UNIVERSIDADE ESTADUAL PAULISTA – UNESP

CÂMPUS DE JABOTICABAL

EFEITOS LUTEOTRÓFICOS DA GONADOTROFINA CORIÔNICA HUMANA ADMINISTRADA 7,5 DIAS APÓS A INDUÇÃO DE ESTRO EM OVELHAS MORADA NOVA

Gabriel Brun Vergani

Médico Veterinário

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CERTIFICADO DE APROVAÇÃO

TÍTULO DA DISSERTAÇÃO: EFEITOS LUTEOTRÓFICOS DA GONADOTROFINA CORIÔNICA HUMANA ADMINISTRADA 7,5 DIAS APÓS A INDUÇÃO DE ESTRO EM OVELHAS MORADA NOVA

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Jaboticabal, 07 de julho de 2020

DADOS CURRICULARES DO AUTOR

Gabriel Brun Vergani, nascido na cidade de Toledo, Paraná - Brasil, em 22 de novembro de 1994. Graduou-se em Medicina Veterinária pela Universidade Federal do Paraná, Setor Palotina em 2018. Participou do Programa de Iniciação à Docência – Monitoria, nas disciplinas de Anatomia Veterinária I, Bovinocultura de Corte e Bovinocultura de Leite. Desenvolveu projeto de pesquisa no Programa de Iniciação Científica. Participou de projetos de extensão e estágios voluntários durante a graduação. Realizou estágio curricular na área de Reprodução Animal na Empresa Brasileira de Pesquisa Agropecuária, sob orientação do pesquisador Dr. Jeferson Ferreira da Fonseca. Em 2018, ingressou no programa de Pós-Graduação em Medicina Veterinária, área de Reprodução Animal, pela UNESP – Câmpus de Jaboticabal, como bolsista CNPq, sob orientação da Profª. Drª. Maria Emilia Franco Oliveira e coorientação do Prof. Dr. Jeferson Ferreira da Fonseca.

“O dinheiro faz homens ricos, o
conhecimento faz homens sábios e a
humildade faz grandes homens”
Mahatma Gandhi

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Aos meus pais, Rafael B. Vergani e Vania M. B. Vergani, ao meu irmão Felipe B. Vergani. Por tudo que fizeram e tem feito para que eu possa alcançar meus sonhos. E a todos os familiares pelo incentivo e apoio.

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Embrapa

Pecuária Sudeste

CERTIFICADO

PRT Nº 09/2017

Certificamos que o projeto de pesquisa intitulado: **Estudo de pontos estratégicos para a elevação da eficiência da superovulação, colheita, criopreservação e transferência de embriões em ovinos**, registrado com o nº 09/2017 sob a responsabilidade do pesquisador científico Alexandre Rosseto Garcia, que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica, encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS DA EMBRAPA PECUÁRIA SUDESTE.

São Carlos, 22 de novembro de 2017.

Dra. Márcia Cristina de Sena Oliveira

Presidente da Comissão de Ética no Uso de Animais

Embrapa Pecuária Sudeste

Reunião de ___/___/____

Finalidade	Pesquisa Científica
Vigência da autorização	Agosto de 2017 a março de 2020
Espécie/linhagem/raça	Ovinos de qualquer raça
Nº de animais	150
Peso/Idade	1 a 8 anos, 45 kg
Sexo	Machos e fêmeas
Origem	CPPSE

Embrapa
Pecuária Sudeste

CEUA PRT Nº 09/2017

Nome do projeto: Estudo de pontos estratégicos para a elevação da eficiência da superovulação, colheita, criopreservação e transferência de embriões em ovinos.

PARECER DO RELATOR 1

APROVADO (X) APROVADO COM RESTRIÇÕES ( ) REPROVADO ( )

Justificativa: o projeto tem por objetivo caracterizar pontos estratégicos para a elevação da eficiência da superovulação, colheita, criopreservação e transferência de embriões em ovinos. Para isso, serão utilizados 150 animais alocados a pasto ou em galpão coberto com alimentação adequada e água a vontade, seguindo procedimentos de rotina que visam as boas práticas animais adotadas no CPPSE. Esporadicamente serão realizados procedimentos com grau de invasividade G2: todos os fármacos aplicados são medicamentos de uso veterinário e aprovados pelo MAPA. A imobilização será necessária para os procedimentos de rotina na área de reprodução de ovinos (Inseminação artificial, colheita de sangue, embrião e sêmen) e conforme protocolos validados. Ao término do experimento, os animais retornarão ao rebanho, pois não haverá efeito deletério na vida reprodutiva ou produtiva dos animais.

Nome: Ana Carolina de Souza Chagas

Assinatura: Ana Carolina Chagas

Data: 10/11/2017

PARECER DO RELATOR 2

APROVADO (X) APROVADO COM RESTRIÇÕES ( ) REPROVADO ( )

Justificativa: Serão usados 150 animais criados de acordo com as normas zootécnicas e de boas práticas. Apesar de alguns procedimentos provocarem algum desconforto aos ovinos (grau 2), todas as precauções serão tomadas para que seja reduzido ao mínimo.

Nome: Márcia Cristina de Sena Oliveira

Assinatura: _____

Data:22/11/2017

PARECER DO RELATOR 3

APROVADO (X) APROVADO COM RESTRIÇÕES ( ) REPROVADO ( )

Justificativa: o projeto busca desenvolver em ovinos uma técnica de coleta de embriões e inovulação por via transcervical em substituição à técnica cirúrgica. Para tanto, justifica-se o uso de 300 ovinos, 150 machos e 150 fêmeas, que serão mantidos em adequadas condições de alojamento, alimentação e sanidade. Os animais serão submetidos a estimulações hormonais (G1 – 2 aplicações por ano em 60 ovelhas), coletas de sangue (G1 – 6 coletas por ano em 60 ovelhas), sêmen (G2) e embriões (G2 – 1 coleta por ano em 72 ovelhas), biópsia de pele (G2) e inovulação (G2 – 60 ovelhas e 30 ovelhas para inovulação cirúrgica), utilizando-se de anestesia e analgesia quando necessário, além de acompanhamento pós-operatório.

Nome: Simone Cristina Meo Niciura

Assinatura: Simone Cristina Meo Niciura

Data: 23/11/17

EFEITOS LUTEOTRÓFICOS DA GONADOTROFINA CORIÔNICA HUMANA ADMINISTRADA 7,5 DIAS APÓS A INDUÇÃO DE ESTRO EM OVELHAS MORADA NOVA

RESUMO - Este estudo foi desenvolvido para avaliar os efeitos da administração da gonadotrofina coriônica humana (hCG) 7,5 dias após protocolo de indução de estro sincronizado em ovelhas Morada Nova, durante o período não reprodutivo, na biometria e vascularização do tecido luteal, concentrações de progesterona (P4) e desempenho reprodutivo (taxa de prenhez, número de partos múltiplos e número de cordeiros nascidos). Foram submetidas ovelhas da raça Morada Nova (n = 115) à indução de estro sincronizado utilizando esponja intravaginal impregnada de medroxiprogesterona (60mg) por 6 dias mais 200 UI de eCG i.m. e 30 µg de d-cloprostenol i.m. 36 h antes da remoção da esponja. Após o acasalamento natural, ovelhas que apresentavam pelo menos um corpo lúteo (CL; n = 108), foram igualmente designadas para receber 1 mL de solução salina (G-Controle; n = 56) ou 300 UI de hCG (G-hCG; n = 57) no dia 7.5 após a remoção da esponja. A avaliação por ultrassonografia ovariana e a coleta de sangue foram realizadas nos dias 7,5, 13,5, 17,5, 21,5 e 30,5 após a remoção da esponja. A presença de corpo lúteo acessório (CLa) foi observado em 0,0% (0/56; G Controle) e 80,7% (46/57; G-hCG) (P = 0,0001). O diâmetro, a área e o volume do tecido luteal foram maiores (P <0,05) em G-hCG em comparação com ovelhas G-Controle do dia 13,5 a 30,5. As concentrações de P4 foram maiores (P <0,05) nos dias 13,5, 17,5, 21,5 e 30,5 para G-hCG. A taxa de prenhez foi semelhante (P = 0,15) entre os grupos (46,4% G-Controle vs 61,4% G-hCG), no entanto, a taxa do número total de cordeiros nascidos pelo número total de ovelhas sincronizadas foi maior (P = 0,005) no G-hCG em comparação ao G-Controle (89,4% (51/57) vs 66,1% (37/56). Em conclusão, a administração de hCG 7,5 dias após a remoção da esponja nas ovelhas Morada Nova na estação não reprodutiva é eficiente para induzir a formação de CLa, melhorar a biometria do tecido lútea e as concentrações de P4, o que aumenta o número total de cordeiros nascidos de ovelhas sincronizadas.

Palavras-chave: aCL, Corpo lúteo acessório, hCG, Progesterona, Ultrassom ovariano, Vascularização Luteal

LUTEOTROPHIC EFFECTS OF HUMAN CHORIONIC GONADOTROPIN ADMINISTERED 7.5 DAYS AFTER SYNCHRONOUS ESTRUS INDUCTION IN MORADA NOVA EWES

ABSTRACT - This study was designed to assess the effects of human chorionic gonadotropin (hCG) administration 7.5 days after the end of synchronous estrus induction protocol in Morada Nova ewes during the non-breeding season on biometrics and vascularization of the luteal tissue, progesterone (P4) concentrations and reproductive performance. Morada Nova ewes (n=115) were submitted to synchronous estrus induction with 60 mg medroxyprogesterone intravaginal sponge for 6 d plus 200 IU of eCG im and 30 µg of d-cloprostenol im 36 h before sponge removal. After natural mating, ewes that had at least one corpus luteum (CL; *n* = 108), were equally assigned to receive either 1 mL of saline solution (G-Control; n = 53) or 300 IU of hCG (G-hCG; n = 55) im on Day 7.5 after sponge removal. Ovarian ultrasound evaluation and blood collection were performed on Days 7.5, 13.5, 17.5, 21.5 and 30.5 after sponge removal. Accessory corpora lutea (aCL) was noted in 0.0% (0/53; G-Control) and 81.8% (46/55; G-hCG ewes) (P = 0.0001). Diameter, area and volume of luteal tissue were greater (P < 0.05) in G-hCG compared with G-Control ewes from Day 13.5 to 30.5. Concentrations of P4 were greater (P < 0.05) on Days 13.5, 17.5, 21.5 and 30.5 for G-hCG. Pregnancy rate was similar (P = 0.15) between groups [47.1% (G-control) vs 60.0% (G-hCG)], although the rate of total number of lambs born by the number of synchronized ewes was greater (*P* = 0.005) in G-hCG (90.9 vs 66.0%). In conclusion, the administration of hCG 7.5 days after sponge removal in Morada Nova ewes in the non-breeding season is efficient to induce aCL formation, improve luteal tissue biometry and P4 concentrations, which increase the total number of lambs born from ewes exposed.

Keywords: aCL, Accessory Corpora Lutea, hCG, Progesterone, Ovarian Ultrasound, Luteal Vascularization

CAPÍTULO 1 – Considerações gerais

1. INTRODUÇÃO

Devido a facilidade de criação e alta adaptabilidade dos ovinos as adversidades climáticas, associado ao curto período entre gerações e possibilidade de mais de um cordeiro por gestação, a ovinocultura pode ser fonte de renda para sistemas familiares e também sistemas sofisticados e intensivos de criação. Com a intensificação na cadeia produtiva de ovinos, há necessidade de aplicação de biotecnologias voltadas a reprodução, contribuindo de forma positiva para o aumento do número de cordeiros nascidos e consequente aumento do rebanho ou de seus produtos (carne, leite, lã e pele).

Os ovinos são animais classificados como poliéstricos estacionais de dia curto, obtendo atividade sexual em respostas à diminuição da disponibilidade de luz (Rosa & Bryant, 2003). Assim é necessário o emprego de tecnologias nestes momentos de sazonalidade reprodutiva para garantir a continuidade da reprodução, atingindo o máximo aproveitamento.

De maneira fisiológica durante este período de anestro estacional não é possível que as fêmeas reestabeleçam a ciclicidade sem interferência humana. Manejos como efeito macho, luz artificial ou indução por meio de protocolos hormonais são necessários para que estas fêmeas possam gerar descendentes durante a estação de anestro. Porém, mesmo com a utilização de hormônios para que estas possam entrar em estro durante este período, as concentrações de progesterona (P4) ao longo da fase luteal induzida não são semelhantes aquelas durante a estação reprodutiva (Theodosiadou et al., 2004), diminuindo assim a possibilidade de manter uma gestação. Desta forma há necessidade de estudos para amenizar estas diferenças e assim otimizar a produção durante está estação crítica.

Buscando elevar as concentrações de P4 após indução de estro, estratégias como indução de corpo lúteo acessório vem sendo estudadas em bovinos (Maillo et al., 2013), caprinos (Fonseca et al., 2006) e ovinos (Allam et al., 2015; Coleson et al.,

2015; Fernandez et al., 2018; Fonseca et al., 2018). Porém há uma grande divergência entre os resultados obtidos em ovinos, devido as metodologias empregadas, quanto ao momento da aplicação, agente indutor (hCG ou GnRH), dose e estação do ano.

2. REVISÃO DE LITERATURA

2.1 Regulação neuroendócrina

A regulação da produção e liberação das gonadotrofinas (FSH e LH) depende de uma complexa interação de fatores extrínsecos e intrínsecos (Rawlings e Bartlewski 2007). Os fatores intrínsecos estão diretamente ligados a disponibilidade de precursores proteicos, hormônios gonadais (progesterona, estrógeno, inibina, activina, folistatina), neurotransmissores e produtos uterinos (prostaglandinas). Já os fatores extrínsecos são fotoperíodo, feromônios masculinos, nutrição e stress e também são responsáveis por afetar o eixo hipotálamo-hipófise-gonadal (Bartlewski et al., 2011).

Após a formação de um corpo lúteo (CL), há uma redução nas concentrações de LH devido ao feedback negativo realizado pela produção e liberação de P4 na corrente sanguínea pelo CL. Por sua vez, o estímulo negativo sobre a liberação de LH acarreta na não possibilidade fisiológica de que ocorra a formação de novos CLs até que ocorra a lise do CL previamente presente no ovário, com consequente queda nas concentrações de P4. Porém esse efeito negativo não é observado na liberação de FSH, assim logo após a ovulação há o recrutamento e crescimento de novas ondas foliculares. Entretanto estes folículos não podem ovular até que ocorra a luteólise (Rawlings & Bartlewski, 2007, Seekallu et al., 2009).

2.2 Indução de corpo lúteo acessório

Protocolos de indução de corpo lúteo acessório (CLa) tem como objetivo a formação de uma nova estrutura lútea funcional, com o objetivo de induzir elevação das concentrações de P4. De maneira indireta as concentrações de P4 elevadas potencialmente afetam positivamente o crescimento e desenvolvimento do(s) concepto(s) por meio de alterações no líquido luminal uterino e estruturas extraembrionárias (Lonergan et al., 2016). Isto porque a P4 é responsável pelo

estabelecimento e manutenção da gestação em mamíferos, também pelo crescimento e desenvolvimento do feto, realizando importante papel na implantação e placentação (Coleson et al., 2015). Para realizar a indução destes CLa é necessário que no momento da aplicação do indutor esteja presente nos ovários pelo menos um folículo, que possua receptores para LH em sua membrana plasmática.

A hCG e GnRH são agentes indutores de ovulação utilizados para indução de CLa, entretanto, estudos demonstram que a aplicação de GnRH pode produzir CLa não funcionais, ou seja, não havendo incremento nas concentrações de P4 (Fernandez et al., 2018). Além deste fator, a hCG possui vantagens relacionadas a sua meia-vida plasmática, que pode ser até seis vezes maior que a do GnRH (Khan et al., 2007). A hCG possui mais de 80% de afinidade aos receptores de LH (Nwabuobi et al., 2017), assim produzindo uma combinação de duração e local de efeito melhor quando comparado com a utilização do GnRH. A aplicação de hCG resultou em aumento do tecido luteal funcional e estímulo do crescimento do concepto, aumento das concentrações de P4 e efeito embriotrófico positivo, estes resultados foram superiores em comparação ao GnRH (Khan et al. 2007).A maior área de comunicação materno-fetal (placentomas) favorece o crescimento do concepto (Khan et al. 2007), conceptos maiores tem maior síntese de interferon-tau (IFN-tau), diminuindo assim a mortalidade embrionária (Nephew et al., 1994). A hCG também possui efeitos angiogênicos, pois o hormônio estimula fatores que são necessários para formação de novos vasos tanto no processo de formação do CL quanto diretamente no endométrio, assim, fornecendo melhor ambiente para o desenvolvimento embrionário e sua implantação, contribuindo para a maior sobrevivência embrionária (Coleson et al. 2015). Enquanto isto, a administração do GnRH pode produzir CL não funcional (Fernandez et al., 2018).

Há relatos da administração do agente luteotrófico na primeira onda folicular que é recrutada logo após a ovulação, ou seja, entre os dias 4 – 5 após a ovulação (Lankford et al., 2010; Coleson et al., 2015; Fernandez et al., 2018; Fonseca et al., 2018), enquanto outros autores preconizaram realizar o tratamento entre os dias 10 – 12 após o estro (Khan et al., 2007; Khan et al., 2009; Catalano et al., 2015). Considerando que fisiologicamente as ovelhas possuem um padrão de ondas de recrutamento folicular, e com ocorrência de três a quatro ondas por ciclo estral (Bartlewski et al., 2011). Somado ao fator do padrão de crescimento destes folículos

principalmente em ovelhas com três a quatro ondas, são esperados folículos de pelo menos 4 mm de diâmetro ao oitavo dia após a ovulação (Evans et al., 2000). Provendo condições para a geração de CLa, pois folículos com este diâmetro (4 mm) já possuem receptores de LH em sua membrana (Driancourt, 2001).

Em ovelha subtropical, o tratamento com hCG nos dias 5 e 7 após o estro aumentou o peso da estrutura lútea e a conversão induzida de células luteais pequenas em grandes células luteais, sendo estas responsáveis por cerca de 80% da produção de progesterona no corpo lúteo, levando a uma maior concentração de P4 no sangue (Farin et al., 1988). Outros estudos em ovinos confirmaram a elevação de P4 plasmática e formação de CLa após aplicações de hCG, quando realizadas 4, 7 e 10 dias após início do estro (Lankford et al. 2010), 4 dias após o acasalamento (Coleson et al., 2015; Fernandez et al. 2018), 7 dias após acasalamento (Fonseca et al. 2018) e 12 dias após o acasalamento (Catalano et al. 2015). Este efeito de elevação das concentrações plasmáticas de P4 está relacionado ao aumento do tecido luteal como dito anteriormente, mas também pelo estímulo direto das células luteais já presentes no animal, por meio da ligação do hCG nos receptores de LH, proporcionando uma maior esteroidogênese (Nwabuobi et al., 2017).

A relação da população folicular no momento da indução ainda não foi estudada, apenas há sugestão do tamanho do folículo capaz de induzir a formação de CLa. Tampouco foi estudada a dinâmica luteal (diâmetro, área e volume) e vascularização do tecido luteal até o momento do diagnóstico de gestação (Dia 30). Preconizando o bem-estar animal, este estudo busca encontrar estas respostas de forma menos invasiva (por meio da ultrassonografia) comparado aos demais trabalhos encontrados na literatura, os quais em sua maioria utilizam da laparoscopia ou laparotomia para averiguar as alterações morfológicas. Com base nos resultados encontrados na literatura, quanto antes for realizada a indução de CLa, são esperados mais benefícios, diretos e indiretos ao embrião, possibilitando assim um maior número de gestações.

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CAPÍTULO 2 - Luteotrophic effects of human chorionic gonadotropin administered 7.5 days after synchronous estrus induction in morada nova ewes

ABSTRACT - This study assessed the effects of human chorionic gonadotropin (hCG) administration after the end of estrus induction protocol during non-breeding season in Morada Nova ewes. Ewes (*n* = 115) received a 60 mg medroxyprogesterone intravaginal sponge for 6 d plus 200 IU of equine chorionic gonadotropin (eCG) im and 37.5 µg of d-cloprostenol im 36 h before sponge removal (Day 0). After natural mating, ewes that had at least one corpus luteum (CL; *n* = 108) were assigned to receive either 1 mL of saline (G-Control; *n* = 53) or 300 IU of hCG (G-hCG; *n* = 55) im on Day 7.5 after sponge removal (Day 0). Ovarian ultrasonography and blood collection were performed on Days 7.5, 13.5, 17.5, 21.5 and 30.5. Accessory CL (aCL) was observed in 81.5% (G-hCG) and 0.0% (G-Control) of ewes (*P* = 0.0001). Diameter, area and volume of luteal tissue were greater (*P* < 0.05) in G-hCG from Day 13.5 to 30.5. Progesterone (P4) concentrations were greater (*P* < 0.05) on Days 13.5, 17.5, 21.5 and 30.5 for G-hCG. Pregnancy rate was similar (*P* = 0.25) between groups [47.1% (G-control) vs 60.0% (G-hCG)], although the rate of total number of lambs born by the number of synchronized ewes was greater (*P* = 0.005) in G-hCG (90.9 vs 66.0%). In conclusion, hCG administration 7.5 days after sponge removal in Morada Nova ewes in the non-breeding season is efficient to induce aCL formation, improve luteal tissue biometry and P4 concentrations, enhancing the total number of lambs born.

Keywords: Accessory Corpora Lutea, hCG, Progesterone, Ovarian Ultrasound, Luteal Blood Flow, Luteal Vascularization.

1. Introduction

The beginning of pregnancy is a critical period when the occurrence of total pregnancy losses can be related to numerous causes, including non-infectious maternal factors such as the hormonal profile (Rhind et al., 1978) and its effect on the uterine environment stands out. Studies in ruminants demonstrated the positive and indirect effect of progesterone (P4) on the uterine environment and, consequently, on early embryo development and quality (Satterfield et al., 2006; Lonergan et al., 2016). These effects are seen by the alteration in the gene expression of the uterine luminal fluid in order to provide better embryonic nutrition conditions and improvement in the uterine receptivity to the embryo (Forde and Lonergan, 2017).

The use of luteotrophic hormones such as human chorionic gonadotropin (hCG) and gonadotrophin-releasing hormone (GnRH) in order to induce the formation of accessory corpora lutea (aCL) and, consequently, to increase the P4 concentration and the conception rate has been investigated in cattle (Fonseca et al., 2000; Fonseca et al., 2001a; Maillo et al., 2013) and goats (Fonseca et al., 2005; Fonseca et al., 2006). Specially in sheep, several studies have reported that hCG administration evoked effects on aCL formation and improved the P4 concentrations (Khan et al., 2009; Allam et al., 2015; Coleson et al., 2015; Fernandez et al., 2018; Fonseca et al., 2018). Despite of these positive effects, no improvements in pregnancy rate have been obtained in ewes (Catalano et al., 2015; Fernandez et al., 2019). In fact, factors influencing the most expected outcome – pregnancy rate increase – are still lacking to be studied. Nonetheless, the ovarian follicular profile at the time of gonadotrophin administration, and the P4 threshold required for pregnancy establishing and maintenance are yet to be determined.

Studies in Santa Inês sheep raised in tropical conditions and subjected to short-term protocol showed interval from sponge removal to ovulation of 58 h (Cavalcanti et al., 2012) and 70 h (Teixeira et al., 2016). The first follicular wave of the estrous cycle emerges near to ovulation and the dominant follicle persists for nine days after ovulation in Corriedale ewes (Viñoles et al., 1999). Thus, the follicle probably reaches its dominance on Day 7 to 8. Formation of aCL was observed in 85.7% of nulliparous

Santa Inês ewes receiving 250 IU hCG on Day 8 (Fonseca et al., 2018) and 100% of Merino ewes receiving 300 IU hCG on Day 7 (Fernandez et al., 2018), both after sponge removal. Although not precisely demonstrated, the latter authors suggested that only follicles ≥ 4 mm of diameter formed aCL; which is logical since at this diameter they already have LH receptors (Driancourt, 2001). Of note, the follicular population present in the ovary at a specific day varies according to the number of waves of each ewe, which are mainly from three to four (Bartlewski et al., 2011). Thus, in ewes presenting three or four waves, follicles ≥ 4 mm of diameter are expected around the eighth day (Evans et al., 2000), which are able to undergo aCL formation.

Another decisive point for the aCL formation success is the “health” of dominant follicle present on the ovary at the day of gonadotrophin administration. Ovarian follicular growing and regressing pattern were initially proposed to dominant follicles preceding ovulation in sheep (Menchaca and Rubianes, 2004) and, recently, early and advanced development pattern was described for largest follicles in goats (Carvalho de Paula et al., 2020). In these studies, the regressing follicles had lesser probability to ovulate in sheep and, in goats, these advanced follicles were associated with abnormal CL function. Therefore, the hormonal treatment regimen chosen may be adequate to promote the favorable follicular status after intravaginal device removal.

In view of the above, the present study was designed to test the hypothesis that the administration of hCG 7.5 days after the end of synchronous estrus induction protocol in Morada Nova ewes during the non-breeding season induces aCL formation, increases the dimensions (diameter, area and volume) and the functionality of luteal tissue (vascularization parameters and P4 concentration), and lastly a positive effect on the pregnancy rate and number of multiple births. The role of antral follicular population present in the ovaries at the time of gonadotropin administration was associated to its efficiency.

2. Material and methods

2.1 Ethics, animals and experimental design

This study was approved by the Animal Use Ethics Committee of EMBRAPA Southeast Livestock (#09/2017). The experiment was carried out in a completely

randomized block design, with three blocks starting one week apart, in the months of October and November (non-reproductive season).

A total of 115 Morada Nova ewes [primiparous (*n* = 37) or pluriparous (*n* = 78)] clinically health, non-pregnant, non-lactating, with 36.6 ± 4.8 kg of body weight (BW) and 3.1 ± 0.2 of body condition score (BCS), on scale of 1 - 5 according to Villaquiran et al. (2007) were used. The animals were kept in an intensive breeding system at the Canchim farm of EMBRAPA Southeast Livestock (21°57'28.5" South and 47°50'36.6" West), remaining in rotated paddocks with cultivated pasture (*Panicum maximum* cv. Aruana) with access to mineral mix and water *ad libitum*. The animals received nutritional supplementation with corn silage and balanced feed in the amount of 200 g/animal/day, provided once daily.

The females received intravaginal sponge containing 60 mg of medroxyprogesterone acetate (MAP; Progespon®, Zoetis, USA) for six days plus 37.5 µg of d-cloprostenol (Veteglan®, Hertape Calier, Barcelona, Spain) im and 200 IU of eCG (Novormon®, Zoetis, USA) im 36 h before sponge removal. Both sponge insertion and removal were performed from 5 to 6 h pm. After sponge removal, ewes were allocated to be mated by four males (male: females 1:10 ratio) previously evaluated for libido and seminal quality and kept together for the following three days. Ewes that had at least one corpus luteum on Day 7.5 (i.e. animals that responded to estrus synchronization protocol, *n* = 108) were homogeneously divided into two experimental groups, according to their BW, BCS, parity order and ram. Females were subjected to treatments and received on Day 7.5 (7 to 8 h am) after sponge removal either 300 IU of hCG (Vetecor®, Hertape-Calier) im (G-hCG, *n* = 55; BW: 36.3 ± 4.0 kg, BCS: 3.1 ± 0.3 and 2.5 ± 1.8 parity order) or 1 mL saline solution (0.9% NaCl, Eurofarma Lab SA, Brazil) im (G-Control, *n* = 53; BW: 36.6 ± 5.5 kg, BCS: 3.1 ± 0.3 and 2.5 ± 1.6 parity order) (Figure 1).

2.2. Ovarian ultrasonography and pregnancy diagnosis

Ultrasonographic evaluations (B-mode and Color Doppler) of the ovaries were performed in all experimental ewes (*n* = 108) on Days 7.5, 13.5, 17.5, 21.5 and 30.5 after sponge removal, using portable equipment equipped with multifrequency linear transrectal transducer (6 to 8 MHz, My Lab Vet 30 Gold, Esaote, Netherlands). The

Color Doppler settings were maintained during all evaluations (Pulse repetition frequency (PRF): 1.4 KHz; Gain 64%; Depth 8 cm; CFM (Frequency): 5 MHz; and Wall Filter (WF): M). The number of antral follicles on Day 7.5 and CL present on all days of evaluation were quantified and measured. For this purpose, the sonographic plane of section which showed the structures in their largest dimensions was selected. The diameter was calculated by the average of two perpendicular measurements. Follicular population was classified in four categories according to diameters: total antral follicles (≥ 2 mm), small (< 3.5 mm); medium (3.5 – 4.49 mm) and large (≥ 4.5 mm) follicles (Oliveira et al., 2016). Percentages of ewes with at least one follicle ≥ 3.5 mm, ≥ 4 mm or ≥ 4.5 mm were determined (considering that 4 mm follicles have LH receptors; Driancourt, 2001). The CL area was determined by delimiting the structure using tools from the ultrasound equipment itself. The estimated CL volume was calculated using the sphere volume formula ($4/3 \times \pi \times r^3$), with r = radius and π = 3.1416. For cavitary CL, the dimensions of the cavity were subtracted. For all measurements, the sum of the CL present in the animal on the day of evaluation were calculated. The area of vascularization present in the CL was determined with the aid of the Image J® software, in number of colored pixels and later transformed into mm^2. The vascularization percentage was calculated using the formula: (number of colored pixels / number of total pixels of the CL) X 100. In the impossibility of individualizing each CL present in the ovary, an evaluation of the ensemble and subsequent division of the value obtained by the number of CL was considered. The vascularization volume was estimated by the formula (average of percentage of vascularization x summed volume of the luteal tissue/100).

Females that on Day 17.5 had CL with no vascularization (i.e. considering the end of the synchronized luteal phase) were not evaluated on Days 21.5 and 30.5. Pregnancy diagnosis was performed on Day 30.5 by means of B-mode ultrasonography of the uterus, confirming the presence of an embryonic vesicle and its heartbeat. The pregnancies were accompanied, and parturition was monitored to identify the number of lambs born in each pregnant female. The lamb(s) body weight was measured right after birth and the litter weight at birth was calculated.

2.3. Blood sampling and serum P4 concentration measurement

Blood samples were taken from 30 ewes (*n* = 15 per treatment, being eight pregnant and seven non-pregnant each) on all ultrasound evaluation days (Days 7.5, 13.5, 17.5, 21.5 and 30.5), by jugular vein puncture in vacuum tubes without anticoagulant. After 30 min of collection, the samples were centrifuged at 3000 X *g* for 10 min and the serum stored at -20 ºC in two aliquots. The measurements of serum P4 concentrations were performed using the solid phase radioimmunoassay (RIA) technique using a commercial kit (ImmuChem, MP Biomedicals, Santa Ana, CA, USA), following the manufacturer's recommendations. The average intra-assay sensitivity and coefficient of variation was 0.05 ng/mL and 11%, respectively.

2.4. Variables and statistical analyses

The following parameters were recorded: Number of antral follicles according to diameter categories; Percentages of ewes with at least one follicle ≥ 3.5 mm, ≥ 4 mm or ≥ 4.5 mm; Percentage of ewes with at least one aCL; Number of CL; Rate of ewes with aCL formed; Sum of diameter (mm), area (mm^2) and volume (mm^3) of luteal tissue; Average percentage of luteal tissue vascularization of luteal tissue (%); Sum of vascularized area of luteal tissue (mm^2); Pregnancy rate (number of pregnant females / number of females X 100) (%); Parturition rate (number of females kidding / number of pregnant females X 100) (%); Total pregnancy loss (number of females that not give birth / number of pregnant females) (%); Multiple birth rate (number of births with at least two lambs / number of ewes that delivered X 100) (%); Total number of lambs born; Prolificacy (number of lambs born / number of ewes that delivered X 100) (%); Number of lambs born / number of synchronized ewes X 100 (%); Lamb weight at birth (kg); Litter weight at birth (kg); and Serum P4 concentration (ng/mL).

Analysis of data was performed using Statistical Analysis System software (SAS). The Cramer-Von Mises test was used to verify the normality and the homoscedasticity of the data. Data were transformed using the Box Cox procedure when needed (Box & Cox, 1964), but data are presented not transformed. Parametric data were analyzed with PROC MIXED procedure with a repeated statement to check the interaction between sequential measurements. Non-parametric data were analyzed by PROC NPAR1WAY with the Wilcoxon test. Data were partitioned into two periods relative to the day of the luteal phase: (1) Days 7.5 to 17.5 (all ewes: both

pregnant and non-pregnant) and (2) Day 17.5 to 30.5 (only data from pregnant ewes). In the former group (all ewes) the effects in the statistical model included treatments, days of evaluation, pregnancy diagnosis and their interactions; for the latter (only pregnant) the effects included treatments, days of evaluation and their interaction. The reproductive performance, number of CLs and follicular population were compared between groups by one-way ANOVA or Chi-square test and post-hoc comparison was performed using Tukey test. Pearson correlation test was used between variables. The level of significance adopted was $P < 0.05$ and tendency was considered for P value between 0.051 and 0.10. The results in the text, tables and figures are represented in means ± standard error of the mean (SEM).

3. Results

The antral follicular population present in the ovaries on Day 7.5 after sponge removal is shown in Table 1. Most (81.5%) of G-hCG ewes had aCL formation compared with none (0.0%) of G-control. On Day 7.5, the percentage of ewes with follicles ≥ 3.5 mm and ≥ 4.5 mm tended to be greater ($P = 0.08$) in G-Control. Number of total antral follicles (≥ 2 mm) and small antral follicles (< 3.5 mm) were greater ($P < 0.03$) in G-Control. Indeed, the number of large follicles (≥ 4.5 mm) tended to be greater ($P = 0.057$) in G-Control. There was no correlation ($P > 0.05$) between the antral follicular population (number of total, small, medium and large follicles and diameter of the largest follicle) with the number of aCL formed.

Within G-hCG, the comparison of the ovarian follicular population on Day 7.5 in ewes either forming or not aCL is shown in Table 2. Percentage of ewes with follicles ≥ 4.0 mm was greater ($P = 0.01$) in ewes with aCL. The number of small antral follicles (< 3.5 mm) was greater ($P = 0.01$) in ewes without aCL, whilst the number of medium follicles tended to be greater ($P = 0.07$) in ewes that formed aCL.

From Days 7.5 to 17.5, interactions ($P < 0.003$) were found between the effects of treatments, pregnancy diagnosis and days of evaluation for the luteal dimensions (diameter, area and volume of luteal tissue) and vascularized dimensions (volume and area of vascularized luteal tissue). From Days 17.5 to 30.5, only effect of treatment ($P < 0.01$) was observed (Figure 2A-E). For CL number and P4 concentration, interactions

were detected between treatments and days of evaluation ($P < 0.01$) and between pregnancy diagnosis and days of evaluation ($P = 0.03$) from Days 7.5 to 17.5; while the treatments were the only effect ($P = 0.007$) found from Days 17.5 to 30.5. For percentage of vascularized luteal tissue difference was only observed between pregnancy diagnosis and days of evaluation ($P = 0.0001$) from Days 7.5 to 17.5 while have only effect of days of evaluation from Days 17.5 to 30.5 ($P = 0.0003$) (Figure 3A-C). The number of CL increased from Day 7.5 to 13.5 and remained with greater until Day 30.5 in G-hCG ewes. In pregnant ewes, this variable was greater on Days 13.5 and 17.5 compared with non-pregnant ewes (Figure 3A). The diameter, area and volume of luteal tissue on Day 7.5 were similar ($P > 0.05$) between all subsets. The diameter and area of the luteal tissue increased ($P = 0.003$) from Day 7.5 to 13.5 in all G-hCG ewes, regardless of their pregnancy status; and only in pregnant ewes, these variables remained greater until Day 17.5 (Figure 2A-B). The volume of luteal tissue increased ($P = 0.0007$) from Day 7.5 to Day 17.5, only in pregnant G-hCG ewes (Figure 2C). In pregnant females, from Day 17.5 to Day 30.5, the diameter, area and volume of luteal tissue were greater in G-hCG ewes (Figure 2A-C).

The G-hCG ewes had increased ($P < 0.001$) P4 concentrations from Day 7.5 to Day 13.5; similarly, G-hCG pregnant females had greater P4 concentrations from Day 17.5 to Day 30.5 compared to G-control. Regardless to the treatment, in both pregnant and non-pregnant ewes, the P4 concentrations increased from Day 7.5 to Day 13.5 and, in pregnant ewes, remained greater on Day 17.5 (Figure 3B). For the percentage of vascularization of the luteal tissue, it was observed difference between the interaction of pregnancy diagnosis and days of evaluation: non-pregnant ewes had the lowest values ($P = 0.0001$) on Day 17.5 (Figure 3C). The vascularized area of luteal tissue increased ($P < 0.01$) from Day 7.5 to 13.5 in both pregnant and non-pregnant ewes from G-hCG while the vascularized volume of luteal tissue increased only in G-hCG pregnant ewes, in which were observed greater values for both variables of vascularization on Day 17.5 compared with other subsets. From Day 17.5 to Day 30.5 greater values were observed to G-hCG compared with G-Control (Figure 2D-E). All biometric variables of functional luteal tissue were positively correlated ($P < 0.02$) with each other, except for the number of CL with the percentage of luteal vascularization

and the latter with volume of luteal tissue (Table 3). The percentage of luteal vascularization showed have the lowest correlations with the other end points.

The pregnancy rate did not differ ($P = 0.25$) between groups (Table 4), however, it was greater ($P = 0.03$) in G-hCG ewes undergoing aCL formation (70.4%, 31/44) compared with those without aCL formation (27.3%, 3/11) or G-Control (47.1%, 25/53). There was positive correlation ($P < 0.01$) between the rate of aCL formation and the pregnancy rate (r = 0.34). For the reproductive performance indices (Table 4), the rate of lambs born / synchronized ewes was greater ($P = 0.003$) in G-hCG ewes.

4. Discussion

This is the first report of aCL induction in sheep with detailed assessment by color Doppler ultrasonography of ovaries. Briefly, the most important findings were that hCG administered 7.5 days after the end of estrus induction protocol was efficient to induce aCL formation, increasing the luteal tissue biometry (number of CL, area and volume) and its functionality (P4 concentration). It must be highlighted that greater numbers of total and small follicles were observed on Day 7.5 in G-Control ewes and it is logical to suppose that aCL formation could have been even greater in G-hCG if there was no difference. Moreover, it is suggested that follicles ≥ 3.5 mm may have formed aCL. The pregnancy rate significantly increased 23.3% in G-hCG ewes undergoing aCL formation compared with G-Control ewes. Finally, the rate of number of lambs born / number of synchronized ewes was significantly increased (24.9%) in G-hCG ewes.

The day of hCG administration in the present study was chosen based on ovulation expected time (Cavalcanti et al., 2012) and ovarian follicular wave emergence (Viñoles et al., 1999) reported in other sheep breeds, because such information was not available for Morada Nova ewes. The great aCL formation rate in this study (81.5%), similar to 85.7% reported in Santa Inês breed (Fonseca et al., 2018), indicates that the moment of hCG administration was adequate. The similarity between the percentage of ewes forming aCL and the percentage of ewes with follicles ≥ 3.5 mm in G-hCG ewes suggest that follicles of this size may have generated aCL. This statement can also be supported by the trend ($P = 0.07$) of a greater number of

medium follicles (3.5 - 4.49 mm) for ewes with aCL in hCG group. This hypothesis is sustained by the hCG half-life (Yen at al., 1968) and follicular growth rate of 0.6 mm per day (Viñoles et al., 1999). Recently, Fernandez et al. (2018) suggested that hCG generated aCL from follicles ≥ 4 mm and it is known that LH receptors are present in 4 mm follicles (Driancourt, 2001). However, there is still no study confirming the diameter of the follicles that give rise to aCL.

Studies of luteal dynamics in response to hCG treatment to induce aCL in ewes are quite scarce and are restricted to the evaluation of the CL area on specific days of luteal phase (Fonseca et al., 2018) or biometrics (ex. weight and diameter) in a single day (*post-mortem*) (Khan et al., 2007; Allam et al., 2015; Catalano et al., 2015). In the present study, the administration of hCG on Day 7.5 ensured an increase in the CL number and dimensions (diameter, area and volume), as well as in the area and volume of luteal vascularization and P4 concentrations on Days 13.5, 17.5, 21.5 and 30.5, specially in pregnant ewes. This set of positive effects was expected and may be directly connected with aCL formation or as a consequence of the hCG luteotrophic effect on the original CL, as reported in cattle (Schmitt et al., 1996; Binelli et al., 2001; Fonseca et al., 2001). The results of the present study do not confirm whether the action of hCG was exclusively by formation of aCL or also on the original CL. The P4 concentrations, however, had greater coefficients of correlation with the dimensions of the luteal tissue ($r \geq 0.57$) than with the CL number ($r = 0.45$). We believe, therefore, there was also a luteotrophic effect on the original CL.

The increase in P4 concentrations from Day 7.5 to 13.5 in G-hCG also confirm the relationship between luteal biometry and its potential for steroidogenesis, as showed in an *in vitro* study (Veenhuizen et al., 1972). Similar results were recorded in several *in vivo* studies (Khan et al., 2007; Lankford et al., 2010; Coleson et al., 2013; Catalano et al., 2015; Fernandez et al., 2018; Fonseca et al., 2018). Among the studies cited, it is possible to observe a difference for the interval between treatment and increase of P4 concentrations, which may be related to the dose used, days of treatment, breeds and seasons. Higher doses seem to have a faster response, and the earlier the treatment is applied, the longer the time in which the concentrations remain high are observed. It is noteworthy that in the present study the P4 concentrations were increased in the moment corresponding to maternal recognition

of pregnancy, from 12 to 13 days after estrus (Ford, 1985). Increasing of plasma P4 concentrations induce positive effects on the uterus, changing the uterine luminal fluid gene expression. Thus, it provides better conditions to nourish the embryo and accelerate its development, as well as favoring uterine receptivity to the embryo (Forde and Lonergan, 2017).

Color Doppler ultrasonography has been shown to be efficient for studying tissue vascularization and its relationship with functionality in sheep, as described in follicular wall (Oliveira et al., 2014; 2017) and luteal tissue (Figueira et al., 2015; Arashiro et al., 2018; Oliveira et al., 2018). This is the probably the first report applying color Doppler in a study of aCL induction in sheep. As expected, the luteal vascularization characteristics (i.e. vascularized area and volume) increased in pregnant hCG ewes and were positively correlated with the functionality of luteal tissue. Similar relation between the area of luteal vascularization and P4 concentrations was reported throughout the sheep estrous cycle (Figueira et al., 2015). In addition, the subjective assessment of the vascularization percentage is proven to be an efficient tool for the early diagnosis of pregnancy in cattle (Pugliesi et al., 2014), sheep (Arashiro et al., 2018) and goats (Consentino et al., 2018). The quantitative (non-subjective) determination of the percentage of vascularization of luteal tissue, however, was not a good tool to detect any effect of the hCG treatment in active CL, although it reliably revealed luteolysis, as demonstrated before (Figueira et al., 2015). The percentage of vascularization is possibly less efficient than vascularized area and volume, because the first does not reflect the dimension of luteal tissue, which is associated to capacity of synthesis of P4 (Schmitt et al., 1996).

Despite all the positive effects of hCG on luteal dynamics of Morada Nova ewes, no significant increase in pregnancy and parturition rates was confirmed. These results corroborate with previous studies testing aCL induction in sheep (Khan et al., 2007; Catalano et al., 2015; Fonseca et al., 2018; Fernandez et al., 2019). Considering that the present study was conducted in ewes during non-breeding season, it can be speculated that a different result could be found in the breeding season. In addition, improvements in the protocol to induce aCL are needed to increase the percentage of aCL formation, since there is a positive correlation in the formation of aCL and an increase in the pregnancy rate.

It is important to highlight that few aspects may have affected the pregnancy rate in the current study, since it was not possible to precisely determine estrous response and mating. Indeed, the total number of females used may also have hidden a significant response in pregnancy rates, even observing a 13% increase in G-hCG treatment. Thus, future studies are necessary, with a larger number of females, better control of fertilization. Interestingly, the pregnancy rate was significantly greater in hCG-treated sheep presenting ≥ 1 aCL. This result indicates that the strategy to further improve hCG responses is by increasing the rate of aCL formation. The efficiency of the current hormonal strategy in the non-breeding season was evidenced in the greater rate of lambs born according to the number of synchronized ewes in G-hCG ewes. Moreover, lambs weight and litter weight at birth were similar between treatments, indicating that lamb uterine development was not affected. This is probably the first study to observe this outcome with the use of hCG as an aCL inducer in farm animals.

5. Conclusions

The administration of hCG 7.5 days after sponge removal in Morada Nova ewes in the non-breeding season is efficient to induce aCL formation, improve luteal tissue biometry and P4 concentrations. Follicles ≥ 3.5 mm of diameter could generate aCL after hCG treatment. Although overall pregnancy rate was not significantly increased, hCG-ewes undergoing aCL formation had increased pregnancy rate. Finally, the hormonal strategy employed ensured a significant increase in the most important feature desired, the number of lambs born per ewes exposed.

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Table 1. Mean ± SEM of the antral follicular population present in the ovaries in Morada Nova ewes receiving either 300 IU of hCG (G-hCG) or 1 mL of saline solution (G-Control) on Day 7.5 after the end of synchronous estrus induction protocol in non-breeding season.

End Points	G-Control	G-hCG	*P* value
Rate of ewes with aCL formed (%)	0.0 (0/53)	81.5 (44/55)	< 0.0001
Ewes with follicles ≥ 3.5 mm (%)	94.3 (50/53)	81.8 (45/55)	0.08
Ewes with follicles ≥ 4.0 mm (%)	77.35 (41/53)	61.8 (34/55)	0.12
Ewes with follicles ≥ 4.5 mm (%)	60.3 (32/53)	41.8 (23/55)	0.08
Nb of total follicles (≥ 2 mm)	5.8 ± 0.2	4.5 ± 0.2	< 0.0001
Nb of small follicles (< 3.5 mm)	3.7 ± 0.2	2.8 ± 0.2	0.002
Nb of medium follicles (3.5 – 4.49 mm)	1.3 ± 0.1	1.2 ± 0.1	0.52
Nb of large follicles (≥ 4.5 mm)	0.6 ± 0.1	0.4 ± 0.1	0.057
Diameter of the largest follicle (mm)	4.5 ± 0.1	4.3 ± 0.1	0.30

aCL: Accessory corpora lutea

Table 2. End points (mean ± SEM and %) according to antral follicular population present in the ovaries of Morada Nova ewes receiving 300 IU of hCG on Day 7.5 after the end of synchronous estrus induction protocol and either formed or not accessory corpora lutea (aCL) in non-breeding season.

	G-hCG		
End points	Without aCL	With aCL	*P* value
Ewes with follicles ≥ 3.5 mm (%)	63.6 (7/11)	86.3 (38/44)	0.18
Ewes with follicles ≥ 4.0 mm (%)	27.2 (3/11)	70.4 (31/44)	0.01
Ewes with follicles ≥ 4.5 mm (%)	27.2 (3/11)	45.4 (20/44)	0.32
Nb of total follicles (≥ 2 mm)	4.9 ± 0.5	4.4 ± 0.2	0.65
Nb of small follicles (< 3.5 mm)	3.9 ± 0.6	2.5 ± 0.2	0.01
Nb of medium follicles (3.5 – 4.49 mm)	0.7 ± 0.3	1.3 ± 0.1	0.07
Nb of large follicles (≥ 4.5 mm)	0.2 ± 0.1	0.5 ± 0.1	0.24
Diameter of the largest follicle (mm)	3.9 ± 0.3	4.4 ± 0.1	0.20

Table 3. Pearson correlation coefficient (r) between the biometry and functionality variables of the luteal tissue of Morada Nova ewes with at least one accessory corpora lutea treated with 300 IU of hCG on Day 7.5 after the end of synchronous estrus induction protocol in non-breeding season.

Variables	Number of CL	Diameter of luteal tissue	Area of luteal tissue	Volume of luteal tissue	Progesterone concentration	Percentage of luteal vascularization	Area of luteal vascularization
Diameter of luteal tissue	0.93	1					
Area of luteal tissue	0.84	0.92	1				
Volume of luteal tissue	0.84	0.91	0.81	1			
Progesterone concentration	0.45	0.59	0.70	0.57	1		
Percentage of luteal vascularization	-	0.16	0.20	-	0.46	1	
Area of luteal vascularization	0.68	0.75	0.76	0.67	0.67	0.61	1
Volume of luteal vascularization	0.78	0.86	0.77	0.93	0.56	0.30	0.80

*All the coefficients presented were significant (P<0.02).

Table 4. Indices of reproductive performance of Morada Nova ewes that on Day 7.5 after the end of the synchronous estrus induction protocol received either 300 IU hCG (G-hCG) or 1 mL of saline solution (G-Control) in the non-breeding season.

End points	G-Control	G-hCG	*P* value
Rate of ewes with aCL formed (%)	0.0 (0/53)	81.5 (44/55)	<.0001
Pregnancy rate (%)	47.1 (25/53)	60.0 (33/55)	0.25
Parturition rate (%)	92.0 (23/25)	96.9 (32/33)	1.00
Total pregnancy loss (%)	8.0 (2/25)	6.1 (2/33)	1.00
Multiple birth rate (%)	56.5 (13/23)	46.8 (15/32)	0.66
Total number of lambs born	35	50	-
Prolificacy	1.5 ± 0.5	1.5 ± 0.7	0.98
Rate of number of lambs born / number of synchronized ewes (%)	66.0 (35/53)	90.9 (50/55)	0.003
Lamb weight at birth (kg)	2.7 ± 0.5	2.6 ± 0.7	0.21
Litter weight at birth (kg)	3.9 ± 1.3	3.8 ± 1.4	0.62

( ) number of animals

Legend Figure:

Fig. 1. Schematic representation of the experiment undertaken to test luteotrophic effects of administration of 300 IU of hCG 7.5 days after the end of synchronous estrus induction protocol in Morada Nova ewes during non-breeding season.

Fig. 2. Mean (± SEM), diameter (A), area (B), volume (C) of luteal tissues, vascularized area (D) and vascularized volume (E) of the corpus luteum on Days 7.5, 13.5, 17.5, 21.5 and 30.5 of non-pregnant and pregnant Morada Nova ewes that received 300 IU hCG (G-hCG) or 1 mL of saline solution (G-Control) on Day 7.5 after the end of synchronous estrus induction protocol. *Different letters indicate statistical difference ($P < 0.05$). **PD = Pregnancy diagnosis.

Fig. 3. Mean (± SEM) of the number of corpus luteum (A), serum progesterone concentrations (B), vascularized percentage (C) of luteal tissue on Days 7.5, 13.5, 17.5, 21.5 and 30.5 of non-pregnant and pregnant Morada Nova ewes that received 300 IU hCG (G-hCG) or 1 mL of saline solution (G-Control) on Day 7.5 after the end of synchronous estrus induction protocol. *Different letters indicate statistical difference ($P < 0.05$). ** PD = Pregnancy diagnosis.

Fig. 1

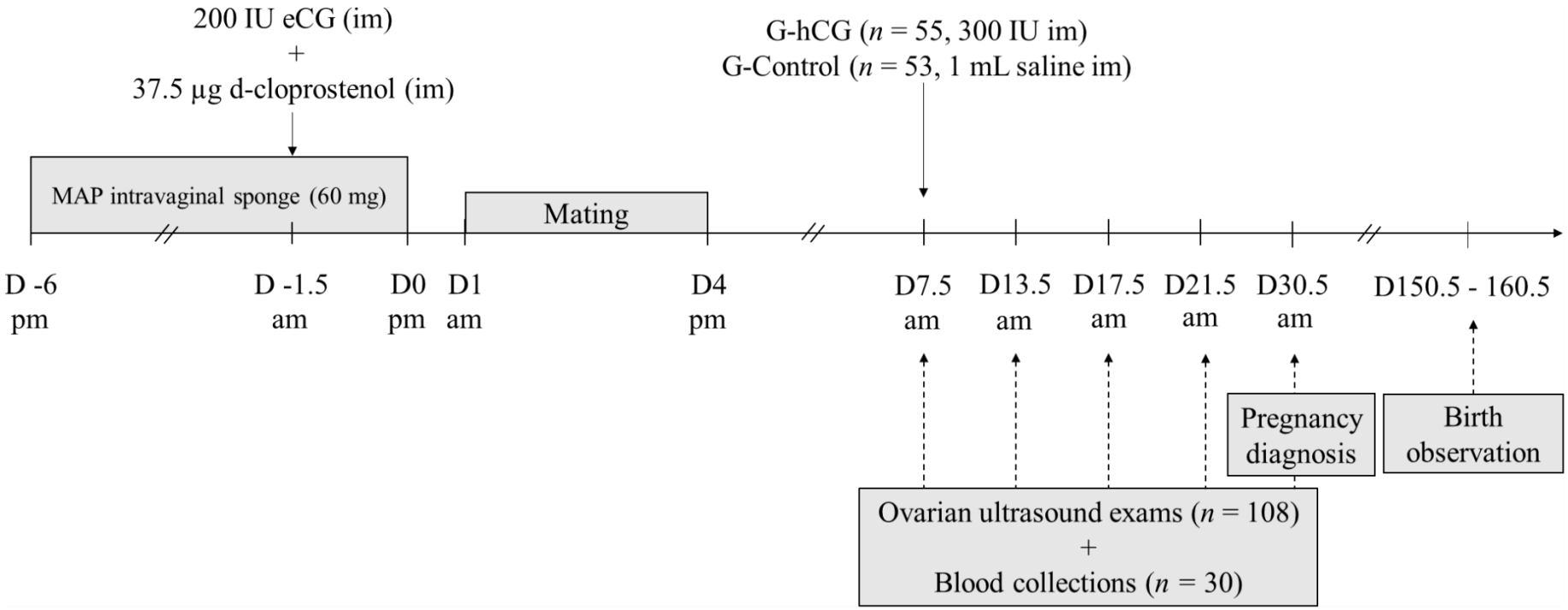

200 IU eCG (im)
+
37.5 µg d-cloprostenol (im)
MAP intravaginal sponge (60 mg)
Mating
G-hCG (n = 55, 300 IU im)
G-Control (n = 53, 1 mL saline im)
D -6 pm
D -1.5 am
D0 pm
D1 am
D4 pm
D7.5 am
D13.5 am
D17.5 am
D21.5 am
D30.5 am
D150.5 - 160.5
Pregnancy diagnosis
Birth observation
Ovarian ultrasound exams (n = 108)
+
Blood collections (n = 30)

Fig. 2

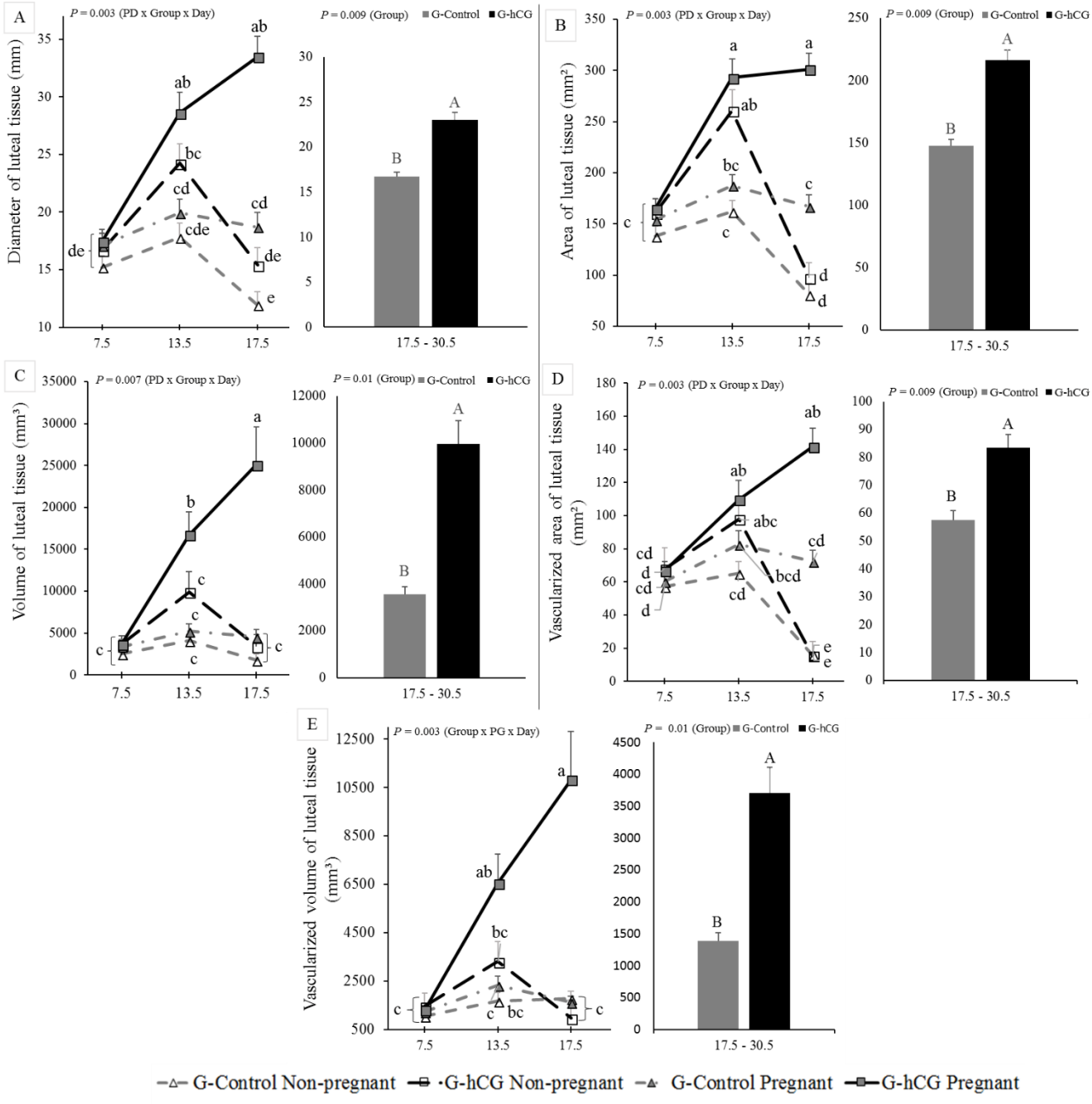
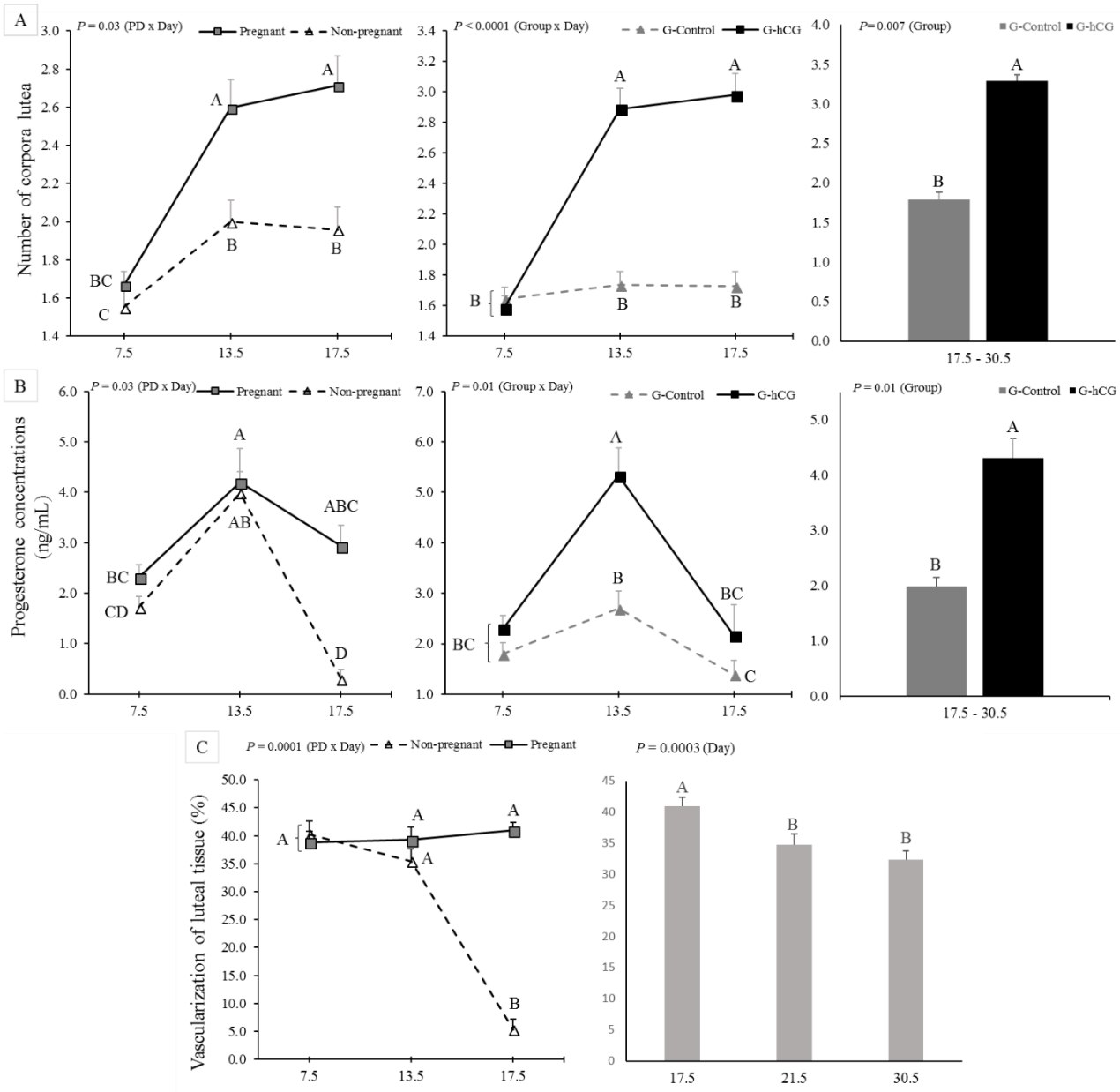

A
P = 0.003 (PD x Group x Day)
Diameter of luteal tissue (mm)
P = 0.009 (Group) G-Control G-hCG
17.5 - 30.5
B
P = 0.003 (PD x Group x Day)
Area of luteal tissue (mm²)
P = 0.009 (Group) G-Control G-hCG
17.5 - 30.5
C
P = 0.007 (PD x Group x Day)
Volume of luteal tissue (mm³)
P = 0.01 (Group) G-Control G-hCG
17.5 - 30.5
D
P = 0.003 (PD x Group x Day)
Vascularized area of luteal tissue (mm²)
P = 0.009 (Group) G-Control G-hCG
17.5 - 30.5
E
P = 0.003 (Group x PG x Day)
Vascularized volume of luteal tissue (mm³)
P = 0.01 (Group) G-Control G-hCG
17.5 - 30.5
G-Control Non-pregnant
G-hCG Non-pregnant
G-Control Pregnant
G-hCG Pregnant

Fig. 3


A
P = 0.03 (PD x Day)
Pregnant
Non-pregnant
Number of corpora lutea
P < 0.0001 (Group x Day)
G-Control
G-hCG
P = 0.007 (Group)
17.5 - 30.5
B
P = 0.03 (PD x Day)
Progesterone concentrations (ng/mL)
P = 0.01 (Group x Day)
P = 0.01 (Group)
C
P = 0.0001 (PD x Day)
Vascularization of luteal tissue (%)
P = 0.0003 (Day)
7.5
13.5
17.5
21.5
30.5