

PERFIL ESTEROIDOGÊNICO DA MEMBRANA CORIOALANTÓIDE DE ÉGUAS GESTANTES DE POTROS MUARES

Thaís Naomi Gonçalves Nesiya

Prof. Dr. Antônio Chaves de Assis Neto

Profa. Dra. Claudia Barbosa Fernandes

FMVZ- Universidade de São Paulo

thais_ngn@hotmail.com

Objetivos

Analisar e comparar o perfil esteroideogênico das membranas corioalantóides a termo, de éguas gestantes de produtos muares x produtos equinos, por meio da imunolocalização das enzimas da cascata esteroideogênica.

Métodos e Procedimentos

Foram utilizadas as membranas corioalantóides a termo de 8 gestações de matrizes da raça Mangalarga, sendo que 4 gestações de produtos muares e 4 de produtos equinos. Os partos foram acompanhados e imediatamente após o delivramento, as membranas fetais foram recolhidas e avaliadas macroscopicamente. Foram coletados fragmentos de 1 cm² da membrana corioalantóide, nas regiões do corpo uterino (CP), corno gravídico (CG) e corno não gravídico (CnG) e armazenados em paraformoldeído tamponado 4% a 5°C. Posteriormente, os tecidos passaram por emblocagem em parafina. Foram realizados cortes histológicos e as lâminas sinalizadas preparadas e armazenadas a 5°C para imunohistoquímica, onde seriam analisadas as enzimas: 17 α -hidroxilase, 17,20-liase/17hidroxilase/ citocromo P450 (P450c17), 17 β -hidroxiesteroide desidrogenase (17 β -HSD), 3 β -hidroxisteróide desidrogenase/ Δ 5-4 isomerase (3 β HSD), 21 β -hidroxilasa, Citocromo P450 aromatase (P450arom) e 5 α - redutase.

Resultados parciais

Foi possível acompanhar os partos, realizar as coletas, avaliações macroscópicas, pesagens dos anexos fetais (**Tabela 1**). Foi realizada também a confecções dos blocos e das lâminas conforme podemos observar na **Tabela 2**.

Identificação da égua	Raça	Número de partos	Idade (anos)	Data do parto	Potro	Sexo do produto	Peso da membrana corioalantóide (kg)	Peso do amnion e cordão umbilical (kg)	Peso total dos anexos placentários (kg)
Fronteira	M.L.	5	13	10/09/2019	Equino	Macho	2,400	2,900	5,300
Isadora	M.L.	4	11	07/12/2019	Equino	Fêmea	3,600	0,900	4,500
Madrid	M.L.	2	7	03/11/2019	Equino	Macho	3,200	1,100	4,300
Mariane	M.L.	3	8	26/12/2019	Equino	Macho	3,500	2,500	6,000
Esfinge	M.L.	5	12	17/12/2019	Muar	Fêmea	2,300	1,400	3,700
Floresta	M.L.	3	10	21/08/2019	Muar	Fêmea	3,200	1,400	3,600
Grécia	M.L.	2	12	23/12/2019	Muar	Fêmea	2,400	1,300	3,700
Mariana	M.L.	2	7	12/12/2019	Muar	Fêmea	2,900	1,700	4,600

Tabela 1 – Identificação materna, data do parto, grupo experimental do produto, sexo do potro e peso dos anexos fetais obtidos de gestações a termo de éguas gestantes de produtos equinos e de produtos híbridos muares.

IDENTIFICAÇÃO DAS LÂMINAS- REGIÃO PLACENTÁRIA			
Corpo do útero (CP)	Corno gravídico (CG)	Corno não gravídico (CnG)	Número total de lâminas
Fronteira - CP (10/09/2019)	Fronteira - CG (10/09/2019)	Fronteira - CnG (10/09/2019)	6
2x (A/B)	2x (A/B)	2x (A/B)	
Isadora - CP (07/12/2019)	Isadora - CG (07/12/2019)	Isadora - CnG (07/12/2019)	6
2x (A/B)	2x (A/B)	2x (A/B)	
Madrid - CP (03/11/2019)	Madrid - CG (03/11/2019)	Madrid - CnG (03/11/2019)	6
2x (A/B)	2x (A/B)	2x (A/B)	
Mariane - CP (26/12/2019)	Mariane - CG (26/12/2019)	Mariane - CnG (26/12/2019)	6
2x (A/B)	2x (A/B)	2x (A/B)	
Esfinge - CP (17/12/2019)	Esfinge - CG (17/12/2019)	Esfinge - CnG (17/12/2019)	6
2x (A/B)	2x (A/B)	2x (A/B)	
Floresta - CP (21/08/2019)	Floresta - CG (21/08/2019)	Floresta - CnG (21/08/2019)	6
2x (A/B)	2x (A/B)	2x (A/B)	
Grécia - CP (23/12/2019)	Grécia - CG (23/12/2019)	Grécia - CnG (23/12/2019)	6
2x (A/B)	2x (A/B)	2x (A/B)	
Mariana - CP (12/12/2019)	Mariana - CG (12/12/2019)	Mariana - CnG (12/12/2019)	6
2x (A/B)	2x (A/B)	2x (A/B)	

Tabela 2- Identificação das lâminas- Região placentária: Nome da égua e nomenclatura da membrana corioalantóide. Cada égua possui lâminas em duplicata (A/B), das 3 diferentes regiões placentárias da membrana corioalantóide (corpo do

útero – CP, corno gravídico – CG, e corno não gravídico - CNG). Somadas, cada égua tem um total de 6 lâminas.

Contudo, devido a pandemia de COVID-19, as etapas da imunohistoquímica, com o objetivo de identificar as enzimas da cascata esteroidogênica, não puderam ser concluídas.

Conclusões

Desta forma, com o final do prazo do programa de IC, infelizmente postergamos as análises imunohistoquímicas para um momento mais adequado, quando possamos acessar os laboratórios colaboradores.

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STEROIDOGENIC PROFILE OF THE CHORIOALLANTOIC MEMBRANE OF MARES PREGNANT WITH MULES' FOALS

Thaís Naomi Gonçalves Nesiya

Prof. Dr. Antônio Chaves de Assis Neto

Prof. Dra. Claudia Barbosa Fernandes

FMVZ- University of São Paulo

thais_ngn@hotmail.com

Objectives

Analyze and compare the steroidogenic profile of term chorioallantoic membranes of pregnant mares of mule x equine products, through the immunolocalization of enzymes of the steroidogenic cascade.

Materials and Methods

Were used chorioallantoic membranes at term from 8 pregnancies from Mangalarga breeders, 4 pregnancies from mule products and 4 from equine products. The deliveries were monitored and immediately after deliverance, fetal membranes were collected and evaluated macroscopically. Fragments of 1 cm² of the chorioallantoic membrane were collected in the regions of the uterine body (CP), pregnant horn (CG) and non-pregnant horn (CnG) and stored in 4% buffered paraformaldehyde at 5°C. Subsequently, the tissues underwent paraffin embedding. Histological sections were performed and the labeled slides prepared and stored at 5°C for immunohistochemistry, to the following enzymes would be analyzed: 17 α -hydroxylase, 17,20-lyase/ 17hydroxylyase/ cytochrome P450 (P450c17), 17 β -hydroxysteroid dehydrogenase (17 β -HSD), 3 β -hydroxysteroid dehydrogenase/ Δ 5-4 isomerase (3 β HSD), 21 β -hydroxylase, Cytochrome P450 aromatase (P450arom) and 5 α -reductase

Parcial Results

It was possible to monitor the births, perform macroscopic evaluation, weighting, and collecting of placentas (Table 1). Furthermore, the making of blocks and slides as shown in (Table 2).

Mare identification	Breed	Number of deliveries	Age	Birth date	Foal species	Foal sex	Weight of chorioallantoic membrane (kg)	Weight of amnion and umbilical cord (kg)	Total weight of placental (kg)
Fronteira	ML	5	13	10/09/2019	Equine	Male	2,400	2,900	5,300
Isadora	ML	4	11	07/12/2019	Equine	Female	3,600	0,900	4,500
Madrid	ML	2	7	03/11/2019	Equine	Male	3,200	1,100	4,300
Mariane	ML	3	8	26/12/2019	Equine	Male	3,500	2,500	6,000
Esfinge	ML	5	12	17/12/2019	Mule	Female	2,300	1,400	3,700
Floresta	ML	3	10	21/08/2019	Mule	Female	3,200	1,400	3,600
Grécia	ML	2	12	23/12/2019	Mule	Female	2,400	1,300	3,700
Mariana	ML	2	7	12/12/2019	Mule	Female	2,900	1,700	4,600

Table 1 - Maternal identification, birth date, experimental product group, foal sex and fetal placental weight obtained from pregnant mares of equine products and mule hybrid products.

IDENTIFICATION OF SLIDES- PLACENTAL REGION			
Uterine body (CP)	Pregnant horn (CG)	Non-pregnant horn (CnG)	Total number of slides
Fronteira - CP (10/09/2019) 2x (A/B)	Fronteira - CG (10/09/2019) 2x (A/B)	Fronteira - CnG (10/09/2019) 2x (A/B)	6
Isadora - CP (07/12/2019) 2x (A/B)	Isadora - CG (07/12/2019) 2x (A/B)	Isadora - CnG (07/12/2019) 2x (A/B)	6
Madrid - CP (03/11/2019) 2x (A/B)	Madrid - CG (03/11/2019) 2x (A/B)	Madrid - CnG (03/11/2019) 2x (A/B)	6
Mariane - CP (26/12/2019) 2x (A/B)	Mariane - CG (26/12/2019) 2x (A/B)	Mariane - CnG (26/12/2019) 2x (A/B)	6
Esfinge - CP (17/12/2019) 2x (A/B)	Esfinge - CG (17/12/2019) 2x (A/B)	Esfinge - CnG (17/12/2019) 2x (A/B)	6
Floresta - CP (21/08/2019) 2x (A/B)	Floresta - CG (21/08/2019) 2x (A/B)	Floresta - CnG (21/08/2019) 2x (A/B)	6
Grécia - CP (23/12/2019) 2x (A/B)	Grécia - CG (23/12/2019) 2x (A/B)	Grécia - CnG (23/12/2019) 2x (A/B)	6
Mariana - CP (12/12/2019) 2x (A/B)	Mariana - CG (12/12/2019) 2x (A/B)	Mariana - CnG (12/12/2019) 2x (A/B)	6

Table 2- Identification of slides- Placental region: Name of the mare and nomenclature of the chorioallantoic membrane. Each mare has duplicate slides (A/B) from the 3 different placental regions of the chorioallantoic membrane (uterine body – CP,

pregnant horn – CG, and non-pregnant horn – CNG). Added together, each mare has a total of 6 slides.

However, due to the COVID-19 pandemic, the steps of immunohistochemistry to identify the enzymes of the steroidogenic cascade could not be completed.

Conclusions

In short, with the deadline for the CI program, we unfortunately postponed all immunohistochemical analyzes to a more appropriate time, when we can access the collaborators' laboratories.

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