JULIANA NASCIMENTO DUARTE RODRIGUES

EFEITOS DA HCG ADMINISTRADA POR DIFERENTES VIAS E DIAS DO CICLO ESTRAL SOBRE A EFICIÊNCIA REPRODUTIVA DE CABRAS LEITEIRAS

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Medicina Veterinária, para obtenção do título de *Doctor Scientiae*.

Orientador: Jeferson Ferreira da Fonseca

Coorientadores: José Domingos Guimarães
Erick Fonseca de Castilho

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Jeferson Ferreira da Fonseca
Orientador

Ao meu velhinho guerreiro de 4 patas,

Aos meus pais e irmãos, que tanto amo,

Dedico.

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"O melhor de tudo é participar, com responsabilidade, da maior parte! Se puder, não falte! Na vida, ou você ouve histórias que viveram, ou você conta histórias que viveu! Melhor viver e contar!".

(Jeferson Fonseca)

RESUMO

RODRIGUES, Juliana Nascimento Duarte, D.Sc., Universidade Federal de Viçosa, dezembro de 2022. **Efeitos da hCG administrada por diferentes vias e dias do ciclo estral sobre a eficiência reprodutiva de cabras leiteiras**. Orientador: Jeferson Ferreira da Fonseca. Coorientadores: José Domingos Guimarães e Erick Fonseca de Castilho.

A Gonadotrofina Coriônica humana (hCG) pode ser utilizada em diversos momentos do ciclo estral de caprinos, com finalidade de melhorar parâmetros reprodutivos. Este estudo objetivou avaliar os efeitos da hCG administrada em diferentes momentos do ciclo e por diferentes vias sobre parâmetros reprodutivos de cabras leiteiras. O primeiro capítulo objetivou avaliar os efeitos da hCG administrada 7 dias após o início do estro sincronizado nos corpos lúteos (CL) originais (oCL) e nos CL acessórios (aCL), bem como nas concentrações plasmáticas de P4 e na fertilidade em cabras leiteiras inseminadas artificialmente durante a estação não reprodutiva. O tratamento resultou na formação de aCL e teve um efeito hipertrófico no CL existente (ovulatório), além de elevar (P<0,05) as concentrações plasmáticas de P4 e a taxa de gestação em 22.5%. O segundo capítulo objetivou avaliar se a hCG administrada por via intramuscular ($hCG_{i.m.)}$ ou intravaginal ($hCG_{i.vag.)}$ pode ser detectada por um teste rápido de β-hCG em amostras de plasma sanguíneo, e documentar os efeitos ovarianos da hCG administrada por ambas as vias no momento da inseminação artificial (IA) em cabras. A hCG pode ser consistentemente detectada em amostras de sangue usando o teste rápido de β-hCG apenas no grupo hCGi.m. e não houve diferenças (P>0,05) na taxa média de prenhez, concentrações circulantes de P4 e vários parâmetros lúteos estudados entre Controle, $hCG_{i.m.}$ e $hCG_{i.vag}$. O terceiro capítulo objetivou foi avaliar os efeitos da administração de hCG nos dias 5 ou 7 após a observação do estro sobre os oCL e aCL, além da concentração plasmática de P4 e taxa de prenhez de cabras leiteiras na estação não reprodutiva. A hCG promoveu o crescimento dos oCL (P<0,05), induziu a formação de aCL, aumentou a concentração de P4 (P<0,05) e apresentou resultados semelhantes entre os grupos que receberam hCG, sendo o uso no sétimo dia mais eficaz na indução de aCL que no quinto dia. O quarto capítulo avaliou o efeito da hCG i.vag. administrada no momento da IA sobre parâmetros ovulatórios, dinâmica e vascularização luteal, concentrações circulantes de P4 e taxa de gestação de cabras na estação reprodutiva. O tratamento não alterou os parâmetros ovulatórios, não impediu a regressão luteal precoce, não aumentou a concentração P4, mas foi capaz de aumentar a área e vascularização luteal (P<0,05) e elevar a taxa de concepção em 13.1% (P<0,05). O quinto

capítulo avaliou o efeito da hCG i.m. ou i.vag. no sétimo dia após a observação do estro oCL e aCL e a concentração de P4 em cabras leiteiras induzidas ao estro pelo protocolo de luz artificial. A hCG administrada por via intravaginal no sétimo dia após a observação do estro teve um efeito luteotrófico limitado e não aumentou a concentração de P4 em cabras leiteiras induzidas ao estro pelo protocolo de luz artificial.

Palavras-chave: hCG. Corpo lúteo original. Corpo lúteo acessório. Progesterona.

ABSTRACT

RODRIGUES, Juliana Nascimento Duarte, D.Sc., Universidade Federal de Viçosa, December, 2022. **Effects of hCG administered by different routes and days of the estrous cycle on the reproductive efficiency of dairy goats**. Adviser: Jeferson Ferreira da Fonseca. Co-advisers: José Domingos Guimarães and Erick Fonseca de Castilho.

Human chorionic gonadotropin (hCG) can be used at different times of the estrous cycle in goats, with the aim of improving reproductive parameters. This study aimed to evaluate the effects of hCG administered at different times of the cycle and by different routes on the reproductive parameters of dairy goats. The first chapter aimed to evaluate the effects of hCG administered 7 days after the onset of synchronized estrus on the original corpus luteum (CL) and on the accessory CL (aCL), as well as on plasma P4 concentrations and fertility in inseminated dairy goats. artificially during the non-breeding season. The treatment resulted in the formation of aCL and had a hypertrophic effect on the existing (ovulatory) CL, in addition to elevating ($P<0.05$) plasma P4 concentrations and the pregnancy rate by 22.5%. The second chapter aimed to assess whether hCG administered intramuscularly ($hCG_{i.m.}$) or intravaginally ($hCG_{i.vag.}$) can be detected by a rapid β-hCG test in blood plasma samples and to document the ovarian effects of hCG administered by both routes at the time of artificial insemination (AI) in goats. hCG could be consistently detected in blood samples using the β-hCG rapid test in the $hCG_{i.m}$ group only. and there were no differences ($P>0.05$) in mean pregnancy rate, circulating P4 concentrations, and various luteal parameters studied between Control, $hCG_{i.m.}$ and $hCG_{i.vag.}$ The third chapter aimed to evaluate the effects of hCG administration on days 5 or 7 after estrus observation on oCL and aCL, in addition to plasma P4 concentration and pregnancy rate in dairy goats in the non-breeding season. hCG promoted the growth of oCL ($P<0.05$), induced the formation of aCL, increased the concentration of P4 ($P<0.05$) and presented similar results between the groups that received hCG, with the use on the seventh day being more effective in inducing of aCL than on the fifth day. The fourth chapter evaluated the effect of hCG i.vag. administered at the time of AI on ovulatory parameters, dynamics and luteal vascularization, circulating P4 concentrations and pregnancy rate of goats in the breeding season. Treatment did not alter ovulatory parameters, did not prevent early luteal regression, did not increase P4 concentration, but was able to increase luteal area and vascularity ($P<0.05$) and raise the conception rate by 13.1% ($P<0.05$). The fifth chapter evaluated the effect of hCG i.m. or i.vag. on the seventh day after the observation of estrus

oCL and aCL and the concentration of P4 in dairy goats induced to estrus by the artificial light protocol. hCG administered intravaginally on the seventh day after estrus observation had a limited luteotropic effect and did not increase P4 concentration in dairy goats induced to estrus by the artificial light protocol.

Keywords: hCG. Original corpus luteum. Accessory corpus luteum. Progesterone.

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SUMÁRIO

1. INTRODUÇÃO

A atividade leiteira tem grande impacto econômico e social no Brasil e a caprinocultura é de suma importância em países em desenvolvimento. Raças caprinas, como a Saanen e a Alpina, são de origem europeia e possuem uma produção de leite rentável. No entanto, estas raças apresentam marcada estacionalidade reprodutiva no sudeste brasileiro e apresentam anestro na primavera (BALARO et al., 2019). Devido esta sazonalidade, o fornecimento de produtos lácteos caprinos ao consumidor é inconstante, o que prejudica a cadeia produtiva (CHEMINEAU et al., 2007). O uso de protocolos hormonais é uma ferramenta comumente utilizada para induzir o estro sincronizado (CARVALHO-DE-PAULA et al., 2020; FONSECA et al., 2017).

A produção de P4 é inferior após estro induzido na estação não reprodutiva que em fêmeas cíclicas em ovinos (RHIND; CHESWORTH; ROBINSON, 1978) e caprinos (BŁASZCZYK; UDAŁA; GĄCZARZEWICZ, 2004). No entanto, um limiar mínimo de progesterona (P4) no sangue é necessário para que ocorra o estabelecimento da gestação em mamíferos (SPENCER et al., 2004) e a produção inadequada é a causa de 30-40% das mortes embrionárias em caprinos e ovinos (D'ALESSANDRO; MARTEMUCCI, 2016; LONERGAN, 2011; NANCARROW; HILL, 1995). Desta forma, protocolos hormonais que elevem a concentração de P4 durante o reconhecimento materno da gestação podem diminuir as perdas embrionárias em pequenos ruminantes. Na cabra, a progesterona é produzida principalmente pelo corpo lúteo durante a gravidez, sem que a placenta faça uma apreciável contribuição (LINZELL; HEAP, 1968), esta produção pode ser influenciada por administrações de gonadotrofinas exógenas como a coriônica humana (hCG; SCHMITT et al., 1996).

A hCG pode ser utilizada em diversos momentos do ciclo estral de cabras e ovelhas com o intuito de melhorar os parâmetros reprodutivos. Khan et al. (2003) administraram de 150 UI de hCG em ovelhas no momento do acasalamento e observaram aumento do comprimento cabeça-nádega, a largura do saco amniótico e o número de placentomas. No entanto, seu uso é mais comum durante a fase lútea, em que pode promover a formação de CL acessório (CÔRTES et al., 2021; FONSECA et al., 2018; VERGANI et al., 2020), aumento do CL original , elevação da progesterona circulante (P4) (FONSECA et al., 2006; VERGANI et al., 2020) e incremento nas taxas de gestação em pequenos ruminantes (AKIF CAM; KURAN, 2004; CÔRTES et al., 2021; GÓMEZ-BRUNET et al., 2007; KHAN; BECK; KHALID, 2009).

A via intravaginal é bastante utilizada na medicina veterinária para administração de progestágenos em ruminantes domésticos (RATHBONE; BURKE, 2013), mas também tem sido explorada como via alternativa para a aplicação de outros hormônios reprodutivos como oxitocina (PRELLWITZ et al., 2019) e estradiol (CHŁOPEK et al., 2008). Esta via é viável porque a parede vaginal contém uma densa rede de vasos sanguíneos capilares que são permeáveis aos hormônios lipossolúveis (ou seja, esteroides) e hidrofílicos/peptidérgicos (HUSSAIN; AHSAN, 2005). Além disso, a deposição intravaginal reduz o metabolismo hepático e a depuração dos hormônios, pois eles desviam do "metabolismo de primeira passagem pelo fígado" para o "efeito de primeira passagem uterina", em que um alto grau de transporte direto da vascularização da vagina para o útero e o ovário (BULLETTI et al., 1997).

2. REVISÃO DE LITERATURA

2.1. Corpo lúteo

O corpo lúteo (CL) é uma glândula endócrina transitória que tem como principal função a manutenção da gestação por meio da produção de progesterona, no entanto, é uma glândula dinâmica que apresenta variações de tamanho, estrutura e atividades esteroidogênicas em diferentes fases do ciclo estral e gestação (FIELDS; FIELDS, 1996). O CL é a continuação da maturação folicular e é formado a partir das células da granulosa e teca interna, que dão origem as grandes e pequenas células luteais, respectivamente. Quando em sua fase ativa, ele é altamente vascularizado (BALARO et al., 2017) e secreta progesterona como o principal hormônio esteroide, além de pequenas quantidades de 17β-estradiol, prostaglandinas e vários hormônios peptídicos, como relaxina, ocitocina, neurofisina-I relacionada à ocitocina, vasopressina e inibina (FIELDS, 1991).

A formação do corpo lúteo ocorre a partir do folículo ovulatório e esta transição envolve diversos mecanismos similares a cicatrização de feridas e formação tumoral. Durante a formação do CL, diversas células sofrem hiperplasia, hipertrofia e/ou migração, além de um intenso processo angiogênico. A preparação para a formação luteal ocorre ainda antes da ovulação. Sabe-se que a luteinização e produção de progesterona pode preceder a ovulação em vacas (KESLER et al., 1981) e ovelhas (MURDOCH; DUNN, 1983) e mecanismos associados a luteinização não dependem da ovulação, assim como a ovulação não garante uma formação e um desenvolvimento luteal normal.

A vida útil do corpo lúteo é marcada por extensa remodelação tecidual e envolve vários processos celulares, incluindo proliferação, migração, diferenciação e apoptose. A ovulação, o desenvolvimento e a luteólise do corpo lúteo são acompanhados por uma extensa remodelação da matriz extracelular (MEC). A ovulação é caracterizada pela degradação localizada da MEC no ápice da parede folicular pré-ovulatória. Após a ruptura do estigma, as células foliculares remanescentes são então reorganizadas em um corpo lúteo (SMITH et al., 2002).

Após a ovulação, a formação do corpo lúteo ocorre devido a vascularização da membrana granulosa, antes avascularizada, simultaneamente com a degeneração da lâmina basal sob a influência de vários fatores angiogênicos (REYNOLDS; REDMER, 1998). Estudo in vitro demonstrou que o LH aumenta a atividade angiogênica em tecidos lúteos, enquanto que a PGF2α bloqueia esta ação do LH (REDMER et al., 1988). O processo de ovulação, com as células da teca degradando a membrana basal folicular e invadindo a camada granulosa envolve as MMPs (WOESSNER et al., 1989). Os capilares sanguíneos formados, invadem as células da granulosa e formam uma extensa rede no interior do CL da cabra (SHARMA; SHARMA, 1998).

Durante a luteinização, os contatos intercelulares entre as células granulosas lúteas em diferenciação são restabelecidos e o epitélio folicular é reorganizado. O LH modula as junções comunicantes (NISWENDER et al., 1985), que estão presentes entre as pequenas células luteais e as grandes células luteais de cabra (SHARMA; SHARMA, 1998) e podem ser importantes para a regulação do crescimento lúteo, diferenciação e regressão no CL na vaca (GRAZUL-BILSKA et al., 1996).

A ação do LH pré ovulatório promove a diferenciação das células foliculares em células lúteas produtoras de progesterona. Estas células começam a expressar um novo conjunto de moléculas para o novo ambiente hormonal, desta forma, o fenótipo final da célula luteal depende de uma combinação específica de genes que codificam proteínas regulatórias, como receptores, fatores de transcrição e proteínas de sinalização, que garantem a expressão apenas dos genes necessários para a função da célula luteal. Uma das mudanças mais importantes durante a luteinização é a alteração na responsividade celular aos sinais externos, permitindo que as células luteais respondam a um novo conjunto de hormônios.

Uma vez que a luteinização ocorre, uma determinada população de células sofre extensa hipertrofia e se diferencia em grandes células luteais esteroidogênicas, enquanto outra população de células permanece muito menor e compreende pequenas células luteais esteroidogênicas. É de grande aceitação que as pequenas células lúteas do CL sejam derivadas

das células da teca do folículo, enquanto que as células grandes são originadas das células da granulosa. Além disso, pequenas células luteais também são transformadas em grandes células luteais à medida que a idade do CL aumenta durante o ciclo estral e a gestação em ovelhas (FITZ et al., 1982) e vacas (ALILA; HANSEL, 1984). As células luteais grandes secretam várias vezes mais progesterona basal por célula do que as células luteais pequenas (WEBER et al., 1987); no entanto, estes últimos apresentam maior resposta à estimulação de LH devido à presença de maior número de receptores de LH em sua membrana (FITZ et al., 1982).

Na cabra, as células luteais grandes possuem 19-25 µm de diâmetro com núcleo localizado centralmente, de aproximadamente 8 µm e nucléolo proeminente, enquanto que as células luteais pequenas possuem aproximadamente 10 µm de diâmetro com núcleo arredondado de aproximadamente 7 µm e nucléolos distintos (AZMI; BONGSO, 1985). Aproximadamente 60% de todas as células nucleadas são células endoteliais e/ou periócitos. As células luteais representaram 27.5% das células contadas, nas quais cerca de dois terços são células luteais pequenas (17.5% do total) e um terço células luteais grandes (10% do total), além de fibroblastos que representam cerca de 10% do corpo lúteo (AZMI; BONGSO, 1985).

Ao avaliar por ultrassonografia a dinâmica luteal durante o ciclo estral em cabras leiteiras, Balaro et al. (2017) observaram que o volume, diâmetro e área lútea aumentaram ao decorrer dos dias e os valores estabilizaram a partir do 9º dia do ciclo (1,24 ± 0,38 cm3; 13,2 ± 1,5 mm; 160,9 ± 40,4 mm2).

Na cabra, a progesterona é produzida principalmente pelo corpo lúteo durante a gravidez, sem que a placenta faça um apreciável contribuição (LINZELL; HEAP, 1968). Ovariectomia bilateral em qualquer fase da gravidez faz a concentração plasmática de progesterona cair e os animais abortam subsequentemente (THORBURN; SCHNEIDER, 1972). Administração de uma dose luteolítica do análogo da prostaglandina provoca aborto em cabras em diversas fases da gestação (HOLST; NANCARROW, 1975). Desta forma, a progesterona ovaria é essencial para a manutenção da gestação em cabras.

Durante o ciclo estral de cabras leiteiras, as concentrações de P4 diferiram ao longo do período de luteogênese e aumentaram no 8º dia (12,3 ± 3,1 ng/mL), com valores máximos no 14º dia (14,9 ± 2,2 ng/mL). A concentração de P4 está diretamente associada ao número de CL ativos (BALARO et al., 2017; RODRIGUES et al., 2022; VERGANI et al., 2020).

Nos animais, a angiogênese não associada a processos patológicos ocorre no interior dos ovários, no útero, nas glândulas mamárias e na placenta. A alta proporção de células

endoteliais ou pericitos indica a alta vascularização dos tecidos lúteos (SHARMA; SHARMA, 1998). O pico de LH inicia uma cascata de eventos para o desenvolvimento luteal, incluindo a migração de células endoteliais (SMITH et al., 2002). As células endoteliais capilares constituem aproximadamente 10% do volume do CL da cabra, mas representam aproximadamente 50% do número total de células. Após a dissociação, essas células têm aproximadamente 10 μm de diâmetro. No entanto, in vivo essas células têm uma forma longa e esbelta e revestem o lúmen dos vasos sanguíneos no CL. Os fibroblastos infiltram-se no CL após a ruptura da membrana basal durante a ovulação e a luteinização e têm uma função luteal desconhecida (SHARMA; SHARMA, 1998). A taxa de crescimento vascular luteal é maior no início do ciclo estral e o CL atinge a máxima vascularização no dia 10 com manutenção desta vascularização até que ocorra a luteólise em cabras não gestantes (BALARO et al., 2017; RODRIGUES et al., 2022).

Malecki et al. (1987) sugeriram que a cabra não necessita de LH hipofisário materno para manter a prenhez entre os dias 50 e 130 de gestação e concluíram que a função do CL pode ser mantida por luteotrofinas ou por um agente antiluteolítico produzido pelos componentes uterinos.

A regressão do CL em cabras é ocasionada pela liberação uterina de PGF2α e está associada com a regressão dos vasos sanguíneos, um aumento no número de corpos lipídicos, lisossomos, surgimento de corpos autofagocitários e diminuição da massa celular luteal (PATE, 1994). Mecanismos propostos para explicar os efeitos luteolíticos do PGF2α incluem uma diminuição rápida e dramática no fluxo sanguíneo luteal, uma diminuição no número de receptores de LH, um desacoplamento dos receptores de LH da adenilato ciclase e um efeito citotóxico (KNICKERBOCKER; WILTBANK; NISWENDER, 1988). Entre os mecanismos moleculares de luteólise mais aceitos está a apoptose (JUENGEL, 1993; SAWYER et al., 1990), em que ocorre a ativação de endonucleases de restrição dependentes de Mg2+ e Ca2+ no interior das células que clivam o DNA nuclear em oligonucleotídeos de 185pb (ARENDS; MORRIS; WYLLIE, 1990; SHARMA, 2000).

O CL é uma glândula dinâmica que secreta progesterona como hormônio principal e outros hormônios em quantidades mínimas. Em ruminantes, os mecanismos envolvidos na formação, funcionamento e luteólise do corpo lúteo são muito complexos e não são totalmente compreendidos. O esclarecimento desses mecanismos pode levar a métodos aprimorados para induzir e/ou sincronizar o estro na estação reprodutiva ou não reprodutiva, reduzir a regressão luteal precoce e a taxa de gestação em cabras.

2.2. Gonadotrofina Coriônica humana: síntese, secreção, metabolismo e receptores

A gonadotrofina coriônica humana (hCG) é um hormônio proteico produzido pelo sinciciotrofoblasto da placenta de mulheres (HANDSCHUH et al., 2007). É uma glicoproteína complexa composta por 244 aminoácidos com massa molecular de 36,7 kDa e formada por duas subunidades glicosiladas que estão associadas de forma não covalente. A subunidade α é idêntica a cadeia α dos hormônios gonadotrofínicos hipofisários: hormônio luteinizante (LH; SAIRAM; PAPKOFF; LI, 1972), hormônio folículo-estimulante (FSH) e hormônio estimulante da tireóide (TSH), contém 92 aminoácidos com dois sítios de N-glicosilação. A subunidade β de hCG contém 145 aminoácidos e é rica em resíduos de prolina no domínio carboxil terminal, o que lhe confere especificidade biológica para interação com seu receptor.

A hCG é caracterizada por ter uma meia-vida biológica estendida comparada ao LH (Yen 1968). Em mulheres, o desaparecimento do hCG sérico segue um decaimento biexponencial após o parto, que é caracterizado por uma queda precoce e rápida de hCG (T0,5 = 6 h) durante as primeiras 72 h, seguida por um desaparecimento tardio e lento (T0,5 = 36 h) do hormônio (COLE, 2015; NISULA et al., 1989), assim, a meia vida biológica do hormônio em mulheres é considerada de 36 h. Estudos da meia vida deste hormônio após aborto induzido e espontâneo ou gravidez ectópica removida mostraram um padrão semelhante (STEIER; BERGSJO; MYKING, 1984).

De acordo com Wehmann e Nisula (1981), em humanos as subunidades da hCG dissociadas são biologicamente inativas ao receptor LH/hCG e são eliminados da circulação muitas vezes mais rapidamente que a hCG, por terem uma meia-vida menor (hCGβ = 41.2 ± 1.7 min; hCGα = 13.0 ± 0.9 min). O hCG é depurado diretamente pelo rim ou fígado, sendo este último responsável por limpar 78% das moléculas relacionadas a hCG (COLE, 2015; NISULA et al., 1989).

Quanto a ruminantes, em bovinos, durante a fase inicial rápida, a hCG possui meia vida de 5 a 9 h e, durante a segunda fase de liberação lenta, há uma meia vida de 1 a 1.3 dias (SCHMITT et al., 1996). Saleh et al. (2012) estudaram a farmacocinética da hCG administrada por via intramuscular em cabras adultas Boer e, além da meia vida biológica de 39,4 ± 5,1 h, observaram tempo de latência de 0,4±0,1 h; constante da taxa de absorção de 0,34 ± 0,002 h; meia-vida de absorção de 2,7 h; constante da taxa de eliminação de 0,02 ± 0,002 h; e volume aparente de distribuição de 16,9 ± 4,3 L/h. O perfil plasmático de hCG em cabras foi caracterizado por absorção rápida (11,6 h) e eliminação lenta (70,0 h) e este padrão

se assemelha ao de bovinos (SCHMITT et al., 1996) e humanos (CHAN et al., 2003; MIDGLEY; JAFFE, 1966).

As subunidades β de LH e hCG estão intimamente relacionados em sequência, e esses dois hormônios se ligam ao mesmo receptor e provocam respostas biológicas semelhantes. A resposta imediata das células-alvo à ligação de LH e hCG é um aumento na atividade da adenilil ciclase mediada por proteínas G intracelulares associadas à membrana. O aumento resultante no AMPc, em última análise, leva a um aumento na síntese e secreção de esteroides (HUNZICKER-DUNN; BIRNBAUMER, 1985).

No ovário, o LH promove a maturação das células foliculares. Após o efeito indutor inicial do FSH na expressão de LHR nos folículos pequenos e médios, o LH aumenta os estágios subsequentes de desenvolvimento folicular e esteroidogênese nas células da granulosa e lúteas (RICHARDS; HEDIN, 1988). Nos ovários de cabras, os receptores de LH/hCG são encontrados em corpos lúteos e células tecais de folículos maduros, enquanto os folículos imaturos são praticamente desprovidos destes receptores (GNANAPRAKASAM; GUPTA; TALWAT, 1976). No entanto, o gene LHR é expresso em uma variedade de tecidos e órgãos não gonadais. Wang et al. (2012) investigaram a presença de LHR em órgãos não gonadais em ovelhas e encontrou este receptor no cérebro (hipófise, medula oblonga, bulbo olfatório e hipotálamo), órgãos digestórios (rúmen, retículo, omaso, abomaso, duodeno, jejuno, íleo, ceco, cólon, reto, pâncreas e fígado) e órgão urogenitais (rin, oviduto e útero). Esta expressão foi alterada em cada órgão de acordo com a fase do ciclo estral e essa mudança em alguns tecidos foi significativamente correlacionada com a expressão dos receptores do hipotálamo, hipófise, oviduto ou útero (WANG et al., 2012).

Mishra et al. (2002) identificou a presença de receptores de LH em oócitos, embriões de oito células, dezesseis células células e blastocistos bovinos, no entanto, não verificou melhora do desenvolvimento embrionário quando adicionada a hCG no meio de cultura. O efeito dessa ligação é desconhecido, mas um tema comum na ação do LH fora das gônadas em todas as espécies é a regulação positiva da síntese de prostaglandinas.

Fields e Shemesh (2004) revisaram a distribuição de receptores LHR em tecidos reprodutivos extragonaidais em mamíferos e relataram a presença deste receptor no oviduto, endométrio, útero intacto, miométrio, vasos uterinos e cérvix (tabela 1). Importante enfatizar a produção de glicoproteína ovidutal (OGP) e protaglandinas em tecidos extragonaidais sob ação do LH/hCG.

As prostaglandinas são essenciais para o alongamento do concepto, pois as infusões intrauterinas de meloxicam impediram o alongamento do concepto em ovelhas prenhes

(DORNIAK; BAZER; SPENCER, 2011; SIMMONS et al., 2010). Os conceptos alongados de ovelhas e bovinos sintetizam e secretam mais PG do que o endométrio subjacente (LEWIS, 1989; LEWIS et al., 1982; LEWIS; WATERMAN, 1983).

A ativação do LHR ovidutal bovino resulta em um aumento da síntese de glicoproteína ovidutal (SUN; LEI; RAO, 1997), que se liga aos embriões para aumentar seu desenvolvimento (NANCARROW; HILL, 1995). Mishra et al. (2002) observaram que no tratamento com hCG em coculturas com células epiteliais do oviduto aumentou ainda mais o desenvolvimento embrionário em blastocistos e a inibição da síntese de OGP, e a prevenção da ativação da proteína quinase A bloquearam o efeito de hCG em coculturas (MISHRA; LEI; RAO, 2002). Desta forma, o estudo sustenta a evidência de que a ação da hCG aumenta a síntese de OGP, que provavelmente aumenta o crescimento e desenvolvimento embrionário precoce. Khan et al. (2003) relataram que o uso de 150 UI de hCG em ovelhas no dia da cobertura melhorou o comprimento do concepto e este achado pode está associado a ação do hormônio no desenvolvimento embrionário precoce.

A presença de LHR em várias células do oviduto e as mudanças na quantidade de receptores dependem do estado hormonal, indicando que o LH pode regular diretamente a função tubária. Em estudo sobre a expressão do RNAm do LHR no oviduto de cabras, Li et al. (2009) verificaram que a expressão do mRNA do LHR foi diferente nas quatro fases do ciclo estral. O RNAm do LHR no infundíbulo foi maior durante o estro, na ampola, foi mais baixa durante o metaestro e, no istmo, foi maior durante o proestro.

As ações da hCG sobre o todo o trato reprodutor feminino (ovidutos, miométrio, endométrio, colo do útero e vasos sanguíneos) é bem evidente. Estudos sobre a sua farmacocinética e que relatam a presença e ação de receptores LHR em diversas espécies, inclusive em pequenos ruminantes, tem auxiliado para melhor entendimento do mecanismo de ação deste hormônio em diferentes órgãos e em diferentes momentos do ciclo estral. Desta forma, a hCG pode ser utilizada em uma gama de estratégias a fim de melhorar os índices reprodutivos de cabras e ovelhas.

Tabela 1 – Distribuição de LHRs e sua função em tecidos não gonadais.

Tecido	Espécie com LHRs	Resposta fisiológica e/ou segundo mensageiro	Produto	Referência
Oviduto	Bovino	↑ contrações	Endotelina-1, glicoproteína ovidutal, PGE_2, $PGF_{2\alpha}$	(MISHRA; LEI; RAO, 2002; SUN; LEI; RAO, 1997; WIJAYAGUNAWARDANE; GABLER; ACOSTA, 2001; WIJAYAGUNAWARDANE; MIYAMOTO; SATO, 1999)
	Humano		PGE_2, PGHS-1e -2, 5-lipoxigenase	(HAN; LEI; RAO, 1996; LEI et al., 1993)
	Suíno	↑ relaxamento		(DERECKA et al., 1995; GAWRONSKA et al., 1999; GAWRONSKA; STEPIEN; ZIECIK, 2000a, 2000b)
	Rato			(ZHENG et al., 2001)
	Peru			(YOU et al., 2000)
Endométrio	Bovino	Indução LHR, Gsα, AMPc, hidrólise de fosfato de inositol	PGHS-2, PGFα	(FREIDMAN; GUREVICH; SHEMESH, 1995; SHEMESH et al., 2001)
	Suíno		PGHS-2, PGE_2 (gestação), PGF2α (ciclo)	(DERECKA et al., 1995; STEPIEN et al., 2000; STEPIEN; SHEMESH; ZIECIK, 1999; ZIECIK; KOTWICA, 2001; ZIECIK; STANCHEV; TILTON, 1986; ZIECIK; STEPIEN; GAWRONSKA, 2000)
	Ovino			(WEEMS et al., 2003)

Útero intacto	Rato		PGE_2, $PGF_{2\alpha}$	(ZHENG et al., 2001)
	Humano	AMPc, PKA, ERK1/2	PGHS-2 e PGE_2	(HAN; LEI; RAO, 1997; LIN et al., 2003; RESHEF et al., 1990; SRISUPARP et al., 2003)
	Baboino	ERK1/2	PGE_2	(SRISUPARP et al., 2003)
	Ramister	AMPc		(BONNAMY; BENHAIM; LEYMARIE, 1989; DERECKA et al., 1995; JENSEN; ODELL, 2016; SAWITZKE; ODELL, 1991)
	Peru			(YOU et al., 2000)
Miométrio	Bovino		PGE_2	(SHEMESH, 2001)
	Suíno	↑ relaxamento		(RZUCIDLO; WEIGL; TILTON, 1998; ZIECIK et al., 1991, 1993; ZIECIK; GOLBA; KISIELEWSKA, 1996; ZIECIK; JEDLINSKA; RZUCIDLO, 1992; ZIECIK; STANCHEV; TILTON, 1986; ZIECIK; STEPIEN; GAWRONSKA, 2000)
Veias uterinas	Humano	PKA, ↑ densidade de células musculares		(KÖRNYEI et al., 1994; RESHEF et al., 1990; ZUO; LEI; RAO, 1994)
	Bovino		PGHS-2, PGE_2, $PGF_{2\alpha}$	(MIZRACHI; SHEMESH, 1999)
	Suíno	Aumento do fluxo sanguíneo		(ZIECIK et al., 1995; ZIECIK; GOLBA; KISIELEWSKA, 1996)

Cérvix	Humano	Promoção de neovascularização	PGHS-1 e -2, PGF2α, inibe PGE_2, thromboxano B_2	(LEI; RESHEF; RAO, 1992; TOTH et al., 1994; ZONDEK; SULMAN; BLACK, 1945; ZYGMUNT et al., 2002)
	Bovino	AMPc, hidrólise de fosfato de inositol	PGHS-2, PGE_2	(MIZRACHI; SHEMESH, 1999)
	Suíno			(STEPIEN et al., 2000)
	Humano	AMPc	PGHS-2, PGE_2	(LIN et al., 2003)

Fonte: Fields e Shemesh (2004).

2.3. Mecanismo de ação da hCG sobre a eficiência reprodutiva

Em protocolos de indução de estro de cabras na estação não reprodutiva, a indução é feita principalmente com o uso de dispositivos vaginais contendo progesterona (CIDR) ou progestágenos (esponjas) por vários dias. O uso de CIDR isoladamente promove a renovação folicular e o surgimento de uma nova onda folicular nos 3 dias seguintes à inserção do dispositivo, com sucesso na indução do estro e ovulação (VILARIÑO; RUBIANES; MENCHACA, 2011). No entanto, apenas o uso de P4/progestágenos não oferece uma boa sincronização do estro/ovulação e, por isso, para a indução de estro sincronizada é essencial o uso de gonadotrofinas que, nesta situação, são utilizadas como indutores de ovulação, promovendo uma maior sincronia no estro e ovulação destes animais. Assim, a indução de estro sincronizada é feita principalmente com o uso de dispositivos vaginais contendo P4/progestágenos de 6 a 18 dias (CARVALHO-DE-PAULA et al., 2020; GREYLING; NIEKERKT; GROBBELAAR, 1985) combinados com prostaglandinas e gonadotrofinas (FONSECA et al., 2005b).

Quando utilizada neste momento, o mecanismo de ação da hCG ocorre sobre o crescimento final dos folículos, ocasionando uma melhor sincronização de estro e ovulação e podendo levar a múltiplas ovulações (CHEMINEAU et al., 1988). Sugere-se ainda que este hormônio poça agir posteriormente sobre o corpo lúteo formado. Há relatos de receptores de LH/hCG (LHR) no oócito de bovinos (MISHRA; LEI; RAO, 2003), no entanto ação que ocorre com esta ligação precisa ser melhor estudada.

Em ovelhas, o uso de hCG no início do estro promoveu um aumento no número de CL e elevou as taxas de parição e prolificidade durante a estação de reprodução (KILLEEN; MOORE, 1970). No entanto, em cabras, o uso de 250 UI de hCG no dia do início do estro após protocolo de duas doses de PGF2α na estação reprodutiva não promoveu melhora na sincronização do estro e ovulação (ESTEVES et al., 2013), necessitando de mais estudos para identificar o melhor momento e dose de utilização deste hormônio na estação reprodutiva associado ao protocolo de duas doses de PGF2α, além dos posteriores efeitos luteotróficos e embriotróficos que não foram explorados esse trabalho.

Quando utilizado no momento do serviço, a ação da hCG ocorre como auxílio no momento do pico pré-ovulatório de LH, que pode melhorar a maturação folicular, com consequente melhoria na qualidade oocitária e posterior viabilidade embrionária, podendo também promover ovulações múltiplas, além de ter uma ação luteotrófica no CL a ser formado (SCHMITT et al., 1996a) e melhorar o desenvolvimento embrionário precoce. A

ativação do LHR ovidutal bovino resulta em um aumento da síntese de glicoproteína ovidutal (OGP; SUN; LEI; RAO, 1997), que se liga aos embriões para aumentar seu desenvolvimento (NANCARROW; HILL, 1995). Mishra et al. (2003) observaram que no tratamento com hCG em coculturas com células epiteliais do oviduto aumentou ainda mais o desenvolvimento embrionário em blastocistos e a inibição da síntese de OGP, e a prevenção da ativação da proteína quinase A bloquearam o efeito de hCG em coculturas (MISHRA; LEI; RAO, 2002). Desta forma, o estudo sustenta a evidência de que a ação da hCG aumenta a síntese de OGP, que provavelmente aumenta o crescimento e desenvolvimento embrionário precoce. A administração de 150 UI de hCG no dia do acasalamento foi estudado em cordeiras e melhorou o crescimento do concepto, a placentação e a taxa de prolificidade (KHAN et al., 2003).

A hCG também pode ser utilizada por dias consecutivos após remoção da esponja com intuito de evitar a regressão luteal precoce em cabras submetidas a protocolos de superovulação. Kelidari et al. (2010) observaram que a aplicação de 500 UI de hCG nos dias 0 e 1 ou 0, 1 e 2 após a remoção da esponja previne a regressão luteal precoce e melhora a função lútea em cabras após tratamento superovulatório com eCG. Neste caso, a prevenção da regressão luteal precoce pode ter ocorrido devido ao aumento do fluxo sanguíneo para os CLs por ação da hCG, visto que Farin et al. (1988) verificaram um aumento de células vasculares em CLs de ovelhas tratadas com LH em comparação ao grupo controle, além da sua ação luteotrófica sobre os receptores de LH (LHR) do CL (SAHARREA et al., 1998).

Diversos efeitos positivos já foram relatados após o uso da hCG na fase luteal de pequenos ruminantes. A tabela 1 destaca estes efeitos e o mecanismo de ação pelo qual a hCG promove tais resultados em ovelhas e cabras.

Devido sua similaridade com o LH, a hCG age sobre os folículos antrais presentes com receptores LHR e pode induzir a formação de corpos lúteos acessórios (Cla), efeito observado em cabras (CÔRTES et al., 2020; RODRIGUES et al., 2022) e ovelhas (AZARI et al., 2020; CATALANO et al., 2015; COLESON et al., 2015; FARIN et al., 1988; FONSECA et al., 2018; GAMBONI et al., 1984; KITTOK; STELLFLUG; LOWRY, 1983; NEPHEW et al., 1991; VERGANI et al., 2020). O mecanismo corre por meio da estimulação da ruptura folicular e luteogênese ou pela luteinização de folículos antrais (DRIANCOURT, 2001). Assim, a hCG pode aumentar a área luteal, promovendo uma elevação na produção de P4.

O estabelecimento da gestação em ruminantes começa no estágio de concepto e inclui sinalização de reconhecimento de prenhez, implantação e placentação (SPENCER et

al., 2007; SPENCER; SANDRA; WOLF, 2008). Como revisado por Hue (2012), o embrião em estágio de mórula entra no útero nos dias 4 a 6 após o acasalamento e então forma um blastocisto. Após a eclosão da zona pelúcida (dias 8 a 10), o blastocisto cresce lentamente para uma forma tubular ou ovoide e é então denominado concepto (embrião e membranas extraembrionárias associadas). Em ovelhas, o concepto ovóide de cerca de 1 mm de comprimento no dia 11, começa a se alongar no dia 12 e forma um concepto filamentoso de 15 a 19 cm ou mais de comprimento no dia 15. Sucessivamente, o concepto alongado inicia o processo de implantação central e placentação por volta do dia 16 em ovelhas (SPENCER et al., 2007).

A P4 ovariana induz a expressão de vários genes, especificamente no epitélio endometrial, que são então estimulados por fatores do concepto (por exemplo, IFNγ, prostaglandinas e cortisol), bem como pelo próprio endométrio (por exemplo, prostaglandinas e cortisol) (DORNIAK; BAZER; SPENCER, 2013). Os genes e funções regulados por esses hormônios e fatores no epitélio endometrial provocam mudanças específicas no histotrofo, como um aumento de aminoácidos selecionados, glicose, citocinas e fatores de crescimento, que são necessárias para o alongamento do concepto (BAZER et al., 2010; DORNIAK; BAZER; SPENCER, 2011; SPENCER et al., 2007; SPENCER; SANDRA; WOLF, 2008).

Tabela 2 – Resumo dos efeitos e mecanismos de ação da administração de Gonadotrofina Coriônica humana (hCG) durante a fase lútea relatados sobre o trato reprodutivo de ovelhas e cabras.

Efeito observado	**Mecanismo de ação da hCG**	**Relatos em ovelha (autores)**	**Relatos em cabra (autores)**
↑ número de CL/ indução de CLa	Ação sobre o crescimento final dos folículos e promoção da ovulação/ luteinização devido sua ação similar ao hormônio luteinizante (LH)	Cam e Kuran (2004); Coleson et al. (2015); Fonseca et al. (2018); Khan et al. (2003); Vergani et al. (2020)	Côrtes et al. (2020); Rodrigues et al. (2022)
↑ número de células grandes/ ↑ proporção células grandes: pequenas	Ação sobre o LHR das células luteais pequenas, promovendo uma posterior conversão destas em células luteais grandes	Farin et al. (1988); Gamboni et al. (1984)	---
↑ peso ou área luteal	A conversão de células luteais pequenas em células luteais grandes, além do aumento de fibroblastos e tecido endotelial, promovem o aumento do tamanho e peso dos CLs	Cam e Kuran (2004); Fonseca et al. (2018); Nephew et al. (1994); Vergani et al. (2020)	Bustamante-Andrade et al. (2021); Rodrigues et al. (2022)
↑ vascularização do CL	Regula a expressão de fatores pró-angiogênicos, como fator de crescimento endotelial vascular (VEGFA) e angiopoietina um (ANGPT1)	Farin et al. (1988)	Rodrigues et al. (2022)
↑ [P4]	Aumento da área luteal pela indução de Cla ou aumento do tamanho do Clo; Ação da hCG sobre os receptores LHR do CL, encontrado em maior quantidade nas células luteais pequenas, que promove um aumento na produção de P4 por essas células e posterior conversão destas células em células luteais grandes, maiores produtoras de P4. A hCG também aumenta a vascularização luteal, fornecendo maior substrato para a esteroidogênese	Azari et al. (2020); Catalano et al. (2015); Coleson et al. (2015); Farin et al. (1988); Fonseca et al. (2018); Gamboni et al. (1984); Kittok et al. (1983); Nephew et al. (1991); Vergani et al. (2020)	Fonseca et al. (2005, 2006); Lashari e Tasawar (2011); Rodrigues et al. (2022)

↑ [proteína total]	O aumento da produção de P4 pelos CLs, melhora o ambiente uterino, com maior síntese de proteínas pelo endométrio	Nephew et al. (1994)	---
↑ comprimento do concepto	A ação luteotrófica que promove um aumento na produção de P4, promove um melhor ambiente uterino que favorece o crescimento do embrião e/ou ação sobre os LHR no oviduto, estimulando produção de OGP, que se liga ao embrião ocasionando um melhor desenvolvimento	Cam e Kuran (2004); Catalano et al. (2015); Fernandez et al. (2019); Khan et al. (2003); Nephew et al. (1994)	Lashari e Tasawar (2011)
↑ [IFNγ]	O melhor desenvolvimento do concepto, gerado por uma maior concentração de P4 e um melhor ambiente uterino e/ou ação sobre os LHR no oviduto, estimulando a produção de OGP. Ambos promovem um melhor desenvolvimento embrionário, que favorece uma maior produção de IFNγ	Nephew et al. (1994)	---
↑ taxa de gestação, taxa de fecundidade e taxa de prolificidade	Por meio dos mecanismos supracitados, a hCG promove uma redução na perda embrionária	Azari et al. (2020); Catalano et al. (2015); Khan et al. (2003); Kittok et al. (1983); Nephew et al. (1994); Vergani et al. (2020)	Bustamante-Andrade et al. (2021); Côrtes et al. (2020); Fonseca et al. (2005); Rodrigues et al. (2022)

P4: progesterona; IFNγ: interferon-tau; CL: corpo lúteo; CLa: corpo lúteo acessório; CLo: corpo lúteo original; LHR: receptor de LH/hCG; OGP: glicoproteína ovidutal

Os corpos lúteos ovulatórios (Clo) atingem seu crescimento máximo no dia 9 em cabras (BALARO et al., 2017) e dia 12 em ovelhas (FIGUEIRA et al., 2015). Quando a administração de hCG ocorre antes desse momento, pode auxiliar no crescimento destes CLs. Rodrigues et al. (2022) comprovaram este aumento de área luteal, além da elevação da vascularização do Clo e na concentração de P4 a partir do dia 10 do ciclo estral em cabras que receberam 300 UI de hCG no dia 7 do ciclo. O mecanismo envolvido no aumento da área é a conversão de células luteais pequenas em células luteais grandes somado ao aumento de fibroblastos e células vasculares provocados pela hCG (FARIN et al., 1988). O aumento da vascularização provocada pela hCG ocorre devido este hormônio regular a expressão de fatores pró-angiogênicos, como fator de crescimento endotelial vascular (VEGFA) e angiopoietina um (ANGPT1) (SUGINO et al., 2000; WULFF et al., 2000). Enquanto que a elevação da P4, ocorre inicialmente pelo estímulo da hCG sobre os receptores LHR nas células luteais pequenas, que produzem mais P4 sob estímulo deste hormônio (FITZ et al., 1982) e posteriormente devido o aumento de área luteal e maior quantidade de células luteais grandes, que produzem P4 em maior quantidade que as células pequenas (FITZ et al., 1982).

A indução de Cla e a ação luteotrófica sobre o Clo promovem uma elevação na P4 plasmática de pequenos ruminantes (FONSECA et al., 2005a, 2006; RODRIGUES et al., 2022; VERGANI et al., 2020). A concentração plasmática de P4 é um fator essencial para o estabelecimento da gestação em mamíferos e a produção inadequada de P4 causa 30-40% das mortes embrionárias em pequenos ruminantes (D'ALESSANDRO; MARTEMUCCI, 2016; LONERGAN, 2011; NANCARROW, 1994).

Em ruminantes, interferon tau (IFNγ) é o sinal de reconhecimento da prenhez secretado pelo concepto em alongamento que atua no endométrio para inibir o desenvolvimento do mecanismo luteolítico (SPENCER et al., 2006). O IFNγ é secretado predominantemente pelo concepto em alongamento antes da implantação (ROBINSON et al., 2006). Nephew et al. (1994), ao estudar ovelhas que receberam ou não 100 UI de hCG no dia 11.5 do ciclo estral, lavaram os cornos e corpo do útero, quantificaram o IFNγ e proteína total e relataram um aumento significativo na produção destes fatores no grupo que recebeu hCG. Logo, a elevação da concentração de P4, ao melhorar o ambiente uterino, pode levar a um maior crescimento do concepto (KHAN; BECK; KHALID, 2007, 2009), que, por sua vez, secreta mais interferon-tau (IFNγ), sinalizando a gestação de forma mais eficaz (NEPHEW et al., 1994), o que reduz a mortalidade embrionária e consequentemente eleva a taxa de gestação em cabras (BUSTAMANTE-ANDRADE et al., 2021; CÔRTES et al., 2021;

RODRIGUES et al., 2022) e ovelhas (AZARI et al., 2020; CATALANO et al., 2015; KHAN et al., 2003; KILLEEN; MOORE, 1970; KITTOK; STELLFLUG; LOWRY, 1983; NEPHEW et al., 1994; VERGANI et al., 2020). Lashari e Tasawar (2010) ao estudarem o efeito de 300 UI de hCG administrado no dia 12 do ciclo estral em cabras, relataram um maior crescimento do concepto e uma redução de 17% na morte embrionária em cabras que receberam o tratamento.

2.4. Vias intramuscular e intravaginal de aplicação da hCG

A aplicação via intramuscular é o método de administração mais confiável e utilizado para alcançar níveis séricos previsíveis de hCG. No entanto, os níveis séricos podem ser inferiores aos níveis teciduais, o que pode influenciar no tempo e intensidade da resposta biológica do órgão-alvo. Visto que a hCG tem sua principal ação nos ovários (corpos lúteos e células tecais dos folículos maduros; GNANAPRAKASAM; GUPTA; TALWAT, 1976), além de possuir receptores nos ovidutos, útero e vagina (LI; WANG; WANG, 2009; SHEN et al., 2009; WANG et al., 2012), pode-se sugerir uma via de administração que favoreça a concentração e velocidade de ação deste hormônio no trato reprodutivo.

A rota intravaginal de administração é amplamente utilizada para a administração de P4/progestágenos em ruminantes (RATHBONE; BURKE, 2013) e tem sido explorada como uma nova alternativa a outros hormônios reprodutivos (CHŁOPEK et al., 2008; NETTO et al., 2019; PRELLWITZ et al., 2019). Essa via farmacológica de administração tem sido cada vez mais explorada porque contém uma densa rede de vasos sanguíneos, é permeável a proteínas e peptídeos (HUSSAIN; AHSAN, 2005), permite facilidade na aplicação de medicamentos, oferece disponibilidade imediata para circulação sistêmica, pois desvia do metabolismo de primeira passagem do fígado, além de ter um "primeiro efeito de passagem uterino", em que o hormônio administrado tem um alto grau de transporte direto da vagina para o útero (BULLETTI et al., 1997).

Einer-Jensen et al. (1993), observaram uma maior concentração de progesterona na artéria uterina de marrãs quando comparada com a concentração na artéria carótida após administração de P4 intravaginal. Resultados similares foram encontrados em mulheres quando comparada a concentração de P4 nas artérias uterina e radial (CICINELLI et al., 1998). Esse fenômeno indica a existência de um sistema de redistribuição local, que seria o “efeito de primeira passagem uterina” demonstrado por Bulletti et al. (1997).

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3. OBJETIVOS

3.1. Objetivo Geral]

Avaliar os efeitos da hCG administrada por diferentes vias e dias do ciclo estral sobre a eficiência reprodutiva de cabras leiteiras.

3.2. Objetivos específicos

- Estudar os efeitos da administração de 300 UI de hCG por via intravaginal no momento da inseminação artificial sobre parâmetros e dinâmica ovulatória e vascularização lútea, bem como concentrações circulantes de progesterona (P4) e taxa de gestação de cabras leiteiras.
- Avaliar se a hCG administrada por via intramuscular ou intravaginal no momento da IA pode ser detectada por um teste comercial rápido de β-hCG em amostras de plasma sanguíneo e se este teste pode ser adaptado como uma abordagem semi-qualitativa para detectar as concentrações de hCG no plasma sanguíneo após tratamento
- Estudar o efeito da administração de 300 UI de hCG pelas vias intramuscular ou intravaginal no sétimo dia após a observação do estro nos corpos lúteos originais e acessórios e a concentração de progesterona em cabras leiteiras induzidas ao estro pelo protocolo de luz artificial.
- Avaliar os efeitos da hCG administrada sete dias após o início do estro sincronizado no corpo lúteo pós-ovulação (oCL) e CL acessório (aCL), bem como nas concentrações plasmáticas de P4 e na fertilidade em cabras leiteiras inseminadas artificialmente durante a época de não reprodução
- Avaliar os efeitos da administração de 300 UI de hCG nos dias 5 ou 7 após a observação do estro sobre o número, área e vascularização dos corpos lúteos originais e acessórios, além da concentração plasmática de progesterona e taxa de gestação de cabras leiteiras induzidas por estro na estação não reprodutiva.

CAPÍTULO 1

Reproductive parameters and luteal dynamics of dairy goats treated with human Chorionic Gonadotropin applied intravaginally at the time of artificial insemination

ABSTRACT

Two experiments were carried out to evaluate the effects of administering 300 IU of hCG intravaginally (i.vag.) at the time of artificial insemination on ovulatory parameters, dynamics and luteal vascularization, circulating progesterone (P4) concentrations and pregnancy rate of Alpine (A) and Saanen (S) goats in the breeding season. All animals received two i.m. injections of 37.5 μg of d-cloprostenol (Prolise®, Tecnopec, São Paulo, Brazil) 11.5 (Exp. 1 and Exp. 2) or 7.5 (Exp. 2) days apart. One day after the onset of estrus (at the time of AI), the goats were randomly allocated to one of the two groups that received: 300 IU of hCG (Vetecor®; Hertape-Calier, São Paulo, Brazil) i.vag. (G-hCG; Exp.1: *n* = 12A; Exp.2: *n* =143A + S) or 0.3 mL or saline solution i.vag. (G-Control; Exp.1: *n* = 12A; Exp. 2: *n* = 147A + S). Sample blood collections were done on days of application of d-cloprostenol and days 3, 7, 10, 13 and 21 (Exp. 1). Transrectal ovarian ultrasonography was done on days 7, 10, 13, 17, and 21 (Exp.1), and pregnancy detection on day 60 (Exp. 1 and 2). in the exp. 1, there was no difference ($P>0.05$) in the timing of ovulation, number of ovulations, diameter of ovulatory follicles and occurrence of early luteal regression between groups. There was an increase ($P<0.05$) of the luteal area in D17 and D21 and greater ($P<0.05$) vascularization in D10 and D13 in G-hCG in relation to G-Control. Exp. 2 the pregnancy rate was higher ($P<0.05$) in G-hCG compared to G-Control (67.3% vs 80.4%). hCG i.vag. administered at the time of AI did not alter ovulatory parameters, did not prevent early luteal regression, did not increase progesterone concentration, but was able to increase the luteal area and vascularization and increase the conception rate by 13.1%.

Keywords: Human Chorionic Gonadotropin. Luteal area. Progesterone**.**

1. INTRODUCTION

Human Chorionic Gonadotropin (hCG) is a glycoprotein hormone that is similar in structure to luteinizing hormone (LH), binds to the same receptor, and has a longer half-life than LH (~39 h vs 6 h, respectively) (COLE, 2010; SALEH et al., 2012).

The hCG can be used at different times of the estrous cycle of goats to improve reproductive rates (FONSECA et al., 2021). The use of hCG on the day of mating/AI has been studied in sheep with satisfactory results (GÓMEZ-BRUNET et al., 2007; KHAN et al., 2003) or not (CATALANO et al., 2006, 2012).

The administration of hCG on the day of the service would have an action mainly at the time of the pre-ovulatory peak of LH, in follicular maturation, with consequent improvement in oocyte quality and subsequent embryonic viability (SCHMITT et al., 1996), besides being able to improve the oviductal environment (MISHRA; LEI; RAO, 2002) and uterine (SHEN et al., 2009). While the use of this hormone during the luteal phase has as its main purpose the luteotrophic action, to increase the production of progesterone (P4) and/or and embryotrophic action to improve conceptus development. These mechanisms help in maternal recognition of pregnancy (NEPHEW et al., 1994) and so the use of hCG can increase the pregnancy rate in goats (CÔRTES et al., 2021; RODRIGUES et al., 2022b) and ewes (AKIF CAM; KURAN, 2004; FERNANDEZ et al., 2019).

The intravaginal route has been explored in the administration of several reproductive hormones, such as P4/progestagens (RATHBONE; BURKE, 2013), estradiol (CHŁOPEK et al., 2008), oxytocin (PRELLWITZ et al., 2019) and hCG (RODRIGUES et al., 2022a).

Rodrigues et al. (2022a) identified, through a rapid β-hCG test, the presence of plasma hCG in 50% of the females that received the application of 300 IU of hCG via the intravaginal route, but no effects were observed on pregnancy rate, circulating P4 concentrations and various luteal parameters.

Thus, this study aimed to study the effects of administering 300 IU of hCG intravaginally at the time of artificial insemination on ovulatory parameters and dynamics and luteal vascularization as well as circulating progesterone (P4) concentrations and pregnancy rate, under the hypothesis that this administration of hCG would prevent early luteal regression and increase the conception rate of dairy goats.

2. MATERIAL AND METHODS

All experimental procedures had been reviewed and approved by the Ethics Committee of the Embrapa Gado de Leite (protocol #5755150721). The present study was conducted from May to June, during the breeding season of goats (BALARO et al., 2019).

2.1. Location and experimental animals

Experiment 1 (n=24 Alpine goats) was conducted in the Embrapa Gado de Leite experimental field station situated in Coronel Pacheco, MG, Brazil (latitude 21° 35' S, longitude 43°15' W, and altitude of 435 m). Experiment 2 (n=290 Alpine and Saanen goats) was conducted in five different farms, MG, Brazil. In both experiments, goats were reared in an intensive system, fed corn silage and Napier grass with concentrate supplementation according to their nutritional needs (National Research Council, 2007); mineral salt licks and water were available ad libitum. All animals had been assessed for clinical health and reproductive status. The mean body condition score (BCS: 1=very thin and 5=very fat) (VILLAQUIRAN et al., 2007) of the present does was 3.3±0.1 and body weight was 60.4±4.2 kg. All goats were in the final third portion of their lactation period.

2.2. Estrus induction and treatments applied

All goats received two i.m. injections of 37.5 μg of d-cloprostenol (Prolise®, Tecnopec LTDA, São Paulo, Brazil) and were given 11.5 days apart (BONATO et al., 2019) in experiment 1 and 7 days (n = 115) or 11.5 days (n = 200) apart (MAIA et al., 2017) in experiment 2, with the administration of the first dose on a random day of the estrous cycle.

In Experiment 1, estrus was then detected twice a day with fertile bucks placed in a pen for 30 min. In both experiments, artificial insemination (AI) was performed by the Embrapa® transcervical technique (FONSECA et al., 2017) using the flexible-timed approach (FxTAI) (CARVALHO-DE-PAULA et al., 2020). Semen samples from seven bucks (three Alpine and four Saanen) aged 2–4 years belonging to the Brazilian progeny tests – CapraGene were donated by Embrapa Goats and Sheep. Frozen french straws (0.25 mL) containing 100 × 106 viable spermatozoa before freezing and with a minimum of 45% progressive motility and 3 spermatic vigor (0–5 variation) after thawing (35ºC for 30 s in water bach) were equally

assigned to each treatment group. All bucks had semen fertility proved during breeding (BONATO et al., 2019) and non-breeding season (CARVALHO-DE-PAULA et al., 2020).

The timing of AI time was based on the detection of behavioral estrus; the does with an early, intermediate or late onset of estrus (relative to the time of the second administration of d-cloprostenol). Goats in estrus at 24 to 48 h, 60 and 72 h were artificially inseminated at 24, 18 and 10 hours after estrus onset 7 days protocol, while for 11.5 days protocol, goats in estrus at 24 to 36 h, 48 and 60 h were artificially inseminated at 24, 18 and 10 hours after estrus onset, respectively as proposed (MAIA et al., 2017). At the time of artificial insemination, the goats of the present study were allocated to one of the two groups based on their parity, body weight (BW), and body condition score (BCS) (Fig 1): (1) goats that received 0.3 mL of saline solution by intravaginal route (G-Control; n=11; BW: 59.3±4.2; BCS: 3.3±0.2); and animals that received 300 IU of hCG (Vetecor®; Hertape-Calier do Brasil Ltda, São Paulo, SP; Brazil) via intravaginal deposition (G-hCG group; n=12; BW: 59.5±3.4; BCS: 3.3±0.2).

2.3. Blood collection and progesterone assay

In Exp. 1, pre-prandial jugular blood samples were drawn from all goats into vacutainers containing lithium heparin (anticoagulant) on days of administration of d-cloprostenol and on days 3 (D3), 7 (D7), 10 (D10), 13 (D13), 17 (D17) and 21 (D21) following treatment (D0 = day of FTAI/ovulation). The collection tubes were immediately placed on ice, transported to the laboratory, and centrifuged in a refrigerated centrifuge for 15 min at 1500 x g.

Plasma progesterone (P4) concentrations were determined with a solid-phase radioimmunoassay technique using commercial kits (ImmuChem; MP Biomedicals, Santa Ana, CA, USA). Sensitivity and intra-assay coefficients of variation were 0.05 ng/mL and 8%, respectively.

2.4. Ultrasonographic assessments

In Exp. 1, transrectal ovarian ultrasonography (B-mode and color Doppler) was conducted one week before the beginning of estrus synchronization to confirm the cyclicity of goats (presence of CL) and on D7, D10, D13, D17, and D21, using a portable ultrasound

scanner connected to a 7.5-MHz transducer (M5 Vet®; Mindray Medical International Limited, Shenzhen, China; Fig. 1). The transducer was taped to a stiffening PVC tube to facilitate external manipulation during the transrectal examinations. Does were examined in a standing position. Fecal pellets were removed manually from the rectum, and 10 mL of carboxymethylcellulose gel (Carbogel UTL; Carbogel Indústria e Comércio LTDA, São Paulo, SP, Brazil) were deposited with a syringe into the rectum and approximately 5 mL were applied onto the surface of the transducer before each ultrasonographic examination.

The diameter and position of all detected luteal structures were sketched on individual ovarian charts. B-mode images were used to measure the total luteal area (cm^2) defined as the sum of cross-sectional areas of all detected luteal structures; the areas of central cavities, if present, were subtracted from the total luteal area (CÔRTES et al., 2021). The Doppler area (DA) of each luteal structure and high-velocity DA (HVDA) were then determined using ImageProPlus® analytical software (Media Cybernetics Inc., San Diego, CA, USA). The HVDA was regarded as an upper and lower quarter of the Doppler scale bar that corresponds to the velocity range of 0.04 m/s to 0.08 m/s). All color pixels were detected using the "Count/size" tool and subsequently converted to SI units (OLIVEIRA et al., 2017).

In Exp. 2, the only ultrasonographic evaluation was pregnancy detection 60 days after insemination using the same equipment and operated by the same experienced technician as in Exp. 1. All examinations were performed at the constant settings of the ultrasound scanner (75% color gain, pulse repetition frequency (PRF) of 1.0 kHz, 6 cm of depth, and wall filter (WF) of 75 MHz).

2.5. Statistical analysis

Data were analyzed using IBM SPSS Statistics software, version 19. The general linear model with repeated measures over time was applied to the data collected between days. The normality assumption was verified using the Shapiro-Wilk test, and Levene test for homogeneity of variances. Parameters and indicators were also analyzed using the Mann-Whitney teste for non-parametric data; when parametric, t test was applied. Frequencies were assessed by Chi-square or Fisher's exact test. Differences were considered as significant when $P<0.05$. Results were presented as mean±standard error (SEM) or percentages.

3. RESULTS

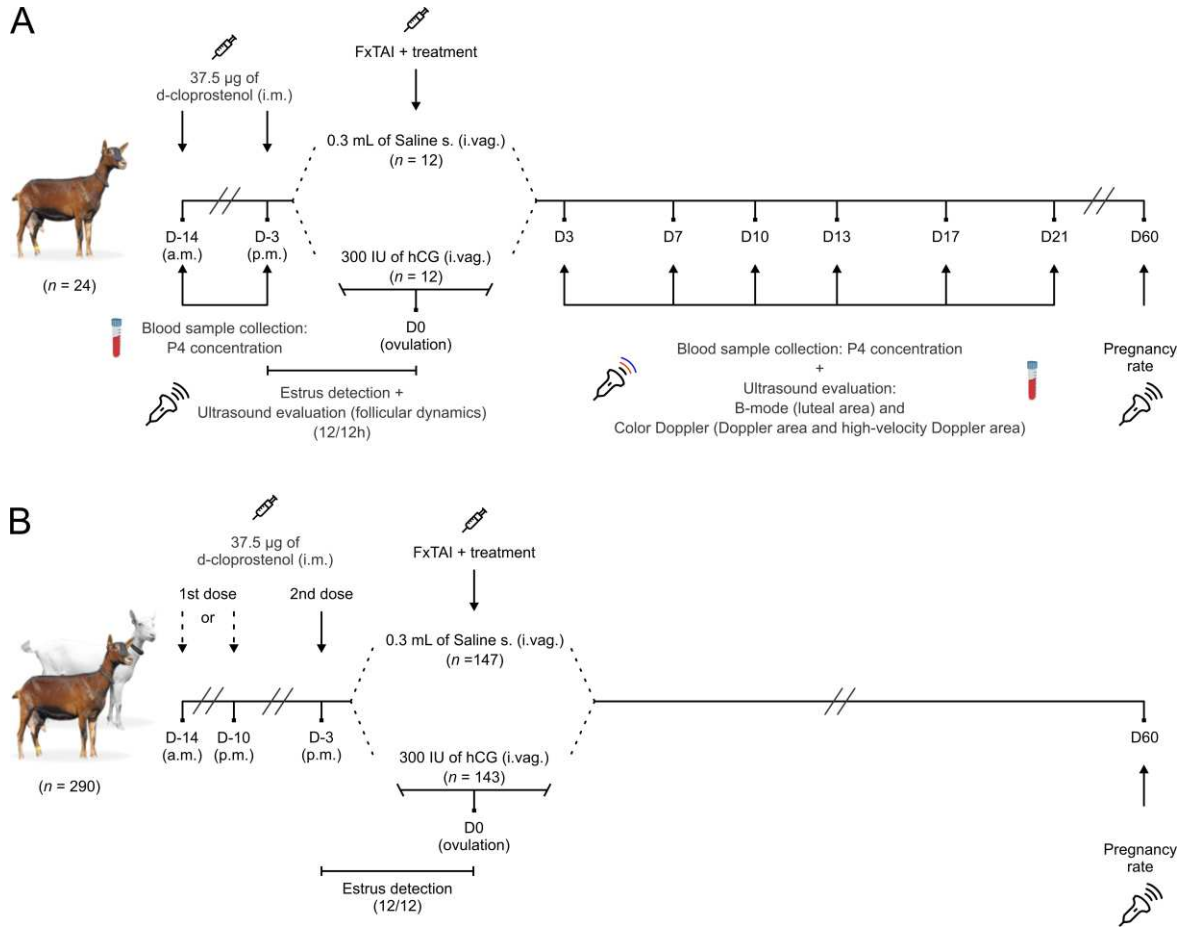
The data of the reproductive variables evaluated in experiment 1 are reported in table 1. All evaluated animals ovulated and there was no difference (P>0.05) in the number of ovulations between groups. After the second dose of cloprostenol, after 60h one goat ovulated in G-hCG, after 72h one goat ovulated in each group and after 96h 2 goats ovulated in G-control and one goat in G-hCG. In most animals, 75.0% (9/12) of the Control-G and 75.0% (9/12) of the G-hCG, ovulated 84h after the second dose of cloprostenol. There was no difference (P>0.05) in the time of ovulation between the groups. Treatment did not change (P>0.05) the size of ovulated follicles and did not change (P<0.05) the number of animals with early luteal regression.

Table 1 – Reproductive outcomes of multiparous goats that underwent flexible time artificial insemination (FxTAI) after estrus synchronization on breeding season, with or without 300 IU of hCG given intravaginally on FxTAI time (Exp. 1)

Endpoints	G-Control (n=12)	G-hCG (n=12)	Total (n=24)	p-value
Interval from second cloprostenol to onset of estrus (h)	48.0 ± 3.3	50.0 ± 2.5	49.0 ± 2.0	0.53
Interval from onset of estrus to ovulation (h)	37.0 ± 3.1	32.0 ± 3.1	34.5 ±2.2	0.24
Interval from second cloprostenol to ovulation (h)	85.0 ± 1.8	82.0 ± 2.5	83.5 ± 1.5	0.53
Interval from second cloprostenol to AI (h)	65.9 ± 0.8	66.8 ± 0.8	66.4 ± 0.6	0.51
Interval from onset of estrus to AI (h)	19.7 ± 2.0	16.8 ± 2.1	18.2 ± 1.5	0.19
Interval from AI to ovulation (h)	19.1 ± 1.7	18.2 ± 3.0	18.6 ± 1.7	0.54
Number of ovulations	2.3 ± 0.2	2.0 ± 0.2	2.1 ± 0.1	0.39
Diameter of largest follicle ovulated (mm)	82.8 ± 3.3	80.9 ± 2.4	82 ± 2.0	0.66
Diameter of second largest follicle ovulated (mm)	77.8 ± 2.4	72.3 ± 2.1	75.2 ± 1.7	0.15
Premature luteal regression (%)	25.0 (3/12)	16.0 (2/12)	20.8 (5/24)	1.00
Conception rate (%)	25.0 (3/12)	50 (6/12)	37.5 (9/24)	0.40

In the luteal dynamics, when comparing the groups, two evaluations were made: comparing all the animals that did not suffer early luteal regression (nG-Control = 9 and nG-hCG = 10; Fig. 2) and comparing only the animals that became pregnant with each group (nG-Control = 3 and nG-hCG = 6; Fig. 3).

Figure 1 – Experimental design of the study. Evaluation of the effect of administering 300 IU of hCG intravaginally (i.vag.) at the time of artificial insemination (AI) in dairy goats during the breeding season. Exp. 1 (A), Alpine goats received 0.3 ml of saline solution (G-Control) or 300 IU of hCG i.vag. (G-hCG) at the time of AI. Females were randomly allocated to the two groups immediately after estrus detection. Jugular blood samples were drawn, and B-mode and color Doppler. Exp. 2 (B) Alpine and Saanen goats received 0.3 ml of saline solution (G-Control) or 300 IU of hCG i.vag. (G-hCG) at the time of AI; an ultrasonographic pregnancy check was done 60 days later. FxTAI = flexible time artificial insemination



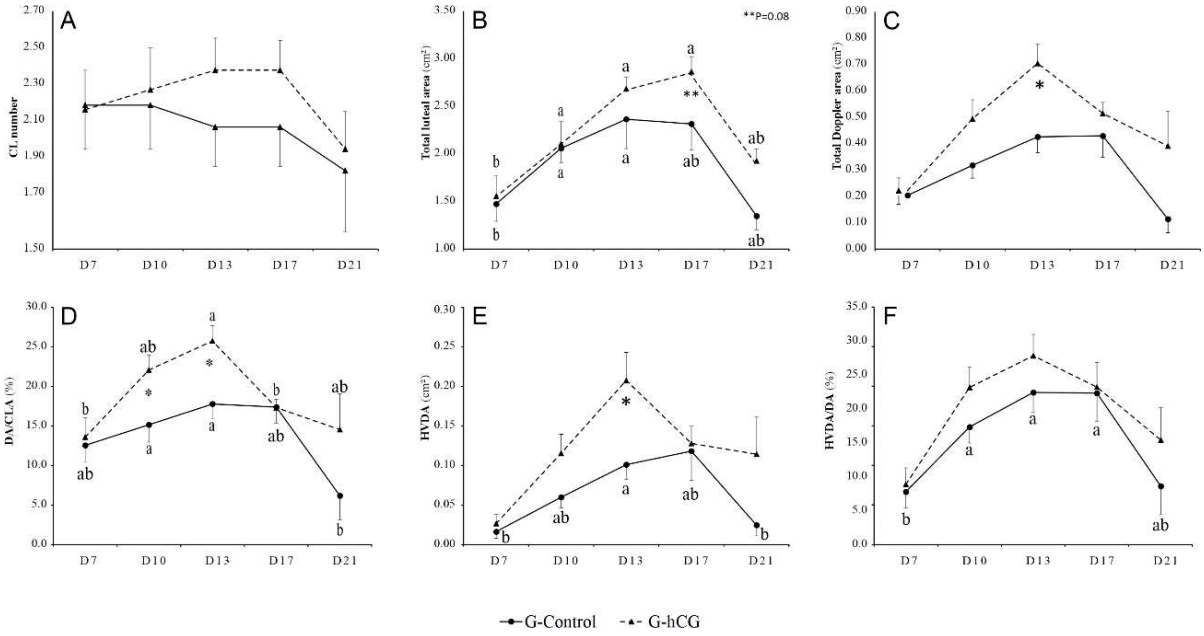
The application of hCG i.vag. induced aCL formation in 16.6% (2/12) of the goats, one developed an aCL at D10 and one developed an aCL at D13. There was no development of aCL in any G-Control animal.

In G-Control, a goat showed partial luteal regression, in which a CL regressed from D10 (3 CLs) to D13 (2 CLs). This phenomenon was not observed in any G-hCG animal, except on D21, which was already expected by luteolysis in non-pregnant animals (Fig. 2A).

When assessing the total luteal area (CLA) in all animals (Fig. 2B), there was a tendency (P=0.08) for a larger luteal area in G-hCG on D17. When evaluating this parameter

only in pregnant animals (Fig. 3B), higher values (P<0.05) of ACL were observed in D17 and D21 in the G-hCG in relation to the G-Control.

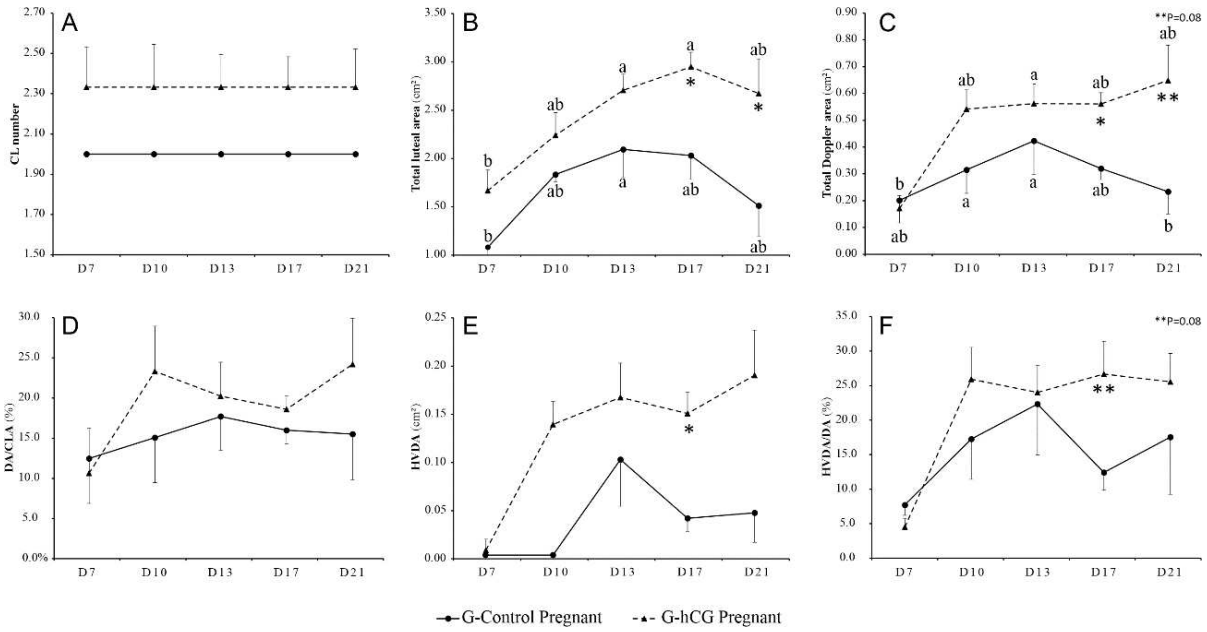
Figure 2 – Mean (±SEM) CL number (A), total luteal area (CLA) (B) total luteal Doppler area (DA) (C), DA/CLA (D) high-velocity DA (HVDA) (E) and HVDA/ DA (F) detected ultrasonographically in Alpine goats with or without (Control) a single i.m. injection of 300 IU of human chorionic gonadotropin (hCG) given intravaginally at the time of artificial insemination. Mean values denoted by different letters vary over time within each group (abc) and one artistic (*) indicates the differences (P<0.05) between groups of does and two asterisks (**) indicate a tendency of differences (P=0.07) between groups of does.



When comparing all animals, the Doppler area (DA) increased (P<0.05) from D7 to D13 in G-hCG (Fig. 2C) while it showed no difference (P<0.05) in G-Control from D7 to D17, only reduced (P<0.05) in D21 compared to D17. This behavior made the AD in G-hCG higher than the G-Control in D13 (P<0.05). When comparing only pregnant animals, AD was higher in G-hCG at D17 (P<0.05) and D21 (P=0.08) compared to G-Control (Fig. 3C).

There was an increase (P<0.05) in the percentage of DA in relation to CLA (DA/CLA – Fig. 2D) from D7 to D13 in G-hCG with a reduction (P<0.05) of this relation in D17. While in the G-control there was no significant increase (P>0.05) in this relationship over the days, only a reduction (P<0.05) in D21. The percentage of DA/CLA was higher (P<0.05) in G-hCG on D10 and D13, compared to G-Control. There were no variations (P>0.05) over the days or between groups for this parameter when only pregnant animals were evaluated (Fig. 3D).

Figure 3 – Mean (±SEM) CL number (A), total luteal area (CLA) (B) total luteal Doppler area (DA) (C), DA/CLA (D) high-velocity DA (HVDA) (E) and HVDA/ DA (F) detected ultrasonographically in Alpine pregnancy goats with or without (Control) a single i.m. injection of 300 IU of human chorionic gonadotropin (hCG) given intravaginally at the time of artificial insemination. Mean values denoted by different letters vary over time within each group (abc) and one artistic (*) indicates the differences (P<0.05) between groups of does and two asterisks (**) indicate a tendency of differences (P=0.07) between groups of does.

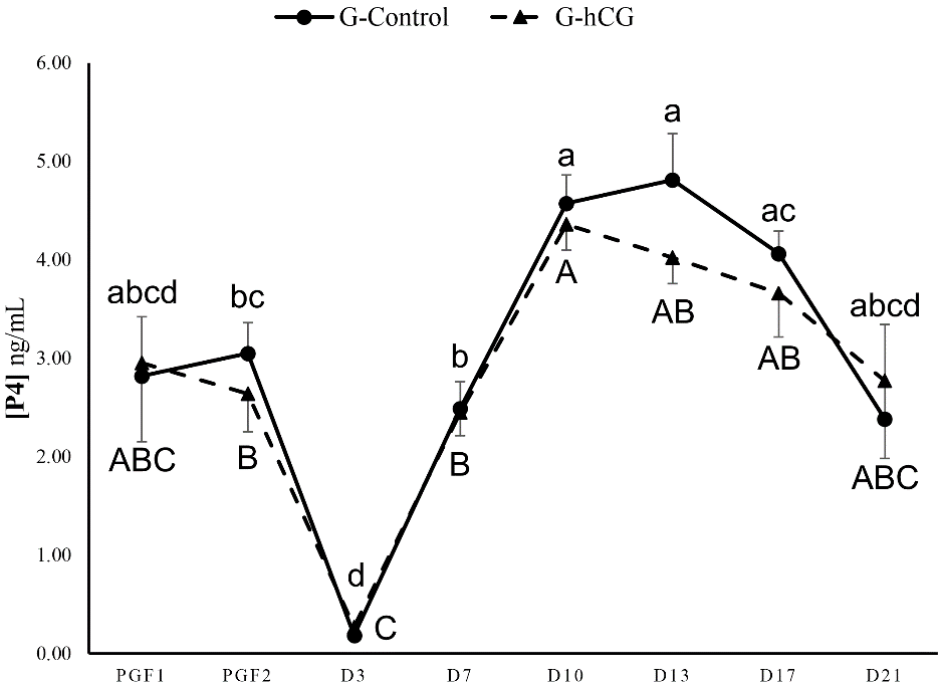


The high-velocity Doppler area (HVDA) was higher (P<0.05) in G-hCG on D13, when all animals were evaluated (Fig. 2E), and was higher (P<0.05) in the same group on D17 compared to G-Control, when comparing only the pregnant animals (Fig. 3E).

There was no difference between groups (P>0.05) in the percentage of HVDA/DA in the evaluation of all animals in the study, however, when only pregnant animals were evaluated, there was a tendency towards a higher mean value (P=0.08) in D17 of the G-hCG group in relation to the G-Control.

In the exp. 2, the pregnancy rate was 67.3% (99/147) in the Control-G and 80.4% (115/143) in the G-hCG, an increase of 13.1% (P< 0.05) in hCG-treated goats than controls.

Figure 4 – Mean (±SEM) circulating progesterone (P4) concentrations in Alpine goats with (hCG) or without (Control) a single intravaginal injection of 300 IU of human chorionic gonadotropin (hCG) given at the time of artificial insemination in goats in the breeding season. Mean values denoted by different letters vary over time within each group and asterisks indicate the differences between the two groups of does



4. DISCUSSION

The conception rate in Exp. 1 was smaller compared to previous studies using the same synchronization protocol, while in Exp. 2 was similar to previous studies in which the same protocol was used. Bonato et al. (2019) reported a conception rate of 78.1% (25/32) in goats given two doses of d-cloprostenol 11.5 days apart and 88.9% (32/36) 7.5 days apart. This lower conception rate in Exp. 1 occurred due to high manipulation of the animals during the estrous cycle for data collection.

The use of hCG at the time of insemination/mating has been studied in sheep. Corroborating with Exp. 2 of this study, Khan et al. (2003) found an increase ($P<0.1$) of 12% in the pregnancy rate in ewes that received 150 IU of hCG i.m. on the day of mating, while Gómez Brunet et al (2007) found an increase ($P<0.1$) of 12.1% in fertility in herds with poor reproductive performance, but no overall increase in fertility or prolificacy in ewes in the breeding and non-breeding seasons that received 500 IU hCG i.m. on the day of artificial insemination, in addition to a trend ($P<0.1$) for higher P4 concentrations from days 8 to 14 in ewes that received hCG but did not conceive on AI. While Catalano et al. (2006), in contrast

to our findings, did not observe an increase in the pregnancy rate in ewes treated with 150 IU of hCG i.m. on the day of mating.

To improve fertility, hCG would have to increase the fertilization rate, assist in the preovulatory LH surge, act on oocyte maturation, change the timing of ovulation or the number and size of ovulatory follicles, or reduce the rate of embryonic death, by a decrease in early luteal regression, increase in luteal area and increase in P4 concentration, or both, or even act by an unknown mechanism, as suggested by Côrtes et al. (2021).

Ovulatory parameters, such as time of ovulation, number of ovulations and size of ovulatory follicles, were not altered by the use of intravaginal hCG in this study. The treatment was also unable to prevent early luteal regression. The action of hCG may have occurred as an aid at the time of the preovulatory peak of LH, which may have improved follicular maturation, with consequent improvement in oocyte quality and subsequent embryonic viability (SCHMITT et al., 1996) and improved early embryonic development. Activation of the bovine oviductal LH receptor (LHR) increases oviductal glycoprotein synthesis (OGP; SUN; LEI; RAO, 1997), which attaches to embryos to enhance their development (NANCARROW; HILL, 1995). Mishra et al. (2002) observed that hCG treatment in cocultures with oviductal epithelial cells further enhanced embryonic development into blastocysts and inhibition of OGP synthesis, and prevention of protein kinase A activation blocked the effect of hCG in cocultures (MISHRA; LEI; RAO, 2002). In this way, the study supports the evidence that the action of hCG increases the synthesis of OGP, which probably increases early embryonic growth and development. (MISHRA; LEI; RAO, 2002).

While in the luteal phase hCG was able to induce the formation of aCL, increase the luteal area on D17 (D17 and D21 in pregnant goats) and improve luteal vascularization on D10 and D13, however, it did not increase the plasma concentration of P4. Since the recognition and establishment of pregnancy in goats occurs between days 10 and 21 of the cycle (SPENCER et al., 2004), and in this period, intravaginal hCG was able to promote luteal alterations, but without increasing the plasmatic concentration of P4, we believe that the same pool decreases embryonic losses by some action other than the elevation of P4. Nephew et al. (1994), when studying ewes that received or not 100 IU of hCG on day 11.5 of the estrous cycle, quantified IFNγ and total protein in the uterine washings and reported an increase in the size of the conceptus and in the production of these factors in the group that received hCG. hCG can act on the oviducts, uterus and vagina, due to having receptors in

these tissues. (LI; WANG; WANG, 2009; SHEN et al., 2009; WANG et al., 2012), stimulate growth and greater secretion of interferon-tau (IFNγ) by the embryo, signaling pregnancy more effectively (NEPHEW et al., 1994), which reduces embryo mortality and consequently increases the pregnancy rate in goats (BUSTAMANTE-ANDRADE et al., 2021; CÔRTES et al., 2021; RODRIGUES et al., 2022b).

Saleh et al. (2012) studied the pharmacokinetics of hCG (500 IU) administered intramuscularly in goats, and determined a relatively rapid absorption rate (approximately 11.6 h) and slow elimination rate (approximately 70.0 h), and traces of hCG were found in the system after 5 days. The pharmacokinetics of hCG administered via the intravaginal route in goats is not known, however, Rodrigues et al. (2022a) identified the presence of hCG in the blood plasma 3h after the administration of 300 IU via the intravaginal route and the rapid β-hCG test identified the presence of the hormone 7 days after treatment in 75% (3/4) and 21 days after in 25% (1/4) of the goats in which the hormone was detected, suggesting that hCG administered by this route may act on the corpora lutea and even promote the development of accessory corpora lutea throughout the cycle, as occurred in 16.6% (2/10) of the goats in our study.

5. CONCLUSION

A single application of 300 IU of hCG intravaginally at the time of AI did not change the time of ovulation, the size or the number of ovulated follicles. It was also unable to prevent early luteal regression, nor to increase the plasmatic progesterone concentration, however, it promoted an increase in the luteal area on days 17 and 21 and increased luteal vascularization on days 10 and 13 of the cycle, in addition to raising the rate of conception at 13.1% in dairy goats synchronized with two doses of d-cloprostenol during the breeding season.

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CAPÍTULO 2

Article: Luteal function in cyclic goats treated with human Chorionic Gonadotropin (hCG) administered by intramuscular or intravaginal routes at the time of artificial insemination

Published in Reproduction in Domestic Animals

ABSTRACT

Human chorionic gonadotropin (hCG) has been used to improve goats reproductive efficiency. This study aimed to: i. evaluate if hCG administered by the intramuscular (i.m.) or intravaginal (i.vag.) route can be detected by a rapid β-hCG test in blood plasma samples, and ii. document ovarian effects of hCG administered by both routes at the time of artificial insemination (AI) performed 60 h after estrus synchronization in goats. Twenty-two Alpine goats received two i.m. injections of 30 μg of d-cloprostenol (Prolise®, Tecnopec, São Paulo, Brazil) 7.5 days apart. One day after the onset of estrus (at the time of AI), the goats were randomly allocated to one of the three groups that received: Control (n=7): 0.3 mL of saline solution intravaginally; $hCG_{i.m.}$ (n=7): 300 IU of hCG (Vetecor®; Hertape-Calier, São Paulo, Brazil) i.m.; and $hCG_{i.vag.}$ (n=8): 300 IU of hCG deposited intravaginally. Blood samples were drawn at −1 h, 3 h, 6 h, 9 h, and 24 h after as well as on days 3, 7, 10, 13, 17, and 21 after hCG treatment/AI. All animals tested negative for hCG (ECO Diagnóstica, Corinto, Brazil) at −1 h, and all animals control tested negative throughout the entire blood collection period. All $hCG_{i.m.}$ animals tested positive from 3 h until D3 post-AI but only 50% of $hCG_{i.vag.}$ goats tested positive during the present study. In all animals studied, mean circulating P4 concentrations increased ($P<0.05$) from D3 to D7 after AI and then declined ($P<0.05$) from D10 to D17 in Control and $hCG_{i.m.}$ groups, and from D17 to D21 in the $hCG_{i.vag.}$ group. Total cross-sectional luteal area (CLA), mean color Doppler area (DA), DA/CLA, mean high-velocity Doppler area, and HVDA/CLA all declined ($P<0.05$) by D17-D21 in all animals studied. In summary: i. human chorionic gonadotropin could consistently be detected in blood samples using the rapid β-hCG test only in the $hCG_{i.m.}$ group; and ii. there were no significant differences in the mean pregnancy rate, circulating P4 concentrations and various luteal parameters studied among Control, $hCG_{i.m.}$ and $hCG_{i.vag.}$ does.

Keywords: Goat. Corpus luteum. Luteal function. hCG. Lltrasonography. Pregnancy rates.

1. INTRODUCTION

Human chorionic gonadotropin (hCG) has been used to improve the reproductive efficiency of small ruminants (Fonseca et al., 2021). Administered in the presence of functional ovulatory corpora lutea (CL), hCG induces the formation of accessory CL (Côrtes et al., 2021; Fonseca et al., 2018; Rodrigues et al., 2022; Vergani et al., 2020), has a luteotropic effect on original CL, increases circulating progesterone (P4) concentrations (Fonseca et al., 2006; Rodrigues et al., 2022; Vergani et al., 2020), and boosts pregnancy rates in small ruminants(Cam & Kuran, 2004; Côrtes et al., 2021; Gómez-Brunet et al., 2007; Khan et al., 2009; Rodrigues et al., 2022). Human chorionic gonadotropin can also be used at the time of mating/artificial insemination. Khan et al. (2003) reported that administration of 150 IU of hCG in ewe lambs at the time of mating increased crown-rump length, amniotic sac width, and the number of placentomes, but did not affect plasma progesterone concentrations in gestating animals or progesterone production from luteal slices cultured in vitro. However, there is a paucity of information on potential luteotropic and pro-gestational effects of hCG given by different routes at the time of artificial insemination (AI) in goats.

The intravaginal route is commonly used for the administration of progestins in domestic ruminants (Rathbone & Burke, 2013), but it has also been explored as an alternative route for the application of other reproductive hormones (Netto et al., 2020; Prellwitz et al., 2019). Intravaginal administration of various hormones is feasible because the vaginal wall contains a dense network of capillary blood vessels that are permeable to fat soluble (i.e., steroids) and hydrophilic/peptidergic hormones (Hussain & Ahsan, 2005). Moreover, intravaginal deposition reduces the hepatic metabolism and clearance of the hormones as they divert from the “first-pass metabolism by the liver” to the "first uterine pass effect", wherein a high degree of direct transport from the vagina to uterine and ovarian vasculature occurs (Bulletti et al., 1997). During the estrus phase, estrogens produced by the ovulatory follicle(s) promote growth and vascularization of the reproductive tract epithelium (Souza-Fabjan et al., 2021), which further increases the rate of transvaginal absorption of drugs/hormones.

Human chorionic gonadotropin can be measured in urine and blood samples. Immunochromatographic analysis (a rapid test for hCG detection in the urine of women) has been the most widely used method. This one-step rapid hCG test is inexpensive ($0.25/rapid test, while the analysis in a diagnostic laboratory cost around $2.50) and easy to perform.

However, for pregnancy diagnosis, this test has only been used for the qualitative determination of hCG.

Thus, the main aim of this study was to: i. evaluate if hCG administered by the intramuscular or intravaginal route at the time of AI can be detected by a commercial rapid β-hCG test in blood plasma samples and if this test could be adapted as a semi-qualitative approach to detect blood plasma hCG concentrations post-treatment; and ii. document the effects of the hCG treatment on the luteal area and vascularization as well as circulating progesterone (P4) concentrations.

2. MATERIAL AND METHODS

All experimental procedures had been reviewed and approved by the Ethics Committee of the Embrapa Gado de Leite (protocol #5755150721). The present study was conducted from May to June, during the breeding season of goats (Balaro et al., 2019).

2.1. Location and experimental animals

The experiment utilized clinically healthy 22 Alpine goats (between 1 and 4 years of age, 13 nulliparous and 9 multiparous) housed in the Embrapa Gado de Leite experimental field station situated in Coronel Pacheco, MG, Brazil (latitude 21°35'S, longitude 43°15'W, and altitude of 435 m.a.s.l.). Goats were reared in an intensive system, fed 50% of corn silage and 50% of Napier grass with concentrate supplementation (soybean, corn, minerals, and vitamins-based mixture; with 16% of crude protein and 68% of total digestible nutrients) according to their nutritional needs (National Research Council, 2007). Mineral salt licks and water were available ad libitum. The mean body condition score (BCS: 1=very thin and 5=very fat (Villaquiran et al., 2007)) was 3.0±0.1, and the mean body weight of does at the beginning of the study was 41.4±3.3 kg. All multiparous goats were in the last third portion of their lactation period (305 days), with a mean annual milk yield of 727 kg (Faco et al., 2020).

2.2. Estrus induction and treatments applied

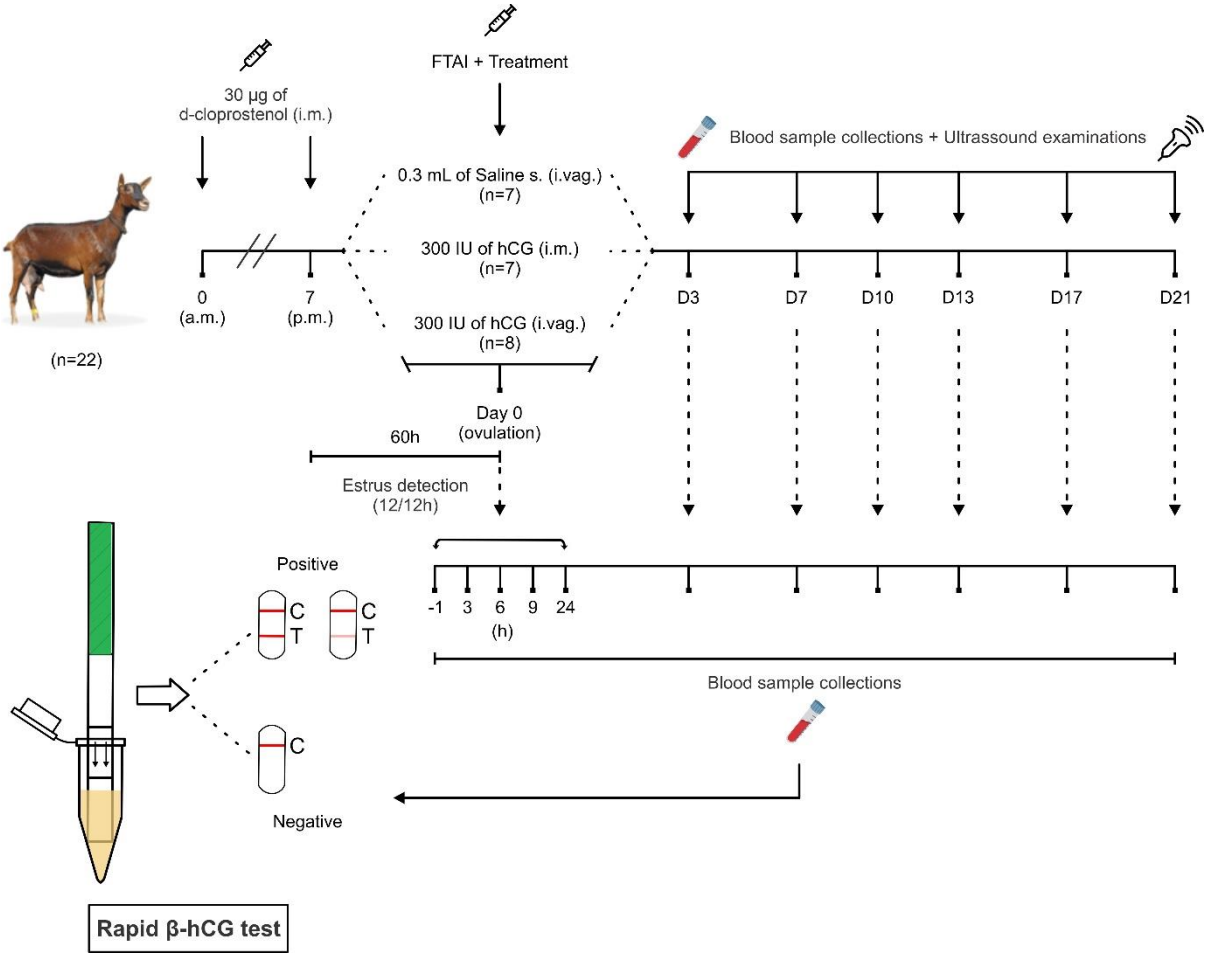
Two i.m. injections of 30 μg of d-cloprostenol (Prolise®, Tecnopec LTDA, São Paulo, Brazil) were given 7.5 days apart (Bonato et al., 2019), with the administration of the

first dose on a random day of the estrous cycle. Artificial insemination (AI) was performed 60 h after the application of the second dose of d-cloprostenol (Bonato et al., 2019) by the Embrapa® transcervical technique (J. F. Fonseca et al., 2017), using a Collin speculum (sizes 0-3). The speculum was opened in the center of the vagina and, with the help of a flashlight, the cervical opening was positioned. Then the cervix was immobilized with forceps (Embrapa® forceps for Cervical Immobilization and Artificial Insemination in Small Ruminants; Brasilia – DF, Brazil), and the semen applicator was inserted into the cervical canal until reaching the body of the uterus, where a semen dose was deposited. The Embrapa Goats and Sheep donated semen obtained from fours bucks (Alpine bucks owned by the Brazilian progeny testing corporation CapraGene). All bucks had been subjected to breeding soundness evaluation protocols during the breeding (Bonato et al., 2019) and non-breeding seasons (Carvalho-de-Paula et al., 2020). Inseminate doses containing 100×10^6 viable spermatozoa, with minimum progressive motility of 45% and spermatic vigor of 3 (range 0 to 5) before freezing, were packed in French straws (0.25 mL) and stored in liquid nitrogen. Semen was thawed in a water bath at 35ºC for 30 s.

At the time of artificial insemination, the goats of the present study were allocated to one of the three groups based on their parity, body weight (BW), and body condition score (BCS) (Fig 1): (1) goats that received 0.3 mL of saline solution by intravaginal route (Control group; n=7; 4 nulliparous and 3 multiparous; BW: 41.0±6.9; BCS: 2.8±0.1); animals that received 300 IU of hCG (Vetecor®; Hertape-Calier do Brasil Ltda, São Paulo, SP; Brazil) via the intramuscular route ($hCG_{i.m.}$ group; n=7; 4 nulliparous and 3 multiparous; BW: 40.7±4.2; BCS: 2.9±0.2); and animals that received 300 IU of hCG via intravaginal deposition ($hCG_{i.vag.}$ group; n=8; 5 nulliparous and 3 multiparous; BW: 39.6±3.4; BCS: 2.9±0.2).

In the $hCG_{i.m.}$ group, 5000 IU of hCG were initially diluted in 16.6 mL of saline solution, to a final concentration of 300 IU/mL, and each goat received an i.m. injection of 1mL of this solution. In the $hCG_{i.vag.}$ group, 5000 IU of hCG were diluted in 5 mL of saline solution, to a final concentration of 1000 IU/mL, before each animal received 0.3 mL of the solution via the intravaginal route. All animals were ultrasonographically checked for pregnancy 60 days after AI.

Figure 1 - Experimental design. Twenty-two Alpine goats were allocated to receive 0.3 mL of saline solution i.m. or 300 IU of hCG by intramuscular (i.m.) or intravaginal (i.vag.) route at the time of artificial insemination performed 60 h after the onset of behavioral estrus. Jugular blood samples were drawn at −1 h, 3 h, 6 h, 9 h, and 24 h (Time 0=hCG treatment or AI), D3, D7, D10, D13, D17, and D21. Rapid β-hCG tests as well as B-mode and color Doppler ultrasonography of ovaries was performed on the days of blood collection. FTAI: fixed-time artificial insemination

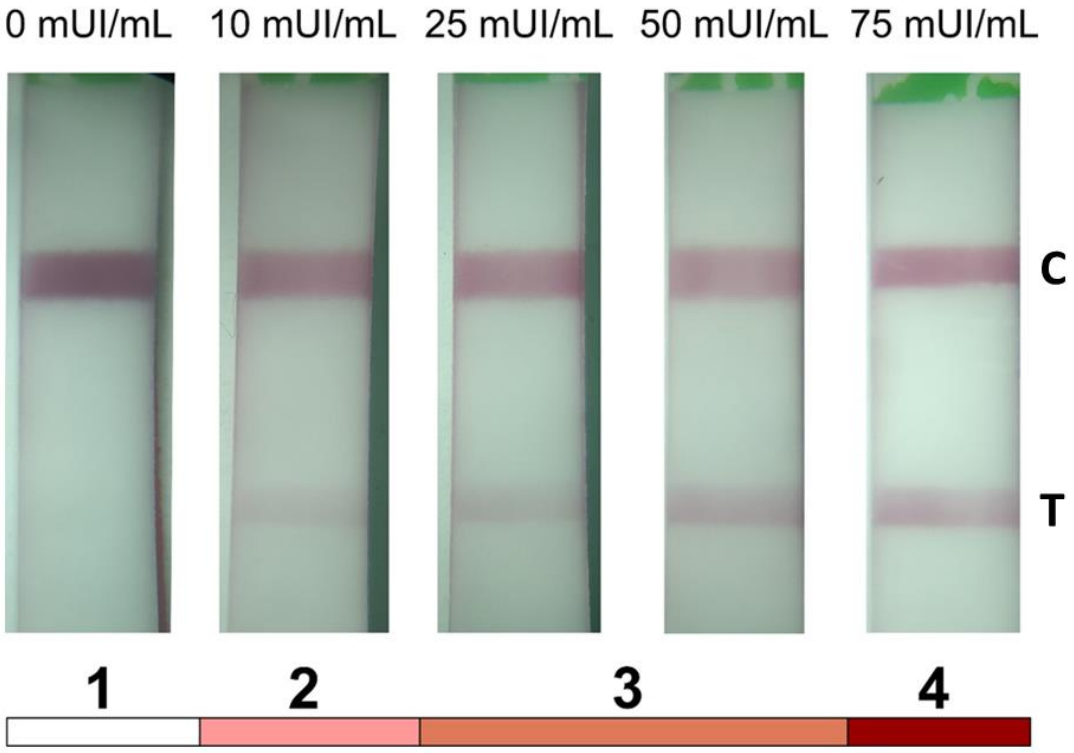


2.3. Blood collection, rapid β-hCG test and progesterone assay

Pre-prandial jugular blood samples were drawn from all goats into vacutainers containing lithium heparin (anticoagulant) one hour before (–1 h) and 3 h, 6 h, 9 h, and 24 h after treatment as well as on days 3 (D3), 7 (D7), 10 (D10), 13 (D13), 17 (D17) and 21 (D21) following hCG administration. The collection tubes were immediately placed on ice, transported to the laboratory, and centrifuged in a refrigerated centrifuge for 15 min at 1500 x

g. After centrifugation, 200 µL of blood plasma was aliquoted in a microtube to perform the β-hCG raster test (hCG 10 mUI ECO Test, ECO Diagnóstica LTDA, Corinto, MG, Brazil). This hCG test utilizing anti-β-hCG monoclonal antibodies for early detection of pregnancy in women uses has >99.0% sensitivity, >99.0% specificity, and a cut-off of 10 mIU/mL. The end of the strip was dipped in 200 µL of blood plasma stored in a microtube for 10 s and then the strip was placed on a flat surface to read the result, as instructed by the manufacturer. The result was recorded after 5-7 minutes. A control line (C) indicates the reaction process on the test strip and the appearance of a test line (T) determines whether the sample contains hCG (Fig. 2).

Figure 2 – Intensity scoring determined with a commercial rapid beta-hCG test and known amounts of hCG dissolved in ovine blood plasma



Initially, ovine plasma was mixed with known concentrations of hCG (10, 25, 50, or 75 mIU/ml) to prepare a set of reference scores according to the intensity of the test line (Fig. 2). The hCG (Vetecor®; Hertape-Calier do Brasil Ltda, São Paulo, SP; Brazil) was diluted in saline solution and then 10, 25, 50 or 75 mIU was added to sheep plasma to obtain plasma with known concentrations of hCG. The strips tested using these concentrations (n=15 strips/hCG concentration) were visually evaluated using a scale ranging from 1 to 4 (1:

undetectable; 2: very weak intensity (similar to or less intense than that for 10 mIU/mL); 3: weak to moderate intensity (similar to that obtained with 25 and 50 mIU/mL, but less intense that for 75 mIU/mL): and 4: strong (similar to or more intense that for 75 mIU/mL). Plasma samples producing scores of ≥1 were considered positive for hCG.

Plasma progesterone ($P_{4)}$ concentrations were determined with a solid-phase radioimmunoassay technique using commercial kits (ImmuChem; MP Biomedicals, Santa Ana, CA, USA). Sensitivity and intra-assay coefficients of variation were 0.05 ng/mL and 8%, respectively (Netto et al., 2020).

2.4. Ultrasonographic assessments

Transrectal ovarian ultrasonography (B-mode and color Doppler) was conducted one week before the beginning of estrus synchronization to confirm the cyclicity of goats (presence of CL) and on days 3, 7, 10, 13, 17, and 21 (day 0 = day of FTAI), using a portable ultrasound scanner connected to a 7.5-MHz transducer (M5 Vet®; Mindray Medical International Limited, Shenzhen, China; Fig. 1). The transducer was taped to a stiffening PVC tube to facilitate external manipulation during the transrectal examinations. Does were examined in a standing position. Fecal pellets were removed manually from the rectum, and 10 mL of carboxymethylcellulose gel (Carbogel UTL; Carbogel Indústria e Comércio LTDA, São Paulo, SP, Brazil) were deposited with a syringe into the rectum and approximately 5 mL were applied onto the surface of the transducer before each ultrasonographic examination.

All examinations were performed by the same experienced operator at the constant settings of the ultrasound scanner (75% color gain, pulse repetition frequency (PRF) of 1.0 kHz, 6 cm of depth, and wall filter (WF) of 75 MHz). The diameter and position of all detected luteal structures were sketched on individual ovarian charts. B-mode images were used to measure the total luteal area (cm^2) defined as the sum of cross-sectional areas of all detected luteal structures; the areas of central cavities, if present, were subtracted from the total luteal area (Côrtes et al., 2021). The Doppler area (DA) of each luteal structure and high-velocity DA (HVDA) were then determined using ImageProPlus® analytical software (Media Cybernetics Inc., San Diego, CA, USA). The HVDA was regarded as an upper and lower quarter of the Doppler scale bar that corresponds to the velocity range of 0.04 m/s to 0.08 m/s). All color pixels were detected using the "Count/size" tool and subsequently converted to SI units (Oliveira et al., 2017).

2.5. Statistical analysis

Serial data were analyzed on a per animal basis using a two-way repeated measure analysis of variance (RM ANOVA) in SigmaPlot® statistical software (Systat Software Inc., Richmond, CA, USA). All data sets were subjected to normality (Shapiro-Wilk) and equal variance tests, and if any of the tests failed, variables were transformed by $\log_n$ prior to ANOVA. If the main effect of Time relative to hCG treatment and of the Treatment group or their interaction were significant, individual mean values were compared with the Holm-Sidak method (All Pairwise Multiple Comparison Procedures). Significance was set at P value <0.05 and all results are given as mean±standard error of the mean (SEM) unless otherwise stated.

3. RESULTS

All animals selected for this study had ultrasonographically detectable luteal structures and show signs of behavioral estrus before AI. On D3, all twenty-two inseminated goats had detectable corpora lutea (CL), but one goat allocated to the $hCG_{i.m.}$ group had regressing CL and non-detectable plasma P_4 concentrations; the data from that doe were removed from ensuing analyses. The mean number of CL did not differ (P>0.05) among the three subsets of goats (Control group: 1.3±0.2; $hCG_{i.m.}$: 1.5±0.2; and $hCG_{i.vag.}$: 1.8±0.3). Accessory corpora lutea (aCL) were detected in two $hCG_{i.m.}$ does (2/6, 33%), in one animal from the $hCG_{i.vag.}$ group (1/8; 12.5%), and in none of the animals control. In the $hCG_{i.m.}$ group, one goat had two aCL detected on D7 and the other doe had an aCL detected on D10, while an aCL in a $hCG_{i.vag.}$ goat was detected on D10.

All animals tested negative (score 1) for hCG 1 h before treatment (Fig. 3A). Moreover, all Control does tested negative throughout the entire blood sampling period. During the entire study period, only 50% (4/8) of goats allocated to the $hCG_{i.vag.}$ group, but all animals in the $hCG_{i.m.}$ group tested positive for hCG. Considering only animals that tested positive at some point between 3 h and 21 days after hCG treatment, all does were found positive on D3, 83.3% of $hCG_{i.m.}$ does (5/6) and 75.0% of $hCG_{i.vag.}$ does (3/4) on D7, and 33.3% of $hCG_{i.m.}$ does (2/6) and 25% in $hCG_{i.vag.}$ does (1/4) on D10. On D17 and D21 there were no hCG-positive animals in the $hCG_{i.m.}$ group (0%), while in the $hCG_{i.vag.}$ group one goat remained positive. In the $hCG_{i.m.}$ group, the animals had the highest hCG test scores at 6 h

after treatment (predominantly score 4), with a gradual decline in scores at 9 h after treatment (predominantly score 3), and another decline at 24 h after treatment (all does with score 2); from 24 h to 13 days post-treatment, all hCG-positive does in this group presented with score 2 (Fig. 3B). In the $hCG_{i.vag.}$ group, all positive animals presented with score 2 regardless of the time of evaluation. Consequently, the averaged hCG test scores were greater ($P<0.05$) in $hCG_{i.m.}$ compared with $hCG_{i.vag.}$ does at 3 h, 6 h, 9 h, and 24 h as well as on D7 post-treatment (Fig. 3C). They increased ($P<0.05$) from one hour before to 3 h after, and again to 6 h after AI, but subsequently declined ($P<0.05$) between 6 h and 9 h, between 9 h and 24 h, and finally between D7 and D17 in the $hCG_{i.m.}$ group, whereas in $hCG_{i.vag.}$ animals, mean scores increased ($P<0.05$) from –1 h to D3.

In all animals studied, mean circulating P_4 concentrations increased ($P<0.05$) from D3 to D7 after AI (Fig. 4). In the Control and $hCG_{i.m.}$ groups, mean P_4 concentrations declined ($P<0.05$) from D10 to D17, whereas in the $hCG_{i.vag.}$ group, mean plasma P_4 concentrations declined ($P<0.05$) from D17 to D21. There were no differences ($P>0.05$) in circulating P_4 concentrations among the three groups of does throughout the entire study period.

Figure 3 – (A) Percentages of goats that tested positive for hCG; (B) summary of intensity scores (rapid β-hCG test); and (C) mean (±SEM) hCG test scores in Alpine goats after administration of 300 IU of hCG by the intramuscular ($hCG_{i.m.}$) or intravaginal ($hCG_{i.vag.}$) route at the time of artificial insemination. Mean values denoted by different letters vary significantly ($P<0.05$; AB: within the $hCG_{i.m.}$ group and ab: within the $hCG_{i.vag.}$ group) and asterisks indicate statistically significant differences between the two groups of goats.

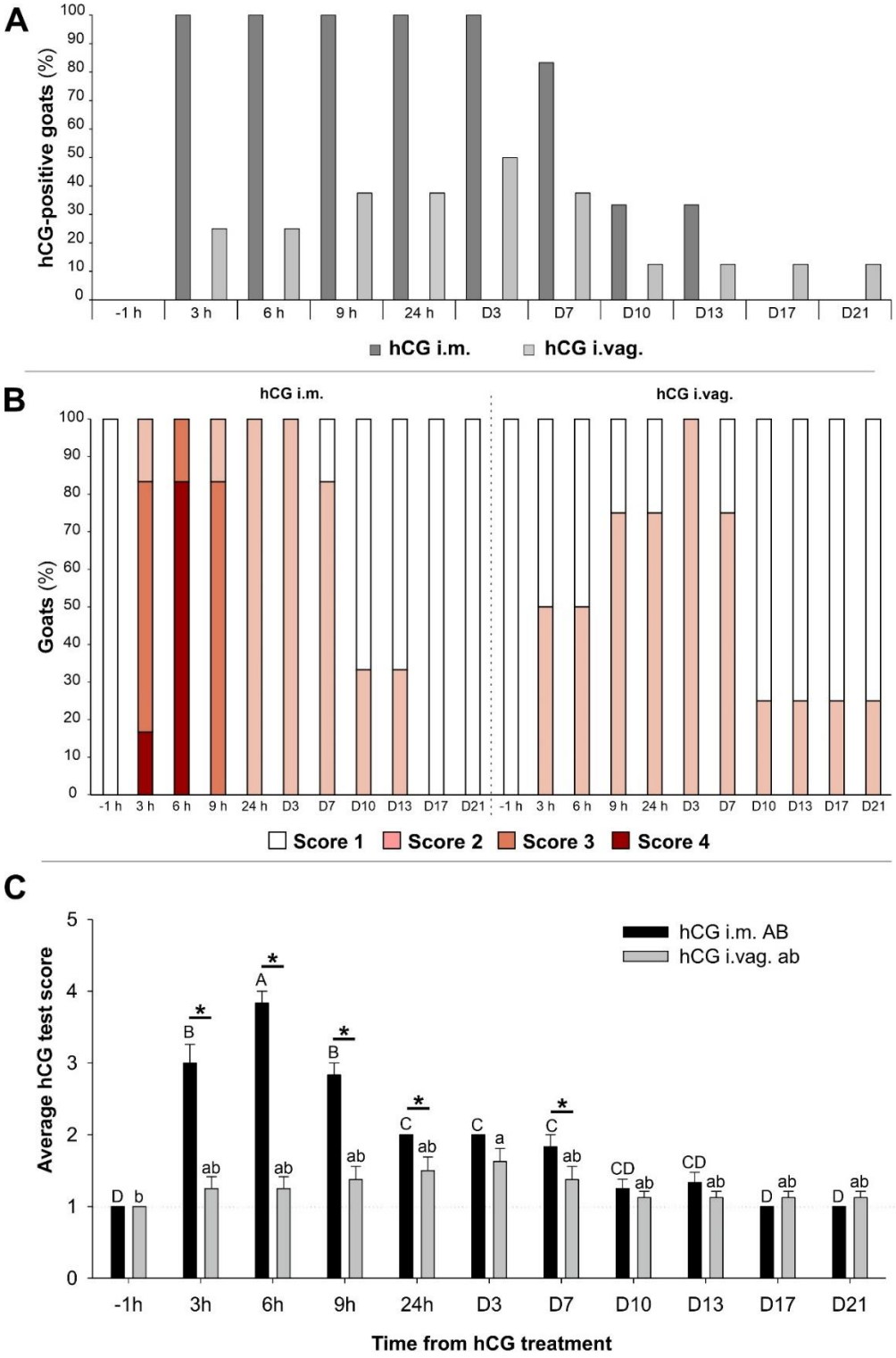
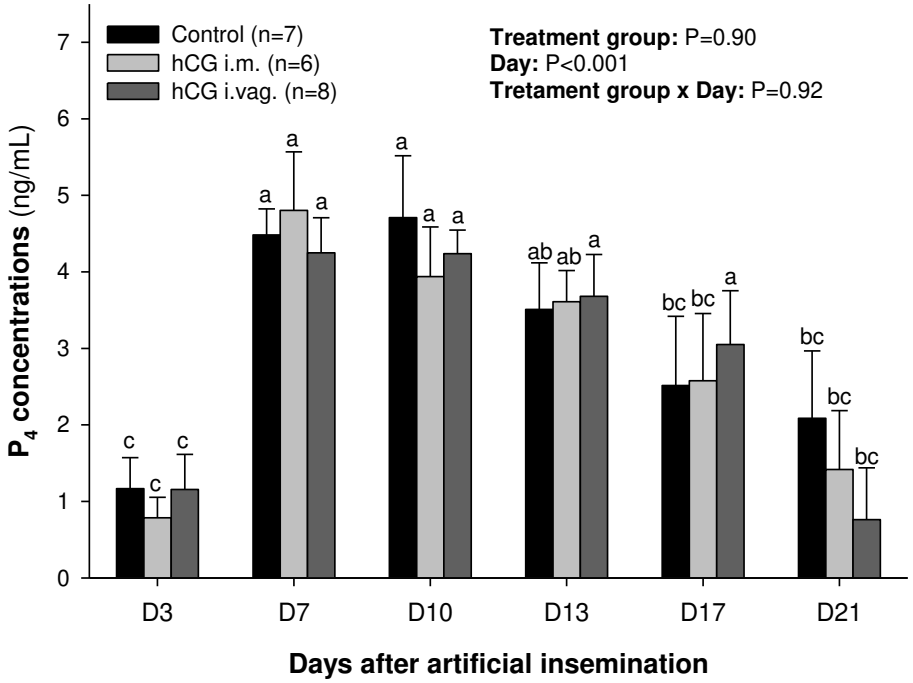


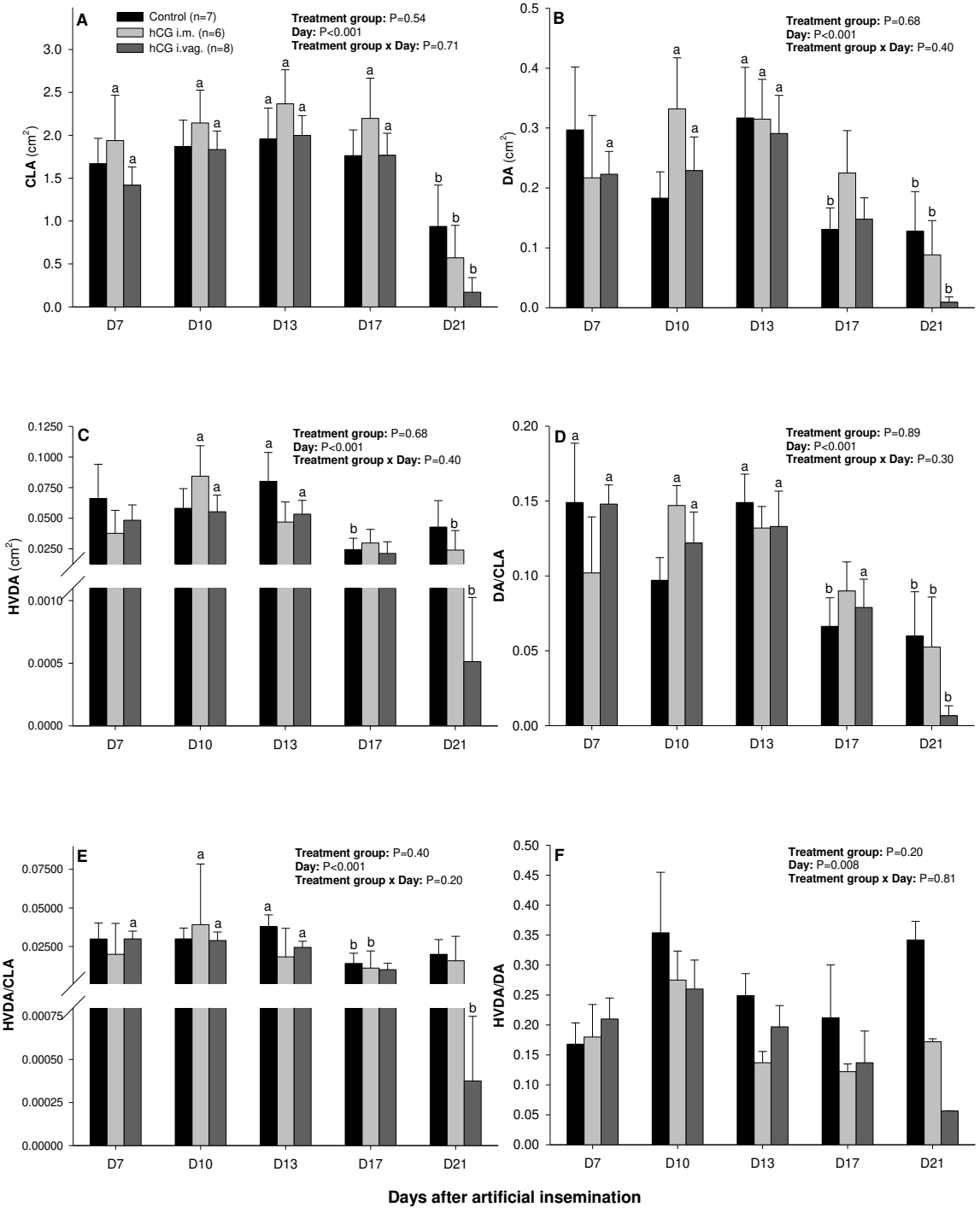
Figure 4 – Circulating (mean±SEM) progesterone (P_4) concentrations in cyclic Alpine goats that received 300 IU of hCG by the intramuscular ($hCG_{i.m.}$) or intravaginal ($hCG_{i.vag.}$) route at the time of artificial insemination (D0). Within each group, mean values denoted by different letters (ab) vary significantly (P<0.05).



Total cross-sectional luteal area (CLA) declined (P<0.05) from D13 to D21 in Control goats, and from D17 to D21 in both the $hCG_{i.m.}$ and $hCG_{i.vag.}$ groups (Fig. 5A). Mean color Doppler area (DA) and DA/CLA declined (P<0.05) from D13 to D17 in Control does, and from D13 to D21 in the $hCG_{i.m.}$ and $hCG_{i.vag.}$ groups (Fig. 5B and D). Mean high-velocity Doppler area (HVDA) declined (P<0.05) transiently from D13 to D17 in Animals control, and it declined (P<0.05) from D10 to D21 in $hCG_{i.m.}$ does and from D13 to D21 in the $hCG_{i.vag.}$ group (Fig. 5C). Mean HVDA/CLA values also declined (P<0.05) transiently from D13 to D17 in the Control group, and they declined (P<0.05) from D10 to D17 in $hCG_{i.m.}$ does and from D13 to D21 in the $hCG_{i.vag.}$ group (Fig. 5E). There were no other significant differences among the three groups of goats or over time for those parameters and no differences for HVDA/DA (Fig. 5F).

Ultrasonographic examinations conducted 60 days after AI revealed that 3/7 does in the Control group, 2/6 does in the $hCG_{i.m.}$ group, and 1/8 does in the $hCG_{i.vag.}$ group were pregnant and carried the kids to term.

Figure 5 – (A) Mean cross-sectional area (CLA) of ultrasonographically detected luteal structures; (B) color Doppler area (CD); (C) high-velocity color Doppler area (HVDA); and relative amounts of color Doppler signals, namely (D) CD/CLA; (E) HVDA/CLA; and (F) HVDA/DA in cyclic Alpine goats that received 300 IU of hCG by the intramuscular (hCGi.m.) or intravaginal (hCGi.vag.) route at the time of artificial insemination (D0). Within each group, mean values denoted by different letters (ab) vary significantly (P<0.05).



4. DISCUSSION

The pregnancy rate in the does of the present study was less compared with similar experiments conducted earlier. Bonato et al. (2019) reported the conception rate of 78.1% in a group of does that received two injections of d-cloprostenol 11.5 days apart, and were subjected to AI following the application of the estrous synchronization protocol.

Human chorionic gonadotropin can be used in animal husbandry to the fertilization rate or reduce early embryo loss. However, the administration of hCG in the present study failed to increase the pregnancy rate in goats. Catalano et al. (2012) reported that hCG administered at the onset of induced estrus in ewes did not alter the growth of large antral follicles or the timing of ovulation. From those indications, hCG injected during the pre- or periovulatory period in small ruminants following the hormonal estrus synchronization appears to have a limited effect on ovarian function and ovulatory responses.

Saleh et al. (2012) studied the pharmacokinetics of hCG (500 IU) administered intramuscularly in goats, and observed individual peak concentrations in systemic circulation ranging from 40 to 85 mIU/mL. They also determined that 500 IU of hCG given intramuscularly in goats had a relatively rapid absorption rate (approximately 11.6 h) and slow elimination rate (approximately 70.0 h). Based on the present results and in particular, on the average hCG scores, the maximum responses after an i.m. injection of 300 IU of hCG occurred approximately 6 h post-treatment and then declined to the pre-treatment levels 10 days later. The latter is difficult to explain, but it could be attributed, at least in part, to the cross-reactivity of hCG. The alpha chains of hCG, thyroid-stimulating hormone (TSH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) are identical (Macri et al., 1993). Different genes code for the unique beta components that display different immunologic characteristics. However, the beta chain of hCG still shares 85% amino acid homology with the beta chain of LH (Macri et al., 1993).

Previous studies have suggested that hCG administered during early gestation in dairy goats could improve the pregnancy rate by increasing the volume and vascularization of ovulatory corpora lutea (CL), inducing accessory CL, and consequently stimulating a rise in circulating progesterone (P_4) concentrations (Côrtes et al., 2021; Rodrigues et al., 2022). In the present experiment, neither the luteal area nor vascularization were affected by the administration of hCG, by different routes, at the time of artificial insemination. However, peripheral concentrations of P_4 declined by D17 in the Control and $hCG_{i.m.}$ group, while in the

$hCG_{i.vag.}$ group, this decline occurred on D21. In addition, the fact that there were hCG-positive goats up to D13 in the $hCG_{i.vag.}$ group and the induction of an accessory CL confirmed in a goat allocated to this group (on D10) may be interpreted to suggest that: i. intermediate metabolism of hCG was delayed in the $hCG_{i.vag.}$ group; and ii. the effects of hCG administered by intravaginal route were more pronounced compared with those seen after intramuscular hCG injections.

Interestingly, only 50% of $hCG_{i.vag.}$ animals tested positive for blood hCG and in all instances, the staining intensity was score 2. This low detection rate may have been due to limited absorption of hCG from the deposition site (vagina) resulting in low plasma concentrations of the hormone (below 10 mIU/mL). Alternatively, lower systemic concentrations of hCG could be a result of the counter-current transfer of the hormone between the vaginal and utero-ovarian circulation (Krzymowski et al., 1981) and/or a greater amount of hCG molecules bound to the target tissues following intravaginal infusion (Saleh et al., 2012). Einer-Jensen et al. (1993) observed a higher concentration of P_4 in the uterine artery of gilts compared with that in the carotid artery after vaginal administration of P_4. Similarly, serum P_4 concentrations were greater in the uterine than radial arteries (Cicinelli et al., 1998), and elevated endometrial concentrations of P_4 were found in women who received the hormone intravaginally rather than via an i.m. route (Miles et al., 1994). These observations confirm the existence of a local redistribution system sometimes referred to as the "first uterine passage effect", the term coined by Bulletti et al. (1997). This phenomenon may also explain, at least in part, the occurrence of hCG-positive goats up to D13 in the $hCG_{i.vag.}$ group.

5. CONCLUSION

It can be concluded that can be detected with the rapid β-hCG test in blood plasma samples following i.m. injections but it was not detectable in 50% of goats that received hCG intravaginally. Human chorionic gonadotropin given at FTAI has a limited effect on the luteal function and fertility of goats. The only difference observed between animals that received hCG via i.m or intravaginal route was that various luteal parameters declined by D17 in control and i.m.

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CAPÍTULO 3

Effect of human Chorionic Gonadotropin administered intravaginally and intramuscularly on the seventh day of the estrous cycle on luteal dynamics and progesterone concentration in dairy goats

ABSTRACT

The objective was to evaluate the effect of the administration of 300 IU of hCG by intramuscular or intravaginal routes on the seventh day after the observation of estrus on the original and accessory corpora lutea and the concentration of progesterone in dairy goats induced to estrus by the artificial light protocol. Saanen goats (n = 26) were subjected to 16 hr of light and 8 hr of darkness for 60 days, starting 10 days after the winter solstice. All goats received 120.5 µg cloprostenol doses on D130 (morning) and D137.5 (afternoon) (7.5 days apart). Oestrus behavior, ovarian dynamics and serum progesterone (P4) analyzes were recorded from the beginning of the induction protocol until the end of the next estrous cycle. On the seventh day (D7) after estrus observation (D0) the goats were randomly allocated to one of the three groups that received: Control (n=8): 0.3 mL of saline solution intravaginally; $hCG_{i.m.}$ (n=9): 300 IU of hCG (Vetecor®; Hertape-Calier, São Paulo, Brazil) i.m.; and $hCG_{i.vag.}$ (n=9): 300 IU of hCG deposited intravaginally. Transrectal ovarian ultrasonography and blood collection were done on days 7, 10, 13, 17, and 21. There was a formation of accessory corpus luteum (aCL) in 88.9% of $hCG_{i.m.}$ animals and 11.1% of $hCG_{i.vag.}$animals. There was an increase ($P<0.05$) of the total luteal area in $hCG_{i.m.}$ on D13 and the original corpora lutea on D10 in relation to $hCG_{i.vag.}$ groups. and Control. After one day of treatment, there was no luteal growth in the control group, while there was a growth of 63.7 and 34.7% in the luteal area in the $hCG_{i.m.}$ and $hCG_{i.vag.}$, respectively. Progesterone concentration was higher ($P<0.05$) in the $hCG_{i.m.}$ in D17 in relation to the Control. hCG administered intravaginally on the seventh day after estrus observation had a limited luteotrophic effect and did not increase P4 concentration in dairy goats induced to estrus by the artificial light protocol.

Keywords: Original corpora lutea. Accessory corpora lutea. Progesterone.

1. INTRODUCTION

Inadequate concentrations of progesterone cause 30-40% of embryonic deaths in goats and sheep, as this hormone is essential for the establishment of pregnancy in mammals (SPENCER et al., 2004). Several strategies are studied to increase the concentration of progesterone and reduce embryonic losses in goats.

Human chorionic gonadotropin (hCG) can be used at different times of the estrous cycle in goats and ewes to improve reproductive rates. Although there are LH/hCG receptors in several extragonadal organs (WANG et al., 2012), the main action of hCG takes place in the ovaries and uterus (MINEGISHI; NAKAMURA; IBUKI, 1993). Its most studied action is luteotrophic, either on the ovulatory corpora lutea (oCL) or on the antral follicles to promote the formation of accessory corpora lutea (aCL) (RODRIGUES et al., 2022b; SCHMITT et al., 1996), and with this stimulus increase the production of progesterone (P4) (FONSECA et al., 2006; RODRIGUES et al., 2022b; VERGANI et al., 2020). It may also promote an embryotrophic action (MISHRA; LEI; RAO, 2002), with the improvement of the development of the conceptus (NEPHEW et al., 1994), either by elevation of serum P4 or by direct action on hCG receptors in the embryo. Thus, hCG promotes better maternal recognition of pregnancy, with a decrease in embryonic loss and a consequent increase in the pregnancy rate in goats.

The intravaginal route of administration is an increasingly explored pharmacological route because it contains a dense network of blood vessels, is permeable to proteins and peptides (HUSSAIN; AHSAN, 2005), allows for ease of medication application, offers immediate availability to the systemic circulation as it bypasses the liver's first-pass metabolism, and has a "uterine first-pass effect" in which the administered hormone has a high degree of direct transport from the vagina to the uterus (BULLETTI et al., 1997).

Thus, the objective of this work was to study the effect of the administration of 300 IU of hCG by intramuscular or intravaginal routes on the seventh day after the observation of estrus on the original and accessory corpora lutea and the concentration of progesterone in dairy goats induced to estrus by the artificial light protocol, under the hypothesis that hCG administered intravaginally has luteotrophic effects similar to hCG administered intramuscularly.

2. MATERIAL AND METHODS

All experimental procedures had been reviewed and approved by the Ethics Committee of the Embrapa Gado de Leite (protocol #5755150721). The present study was conducted from November to December, during the non-breeding season of goats in Brazil (BALARO et al., 2019).

2.1. Location and experimental animals

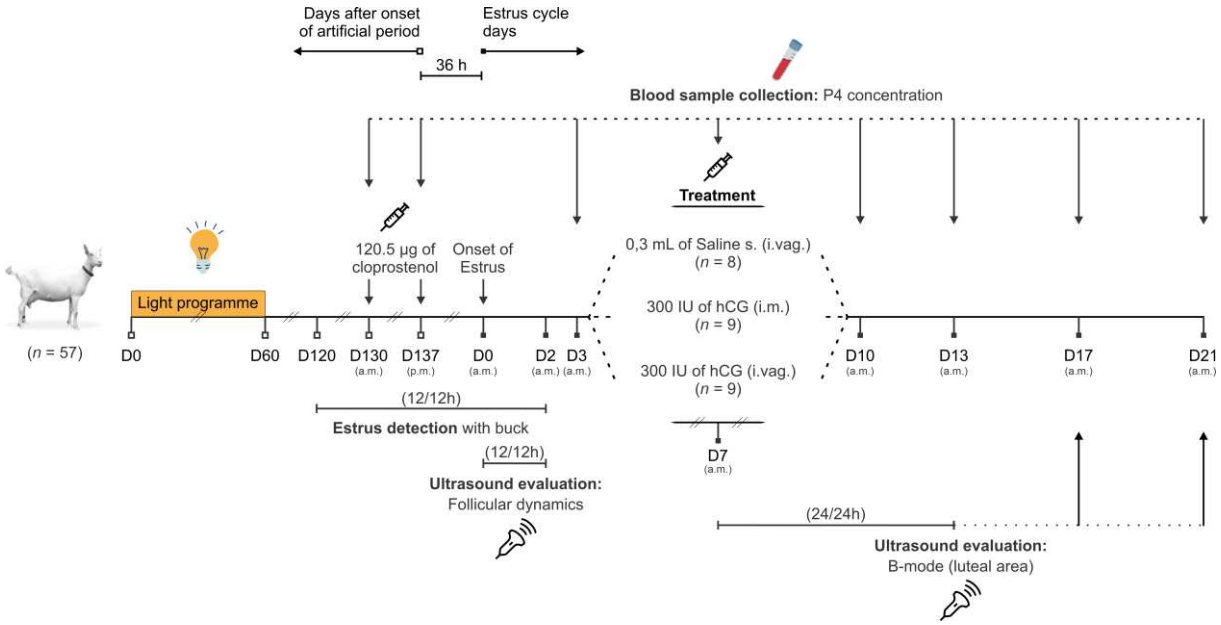
The experiment utilized clinically healthy 57 Saanen goats (between 1 and 4 years of age, 22 nulliparous, 15 primiparous and 20 multiparous) housed in Santo Antônio do Aventureiro (21°45 'S and 42° 48' W), Minas Gerais State, Brazil. Goats were reared in an intensive system, fed 50% of corn silage and 50% of Napier grass with concentrate supplementation (soybean, corn, minerals and vitamins based mixture; with 16% of crude protein and 68% of total digestible nutrients) according to their nutritional needs (National Research Council, 2007). Mineral salt licks and water were available ad libitum. The mean body condition score (BCS: 1=very thin and 5=very fat (Villaquiran et al., 2007)) was 2.9±0.1, and mean body weight of does at the beginning of the study was 41.4±3.3 kg. All primiparous and multiparous goats were in the last third portion of their lactation period, producing an average of 2.4 kg milk per goat (200 to 230 days lactation).

2.2. Estrus induction and treatments applied

The experimental design is shown in Fig.1. To estrus induction, all goats were subjected to the artificial photoperiod protocol (NETTO et al., 2020), which consisted of 16 h of light and 8 h of darkness, starting 10 days after the winter solstice and lasting 60 days.

To synchronization of estrus, all animals received two i.m. administrations of 120.5 µg of cloprostenol (Estron®, Agener União, São Paulo, Brazil) in an interval of 7.5 days (BONATO et al., 2019), with the administration of the first dose on D130 after artificial photoperiod. The estrus of all goats was monitored twice daily from D120 to D137 (second administration of cloprostenol) and continuous two days after the onset of estrus using photostimulated bucks (onset of estrus = D0).

Figure 1 – Schematic representation of the experiment design with artificial photoperiod treatment (light programme) consisting of 16 hr of light and 8 hr of darkness, starting 10 days after the winter solstice and lasting 60 days followed by estrous synchronization with two doses of cloprostenol 7.5 days apart. . Twenty-six Saanen goats were allocated to receive 0.3 mL of saline solution i.m. or 300 IU of hCG by intramuscular (i.m.) or intravaginal (i.vag.) route seven days after onset of estrus. Were realized ultrasonography evaluation and collected sample blood collection for progesterone measurement.



Seven days (D7) after the onset of estrus, 26 goats of the present study were allocated to one of the three groups based on their parity and body condition score (BCS) (Fig 1): (1) goats that received 0.3 mL of saline solution by intravaginal route (Control group; n=8; 4 nulliparous, 1 primiparous and 3 multiparous; BCS: 2.9±0.1); animals that received 300 IU of hCG (Vetecor®; Hertape-Calier do Brasil Ltda, São Paulo, SP; Brazil) via the intramuscular route ($hCG_{i.m.}$ group; n=9; 3 nulliparous, 2 primiparous and 4 multiparous; BCS: 2.9±0.2); and animals that received 300 IU of hCG via intravaginal deposition ($hCG_{i.vag.}$group; n=9; 4 nulliparous, 2 primiparous and 3 multiparous; BCS: 3.1±0.2).

In the $hCG_{i.m.}$ group, 5000 IU of hCG were initially diluted in 16.6 mL of saline solution, to a final concentration of 300 IU/mL, and each goat received an i.m. injection of 1mL of this solution. In the $hCG_{i.vag.}$ group, 5000 IU of hCG were diluted in 5 mL of saline solution, to a final concentration of 1000 IU/mL, before each animal received 0.3 mL of the solution via the intravaginal route.

2.3. Ultrasound evaluation

Transrectal ovarian ultrasonography (B-mode) was conducted daily by D0 (onset of estrus) to D13 and on D17, and D21, using a portable ultrasound scanner connected to a 7.5-MHz transducer (M5 Vet®; Mindray Medical International Limited, Shenzhen, China; Fig. 1). From D0 to D2, the evaluations occurred twice a day, to follow the follicular dynamic and ovulation and from D3 onwards the evaluations occurred once a day to follow the luteal dynamics.

The transducer was taped to a stiffening PVC tube to facilitate external manipulation during the transrectal examinations. Does were examined in a standing position. Fecal pellets were removed manually from the rectum, and 10 mL of carboxymethylcellulose gel (Carbogel UTL; Carbogel Indústria e Comércio LTDA, São Paulo, SP, Brazil) were deposited with a syringe into the rectum and approximately 5 mL were applied onto the surface of the transducer before each ultrasonographic examination.

The diameter and position of all detected luteal structures were sketched on individual ovarian charts. B-mode images were used to measure the total luteal area (cm^2) defined as the sum of cross-sectional areas of all detected luteal structures; the areas of central cavities, if present, were subtracted from the total luteal area (CÔRTES et al., 2021). The ultrasonographic evaluation was performed to pregnancy detection 60 days after insemination.

2.4. Blood sample collection and progesterone assay

Pre-prandial jugular blood samples were drawn from all goats into vacutainers containing lithium heparin (anticoagulant) on days of administration of cloprostenol and days 3 (D3), 7 (D7), 10 (D10), 13 (D13), 17 (D17) and 21 (D21) (D0 = onset of estrus). The collection tubes were immediately placed on ice, transported to the laboratory, and centrifuged in a refrigerated centrifuge for 15 min at 1500 x g.

Plasma progesterone (P4) concentrations were determined with a solid-phase radioimmunoassay technique using commercial kits (ImmuChem; MP Biomedicals, Santa Ana, CA, USA). Sensitivity and intra-assay coefficients of variation were 0.05 ng/mL and 8%, respectively.

2.5. Statistical analysis

Data were analyzed using IBM SPSS Statistics software, version 19. The general linear model with repeated measures over time was applied to the data collected between days. The normality assumption was verified using the Shapiro-Wilk test, and Levene test for homogeneity of variances. Parameters and indicators were also analyzed using the Mann-Whitney or Kruskal Wallis tests followed by Dunn's post hoc for non-parametric data; when parametric, t test or ANOVA was applied followed by Tukey's post hoc. Frequencies were assessed by Chi-square or Fisher's exact test. Differences were considered as significant when $P<0.05$. Results were presented as mean±standard error (SEM) or percentages.

3. RESULTS

The data of the reproductive variables of the nulliparous and non-nulliparous goats used in the study are described in table 1.

Table 1 – Data (mean ± SEM or %) of estrus indicators in the administration phases of the protocol under study between the categories (nulliparous and non-nulliparous).

Variables	Nulliparous	Non-nulliparous	Total
Animals (n)	22	35	57
Estrus before the first application of cloprostenol (%)	45.5[a] (10/22)	22.9[a] (8/35)	31.6 (18/57)
Estrus between doses of cloprostenol (%)	68.2[a] (15/22)	22.9[b] (8/35)	40.4 (23/57)
Estrus after the second dose of cloprostenol (%)	77.3[a] (17/22)	40.0[b] (14/35)	54.4 (31/57)
Interval of the second dose of cloprostenol to estrus (h)	41.3[a] ± 2.4	47.2[a] ± 3.8	45.6 ± 3.1
Ovulation detection (%)	77.3[a] (17/22)	80.0[a] (28/35)	78.9 (45/57)
Occurrence of early luteal regression (%)	35.3[a] (6/17)	42.9[a] (12/28)	31.6 (18/57)
Interval of the second dose of cloprostenol to ovulation (h)	81.7[a] ± 5,3	81.5[a] ± 4,2	81.6 ± 4.7
Single Ovulation (%)	76.5[a] (13/17)	17.9[b] (5/28)	31.6 (18/57)
Double ovulations (%)	17.7[b] (3/17)	57.1[a] (16/28)	33.3 (19/57)
Tree or more ovulations (%)	5.9[a] (1/17)	25.0[a] (7/28)	14.0 (8/57)

[a, b] Difference between columns (nulliparous vs. non-nulliparous) ($P < 0.05$)

Nulliparous goats had a better response to estrus induction by the light protocol, when compared to non-nulliparous goats, with a higher percentage of nulliparous goats ($P<0.05$) with signs of estrus between doses of cloprostenol and after the second dose.

However, there was no difference (P>0.05) in the percentage of goats in which ovulations were detected, which suggests a "silent heat" in some non-nulliparous goats.

Nulliparous goats had a higher incidence of single ovulation (P<0.05), while non-nulliparous goats had a higher incidence of multiple ovulations (P<0.05).

hCG administered intramuscularly induced aCL formation in 88.9% (8/9) of $hCG_{i.m.}$. While hCG administered intravaginally caused aCL induction in only 11.11% (1/9) of $hCG_{i.vag.}$ animals. In $hCG_{i.m.}$ only one animal presented aCL in D10, while the other 7 presented aCL from D13, these 2 animals presented new CLs (aCL) in D13 and D17. None of the Animals control developed aCL throughout the cycle.

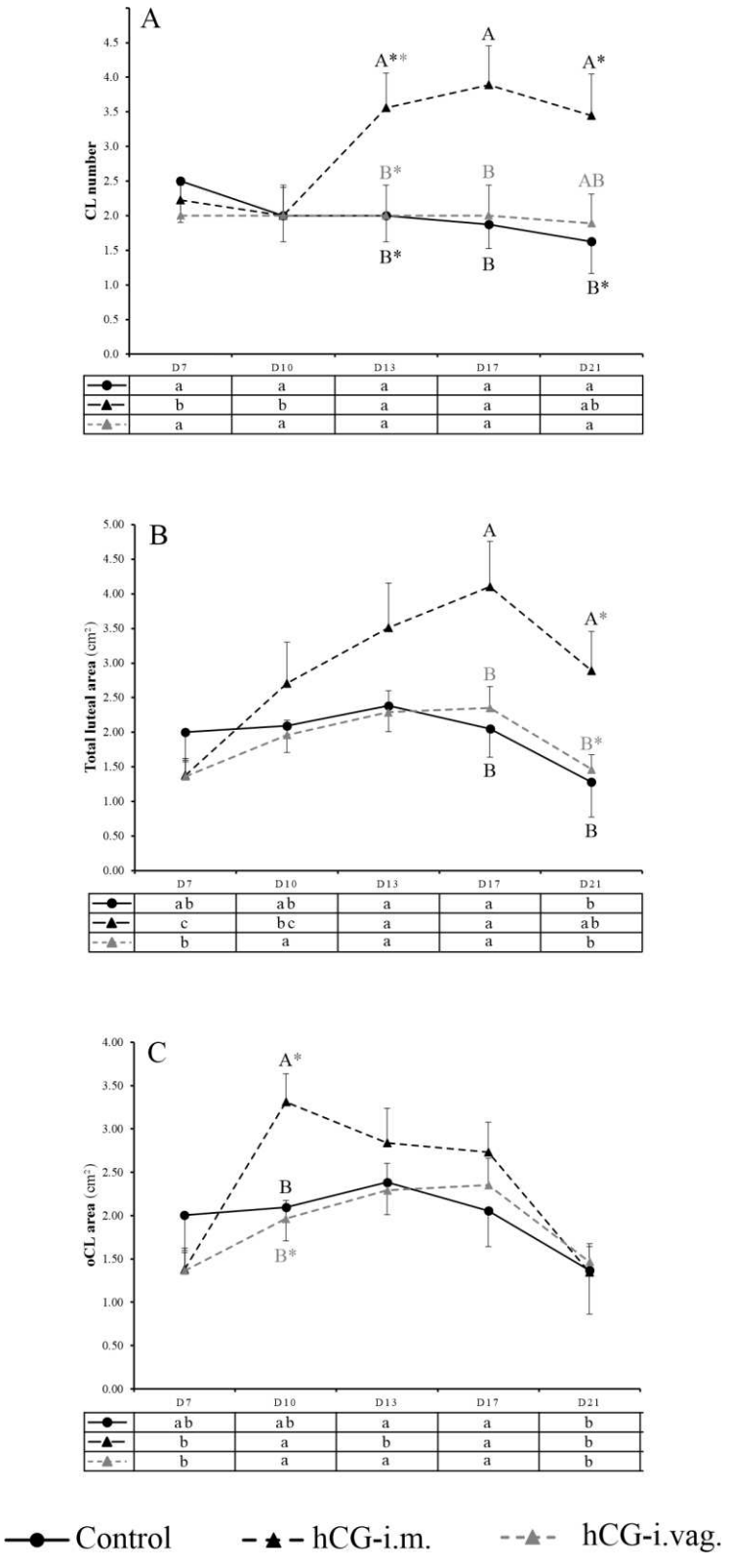
Two Animals control showed partial luteal regression: one animal went from 6 CL on D7 to 3 CL on D10-D21, while one animal went from 2 CL on D7 to 1 CL on D10-D21. One animal on $hCG_{i.m.}$ had early luteal regression and went from 2 CL on D7 to 0 CL on D10, however, the same animal developed 3 aCL on D13 that remained until D21. There was partial luteal regression in one goat on $hCG_{i.vag.}$ which regressed from 3 CL on D7 to 2 CL on D10-21.

Figure 2 shows the results of the ultrasonographic evaluation of the luteal dynamics throughout the estrous cycle. When evaluating the groups over the days, only $hCG_{i.m.}$ increased (P<0.05) the number of CL at D13 and remained high (P<0.05) until D21 (Fig. 2A). This elevation caused the CL number of $hCG_{i.m.}$ was superior (P<0.05) to Control from D13 to D21 and superior (P<0.05) to $hCG_{i.vag.}$ in D10 and D17.

There was no significant area increase (P<0.05) on any day in the Control (Fig. 2B), only a decrease in D21 relative to D13 and D17. In the $hCG_{i.m.}$ there was an increase (P<0.05) in the luteal area from D7 to D10 and from D10 to D13 without significant reduction (P>0.05) in the subsequent days. In the $hCG_{i.vag.}$ group. there was an increase (P<0.05) in the luteal area from D7 to 10, which was maintained until D17 and reduced (P<0.05) in D21 compared to D17. The luteal area differed between the groups at D17 and D21, where $hCG_{i.m.}$ was superior (P<0.05) to Control and $hCG_{i.vag.}$.

When evaluating only the luteal area of the original corpora lutea (Fig. 2C), the elevation of the luteal area in $hCG_{i.m.}$ occurs from D7 to D10 (P<0.05). The same occurs in $hCG_{i.vag.}$ (P<0.05), while in Control this increase only occurs from D7 to D13 (P<0.05). In

Figure 2 – (A) Number of corpora lutea (CL) ultrasonographically detected; (B) Total luteal area; and (C) ovulatory CL (oCL) area in cyclic Saanen goats that received 300 IU of hCG by the intramuscular ($hCG_{i.m.}$) or intravaginal ($hCG_{i.vag.}$) route seven days after onset of estrus. . Mean values denoted by different letters vary over time within each group (ab) and indicate the differences (P<0.05) between groups of does (AB). *P=0.07.



Control there is reduction (P<0.05) of the area in D21 in relation to D17, in $hCG_{i.m.}$ there is a reduction in D13 (P<0.05) with a posterior elevation (P<0.05) in D17 and another reduction (P<0.05) in D21. Whereas in $hCG_{i.vag.}$ the elevation (P<0.05) of D10 is maintained until D17(P<0.05) and regresses in D21 (P<0.05). In the evaluation of the luteal area of the oCL, the $hCG_{i.m.}$ was superior to the Control and $hCG_{i.vag.}$ groups. in D10.

Table 2 shows the daily growth rate of total luteal and original corpora lutea according to each group.

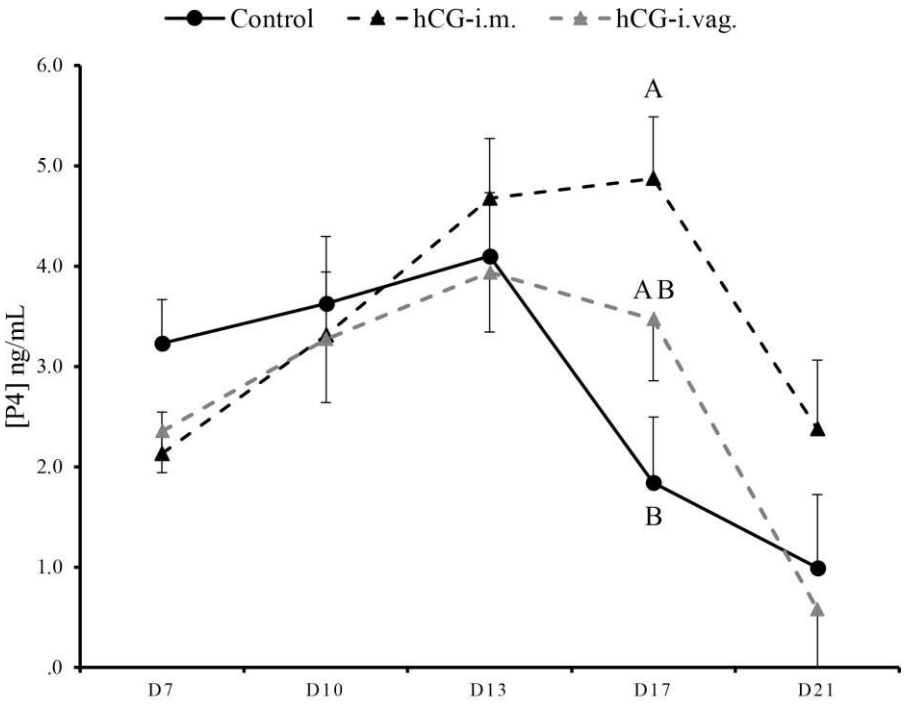
Table 2 – Daily rate of total luteal growth and original corpora lutea (oCL) of dairy goats that received or not (Control) 300 IU of hCG by intramuscular ($hCG_{i.m.}$) or intravaginal ($hCG_{i.vag.}$) in the day 7 of the estrous cycle (D0 = onset of estrous).

	Daily growth rate of luteal area (%)								
	D7	D8	D9	D10	D11	D12	D13	D17	D21
Control	-	-2.0%	8.0%	-0.2%	-0.1%	23.8%	-9.4%	-3.7%	-11.1%
$hCG_{i.m.}$ (Total)	-	63.7%	28.5%	-6.5%	8.4%	25.9%	-5.0%	3.9%	-8.4%
$hCG_{i.m.}$ (oCL)	-	63.7%	26.5%	-7.7%	-5.4%	-1.3%	-8.1%	-1.0%	-14.7%
$hCG_{i.vag.}$	-	34.7%	3.0%	3.8%	17.7%	3.0%	-3.6%	0.7%	-11.2%

The Control shows a more accentuated growth from D11 to D12, while in the treated groups, this growth was anticipated. In $hCG_{i.m.}$ great growth was already noticed in D8, and the same was noticed in $hCG_{i.vag.}$ However, luteal growth of $hCG_{i.vag.}$ was more discreet than $hCG_{i.m.}$ (34.7 Vs 63.7 %, respectively. From D10 onwards, the elevation of the luteal area in $hCG_{i.m.}$ occurs due to the appearance of the aCL, since there is no growth of the oCL area.

Progesterone concentration (Fig. 3) was higher (P<0.05) in the $hCG_{i.m.}$ in D17 in relation to the Control. Over the days, the Control group did not suffer an increase in the concentration of P4, but it suffered a decrease (P<0.05) from D13 to D17 and D21. In the $hCG_{i.m.}$ there was an increase (P<0.05) from D7 to D10 and again to D13. While in the $hCG_{i.vag.}$ group. there was an increase (P<0.05) from D7 to D13, with a decrease in D21.

Figure 3 – Circulating (mean±SEM) progesterone (P4) concentrations in cyclic Saanen goats that received 300 IU of hCG by the intramuscular ($hCG_{i.m}$) or intravaginal ($hCG_{i.vag.}$) route seven days after onset of estrus. Mean values denoted by different letters vary over time within each group (ab) and indicate the differences (P<0.05) between groups of does (AB).



●	ab	ab	a	b	b
▲	c	b	a	ab	abc
▲	bc	abc	a	ab	c

4. DISCUSSION

To our knowledge, this is the first study that evaluates the effect of hCG administered intravaginally on the seventh day of the estrous cycle on the corpora lutea of cyclical dairy goats.

In reproductive parameters after the light protocol for estrus induction, nulliparous goats showed better estrus responses during the synchronization protocol, however, there was no difference in ovulation detection between groups, which represents the occurrence of silent ovulations in the category non-nulliparous. The occurrence of silent ovulation at the beginning of the breeding season in goats is common. (RIVERA et al., 2003), and the difference between categories occurred due to the lower manifestation of estrus in primiparous females, corroborating the findings of Netto et al. (2020), in which the category of primiparous goats had a lower estrus response and pregnancy rate than multiparous goats

after estrus synchronization with two doses of d-cloprostenol with an interval of 11.5 days in estrus-induced goats by artificial light protocol.

The induction of aCL in the $hCG_{i.m.}$ was satisfactory (88.9%) and similar (RODRIGUES et al., 2022b) or superior (CÔRTES et al., 2021) to previous studies that used 300 IU of hCG on the seventh day after estrus observation, where the development of aCL was observed in 47% of the goats in the transition to the breeding season (CÔRTES et al., 2021) and 100% and estrus induced goats in the non-breeding season (RODRIGUES et al., 2022b). The induction of aCL in the $hCG_{i.vag.}$ (11.1% - 1/9) was similar to that found by Rodrigues et al. (2022a), in which the induction of new CL was observed in 16.6% (2/12) of the goats that received 300 IU intravaginally at the time of artificial insemination during the breeding season.

Partial luteal regression has been reported in goats (CARVALHO-DE-PAULA et al., 2020) and ewes (VIÑOLES; MEIKLE; FORSBERG, 2004) before and has not been prevented by the use of hCG by any route. However, hCG i.m. was able to induce the formation of new CL on D13 in a female that underwent total premature luteal regression (PLR) of corpora lutea from D7 to D10, allowing the maintenance of progesterone concentration at adequate levels to ensure the appropriate uterine environment and development embryonic stage, which may allow maternal recognition of pregnancy to occur (SPENCER et al., 2004), which opens new horizons on the use of hCG in the luteal phase in females that have suffered PLR, because even if hCG is not able to prevent PLR, it can compensate the production of P4 with the induction of aCL and reduce embryonic losses.

Even though there was no difference in the luteal area between the $hCG_{i.vag.}$ groups. and Control in no day, it is interesting to note the behavior of each group throughout the cycle. The Control group had no significant elevation in the luteal area during the cycle, while the $hCG_{i.vag.}$ increased ($P<0.05$) after treatment, from D7 to D10. Differences between groups may not have been significant ($P>0.05$) because the control group started with a luteal area about 30% larger than the treated groups.

Rodrigues et al. (2022a), when using a rapid test to identify the presence of hCG in the blood plasma of goats after treatment with 300 IU of hCG administered intravaginally, detected plasma hCG in 50% of the treated animals. The possibility of non-absorption of vaginal hCG in all animals may be a limiting factor for this treatment and may have been the reason for the more discreet elevation of the luteal area from D7 to D10 when compared to the elevation that occurred in the hCG-i.m group.

The results for the luteal area of the original corpora lutea corroborate the findings by Rodrigues et al. (2022), in which there was an increase in area from D10 onwards in goats that received 300 IU of hCG on the seventh day after estrus observation.

Since hCG applied intramuscularly in goats has an absorption half-life of 2.7 h (SALEH et al., 2012), its effect on luteal cells is observable 24 hours after administration. In table 2 the growth rate of the luteal area, it is noted that on the day after treatment (D8) there was no luteal growth in the control group, while there was an increase of 63.7 and 34.7% in the hCG-i.m groups. and $hCG_{i.vag.,}$ respectively.

5. CONCLUSION

It can be concluded that 300 IU of Human chorionic gonadotropin given on the seventh day after estrus observation by the intravaginal route has a limited luteotrophic effect and was not able to raise plasma concentrations of progesterone, as occurs in goats treated with hCG by the intramuscular route.

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CAPÍTULO 4

Article: Human Chorionic gonadotropin affects original (ovulatory) and induced (accessory) corpora lutea, progesterone concentrations and pregnancy rates in anestrous dairy goats

Published in Reproductive biology

ABSTRACT

Two experiments were conducted in acyclic Alpine (A) and Saanen (S) goats that received intravaginal sponges containing 60 mg of medroxyprogesterone acetate for 6 days, as well as 200 IU of eCG and 30 μg of d-cloprostenol i.m. 24 h before sponge removal. On day 7 (day 0=onset of synchronized estrus), all goats were randomly divided into two groups: animals treated with 300 IU of hCG i.m. (hCG; Exp.1: n=8A; Exp.2: n=75A+S) and untreated controls (Control; Exp.1: n=8A; Exp. 2: n=70A+S). In Exp.2, all goats were artificially inseminated. Transrectal ovarian ultrasonography and blood collection were done on days 7, 10, 13, 17, and 21 (Exp.1), and pregnancy detection on day 60 (Exp.2). Estrus and ovulations occurred in five hCG and seven Animals control. Accessory CL (aCL) were detected in all hCG does. The total luteal area of ovulatory corpora lutea (oCL) increased ($P<0.05$) on day 10 in hCG does and remained greater ($P<0.05$) than in Control until day 21. Total and high-velocity color Doppler area were greater ($P<0.05$) for oCL of hCG does on days 13 and 17. Progesterone concentrations were greater ($P<0.05$) in hCG does from days 13 to 21 and related directly to the total luteal and oCL area for the duration of the study in all does. The pregnancy rate was higher ($P<0.05$) in hCG than in Control by 22.5%. Human chorionic gonadotropin given on day 7 of the synchronized estrous cycle positively affected CL function and pregnancy rates in seasonally anovular dairy goats.

Keywords: Corpus luteum. Pregnancy. Progesterone. Human chorionic gonadotropin. Ovarian ultrasonography.

1. INTRODUCTION

An attainment of a threshold in blood progesterone (P_4) concentration is necessary for the establishment of pregnancy in mammalian species. Inadequate P_4 production causes 30 to 40% of embryonic deaths in goats and sheep [1–3], resulting in significantly reduced pregnancy rates in small ruminants. Reduced luteal function occurs mainly in the non-breeding season of small ruminants [4]. Therefore, strategies must be employed to boost luteal function after estrus induction protocols in seasonally anestrous goats. The administration of luteotrophic hormones is a strategy to raise the concentration of P_4 and can be used in the early or late luteal phase. The use in the early phase is based on the induction of accessory corpora lutea (aCL) and initial luteotrophic action on the post-ovulation corpus luteum (oCL), while the late phase has action in increasing ovarian function, conceptus growth, and placental attachment [5].

Administration of human chorionic gonadotropin (hCG) during the period when growing antral follicles have sufficient amounts of luteinizing hormone (LH) receptors can induce accessory corpora lutea (aCL) via stimulating follicle rupture and luteogenesis or by luteinization of large antral follicles [6]. Exogenous hCG given on day 5 of the estrous cycle (day 0=onset of estrous) induces aCL formation and an increase in serum P_4 concentrations in heifers [7] and goats [8,9]. Accessory CL also formed in hair sheep that received hCG on day 7 of the estrous cycle [10]. A significant increase in luteal area and a rise in blood P_4 concentrations were observed; however, neither blood P_4 concentrations nor total luteal tissue changed in Toggenburg goats injected with hCG seven days after the onset of estrus [11], leading the authors to speculate that P_4 mediated the increased pregnancy rates in the hCG-treated goats.

In addition to inducing the formation of aCL, human chorionic gonadotropin also enhances the functionality of the post-ovulation corpora lutea (oCL) [12], because hCG exerts luteotropic effects in LH-responsive small luteal cells [13,14]. Heifers not forming aCL after the administration of hCG five days after the onset of estrus still show a significant increase in circulating P_4 concentrations [12]. However, the influence of hCG on the size and vascularity of post-ovulation CL in small ruminants has yet to be described because all previous ultrasonographic studies of luteal function in hCG-treated ewes [10,15,16] and goats [11,17] only reported the changes in cross-sectional area and blood supply of all detectable luteal structures.

Hence, the main objective of this study was to assess the effects of hCG administrated seven days after the onset of the synchronized estrus on post-ovulation corpus luteum (oCL) and accessory CL (aCL) as well as plasma P_4 concentrations and fertility in artificially inseminated dairy goats during the non-breeding season. We expected that transrectal ovarian ultrasonography (B-mode and color Doppler) would help us delineate the influences of hCG on oCL and aCL in does.

2. MATERIAL AND METHODS

All experimental procedures were reviewed and approved by the Ethics Committee of Embrapa Gado de Leite (protocol #3050060218). The present study was conducted from October to November, during the middle portion of the non-breeding season that spans a period from September to November [18].

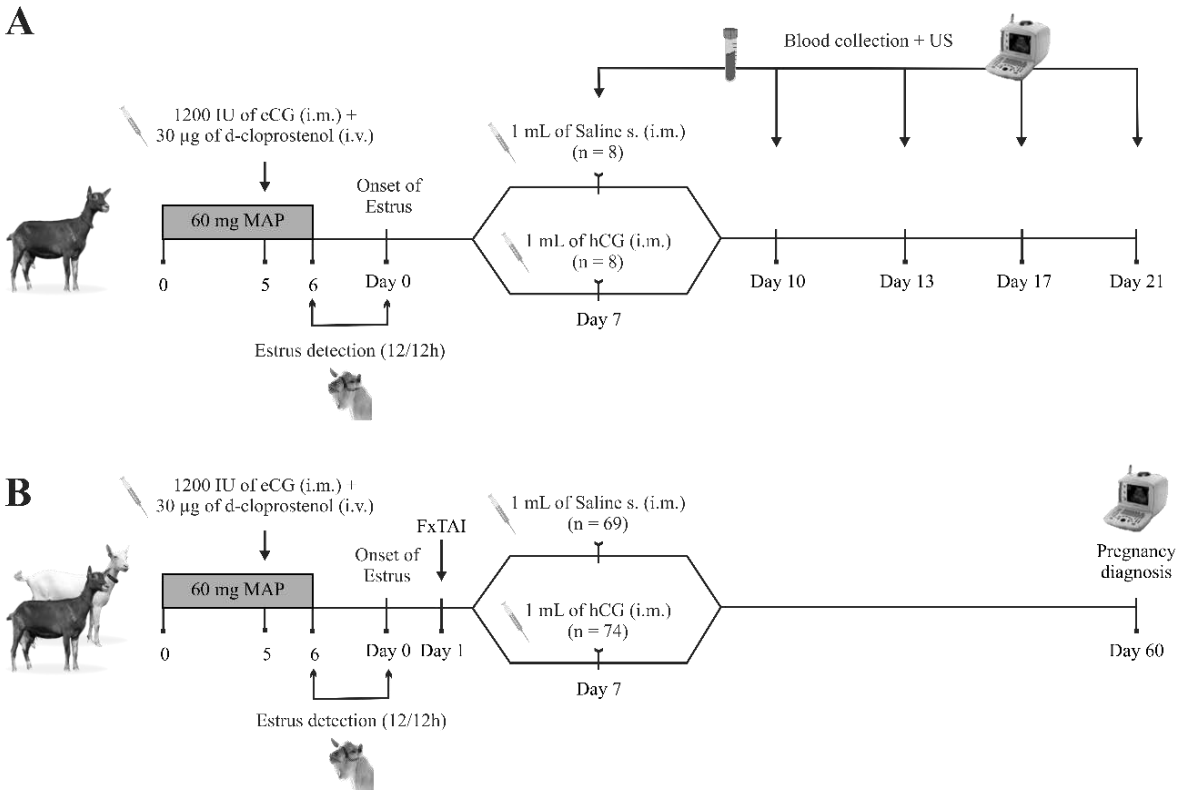
2.1. Location and experimental animals

Experiment 1 (n=16 Alpine goats) was conducted in the Embrapa Gado de Leite experimental field station situated in Coronel Pacheco, MG, Brazil (latitude 21°35'S, longitude 43°15'W, and altitude of 435 masl). Experiment 2 (n=60 Alpine goats and n=85 Saanen goats) was conducted in a commercial dairy goat farm in Ouro Fino, MG, Brazil (22º16' S and 46º22' W, and altitude of 908 masl). In both experiments, goats were reared in an intensive system, fed 50% of corn silage and 50% of Napier grass with concentrate supplementation (soybean, corn, and mineral nucleus-based mixture; with 16% of crude protein and 68% of total digestible nutrients) according to their nutritional needs (National Research Council, 2007). Mineral salt licks and water were available *ad libitum.* All goats were clinically healthy and free of reproductive disorders. The mean body condition score (BCS: 1=very thin and 5=very fat [20]) was 3.0±0.1, and body weight was 56.8±4.2 kg. All goats were in the last third portion of their lactation period, with a mean annual milk yield (305 days of lactation) of 727 kg [21].

2.2. Estrus induction and treatments applied

All goats received intravaginal sponges containing 60 mg of medroxyprogesterone acetate (MAP; Progespon®, Zoetis, São Paulo, SP, Brazil), which were inserted in the late afternoon (1700 to 1800 h) and were left in place for six days. One day before sponge removal, all goats received an i.m. injection of 200 IU of equine chorionic gonadotropin (eCG; Novormon 5000®, Zoetis, São Paulo, SP, Brazil) plus 30 μg of d-cloprostenol (Prolise®: Tecnopec, São Paulo, Brazil), late in the afternoon (1700 to 1800 h). In both experiments, estrus was detected twice daily with fertile bucks placed in a pen with the does for 30 min. In Experiment 2, artificial insemination (AI) was performed by the Embrapa® transcervical technique [22] using the flexible-timed approach (FxTAI) [23]. Semen from seven bucks (three Alpine and four Saanen bucks owned by the Brazilian progeny testing corporation CapraGene) was donated by Embrapa Goats and Sheep. Inseminate doses stored frozen in French straws (0.25 mL) contained 100×10^6 viable spermatozoa before freezing, with minimum progressive motility of 45% and spermatic vigor of 3 (range 0 to 5) were thawed in a water bath at 35ºC for 30 s. Semen quality of all bucks was subjected to semen soundness evaluation protocols and was assessed during the breeding [24] and non-breeding seasons [23]. The time of AI time was based on the detection of estrus; does with an early, intermediate, or late onset of estrus (relative to the time of MAP sponge removal) were inseminated 24, 18, or 10 h after the onset of estrus, respectively. Following the onset of behavioral estrus (Experiment 1) or FxTAI (Experiment 2), goats were divided by breed, body weight, BCS, age, parity, and time elapsed from intravaginal device removal to estrus into two equinumerous groups: goats that received i.m. injections of 300 IU of hCG (Vetecor®; Hertape-Calier do Brasil Ltda, São Paulo, SP; Brazil) (hCG; n=83) and animals injected with 1 mL of saline solution (Control; n=78) on day 7 after the onset of estrus (Fig. 1).

Figure 1 – Experimental design of the study. Evaluation of the effect of administering 300 IU of hCG i.m. on day 7 after the onset of estrus (day 0) in dairy goats during the seasonal anestrus. In Exp. 1 (A), Alpine goats received 1 ml of saline or 300 IU hCG. Females were allocated randomly to the two groups immediately after estrus detection. Jugular blood samples were drawn, and B-mode and color Doppler Alpine and Saanen the goats received 1 mL of saline or 300 IU hCG on day 7, and were artificially inseminated based on the timing of behavioral estrus (flexible time artificial insemination, FxTAI); ultrasonographic pregnancy check was done 60 days later. US: transrectal ovarian ultrasonography; MAP: medroxyprogesterone acetate (progestin)-soaked sponges



2.3. Ultrasound and luteal evaluation

In Exp. 1, transrectal ovarian ultrasonography (B-mode and color Doppler) was conducted one week before estrous induction to assure seasonal anestrus (CL absence) and on days 7, 10, 13, 17, and 21 (day 0=onset of behavioral estrus) using a portable ultrasound scanner equipped with a 7.5-MHz transducer (M5 Vet®; Mindray Medical International Limited, Shenzhen, China) (Fig. 1). The transducer was taped to a PVC tube to facilitate external manipulation during the transrectal exam. Does were examined in a standing position. Fecal pellets were removed manually from the rectum, and 10 mL of carboxymethylcellulose gel (Carbogel UTL; Carbogel Indústria e Comércio LTDA, São

Paulo, SP, Brazil) were deposited with a syringe into the rectum and approximately 5 mL were applied to the transducer before each ultrasonographic examination. Corpora lutea forming after initial ovulations (oCL) were observed on day 7, whereas the accessory corpora lutea (aCL) were recorded from day 10 onwards. The diameter, position, and vascularization of all detected luteal structures were sketched on individual ovarian charts. B-mode images were used to measure the luteal area (cm^2) defined as the sum of the cross-sectional regions of all detected luteal structures (oCL and aCL); the areas of central cavities, if present, were subtracted from the total luteal area [11]. The Doppler area (DA) of each corpus luteum was determined using ImageJ® software, with the number of color pixels ultimately converted to cm^2 [25]. The high-velocity DA (HVDA) was then determined using ImageProPlus® analytical software (Media Cybernetics Inc., San Diego, CA, USA); HVDA color pixels (an upper and lower quarter of the Doppler scale bar, that corresponds to the velocity range of 0.04 m/s to 0.08 m/s) were counted with the "Count/size" tool and converted to cm^2 [26]. In Exp. 2, pregnancy was detected with ultrasonography 60 days after FxTAI, using the same equipment and operated by the same experienced technician as in Exp. 1. All examinations were performed at the constant settings of the ultrasound scanner (75% color gain, pulse repetition frequency (PRF) of 1.0 kHz, 6 cm of depth, and wall filter (WF) of 75 MHz).

2.4. Blood collection and progesterone measurements

In Exp. 1, pre-prandial jugular blood samples were drawn from all goats into vacutainers containing lithium heparin (anticoagulant) on each day of the ultrasonographic examination between 0600 and 0700 h. The collection tubes were immediately placed on ice, transported to the laboratory, and centrifuged in a refrigerated centrifuge for 15 min at 1500 x *g*. After centrifugation, blood plasma was aspirated and stored at −20 °C in 1.5-mL microtubes until P_4 analysis at a later date. Plasma P_4 concentrations were determined by a solid-phase radioimmunoassay technique using commercial kits (ImmuChem, MP Biomedicals, Santa Ana, CA, USA). Sensitivity and intra-assay coefficients of variation were 0.05 ng/mL and 11%, respectively [17].

2.5. Statistical analysis

For statistical analyses in Exp. 1, only goats with active CL on day 7 were included (Control=7 does and hCG=5 does). Luteal data were analyzed for the entire observation period (days 7 to 21) except for various characteristics of aCL that were evaluated from days 13 to 21 (period of ultrasonographic detection of aCL). In Exp. 2, values for the following variables were determined: (after the onset of estrus)-estrus response (number of does in estrus/total number of does×100); interval from MAP sponge removal to the onset of estrus (h); interval from MAP sponge removal to FxTAI (h); interval from the onset of estrus to FxTAI (h); (on days 7, 10, 13, 17 and 21)-numbers of oCL and aCL corpora lutea; total luteal area (TA, cm^2); oCL and aCL area (cm^2); color Doppler area of oCL and aCL (oCL DA and aCL DA, cm2); relative DA areas (oCL DA/oCL area x 100% and aCL DA/aCL area x 100%); high-velocity DA for oCL and aCL (oCL HVDA and aCL HVDA, cm2); relative HVDA for oCL and aCL (oCL HVDA/oCL DA x 100% and aCL HVDA/aCL DA x 100%; plasma P_4 concentrations (ng/mL); (at the time of pregnancy detection)-pregnancy rate (number of pregnant does/number of does artificially inseminated×100); and proportion of inseminated goats with hydrometra (number of does with hydrometra/number of inseminated does×100).

Data analysis was performed using the libraries of the "car," "stats," "geepack," and "emmeans" packages of the R software (version 3.6.3, The R Foundation for Statistical Computing). The Shapiro-Wilk test was used to evaluate the normality of the residual. The Box-Cox transformation of the data was performed whenever necessary. Luteal dynamics data were analyzed by a repeated measurement statement, using generalized estimation equations (GHG) with a logit link to the counting data; the main effects of the treatment group, day and their interaction were included in the statistical model. Different covariance structures were evaluated, and the self-regressive one was selected for presenting the lowest value for Akaike's criterion (AIC). Fisher's exact test was used for nonparametric analyses and the analysis of variance (ANOVA) was used for parametric data. The Tukey test was used to compare the means of the treatments. Correlational analyses utilized simple linear regression. The significance level used for all analyses was 5%. The values are presented as mean ± SEM.

3. RESULTS

In Exp. 1, twelve out of 16 goats (75%) that showed estrus had ultrasonographically detectable corpora lutea (CL) on day 7 (hCG: n=5, and Control: n=7). Mean CL count on day 7 was similar ($P>0.05$) to hCG (1.8±0.1) and Control (2.1±0.1) goats, while on day 13 it was superior ($P<0.05$) in hCG (2.1±0.1) than in Control (1.7±0.1) goats. Accessory CL (aCL) were detected in all hCG treated does and in none of the Animals control. In one hCG goat, an aCL was detected on day 10, while in the remaining does aCL were observed from day 13 onwards. Three of the hCG-treated does had aCL. One doe had a single aCL, and two does formed two aCL.

There were significant effects of day and group and significant day x group interaction for total luteal area and ovulatory CL (oCL) area in goats of the present study (Figs. 2 A and B). Total luteal area increased ($P<0.05$) in hCG goats from day 7 to day 10 and again from day 10 to day 17 (Fig. 2A), whereas the mean oCL area in this subset of goats increased ($P<0.05$) from day 7 to day 10 but then did not change ($P>0.05$) until day 21 (Fig. 2B). Both variables were greater ($P<0.05$) in hCG compared with Control (in Control group, the total luteal area was equal to oCL area) from day 10 to 21. The mean cross-sectional area of aCL in hCG-treated goats increased ($P<0.05$) from day 13 to 21 (Fig. 2C).

There was a significant effect of day post-estrus for the color Doppler area (oCL DA) as well as high-velocity DA of oCL (oCL HVDA; Fig. 3). In addition, there was a significant effect of day and day x group interaction for oCL DA (Fig. 3A) and oCL HVDA (Fig. 3C), and a significant main effect of the treatment (group) for oCL HVDA/oCL DA x 100% (Fig. 3D). Mean oCL DA increased ($P<0.05$) from day 10 to day 13 and then declined ($P<0.05$) from day 17 to day 21 in hCG goats (Fig. 3A). Mean oCL DA declined from day 13 to day 21 in Animals control (Fig. 3A). Mean OCL DA was greater ($P<0.05$) in hCG compared with Control goats on days 13 and 17 (Fig. 3A). Finally, hCG animals exceeded ($P<0.05$) their control counterparts in mean oCL HVDA on days 13 and 17, and mean oCL HVDA values increased ($P<0.05$) in hCG goats from day 7 to day 13 (Fig. 3C). Overall, mean oCL HVDA/oCL DA x 100% values were greater in hCG than in Control does (There were no significant fluctuations in color Doppler characteristics of accessory CL recorded in hCG goats (Fig. 4).

Figure 2 – Mean (±SEM) total luteal area (A) as well as mean cross-sectional areas of ovulatory (oCL) (B) and accessory corpora lutea (aCL) (C) detected ultrasonographically in seasonally anovular Alpine goats with (hCG) or without (Control) a single i.m. injection of 300 IU of human chorionic gonadotropin (hCG) given seven days after the onset of induced estrus. Numbers of goats with detectable aCL are given in parentheses (C). Mean values denoted by different letters vary over time within each group and asterisks indicate the differences between groups of does. Note: total luteal area=oCL area in Control does

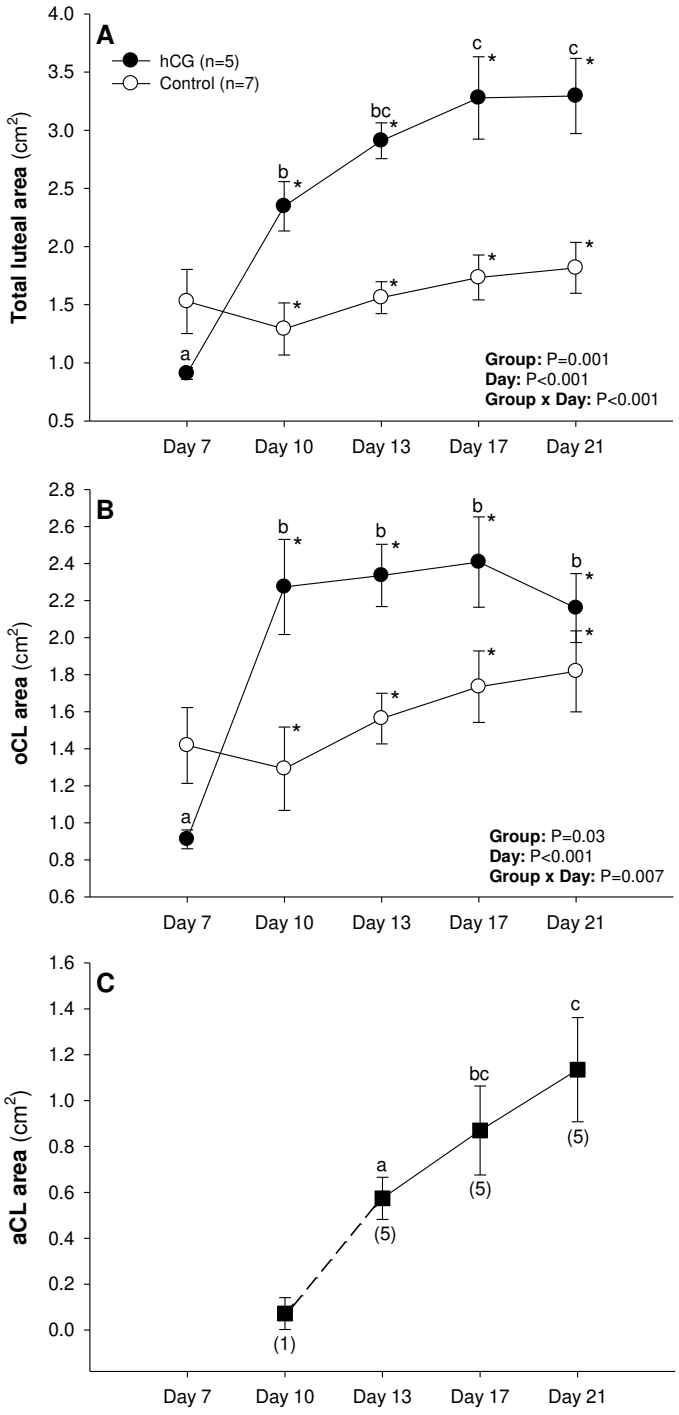


Figure 3 – Summary of the absolute and relative color Doppler area (DA; panels A and B) and high-velocity DA (HVDA) (panels C and D) determined in ovulatory corpora lutea (oCL) in Alpine goats that received a single i.m. injection of 300 IU of human chorionic gonadotropin (hCG) seven days after the onset of induced estrus (hCG) and their respective controls (Control). Mean values denoted by different letters vary over time within each group (ab-hCG and AB-Control) and asterisks indicate the differences between the two groups of does

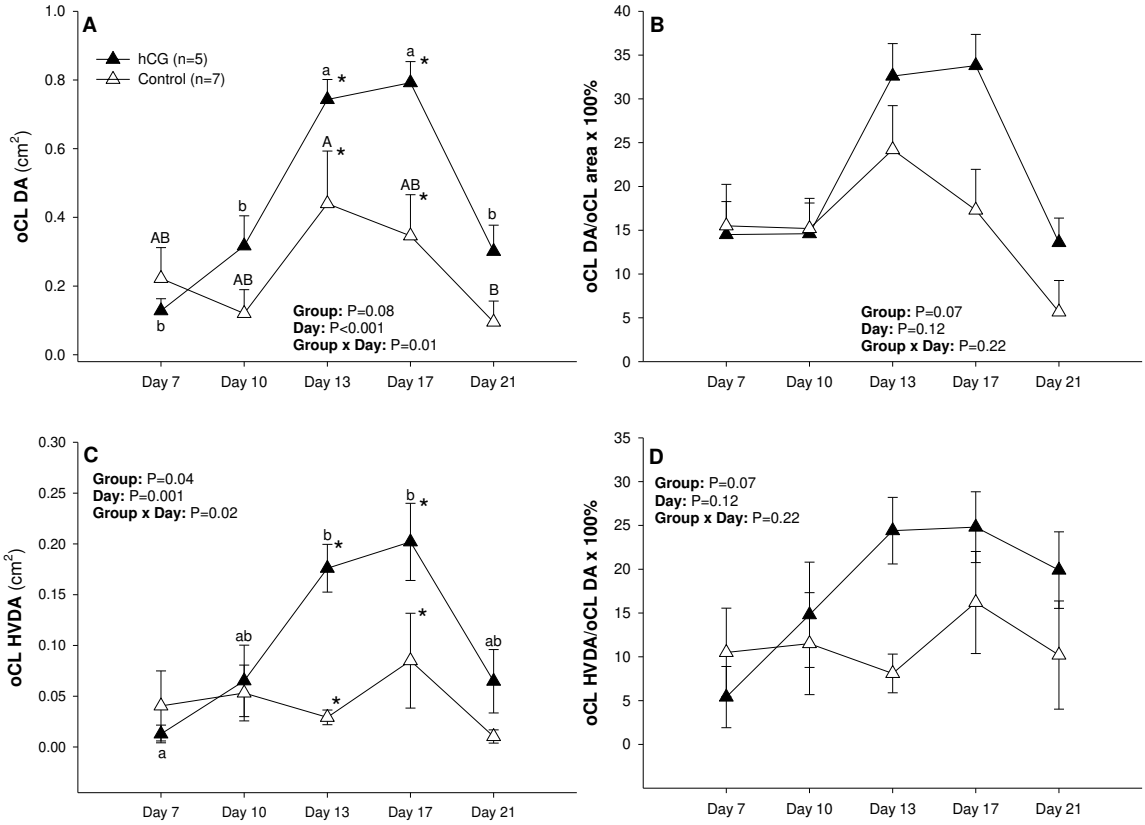
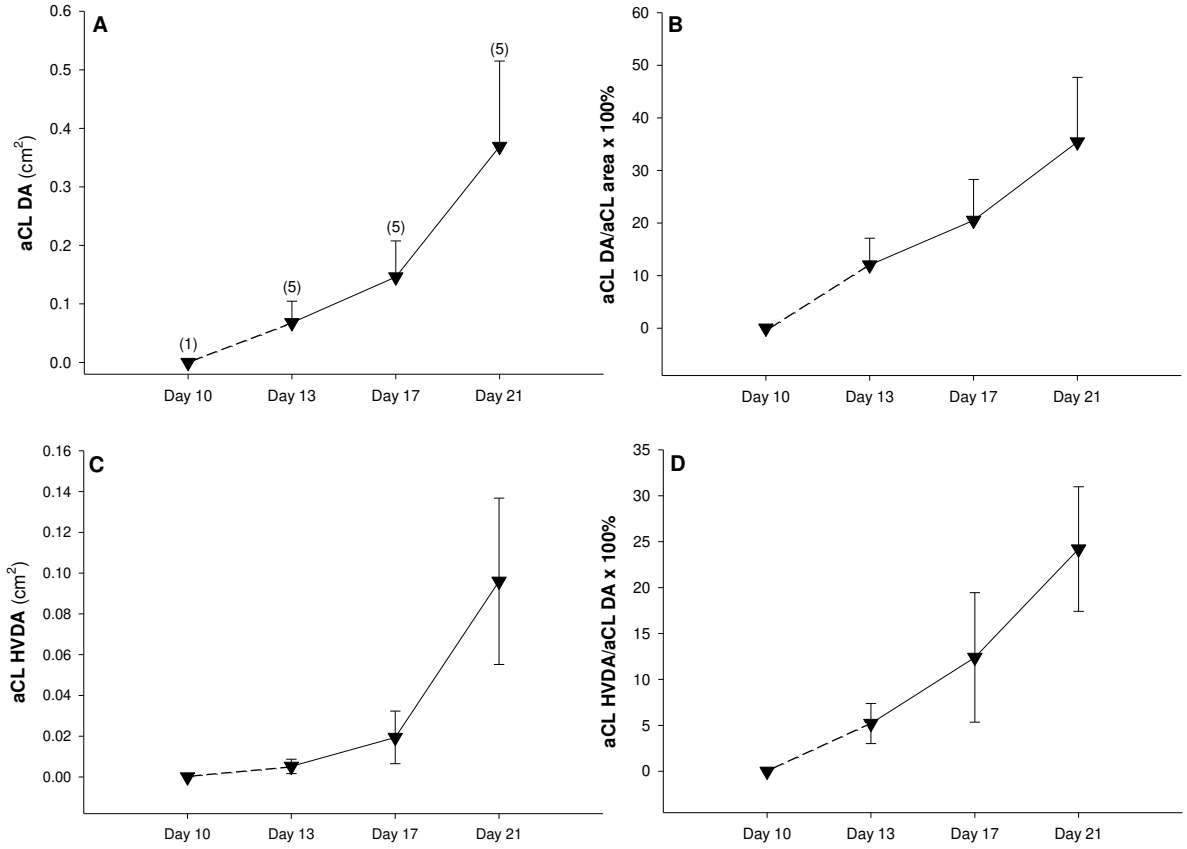


Figure 4 – Summary of the absolute and relative color Doppler area (DA; panels A and B) and high-velocity DA (HVDA) (panels C and D) determined in accessory corpora lutea (aCL) detected in Alpine goats that received a single i.m. injection of 300 IU of human chorionic gonadotropin (hCG) seven days after the onset of induced estrus. Numbers of goats with detectable aCL are given in parentheses (A)



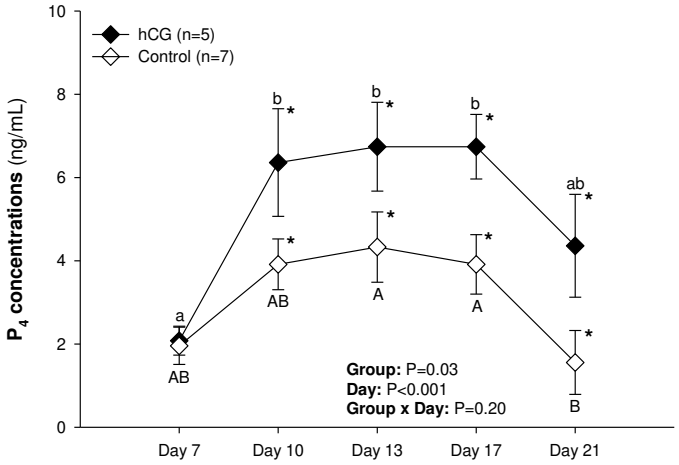
Plasma P_4 concentrations increased ($P<0.05$) from day 7 to day 10 in hCG group and from day 10 to day 13 in Control goats (Fig. 5). In animals control, plasma P_4 concentrations declined ($P<0.05$) from day 17 to 21. Plasma P_4 concentrations were greater ($P<0.05$) in hCG compared with control goats from day 13 to 21. Plasma P_4 concentrations decreased to basal or non-detectable levels on day 21 in one of five hCG goats (20%) and four of seven animals control (57%). During the entire blood collection period (day 7 to 21), plasma P_4 concentrations were positively correlated ($P<0.05$) with total luteal area, oCL area, oCL DA, and oCL DA/oCL area x 100 in both groups of goats (Table 1).

Table 1 – Summary of significant correlations between plasma progesterone (P_4) concentrations and quantitative ultrasonographic variables determined in hCG-treated and control Alpine goats on days 7, 10, 13, 17, and 21 (day 0=onset of estrus and day 7=day of hCG administration)

Dependent variable (y) vs. independent variable (x)	Coefficient of correlation (r)	P value	Regression equation
	hCG		
P_4* vs. total luteal area (cm^2)	0.60	0.002	y=–0.92+3.05x
P_4 vs. oCL area (cm^2)	0.75	<0.001	y=1.10+1.64x
P_4 vs. oCL DA (cm^2)	0.63	<0.001	y=2.55+6.00x
P_4 vs. oCL DA (%)	0.42	0.04	y=3.30+0.09x
P_4 vs. oCL HVDA (cm^2)	0.43	0.03	y=4.07+12.40x
	Control		
P_4 vs. total luteal area (cm^2) or oCL area (cm^2)	0.69	<0.001	y=–0.94+2.66x
P_4 vs. oCL DA (cm^2)	0.76	<0.001	y=2.03+4.84x
P_4 vs. oCL DA (%)	0.65	<0.001	y=1.23+0.12x
P_4 vs. oCL HVDA (cm^2)	0.52	0.005	y=2.44+16.01x
P_4 vs. oCL HVDA/oCL DA x 100%	0.42	0.02	y=2.41+7.34x

*P_4: plasma progesterone concentrations (ng/mL); DA: color Doppler area; HVDA: high-velocity Doppler area; oCL: ovulatory corpora lutea

Figure 5 – Mean (±SEM) circulating progesterone (P_4) concentrations in seasonally anovular Alpine goats with (hCG) or without (Control) a single i.m. injection of 300 IU of human chorionic gonadotropin (hCG) given seven days after the onset of induced estrus. Numbers of goats with detectable aCL are given in parentheses (C). Mean values denoted by different letters vary over time within each group and asterisks indicate the differences between the two groups of does



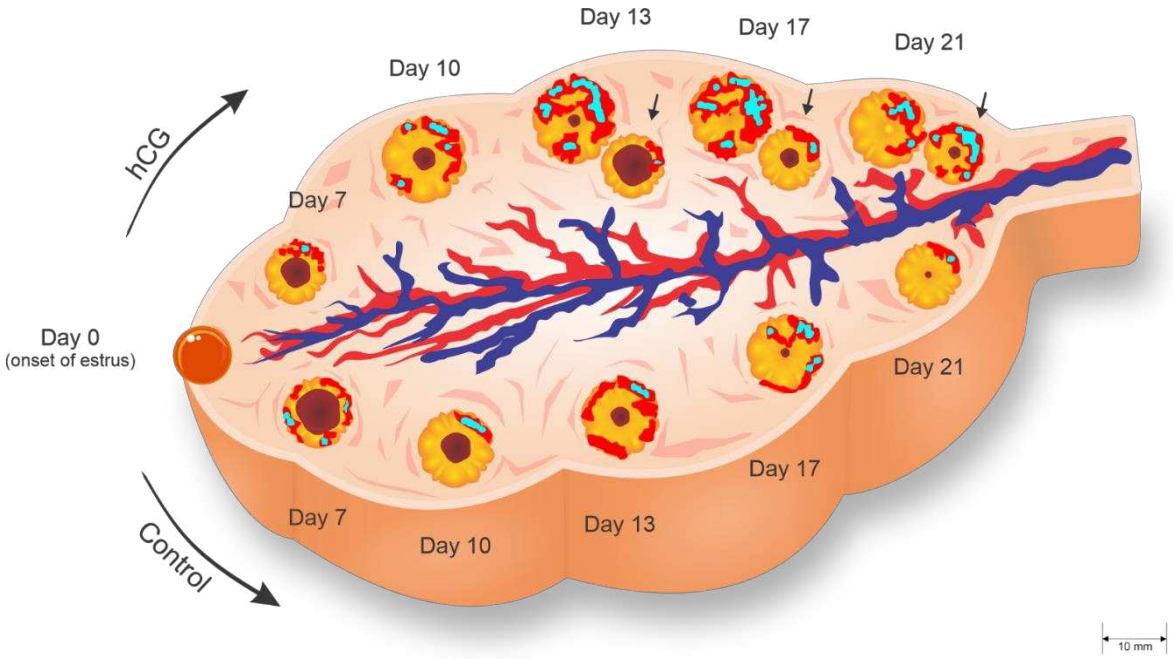
There was not a breed x group interaction in Exp. 2 (P>0.05) for any of the variables analyzed. The overall estrus response was 98.6% (143/145) and mean pregnancy rates were 56.7% in Alpine and 58.0% in Saanen goats. The pregnancy rate, however, was 22.5% higher (P<0.05) in hCG-treated goats than controls (Table 2). All goats showing hydrometra presented uterine US features with early fetal loss.

Table 2 – Reproductive performance of Alpine and Saanen goats that underwent flexible time artificial insemination (FxTAI) after estrus induction in the non-breeding season, with or without 300 IU of hCG given i.m. seven days after the onset of behavioral estrus (Exp. 2)

Variable	Control (n=69)	hCG (n=74)
Estrus response (%)		
36 h after device removal (early)	92.8 (64/69)	93.2 (69/74)
48 h after device removal (intermediate)	4.3 (3/69)	6.8 (5/74)
60 h after device removal (late)	2.9 (2/69)	0.0 (0/74)
Total	98.6 (69/70)	98.7 (74/75)
Interval from device removal to the onset of estrus (h)	29.2±1.2	28.5±1.0
Interval from the onset of estrus to FxTAI (h)	25.3±0.8	25.1±0.7
Interval from intravaginal device removal to FxTAI (h)	55.0±1.0	54.6±0.9
Goats with hydrometra (%)	2.9 (2/69)	1.4 (1/74)
Pregnancy rate (%)		
AI 24 h after the onset of estrus	46.4 (32/69)	64.9 (48/74)
AI 18 h after the onset of estrus	0.0 (0/69)	5.4 (4/74)
AI 10 h after the onset of estrus	1.4 (1/69)	0.0 (0/74)
Total	47.8 (33/69)a	70.3 (52/74)b

ab P<0.05

Figure 6 – A diagram of the main morphological and hemodynamic changes observed in the original (oCL) and accessory (aCL, ↓) corpora lutea, and determined with B-mode and color Doppler transrectal ovarian ultrasonography in Alpine goats that received 300 IU of hCG (hCG) or 1.0 mL of saline solution (Control) on day 7 of the synchronized estrous cycle (day 0=onset of estrus). Both red and blue colors within luteal tissue represent color Doppler area, but blue specifically corresponds to the approximate content of high-velocity Doppler signal



3. DISCUSSION

To the best of authors' knowledge, this is the first study documenting different luteotropic effects of hCG on oCL and aCL using B-mode and color Doppler ultrasonographic techniques. In addition, similar to previous studies, the present study confirmed significant effects of hCG on plasma P_4 concentrations [27] and pregnancy rate in goats [11]. Our present results indicate that hCG administration on day 7 after synchronized estrus can effectively be used in the reproductive management of goat herds in an intensive breeding system, increasing the pregnancy rates during the anestrous period.

In Alpine and Saanen goats, estrus can be induced hormonally during seasonal anestrus [18]. High estrus responses in Exp. 1 (100%) and 2 (98.6%) confirm the efficacy of the protocol used to induce estrus, which can be employed for both FxTAI [23] and intensive natural mating [11] in seasonally anovular lactating goats. However, four out of sixteen

Alpine goats (25%) did not have detectable CL on day 7 (day 0=onset of estrus). Application of the same estrus induction protocol resulted in an average ovulation rate of 81% in Toggenburg goats in the non-breeding season [28].

Interestingly, a single dose of hCG given 7 days after the onset of estrus induced CL formation in all ovulating goats in this study. In a previous study using Toggenburg goats during the transitional period (December to January), only 46.5% of hCG-treated goats formed aCL [11]. This difference in the ovarian response to hCG could be due to breed-specific differences in the wave-like pattern of antral follicular development, season, or both of these factors. Two follicular waves per interovulatory interval is a predominant pattern of antral follicular kinetics in cyclic Toggenburg goats [29], but four emerging waves were more commonly in Saanen [30] and Alpine goats [31]. The first wave of the estrous cycle invariably emerges at or around the time of ovulation and the largest (dominant) follicles of the wave attain their maximum diameter approximately 5-6 days later [23,30]. Depending on the periodicity of wave emergence, hCG administered 7 days after the onset of estrus may cause ovulation/luteinization of follicles developing in the first (static phase follicles) and/or second follicular wave after ovulation (growing phase follicles) as long as the follicles present are responsive to LH [32]. However, the days of follicle wave emergence in small ruminants may vary over the year [33] as ovarian follicular responsiveness to gonadotropic stimuli in seasonal breeders is significantly diminished outside of the breeding season [34–37].

The timing of ultrasonographic detection of aCL in does of the present study (days 10-13) agrees with those reported by Côrtes et al. [11] and Fonseca et al. [10] in estrus-synchronized Toggenburg goats and Santa Inês ewes, respectively, which received 300 IU of hCG on day 7 in the non-breeding season. Based on those studies, the average time to induce ovulation and/or luteinization of LH-responsive antral follicles is 108 h (4.5 days) following an application of hCG [38].

In Exp. 1, a sudden decline in plasma P_4 concentrations to a basal or non-detectable level occurred on day 21 in 57% of control does, whereas the proportion of such does in the hCG group was 20%. Thus, hCG administration on day 7 apparently did not prevent functional luteal regression in all synchronized goats in the non-breeding season, despite transiently improving oCL functionality and facilitating aCL formation, which ultimately led to an increase in total luteal tissue content and at least a transient rise in plasma P_4 concentration in individual goats. Declining blood P_4 concentrations herald pregnancy failure [11]. In Exp. 2, a lack of ovulation and premature luteolysis likely resulted in 52% and 30%

of non-pregnant Alpine and Saanen goats, in the control and hCG-treated groups, respectively. In the present study, plasma P_4 concentrations remained elevated for 14 days following hCG administration (day 10 to day 21), which includes the period of maternal recognition of pregnancy in goats [39]. Because such a shift in P_4 secretion did not occur in Toggenburg goats treated with hCG during the transitional period [11], it can be speculated that the mechanisms governing the effects of hCG vary between the reproductive seasons in goats. While during the seasonal anestrus, hCG appears to primarily enhance P_4 secretion of the luteal structures [10,25], during the transitional period its actions may be mainly associated with embryotropic and uterine effects [11,40]. As this is only a speculation based on our earlier and present observations, more research is needed to elucidate these mechanisms.

No previous study looking at the effects of hCG on luteal morphology and function in sheep or goats entailed ultrasonographic evaluation of ovulatory and induced CL. In the first experiment, the luteotropic effect of hCG on oCL size was observed as early as 3 days post-treatment (day 10), and it was mediated by LH receptors on luteal cells [41–43] promoting cellular hypertrophy [12]. A nearly 2.5-fold increase in oCL area was noted after day 7 in the hCG-treated group. This stimulatory effect of hCG was sustained up until day 21, resulting in a greater oCL area in hCG-treated compared with control does; no fluctuations in the mean oCL area (or total luteal area) occurred in animals control during the entire observation period. Concurrently, the mean cross-sectional area of aCL nearly doubled from day 13 to day 21 in hCG-treated does.

Present Doppler data and correlational analyses shed a new light on the role of luteal vascularity in sustaining elevated blood P_4 concentrations during early pregnancy in does. While luteal vascularity in untreated animals tended to increase after day 13 (based on the percentage of color Doppler area), the mean Doppler area within oCL of hCG-treated does declined significantly from days 13 to 21. High-velocity blood flow area (indicative of elevated steroidogenic activity of ovarian structures; [26]) increased significantly in oCL of hCG-treated animals. Moreover, the analyses of correlation equations for significant linear relationships between circulating P_4 concentrations and total/color Doppler area of oCL revealed that during days 7 to 21, the same amounts of luteal tissue and blood vessels were "generating" 1.6 times (2.76/1.72) and 1.2 times (8.55/6.87) more plasma P_4 in hCG-treated than in control does, respectively. Therefore, it may be suggested that hCG treatment not only increased the amount of luteal tissue while suppressing oCL blood perfusion, but it also

"improved the efficiency" of luteal cells and blood vessels in synthesizing and releasing luteal P_4 in early pregnant does. The latter appears to be supported by a rise in high-velocity blood flow in the ovulatory CL of hCG-treated goats. This is intriguing, but the specific underlying mechanisms of these effects of hCG remain to be elucidated.

Hydrometra was diagnosed in Exp.2 in three of the inseminated goats (two Control and one hCG doe), which showed early gestational loss ~ 60 days post-AI. Thus, in the present study, fetal loss that occurred despite CL maintenance was probably the cause of hydrometra [44]. Hydrometra is the main cause of reproductive disorders in dairy goats managed in the production systems like that in the present study [44], and the etiology of hydrometra remains complex [45]. Hormonal treatments used to synchronize estrus in cyclic and anovular goats [46] are not a likely cause of hydrometra since this disorder has also been identified in goats with estrus synchronized by the light program [17,47] as well as non-synchronized animals (i.e., without estrus induction) [47].

4. CONCLUSION

The administration of 300 IU of hCG seven days after the beginning of estrus in anestrous dairy goats resulted in the formation of aCL and had a hypertrophic effect on the existing (ovulatory) CL. Consequently, mean plasma P4 concentrations were greater in hCG-treated does during the period of maternal recognition of pregnancy. A single dose of hCG increased the pregnancy rate in seasonally anovular estrus-induced dairy goats subjected to FxTAI by 22.5%. The present study provides novel information on the luteotropic effects of exogenous hCG in gestating goats. It also suggests that the mechanism whereby hCG increases pregnancy rates in dairy goats in the non-breeding season appears to be mainly luteotropic.

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CAPÍTULO 5

Effects of hCG applied on days 5 or 7 of the estrous cycle on the area and vascularization of accessory and original corpora lutea of acyclic dairy goats

ABSTRACT

Human Chorionic Gonadotropin (hCG) affects the growth of the original corpora lutea (oCL) and accessory (aCL) of goats. The objective was to evaluate the effects of the administration of 300 IU of hCG on days 5 or 7 after observation of estrus on the number, area and vascularization of oCL and aCL, in addition to the plasma concentration of progesterone (P4) and pregnancy rate of estrus-induced dairy goats in the non-breeding season. Alpine goats received intravaginal sponges containing 60 mg of medroxyprogesterone acetate for 6 days, as well as 200 IU of eCG and 30 mg d-cloprostenol i.m. 24 h before sponge removal. On day 5 (day 0 = onset of synchronized estrus), all goats were randomly divided into three groups: animals treated with 300 IU of hCG i.m. on D5 (hCG_D5; n = 12); animals treated with 300 IU of hCG i.m. on D7 (hCG_D7; n = 11) and untreated controls (Control; n = 11). Transrectal ovarian ultrasound and blood collection were done on D5, D7, D10, D13, D17, and D21, and pregnancy detection on D60. aCL were detected in animals 66.6% in hCG_D5 and 90.9% in hCG_D7. The total luteal area increased ($P<0.05$) on day 10 in hCG_D7 and remained greater ($P<0.05$) than in Control D21. The luteal area growth rate of hCG_D5 was higher ($P<0.05$) than control a from D7 to D21, while hCG_D7 was superior ($P<0.05$) to the control from D10 to D21. The oCL area was higher ($P<0.05$) in hCG_D7 at D10 compared to Control, but the oCL luteal growth rate was higher ($P<0.05$) in hCG_D5 from D7 to D21 compared to Control and higher ($P<0.05$) in hCG_D7 from D10 to D21 compared to Control. hCG applied on days 5 or 7 increased plasma P4 concentration from days 10 to 21 ($P<0.1$) and there was no difference in pregnancy rate between groups. The application of 300 IU of hCG on days 5 or 7 of the estrous cycle promotes the growth of oCL, induces the formation of aCL, increases the concentration of P4, and presents similar results among themselves, with the use on the seventh day more effective. in the induction of aCL.

Keywords: Human Chorionic Gonadotropin. Progesterone. Luteal area.

1. INTRODUCTION

The establishment of pregnancy in goats begins at the conceptus stage and includes pregnancy recognition signaling, implantation, and placentation. (SPENCER et al., 2007; SPENCER; SANDRA; WOLF, 2008). Progesterone plays an important role in this process because a uterine environment with high concentrations of P4 can lead to greater growth of the conceptus (KHAN; BECK; KHALID, 2007, 2009), which, in turn, secretes more interferon-tau (IFNγ), signaling pregnancy more effectively (NEPHEW et al., 1994), which reduces embryonic mortality and consequently increases the pregnancy rate in goats (BUSTAMANTE-ANDRADE et al., 2021; CÔRTES et al., 2021; RODRIGUES et al., 2022) and ewes (AZARI et al., 2020; CATALANO et al., 2015; KHAN et al., 2003; KILLEEN; MOORE, 1970; KITTOK; STELLFLUG; LOWRY, 1983; NEPHEW et al., 1994; VERGANI et al., 2020).

In the goat, progesterone (P4) is produced mainly by the corpus luteum (CL) during pregnancy, without the placenta making an appreciable contribution (LINZELL; HEAP, 1968). In goats, studies have proven the effectiveness of Human Chorionic Gonadotropin (hCG) in increasing the weight or luteal area (BUSTAMANTE-ANDRADE et al., 2021; RODRIGUES et al., 2022), in inducing the formation of accessory corpus luteum (CÔRTES et al., 2021; RODRIGUES et al., 2022), to increase luteal vascularization (RODRIGUES et al., 2022), raise the concentration of P4 (FONSECA et al., 2005a, 2006; LASHARI; TASAWAR, 2010; RODRIGUES et al., 2022), increase the length of the conceptus (LASHARI; TASAWAR, 2010) and improve the pregnancy rate (BUSTAMANTE-ANDRADE et al., 2021; CÔRTES et al., 2021; FONSECA et al., 2005a; RODRIGUES et al., 2022).

Rodrigues et al. (2022) demonstrated that a single dose of 300 IU of hCG applied on day 7 of the estrous cycle of estrus-induced dairy goats has a luteotrophic action mainly on the original corpora lutea (oCL), increasing their area and plasma P4 concentration from the day 13 of the cycle.

Thus, the objective of this study was to evaluate the effects of the administration of 300 IU of hCG on days 5 or 7 after observation of estrus on the number, area and vascularization of original and accessory corpora lutea, in addition to the plasma concentration of progesterone. and pregnancy rate of estrus-induced dairy goats in the non-breeding season, under the hypothesis that a two-day anticipation of hCG application (day 5)

could have better effects on the luteal growth of the original corpora lutea and thus increase the P4 concentration and pregnancy rate in relation to day 7 application.

2. MATERIAL AND METHODS

All experimental procedures had been reviewed and approved by the Ethics Committee of the Embrapa Gado de Leite (protocol #5755150721). The present study was conducted from October to November, during the non-breeding season of goats in Brazil (BALARO et al., 2019).

2.1. Location and experimental animals

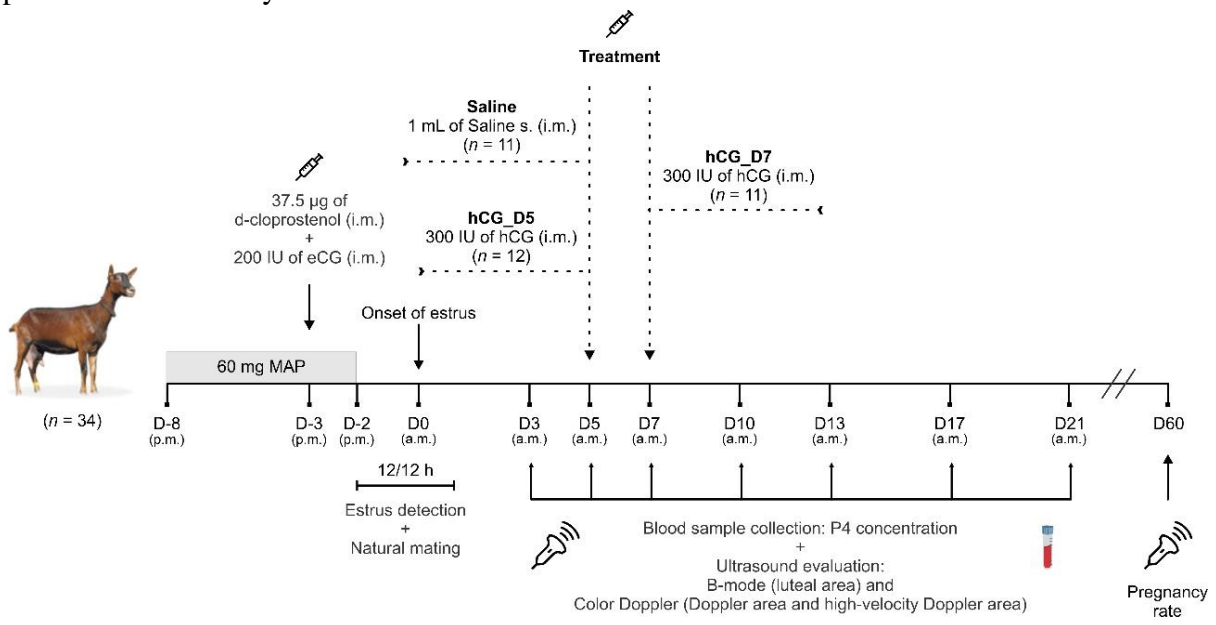
The experiment utilized clinically healthy 34 Alpine goats (between 1 and 4 years of age, 13 nulliparous and 21 multiparous) housed in the Embrapa Gado de Leite experimental field station situated in Coronel Pacheco, MG, Brazil (latitude 21°35'S, longitude 43°15'W, and altitude of 435 m.a.s.l.). Goats were reared in an intensive system, fed 50% of corn silage and 50% of Napier grass with concentrate supplementation (soybean, corn, minerals and vitamins based mixture; with 16% of crude protein and 68% of total digestible nutrients) according to their nutritional needs (National Research Council, 2007). Mineral salt licks and water were available ad libitum. The mean body condition score (BCS: 1=very thin and 5=very fat (Villaquiran et al., 2007)) was 3.2±0.1, and mean body weight of does at the beginning of the study was 54.5±3.3 kg. All multiparous goats were in the last third portion of their lactation period (305 days), with a mean annual milk yield of 727 kg (FACO et al., 2020).

2.2. Hormonal protocol to estrus induction and hCG treatment

To estrus induction, all goats received intravaginal sponges containing 60 mg of medroxyprogesterone acetate (MAP; Progespon®, Zoetis, São Paulo, SP, Brazil), which were inserted in the late afternoon (5 p.m. to 6 p.m.) and were left in place for six days. One day before sponge removal, all goats received an i.m. injection of 200 IU of equine chorionic gonadotropin (eCG; Novormon 5000®, Zoetis, São Paulo, SP, Brazil) plus 30 μg of d-cloprostenol (Prolise®: Tecnopec, São Paulo, Brazil), late in the afternoon (5 p.m. to 6 p.m.).

Estrus was detected twice daily with fertile bucks placed in a pen with the does for 30 min. After estrus onset, goats were mated twice, at the time of observation of the estrus onset and 12 h later. The onset of estrus was Day 0 (D0). On D5, goats that had one or more active corpora lutea were randomly allocated into one of three treatment groups: Control goats (n = 11, 39.36 ± 0.64 kg and 3.14 ± 0.07 of BCS) received 1.0 mL of saline solution on D5; hCG D5 goats (n = 12, 39.36 ± 0.64 kg and 3.14 ± 0.07 of BCS) received 300IU of hCG i.m. (Vetecor®, Hertape-Calier do Brasil Ltda, São Paulo, Brazil) on D5, while the hCG D7 goats (n = 11; 39.24 ± 0.52 kg and 3.25 ± 0.13 of BCS) received 300IU of hCG i.m. on D7 (Fig. 1).

Figure 1 – Experimental design. Thirty-four Alpine goats estrus induced were allocated to receive 1 mL of saline solution i.m. or 300 IU of hCG i.m. on day 5 (D5) or day 7 (D7) after the onset of estrus. Natural mating was realized. Jugular blood samples were drawn at D3, D5, D7, D10, D13, D17, and D21. B-mode and color Doppler ultrasonography of ovaries was performed on the days of blood collection.



2.3. Ovarian ultrasonography and luteal evaluation

The transrectal ovarian ultrasonography (B-mode and color Doppler) was conducted one week before estrous induction to assure seasonal anestrus (CL absence) and on days 5, 7, 10, 13, 17, and 21 (day 0=onset of behavioral estrus) using a portable ultrasound scanner equipped with a 7.5-MHz transducer (M5 Vet®; Mindray Medical International Limited, Shenzhen, China) (Fig. 1). The transducer was taped to a PVC tube to facilitate external manipulation during the transrectal exam. Does were examined in a standing position. Fecal pellets were removed manually from the rectum, and 10 mL of carboxymethylcellulose gel

(Carbogel UTL; Carbogel Indústria e Comércio LTDA, São Paulo, SP, Brazil) were deposited with a syringe into the rectum and approximately 5 mL were applied to the transducer before each ultrasonographic examination. Corpora lutea forming after initial ovulations (oCL) were observed on day 5, according to diameter, position, and vascularization (RODRIGUES et al., 2022), whereas the accessory corpora lutea (aCL) were recorded from day 10 onwards. B-mode images were used to measure the luteal area (cm²) defined as the sum of the cross-sectional regions of all detected luteal structures (oCL and aCL); the areas of central cavities, if present, were subtracted from the total luteal area (CÔRTES et al., 2021).

The Doppler area (DA) and the high-velocity DA (HVDA) were determined using ImageProPlus® analytical software (Media Cybernetics Inc., San Diego, CA, USA); HVDA color pixels (an upper and lower quarter of the Doppler scale bar, that corresponds to the velocity range of 0.04 m/s to 0.08 m/s) were counted with the "Count/size" tool and converted to cm² (OLIVEIRA et al., 2017). The pregnancy was detected with ultrasonography 60 days after breeding, using the same equipment and operated by the same experienced technician. All examinations were performed at the constant settings of the ultrasound scanner (75% color gain, pulse repetition frequency (PRF) of 1.0 kHz, 6 cm of depth, and wall filter (WF) of 75 MHz).

2.4. Blood sampling and plasma P4 measurement

On Days 3, 5, 7, 10, 13, 17 and 21, Before each US exam, blood samples were collected via jugular vein puncture from all goats into vacutainers containing lithium heparin (anticoagulant). The collection tubes were immediately placed on ice, transported to the laboratory, and centrifuged in a refrigerated centrifuge for 15 min at 1500 x g. After centrifugation, blood plasma was aspirated and stored at −20 °C in 1.5-mL microtubes until P4 analysis at a later date.

Plasma progesterone (P4) concentrations were determined with a solid-phase radioimmunoassay technique using commercial kits (ImmuChem; MP Biomedicals, Santa Ana, CA, USA). Sensitivity and intra-assay coefficients of variation were 0.05 ng/mL and 8%, respectively.

2.5. Statistical analysis

Data were analyzed using IBM SPSS Statistics software, version 19. The general linear model with repeated measures over time was applied to the data collected between days. The normality assumption was verified using the Shapiro-Wilk test, and Levene test for homogeneity of variances. Parameters and indicators were also analyzed using Kruskal Wallis tests followed by Dunn's post hoc for non-parametric data; when parametric, ANOVA was applied followed by Tukey's post hoc. Differences were considered as significant when $P<0.05$. Results were presented as mean±standard error (SEM).

3. RESULTS

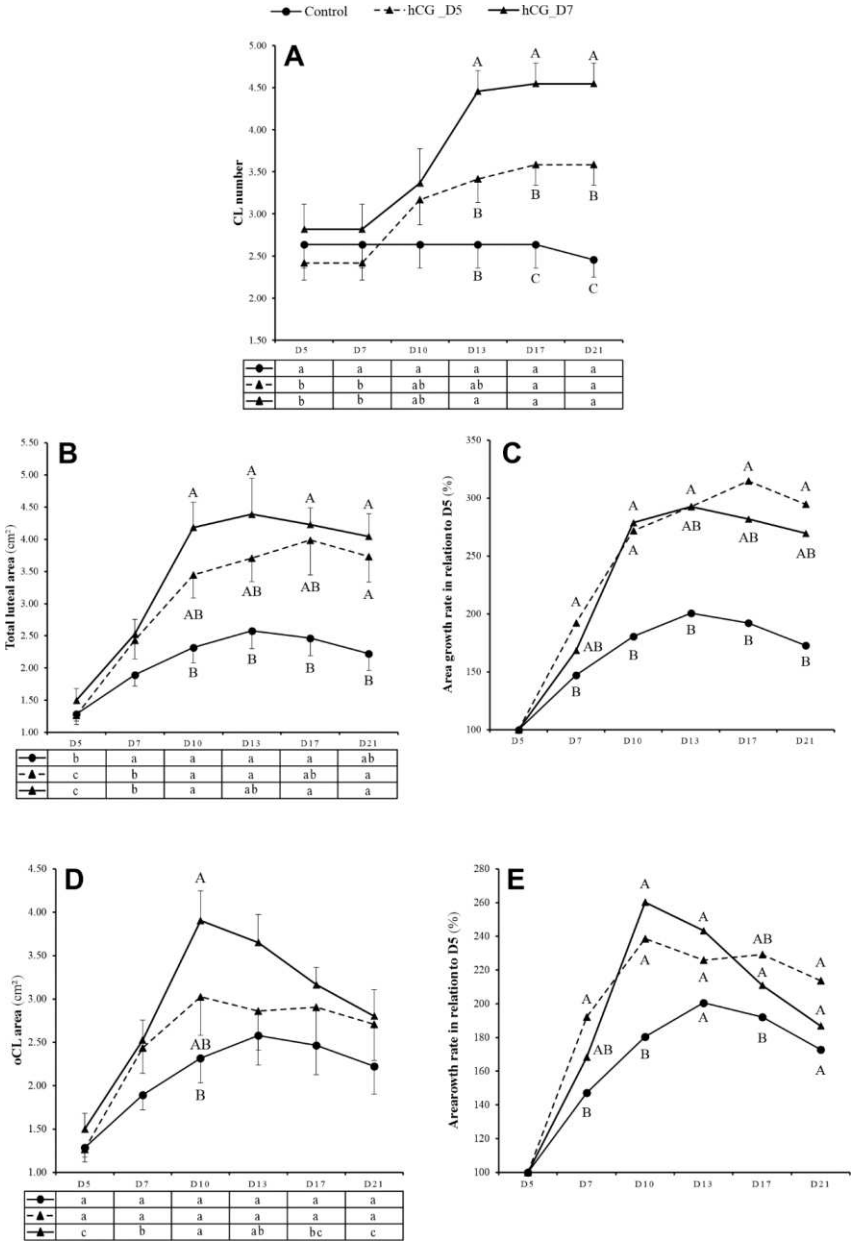
On D5 and D7 there was no difference ($P>0.05$) in the number of CL between the groups (Fig. 2A). aCL were detected in both treated groups, both with onset after D10. In hCG_D5 66.6% (8/12) of the animals developed aCL, 50% (6/12) from D10 onwards, 8.33% (1/12) from D13 onwards and 8.33% (1/12) from D17. In hCG_D7 90.9% developed aCL, 36.4% (4/11) from D10 and 54.5% (6/11) from D13. ACL was not detected in the control group. In hCG_D5, two animals developed aCL on different days, one presented an aCL on D10 and a new aCL on D13, and another animal presented an aCL on D13 and a new aCL on D17, and in the hCG_D7 group the same occurred with two animals, in that one goat presented two aCL in D10 and a new aCL in D13 and another goat presented two aCL in D13 and a new aCL in D17.

When evaluating the mean values of luteal area (Fig. 2B), the hCG_D7 group was superior ($P<0.05$) to the control from D10 to D21, while the hCG_D5 was superior ($P<0.05$) to the control only in D21. However, when evaluating the growth rate of the luteal area (Fig. 2C) from D5, it was noted that the growth of the luteal area of hCG_D5 was higher ($P<0.05$) from D7 compared to the control.

When evaluating the effect of hCG on the original corpora lutea, a superior luteal area ($P<0.05$) of hCG_D7 in relation to the control was observed only on D10 (Fig. 2D) and no difference ($P<0.05$) throughout the days. between hCG_D5 and control groups. Again, when evaluating the luteal growth rate in relation to D5 (Fig. 2E) the effect on the luteal area of the oCL becomes clearer: higher growth ($P<0.05$) of the oCL of hCG_D5 in relation to the control at D7 and D10, while the hCG_D7 was superior ($P<0.05$) to the control in D10 and

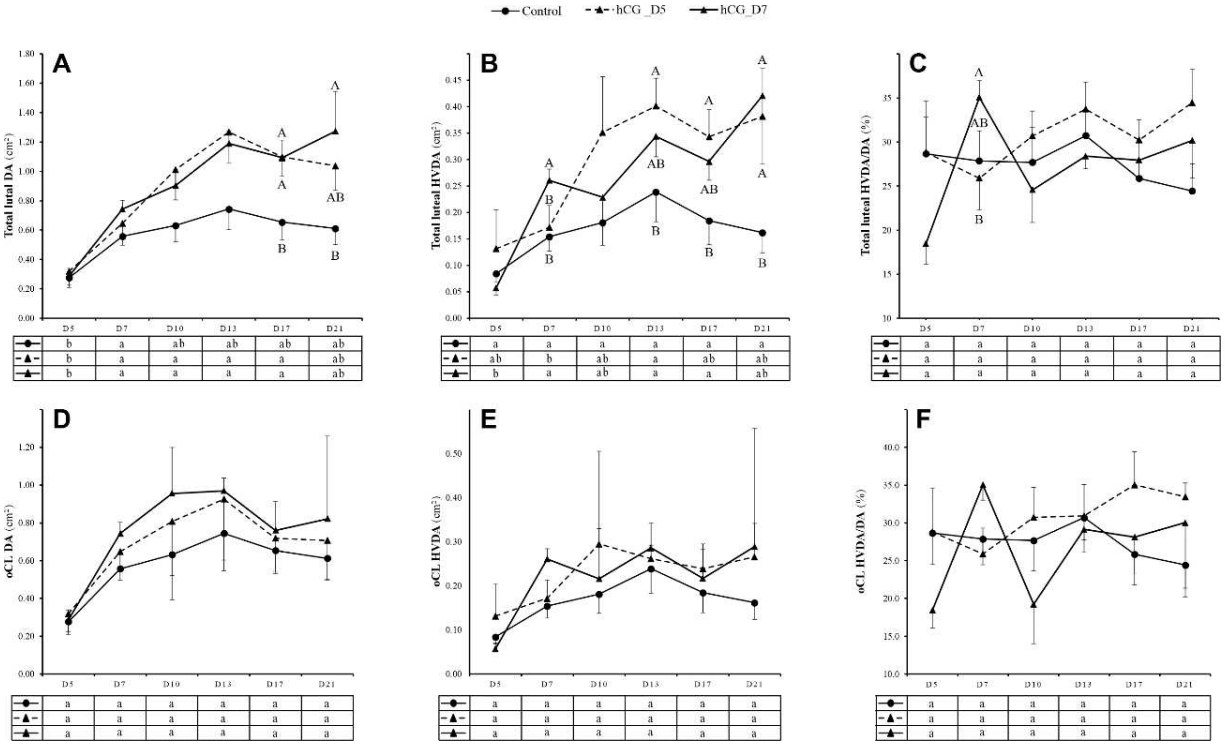
D17. The behavior of luteal area of the oCL had different behavior over the days in each group, in which the luteal area decreased from D10 to D17 (P>0.0.5) and again to D21 (P>0.0.5), while in the hCG_D5 was maintained from D7 to D21 (P>0.05).

Figure 2 – Mean (±SEM) CL number (A), total luteal area (B) area growth rate in relation to D5 (C), original CL (oCL) area (D) and oCL area growth rate in relation to D5 (E) detected ultrasonographically in seasonally anovular Alpine goats with or without (Control) a single i.m. injection of 300 IU of human chorionic gonadotropin (hCG) given five (hCG_D5) or seven days (hCG_D7) after the onset of induced estrus. Mean values denoted by different letters vary over time within each group (abc) and indicate the differences between groups of does (ABC).



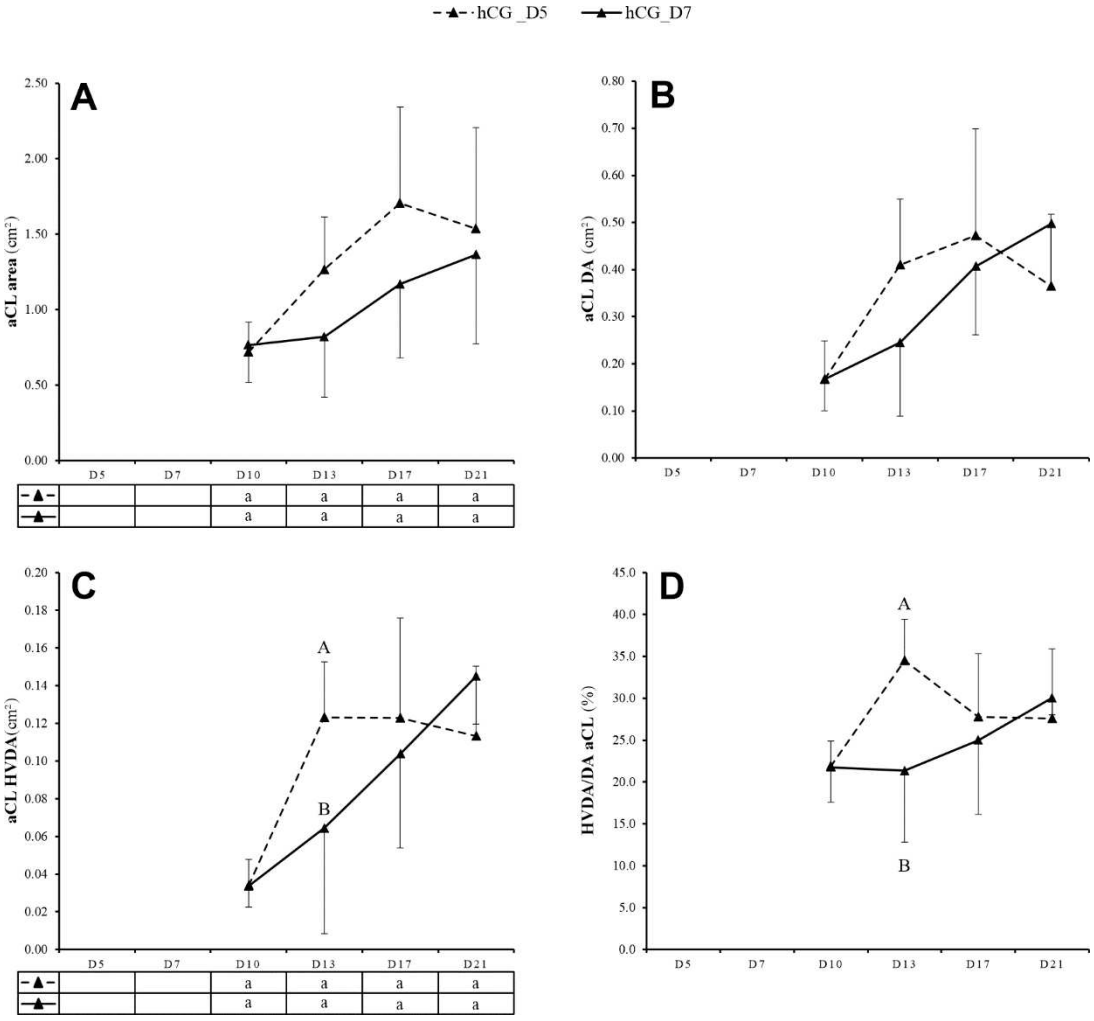
Comparing the hCG_D7 and hCG_D5 groups in the luteal area, there was only a difference in the total (Fig. 2C) and oCL (Fig. 2E) growth rate at D7, with higher mean values (P<0.05) in hCG_D5 and no significant difference in the luteal area of aCL (Fig. 3A).

Figure 3 – Mean (±SEM) total luteal Doppler area (DA) (A), total luteal high-velocity DA (HVDA) (B), total luteal HVD/DA (C) original corpora lutea (oCL) DA (D), oCL HVDA (E) and oCL HVDA/DA (F) detected ultrasonographically in seasonally anovular Alpine goats with or without (Control) a single i.m. injection of 300 IU of human chorionic gonadotropin (hCG) given five (hCG_D5) or seven days (hCG_D7) after the onset of induced estrus. Mean values denoted by different letters vary over time within each group (ab) and indicate the differences between groups of does (AB).



In the Doppler evaluation, the total Doppler area (AD – Fig. 4A) was higher (P<0.05) in the two treated groups in relation to the control in D17 and in the hCG_D7 in relation to the control in D21. While HVDA (Fig. 4B) was higher (P<0.0.5) in hCG_D5 on D13 to D21 compared to control, in hCG_D7 it was higher (P<0.05) than control only on D7 and D21 and the HVDA/DA ratio was superior in hCG_D7 compared to the control only in D7. Comparing the treated groups, there was a significant difference only in HVDA (Fig. 4B), where hCG_D7 was superior to hCG_D5 on D7. There was no difference in the mean values of the Doppler parameters for the oCL assessments (Fig. 4 A, B and C).

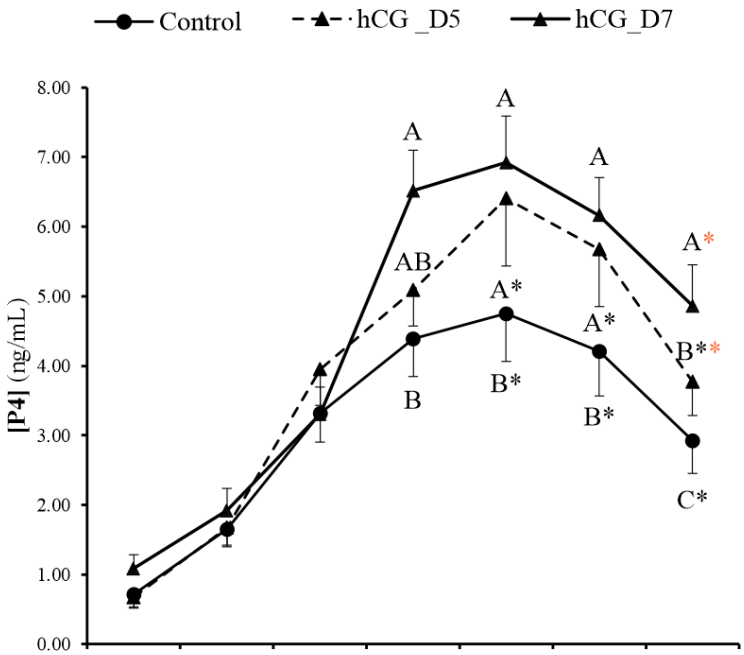
Figure 4 – Mean (±SEM) accessory CL area (A), aCL Doppler area (DA) (B), aCL high-velocity DA (HVDA) (C) and aCL HVD/DA (D) detected ultrasonographically in seasonally anovular Alpine goats with or without (Control) a single i.m. injection of 300 IU of human chorionic gonadotropin (hCG) given five (hCG_D5) or seven days (hCG_D7) after the onset of induced estrus. Mean values denoted by different letters vary over time within each group (ab) and indicate the differences between groups of does (AB).



Plasma concentration of progesterone (Fig. 5) showed a similar behavior between the groups over the days and was higher (P>0.05) and tended to increase (P>0.05 and <0.1) from D10 onwards in the hCG_D7 and hCG_D5, respectively, compared to the control.

In the ultrasound evaluation performed on D21, it was possible to identify anechoic uterine content associated with one or more CL with good vascularization in 100% of the animals hCG_D5 (12/12) and hCG_D7 (11/11) and 90.9% (10/11) of the animals' control. In a control animal, anechoic content was identified in the uterus with LC of low vascularization. The pregnancy rate at D60 was: 100% in hCG_D5, 100% in hCG_D7 and 90.9% in Control, with no statistical difference (P>0.05) between groups.

Figure 5 – Mean (±SEM) circulating progesterone (P4) concentrations in seasonally anovular Alpine goats with or without (Control) a single i.m. injection of 300 IU of human chorionic gonadotropin (hCG) given five (hCG_D5) or seven days (hCG_D7) after the onset of induced estrus. Mean values denoted by different letters vary over time within each group (ab) and indicate the differences between groups of does (AB). Asterisks of the same color indicate a tendency (P>0.05 and <0.1) to the differences between the groups on the graphic and differences over time within each group below the graphic.



	D3	D5	D7	D10	D13	D17	D21
●—	c	b	a	a	a	ab	ab
-▲-	c	b*	a	a	a	a	a*
—▲—	d	c	b*	a*	a	a	ab

4. DISCUSSION

Knowing the luteotrophic action of hCG on the original corpora lutea of goats, this is the first study that sought to identify the best day of the initial luteal phase for hormone administration, aiming at a greater luteal area and P4 production throughout the cycle. In addition, similar to previous studies, the present study confirmed the efficacy of hCG administration on increasing luteal area by growing the original corpora lutea (RODRIGUES et al., 2022) and induction of accessory corpora lutea (CÔRTES et al., 2021; RODRIGUES et al., 2022) of goats.

The moment of detection of aCL in this study (from 3 days after application) corroborates with previous studies in goats (CÔRTES et al., 2021; RODRIGUES et al., 2022) and ewes (FONSECA et al., 2018). No aCL was observed on D7 in the hCG_D5 group

because the average time to induce ovulation and/ or luteinization of LH-responsive antral follicles is 108 h (4.5 days) following an application of hCG (ALVARADO-ESPINO et al., 2016).

Formation of aCL by hCG administration occurs through stimulation of follicular rupture and luteogenesis or by luteinization of antral follicles (DRIANCOURT, 2001). In this study, the administration of hCG on D7 was more effective in forming aCL than on D5. Since the dominant follicles of the first follicular wave reach their maximum size between days 5 and 6 after ovulation (D6 and 7 after the onset of estrus) and the second follicular wave begins between days 5 and 8 after ovulation (D6 and 9 after the onset of estrus) (DE CASTRO et al., 1999), we believe that hCG administered on D7 can also act on ovulation/luteinization of follicles of the second follicular wave, since hCG administered intramuscularly has a biological half-life of 39.4 ± 5.1 h in goats and traces of hCG were found in the system 5 days after administration (SALEH et al., 2012).

In previous studies, the use of hCG on D7 promoted aCL in 100% of Alpine goats in the non-breeding season (RODRIGUES et al., 2022) and 46.5% of Toggenburg goats in the transition to the breeding season (CÔRTES et al., 2021). Studies in goats with hCG administration on D5 did not study the development of aCL (FONSECA et al., 2005a, 2005b, 2006; FONSECA; TORRES, 2005).

Even with a higher number of aCL in hCG_D7 from D13 onwards, this group did not present a larger luteal area in relation to hCG_D5 in these days, due to the main luteotrophic action of hCG being on the oCL in both groups. This phenomenon occurs because on days 5 and 7 the CL of the goats is in the growth phase (BALARO et al., 2017) and has LH/hCG responsive receptors, mainly on small luteal cells (FITZ et al., 1982). hCG promotes the conversion of small luteal cells into large luteal cells, in addition to increasing the number of fibroblasts and vascular cells (FARIN et al., 1988).

Rodrigues et al. (2022) confirmed this increase in total luteal area and oCL, in addition to the increase in oCL vascularization and P4 concentration from day 10 of the estrous cycle in dairy goats that received 300 IU of hCG on day 7 of the cycle. The anticipation of 2 days in the administration of hCG (hCG_D5) in this study promoted a gain in the total luteal area of oCL at D7 in the hCG_D5 group in relation to hCG_D7, but the groups were equal in D10. In addition, the oCL area was more stable throughout the cycle in hCG_D5, however, this decreases in the oCL area in hCG_D7 did not cause a decrease in P4 concentration or the gestational rate.

hCG regulates the expression of pro-angiogenic factors (SUGINO et al., 2000; WULFF et al., 2000), which may increase luteal vascularization. In this study, there was greater vascularization in the groups treated from D17 onwards, in addition to greater HVDA in the hCG_D5 group from D13 and D21 in hCG_D7 compared to the control, indicative of high steroidogenic activity of ovarian structures (OLIVEIRA et al., 2017).

One application of 300 IU of hCG on day 5 after observation of estrus promoted an increase in plasma P4 concentration from D13 onwards and the same application on day 7 promoted an increase in plasma P4 concentration from D10 onwards. We believe that this difference in response on D10 occurred because the hCG_D7 group started the study with a higher mean number of CL and, therefore, had a better response to hCG and greater production of P4 on D10. In any case, it is important to highlight the elevation of P4 in both groups (D13 to D21) in the maternal recognition phase of pregnancy in goats (SPENCER et al., 2004).

5. CONCLUSION

The administration of 300 IU of intramuscular hCG on days 5 or 7 of the estrous cycle of estrus-induced goats in the non-breeding season was able to induce the formation of accessory corpora lutea, promote an increase in the luteal area of original corpora lutea and increase the plasma concentration. of progesterone, however, there was no difference in these effects between the days of application.

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CONCLUSÕES

No capítulo 1, uma única aplicação de 300 UI de hCG por via intravaginal no momento da IA não alterou o tempo de ovulação, o tamanho ou o número de folículos ovulados. Também não foi capaz de prevenir a regressão lútea precoce, nem aumentar a concentração plasmática de progesterona, porém promoveu aumento da área lútea nos dias 17 e 21 e aumento da vascularização lútea nos dias 10 e 13 do ciclo, além de elevar a taxa de concepção em 13,1% em cabras leiteiras sincronizadas com duas doses de d-cloprostenol durante a estação reprodutiva.

No capítulo 2, pôde ser concluído que a hCG pôde ser detectada com o teste rápido de β-hCG em amostras de plasma sanguíneo após administração i.m., mas não foi detectável em 50% das cabras que receberam hCG por via intravaginal. A gonadotrofina coriônica humana administrada na IATF tem efeito limitado na função lútea e na fertilidade de caprinos. A única diferença observada entre os animais que receberam hCG por via i.m ou intravaginal foi que vários parâmetros lúteos diminuíram em D17 no controle e i.m.

No capítulo 3, concluiu-se que 300 UI de gonadotrofina coriônica humana administrada no sétimo dia após a observação do estro pela via intravaginal tem um efeito luteotrófico limitado e não foi capaz de elevar as concentrações plasmáticas de progesterona, como ocorre em cabras tratadas com hCG pela via intramuscular.

No capítulo 4, a administração de 300 UI de hCG sete dias após o início do estro em cabras leiteiras em anestro resultou na formação de aCL e teve efeito hipertrófico no CL existente (ovulatório). Consequentemente, as concentrações plasmáticas médias de P4 foram maiores em coelhas tratadas com hCG durante o período de reconhecimento materno da gestação. Uma única dose de hCG aumentou a taxa de gestação em cabras leiteiras induzidas por estro sazonalmente anovular submetidas a FxTAI em 22,5%.

No capítulo 5, a administração de 300 UI de hCG intramuscular nos dias 5 ou 7 do ciclo estral de cabras induzidas por estro na estação não reprodutiva foi capaz de induzir a formação de corpos lúteos acessórios, promover aumento da área lútea dos corpos lúteos originais e aumentar a concentração plasmática. de progesterona, porém, não houve diferença nesses efeitos entre os dias de aplicação.

Desta forma, uso da hCG se mostrou promissor para melhorar a eficiência reprodutiva de cabras leiteiras, no entanto, os resultados variam de acordo com a via e momento do ciclo estral em que o hormônio é administrado.