^{1,*}



M. L. Newman et al.

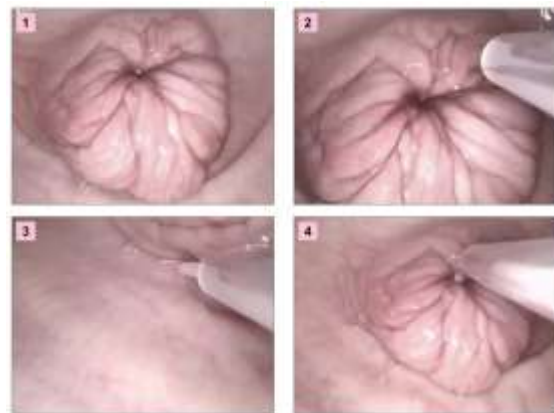
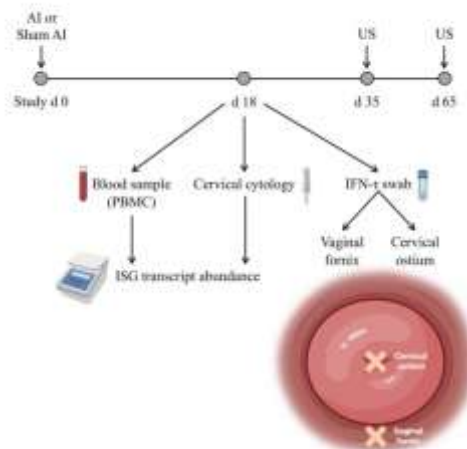


Figure 1. Diagram of study procedures. AI = artificial insemination; IFNT = interferon-tau; ISG = interferon-stimulated genes; PBMC = peripheral blood mononuclear cells; US = transrectal ultrasonography for pregnancy diagnosis.

Table 1. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of early pregnancy diagnosis based on transrectal ultrasonography (US) on days 35 and 65 after AI (n = 238).

Parameter ²	US day 35 after AI		US day 65 after AI	
	Value	95% CI ¹	Value	95% CI ¹
Sensitivity	0.84	0.77 to 0.91	0.84	0.76 to 0.91
Specificity	0.84	0.78 to 0.90	0.82	0.76 to 0.88
PPV	0.81	0.73 to 0.88	0.77	0.69 to 0.85
NPV	0.87	0.81 to 0.93	0.88	0.82 to 0.93
Accuracy	0.84	0.79 to 0.89	0.83	0.78 to 0.88

¹95% confidence interval. ²Sensitivity ($\frac{TP}{TP+FN}$), specificity ($\frac{TN}{TN+FP}$), negative predictive value (NPV; $\frac{FN}{FN+TN}$), positive predictive value (PPV; $\frac{TP}{TP+FP}$), accuracy ($\frac{TP+TN}{TP+FP+FN+TN}$).

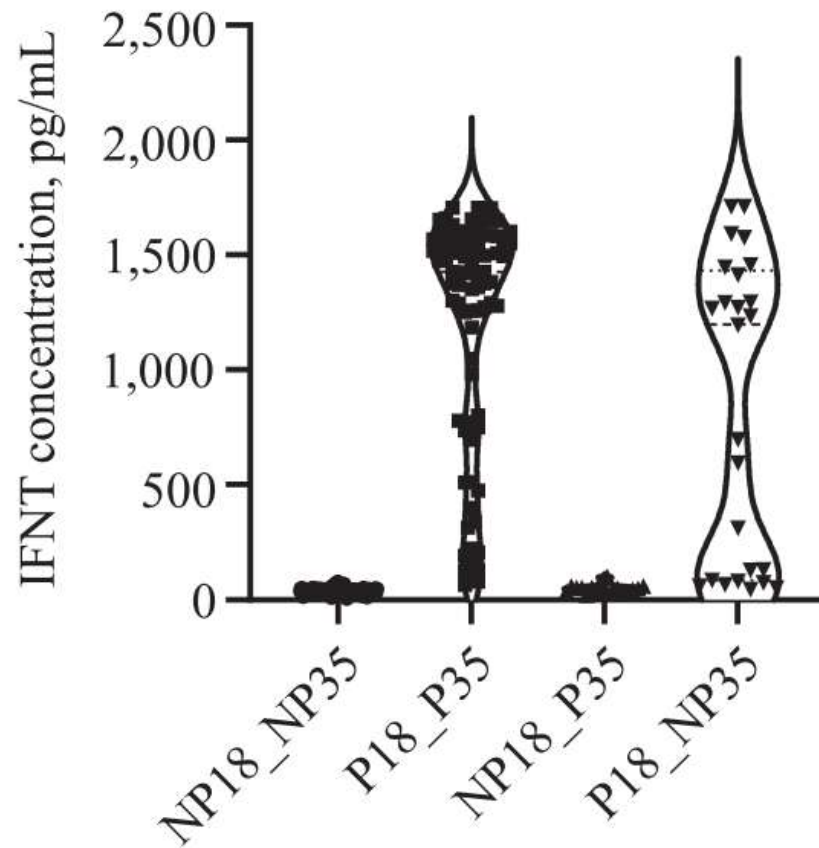


Figure 4. Violin plot depicting interferon-tau (IFNT) concentration in cervical swabs according to pregnancy category (n = 238):



Falso-Positivo

- Mortalidade embrionária precoce;
- Indução de ISGs por outros estímulos (infecções virais).

Falso-Negativo

- IFN- τ não sinalizando o suficiente para estimular a expressão de ISGs
- Ineficácia da técnica de amostragem

Perspectivas futuras

FIELD PHASE: SYNCHRONIZATION & SAMPLE COLLECTION



Day -18: Synchronization Start
(CIDR + Oestradiol Benzestete)
Day -2: Desiwe Removal
(Oloproatesni + Oestradiol
Cypionate + eCB)



Step 1: Hormonal Synchronization (D-10 to D-2)

Females received progesterone (CIDR) and oestradiol treatments to synchronize ovulation before insemination.



Step 2: Fixed-Time Artificial Insemination (D0)

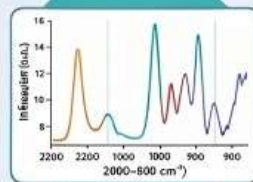
Eighty Nellore females were inseminated with frozen-thawed semen from a single bull.



Step 3: Diagnosis & Serum Collection (D30)

Pregnancy was confirmed via ultrasound, and blood was collected for lab-based spectral analysis.

LABORATORY PHASE: MOLECULAR ANALYSIS & CLASSIFICATION



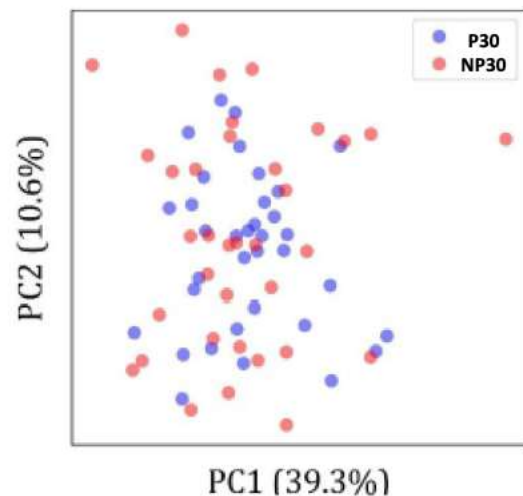
FTIR Molecular Fingerprinting

Serum was analyzed via infrared spectroscopy (2000–900 cm⁻¹) to identify protein and lipid changes.



Machine Learning Classification

Spectral data was processed using Support Vector Machines (SVM) with cubic kernel functions.



LOOCV (72.2%)
SVM Cubic (5 PCs)

ACTUAL CLASS	P30	NP30
P30	66.7% 12	33.3% 6
NP30	22.2% 4	77.8% 4
PREDICTED CLASS		

External Validation (62.5%)
SVM Linear (5 PCs)

ACTUAL CLASS	P30	NP30
P30	37.5% 3	62.5% 5
NP30	12.5% 1	87.5% 7
PREDICTED CLASS		

Perspectivas futuras

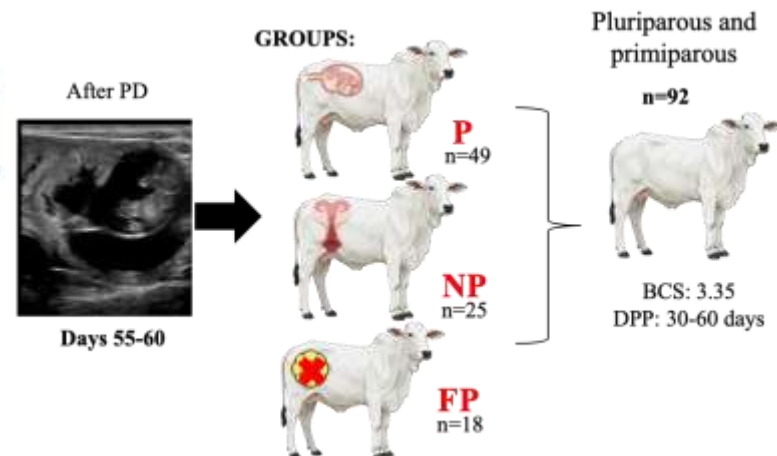
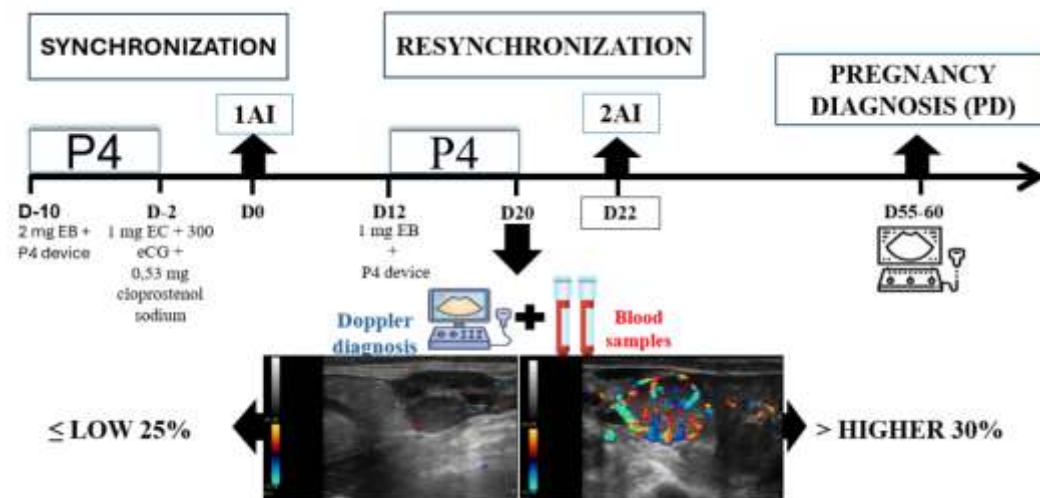
■ Metabolômica no soro



Investigation of the metabolomic profile of Nelore cows for anticipation of pregnancy diagnosis

Ana Laura dos Santos Munhoz Gôngora¹, Samuel Souza Volpe², Vinicius Laerte Silva Herreira¹, Eduardo Solano Pina Santos¹, Lauro César Ferreira Beltrão¹, Maria Angélica dos Santos¹, Fernanda Maria Marins Ocampos³, Luiz Alberto Conalço³, Amanda Guimarães Silva², Isabella Rio Feltrin², Mario Binelli⁴, Guilherme Pugliesi², Nara Regina Brandão

Cônsolo¹



Perspectivas futuras

■ Metabolômica no soro

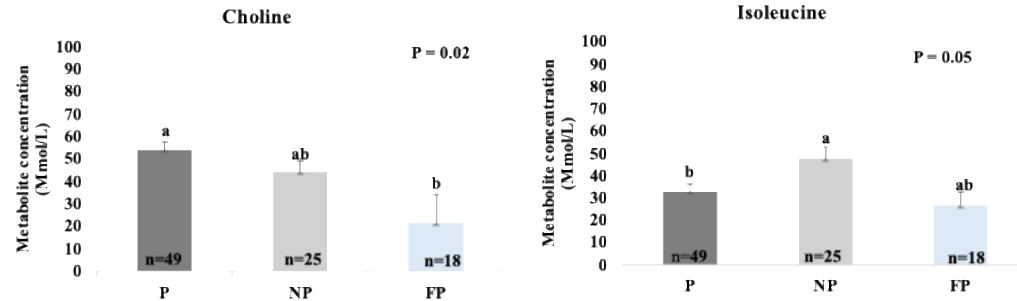
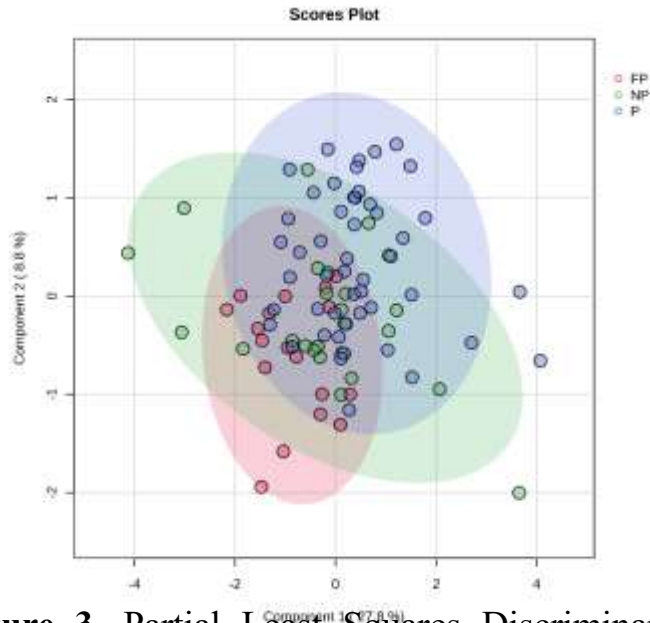


Figure 3. Partial Least Squares Discriminant Analysis (PLS-DA) of serum metabolites treatments. FP: false-positive; P: pregnant; NP: non-pregnant.



■ Metabolômica no soro

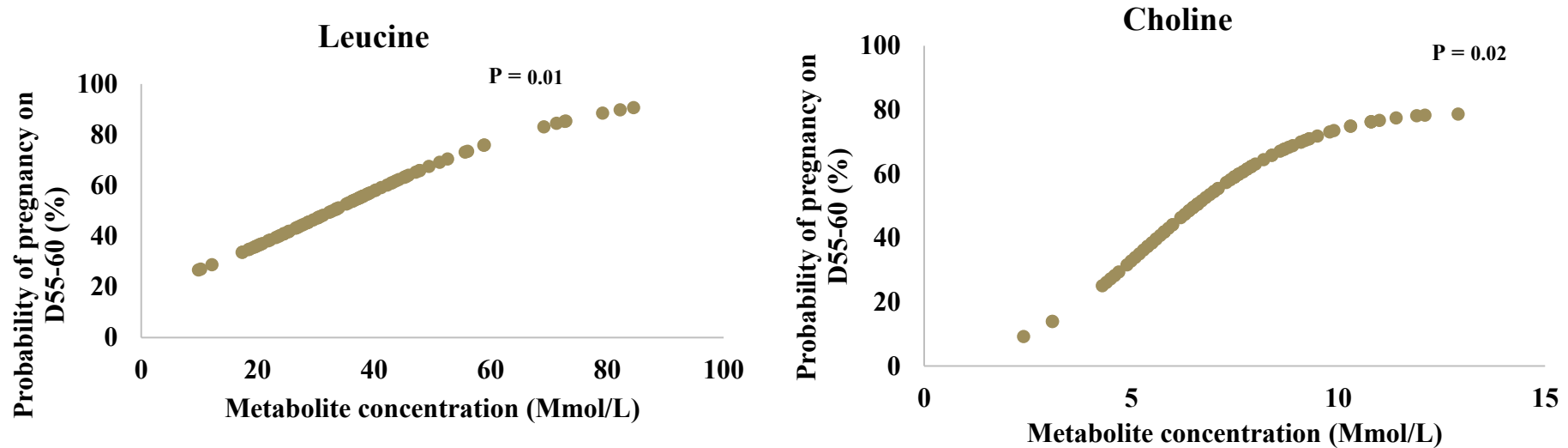
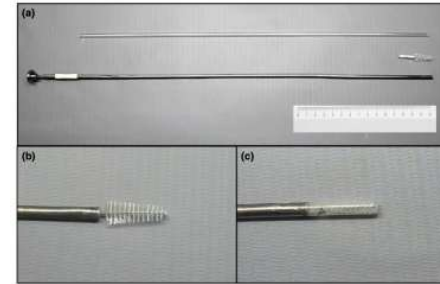
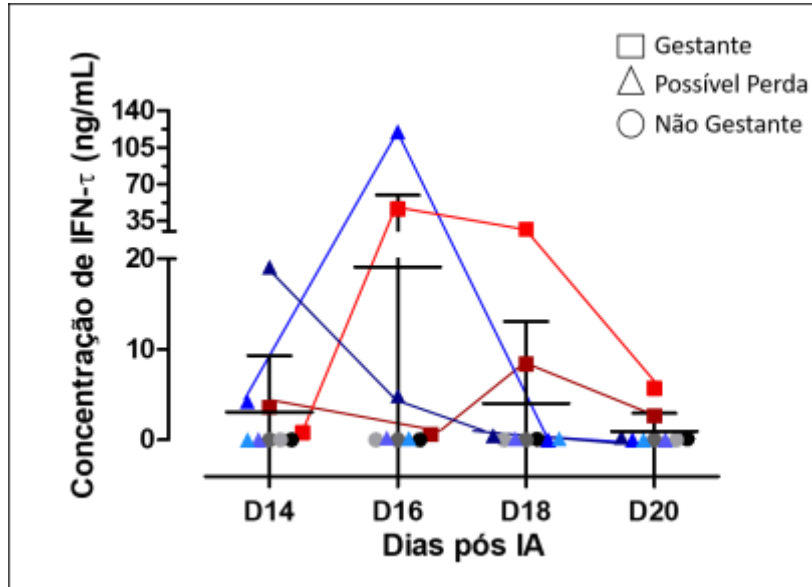


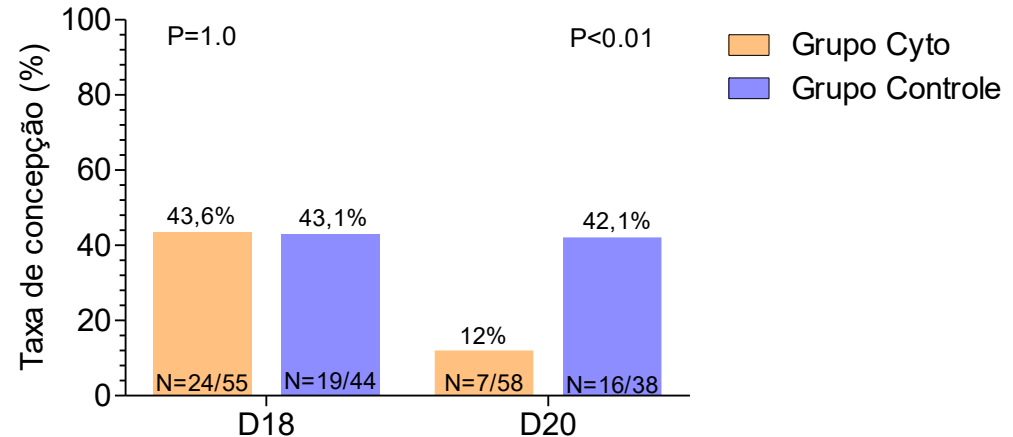
Figure 9. Probability of being pregnant at confirmatory diagnosis (D55–60) based on the concentration of significant metabolites.

Perspectivas futuras

■ Mensuração de IFN-tau no útero



Fêmeas Inseminadas DG 60



Dispositivos de Respiração ("Bafômetro para Vacas")



Home > Artigos > "Bafômetro para vacas" com tecnologia da NASA detecta prenhez em segundos

"Bafômetro para vacas" com tecnologia da NASA detecta prenhez em segundos

Escrito por Compre Rural Notícias

19 de setembro de 2025 - 15h01

Atualizado em 19 de setembro de 2025 - 15h01

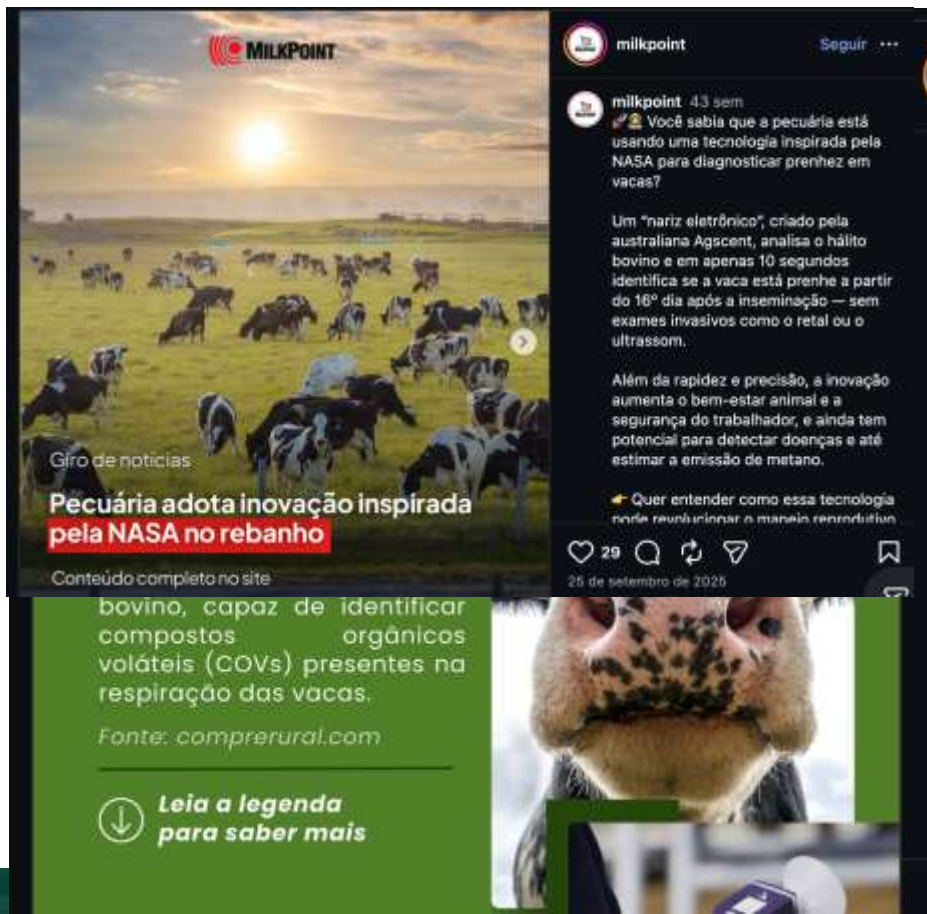


Pecuária adota inovação inspirada pela NASA no rebanho

O "nariz eletrônico" da Agscent realiza diagnósticos rápidos e seguros via hálito bovino, abrindo espaço para maior eficiência e sustentabilidade no agro.

Publicado por: MilkPoint

Publicado em: 25/09/2025 - 2 minutos de leitura



Dispositivos de Respiração ("Bafômetro para Vacas")

- "Breathomics" → Análise do Metaboloma do Hálito
- Mudanças metabólicas alteram a assinatura química de **Compostos Orgânicos Voláteis (VOCs)** na circulação sanguínea.
- Troca gasosa entre pulmão e sangue periférico: VOCs alterados são excretados continuamente por meio da respiração do animal.
- Detecção por nano sensores (NASA)



Dispositivos de Respiração ("Bafômetro para Vacas")

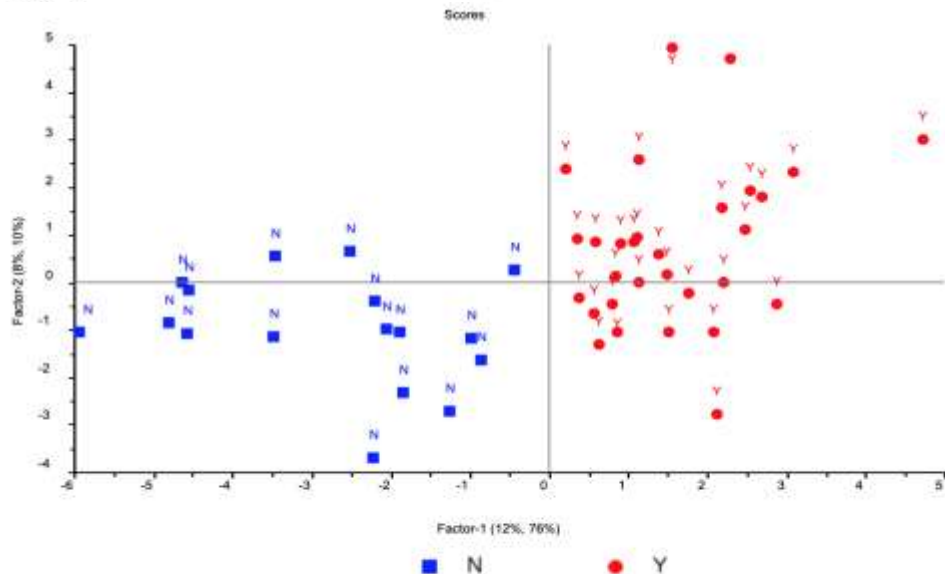


Final report

Agscent Cow Breath Sampling: pregnancy diagnosis proof of concept

Project code: B.AWW.0007
Prepared by: Dr Bronwyn Darlington
Agscent Pty Ltd

Date published: 4 May 2021



Validated model differentiating pregnant/empty cows at day 40 (100%)

Dispositivos de Respiração ("Bafômetro para Vacas")



Research Note

Automatic detection of oestrus cows via breath sampling with an electronic nose: A pilot study

Francis E.P. Sanderink^a, Jan Willem Gerritsen^b,
Peter W.G. Groot Koerkamp^{a,c}, Simon van Mourik^{a,*}

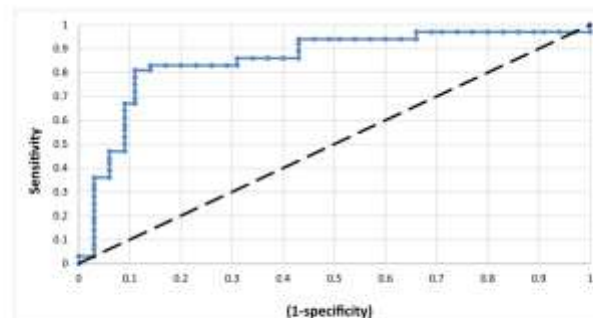
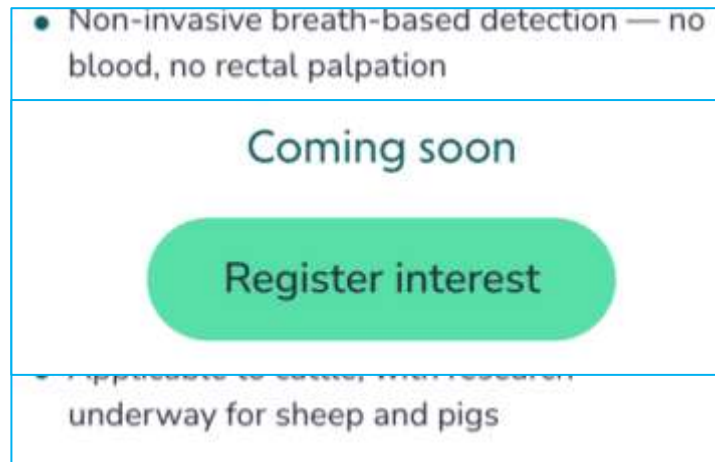


Fig. 3 – ROC curve relating sensitivity (true positives) and 1-specificity (1- true negative) under varying threshold values. The dashed diagonal line represents no predictive value. The AUC is 0.86.

Dispositivos de Respiração ("Bafômetro para Vacas")

Promessa:

- US\$ 10 mil
- A partir de 18 dias pós-IA
- 30 segundos



• Non-invasive breath-based detection — no blood, no rectal palpation

Coming soon

Register interest

• Appropriate to collect from pregnant
underway for sheep and pigs

Realidade:

- Sem comprovação científica
- Só relatório técnico de prova de conceito publicado

Limitantes:

- Validar em diferentes condições
- Disponibilidade do equipamento

Tópicos

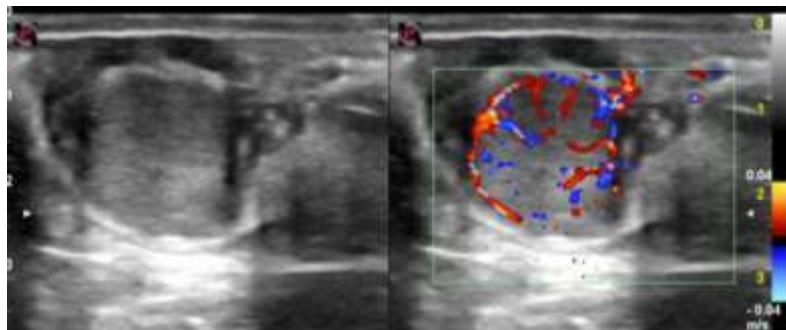
**Métodos de
Diagnóstico de
Gestação
Convencionais**

**Ferramentas para
Antecipar a
Detecção do
Status Gestacional**

**Marcadores
Gestacionais e
Potenciais
Métodos**

**Considerações
Finais**

A campo (em tempo real):
US Modo B e Doppler



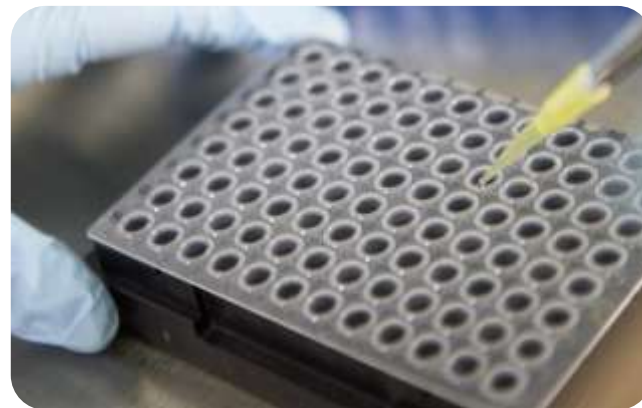
Laboratório (moleculares):

PAGs

Genes estimulados por IFN-tau (ISGs)

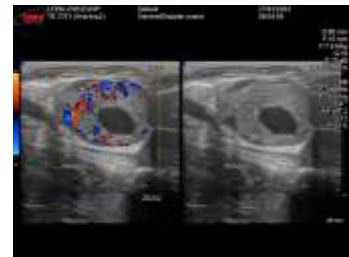
IFN-tau

Novas moléculas ou Assinatura (fingerprint)



Considerações Finais:

- Ferramentas a campo
- Ultrassonografia Doppler (realidade a campo)
 - Antecipa detecção da prenhez em programas em Tempo Fixo
- Ferramenta que permite com alta acurácia identificar as fêmeas não-gestantes com 20-22 pós-inseminação
- Limitações
 - Veterinários especializados
 - Equipamentos regulados
 - Interpretação → Inteligência Artificial



Machine learning e Doppler

Journal of Animal Science, 2025, 163, skaf168
<https://doi.org/10.1093/jas/skaf168>
Advance access publication 13 May 2025
Technology in Animal Science



Computer vision analysis of luteal color Doppler ultrasonography for early and automated pregnancy diagnosis in *Bos taurus* beef cows

Lucas Melo Gonçalves,¹ Pedro Levy Piza Fontes,^{2, A} and Anderson Antonio Carvalho Alves^{1, A, B}

¹Department of Animal and Dairy Science, University of Georgia, Athens, GA, 30602, USA

²Institute for Integrative Precision Agriculture – University of Georgia, Athens, GA 30602, USA

^ACorresponding author: alvesand@uga.edu

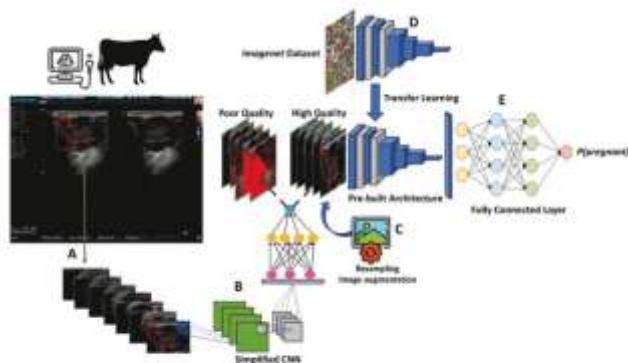


Figure 1. Schematic representation of data acquisition, preprocessing, and analysis steps for classifying pregnancy status in beef cattle using color Doppler ultrasonography. Angus cows were scanned with ultrasound on days 20 and 22 after fixed-time artificial insemination. Frames were extracted from recorded videos using bounding boxes created by the ultrasound software (B). The extracted images were then filtered using a simplified conventional neural network (CNN) (A). During training, segmentation and image enhancement were applied (C). Various pre-built architectures, initialized with ImageNet transferred weights (E), were connected to fully connected layers for classification (F).

Tropical Animal Health and Production (2026) 58:162

<https://doi.org/10.1007/s11250-026-04964-4>

REGULAR ARTICLES

Prediction of gestational status nineteen days after artificial insemination using color ultrasonography and machine learning strategies in beef heifers

Pablo Sebastián Reineri^{1,2} · Sergio Daniel Roldán Bernhard¹ · Santiago Alberto Principi³ · Juan Florencio Aller⁴

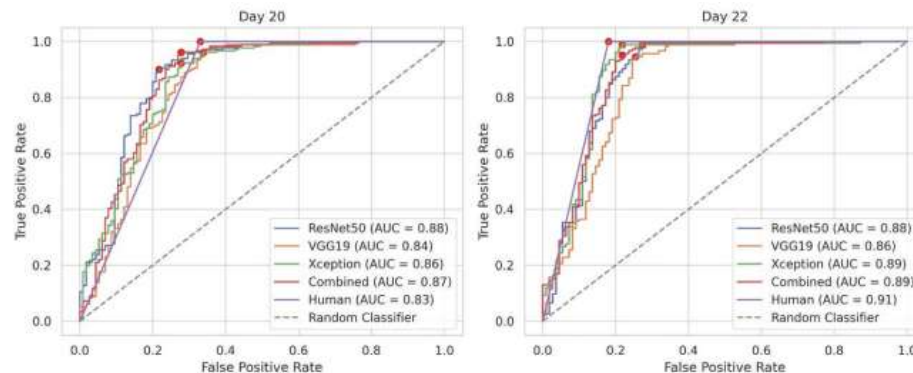


Figure 3. Receiver Operating Characteristic (ROC) curves and area under the curve (AUC) for pregnancy classification in beef cows, comparing human evaluation with different convolutional neural network (CNN) architectures. The pregnancy assessments were based on Doppler ultrasound data collected 20 and 22 d after fixed-time artificial insemination. Circles on the ROC curves indicate the optimal balance between sensitivity and specificity, determined using Youden's Index.

Ainda sem acurácia superior ao veterinário treinado

Machine learning e Doppler



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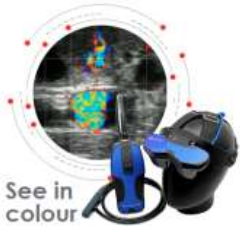
Perspectivas Futuras



Mais pesquisas usando IFN- τ , PAGs e ISGs como biomarcadores de prenhez;



Utilização de metodologias mais rápidas e sensíveis (PCR digital);



Associação de técnicas moleculares com Ultrassonográficas.

Cuidado com as notícias...

MÍDIAS SOCIAIS E SITE



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13

O QUE AS REDES SOCIAIS VENDEM:

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- SENSÇÃO DE FACILIDADE (Custo Baixo)
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Agradecimentos

Pesquisadores, empresas e fazendas parceiras





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Comenta o que achou?



Salve para consultar depois



Compartilhe nos stories e ajude essa informação chegar mais longe



Deixa um like pra gente hehe



Contato: gpugliesi@usp.br

